1 INTRODUCTION

Nature has along with the disease created their cure in the form of vegetables and minerals. According to the Eccleriates “The lord created medicines out of the earth and a wise man will not adore them”¹.

Indian subcontinent is enriched by a variety of flora—both aromatic and medicinal—because of wide variety of climatic factors. Right from the high ranges of Himalayan tract upto the seashore of Kanya Kumari numerous types of plants well recognized by botanists and the local people are actually the living treasure of this country.

The wonderful reference and treatises on herbal cure that have been available in India mention the work of Dhanwantri, Nagarjun and Charak. Most of the Bhikshus of Buddhists Mona steries actually maintained the nursery of medicinal herbs around them and they went abroad they carried these herbs around them and when they went abroad they carried these herbs for the welfare of common man in those lands².

The therapeutic uses of medicinal plants have gained considerable momentum in the world during the past decade. The over use of synthetic drugs with impurities, resulting in higher incidence of adverse drug reactions, has motivated mankind to go back to nature for safer remedies. However, it should be ensured that commercial formulations based on medicinal plants are safe, effective and of less side effects³.

About one third of all pharmaceuticals are derived from plants and over 60% pharmaceutical preparations are plant origin based. Side effects of plants derived drugs are less than synthetic substances. Vane has pointed out that out of every 3000 chemicals synthesized only one is likely to be successful in its clinical trials. India is known to possess about 2000 types of medicinal plants and those containing essential oils⁴.
Earlier days the drugs from the vegetable kingdom were used in the form in which they occurred as fresh or dried plants. Later these were used in the form of extracts. Presently isolation of active and constituents their application in pure form in therapeutic became more and more common. It is known that pure compounds have constant biological effects, which is not influenced by other components. It is also easier to obtain the exact dose with the pure compound and the analytical control is also easier to perform.

The development of spectroscopic methods for the elucidation of chemical structure of naturally occurring compounds together with the development of biological sciences has opened new ways to study the relationship between chemical structure and biological activity. It has become possible to prepare derivatives, to use such compounds as starting materials for semi synthetic new drugs. A recent study conducted by world Federation of proprietary manufacturer, shows that large number of people increasingly prefers plant based medicines for curing their ailments. Medicinal plants are widely used in the pharmaceutical industry as therapeutic agent or as building blocks for semi-synthetic drugs.

India had a rich heritage of medicinal plants in the Ayurvedic and Unani systems of medicine besides use of many plants in folk remedies. The major population of India relives heavily on the use of herbal remedies for treatment of diseases an active interest in the chemistry and pharmacology of plant drugs started in early fifties of and has been gaining momentum steadily. In view of the extensive use of plant derived remedies, there has been the subject of research in India in recent years to make better use of these remedies and to use them as resource material. The general research approaches of crude extracts, bio investigations and identification of active principles, toxicological and clinical studies, standardization and uses of active moiety as the lead molecule for the drug design. Two approaches have been followed with view to verify scientifically the claims of traditional system of medicine and to develop new drugs which would
be acceptable to modern system of medicine. The first approach involves the
direct clinical trials of the more commonly used traditional remedies followed by
chemical and pharmacological studies. In the second there is broad spectrum
screening of individual plants followed by chemical, pharmacological, pre-clinical
and clinical drug development studies. Sir Ram Nath Chopra, a pharmacologist at
the school of tropical medicine, Calcutta, was a pioneer in research in plants used
in the traditional system of medicine.

**Hoary past of herbal drugs:**

During the thousands of years human existence many natural material were
identified for combating human ailments either by instinct or intuition or trial and
error the earliest mention of the medicinal uses of plants has been found in
“Rig Veda”, which were written in between 4000 and 1600 B.C. In the
“Atharva Veda”, we find the more varied uses of drugs. It is in the “Ayurveda”,
which is considered as an “Upa Veda” that definite properties of drugs and their
uses have been given in great detail. “Charak Samhita” is another earliest treaties
on Ayurveda (600 B.C.), which lists total of 341 plants and plant products for use
in health management. “Susruta Samhita” also dealt with plants related to
medicine. Dhanvantri and Nagarjuna were well known persons with an intimate
knowledge of the characteristics of medicinal plants.

**Why herbal Remedies?**

Their effectiveness, easy availability, low cost, and comparatively being
devoid of serious toxic effects (time tasted) popularized herbal remedies.
Need and scope of Herbal Therapy:

Today we are more concentrated with the life style disease like depression, cancer and heart troubles caused by faulty nutrition and stress. The need of alternative therapy, cover will be one of the best practices to over the illness.

Traditional India Practice held that certain drugs should be formulated through the addition of chosen substances that enhance bioavailability of the drug. Recent work particularly in two Indian modern biology labs, has confirmed this bioavailability enhances ability to pepper and point to the active component as the molecule piperine.

An anti-TB drug Rifapicin has to be given at higher dose that required, in order compensating for losses on the way to the target site. Formulation of piperine with rifampicine will have the drug and counter effects.

Herbal oriented Pharmaceutical companies like Dabar, Zandu and the Himalaya Drug Company are investing crores of rupees on researching, developing and popularizing OTC remedies 7.

Efficacy, Self-Medication and Safety:

Efficacy of traditional medicine is one of the most debated issues there are Philosophical, cultural, technical, methodological, and practical aspects involved in efficacy evaluation. For herbal medicine, some of the best known evidences for efficacy of an herbal drug include Artemisia annua for the treatment of malaria, St John’s wort for the management of mild to moderate depression. Patient usually experiences fewer side effects than when treated with anti –depressant, such as amtriptyline 8.

Many of the plants used in herbal medicine contain principles whose effect can be demonstrated pharmacologically and the action of the whole plant extract can usually be related to that of the isolated constituents. However, for most of the
herbal remedies it is not possible to demonstrate or evaluate their pharmacological activity, and the situation is complicated by the frequent use of a number of drugs in combination. The supposed active constituents of which to disregard many such “Polypharmaceutical” preparation and concentrated research on the isolation of individual components having demonstrable activity like anti-cancer, hypotensive and anti-microbial properties.

Herbal remedies, being natural products, are inherently safer than the potent synthetic drugs, which often produce undesirable side effects. Thus, comfrey always considered a ‘safe’ herb, has been found to contain small quantities of pyrrolizidine alkaloids which are known to be hepatotoxic and which when administered to rats, causes liver cancer, reserpine, an alkaloid of *Rauwolfia serpentina* has been associated with breast cancer.\(^8\)

*Actiniopteris australis* (Syn: *A. radiata; A. dichotoma*), a true fern, listed among endangered and fast disappearing plants and *Caralluma adscendens*, found to be little explored with regards to its medicinal uses, reproductive methods and chemical constituents. Even though some pharmacological studies had been reported earlier, they were found to be inadequate to establish beyond doubt, biological activities and medicinal uses this plant, due to contradictions and non-uniformity in their reports. For example, anthelmintic activity of plant extract reported by Bhatnagar, et al.,\(^9\) was later contradicted by K. C. Singhal’s studies in mice.\(^10\) Similarly, antifertility activity reported by Dixit and Bhatt\(^11\) by studies in human volunteers was contradicted just in a year’s span by B. N. Dhawan et al’s.,\(^12\) studies in rats. Adding to this, the fact that most of the activity studies had been reported in 1970s, underlines the significance of elaborate pharmacological evaluation of this plant for more precise and accurate findings.

Although preliminary chemical studies are reported in 1970s\(^{13-15}\) an extensive study is needed in this area for isolation and characterization of chemical constituents. Most of the chemical characterization studies reported was almost 30
years back. These studies used simple extraction and column chromatography for isolation of chemical constituents and M.P, I.R and routine qualitative tests for identification and characterization. An extensive study using sophisticated equipments like LC-MS, HPLC, HPTLC, NMR etc. may bring out the presence of various other constituents also.

Another area of interest is the heavy metal content in this plant and its relation to pharmacological activity and other parameters like LD$_{50}$. The plant has shown to absorb heavy metals like Arsenic from the atmosphere, which have deleterious effects in human beings$^{16}$. Also this plant have shown uptake of Selinium$^{17}$. Since $A$. radiata is an endangered species, detailed study on other aspects like xerophytic adaptation, reproductive methods, resurrection habit etc., may also be appreciated.

Much exploration still needed for this plant, signifies the selection of this topic.

1.1 Taxonomical position of plant:

1.1.1 *Actiniopteris Radiata* (*Actiniopteris australis*):

A simpler classification can be given as under to give an outlook into the taxonomical position of *Actiniopteris radiata*.

<table>
<thead>
<tr>
<th>Superkingdom</th>
<th>Eukaryota</th>
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<tbody>
<tr>
<td>Kingdom</td>
<td>Viridiplantae</td>
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<tr>
<td>Phylum</td>
<td>Embryophyta</td>
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<tr>
<td>Class</td>
<td>Filicopsida</td>
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<tr>
<td>Order</td>
<td>Filicales</td>
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<tr>
<td>Family</td>
<td>Pteridaceae</td>
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<tr>
<td>Genus</td>
<td><em>Actiniopteris</em></td>
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<tr>
<td>Species</td>
<td><em>Radiata</em></td>
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Classification: (According to: *NCBI Taxonomy*) is as follows\(^{18}\).

Kingdom                  Viridiplantae  
Subkingom              Streptophyta  
Phylum                    Embryophyta  
Division                Moniliformopses  
Class                        Filicopsida  
Subclass                  Pteridaceae  
Genus Actiniopteris  
Species Actiniopteris australis

Michael Hassler and Brian Swale have given further details about the class *Filigopsida*, order *Pteridales*, family *Pteridaceae*, Subfamily *Actiniopteridoideae*, genus *Actiniopteris* and various species coming under that genus in the world species list\(^{19}\). Some relevant data from the above said source is given below.

### 1.1.2 Caralluma adscendens:

Kingdom Plantae - Plants  
Subkingdom Tracheobionta – Vascular plants  
Superdivision Spermatophyta – Seed plants  
Division Magnoliophyta – Flowering plants  
Class Magnoliopsida – Dicotyledons  
Subclass Asteridae  
Order Gentianales  
Family Asclepiadaceae – Milkweed family  
Genus Caralluma R. Br. – caralluma  
Species Caralluma adscendens
1.2 An introduction to Pteridophyta:

a) Fern:

A fern is any one of a group of about 20,000 species of plants classified in the division Pteridophyta, also known as Filicophyta. The group is also referred to as polypodiophyta, or polypodiopsida when treated as a subdivision of tracheophyta (vascular plants). The study of ferns is called pteridology; one who studies ferns is called a pteridologist. The term pteridophytes has traditionally been used to describe all seedless vascular plants so is synonymous with "ferns and fern allies". This can be confusing given that the fern phylum Pteridophyta is also sometimes referred to as pteridophytes.

The pteridophytes are a collection of several major groups at the division or class level that rank alongside the Bryophyta (mosses and liverworts) and the Spermatophyta (seed-plants). There are 4 such groups, one of which is the true ferns, the remainder the fern allies.

- Psilotophyta (or Psilotopsida or Psilopsida)
  - Psilotaceae
- Lycopodiophyta (or Lycopodiopsida or Lycopsida)
  - Lycopodiaceae
  - Selaginellaceae
  - Isoetaceae
- Equisetophyta (or Equisetopsida or Sphenopsida)
  - Equisetaceae
- Polypodiophyta (or Polypodiopsida or Filicopsida)
  - Families of true ferns

Key to the major groups is given below.
1 Leaves microphyllous, simple or once-divided, with a single vein and no petiole; sporangia solitary or in 2 - 3-celled synangia, axillary or on peltate sporophylls (fern allies)...

Leaves megaphyllous, variously divided with several to many veins; sporangia mostly in clusters of several to many, abaxial or on special outgrowth of the lamina (true ferns - Polypodiophyta )...

2 2 – 3 (rarely 4) sporangia fused into a single synangium, and borne in the axils of minute and bract-like or larger and leaf-like bifid sporophylls; homosporous; axis not differentiated into roots (Psilotophyta)...

Sporangia free, borne axillary on sporophylls which are often condensed into strobili; homosporous or heterosporous; axis with differentiated roots.

Psilotaceae

3 Plants cylindrical with regular sheathed nodes, scariose, bract-like leaves borne in a single whorl at each node, the stem photosynthetic, hollow, containing many silica crystals; homosporous, spores chlorophyllums (Equisetophyta) Equisitaceae

Plants not cylindrical, bearing expanded photosynthetic leaves; homosporous or heterosporous, spores not chlorophyllous (Lycopodiophyta)...

4 Aquatic (occasionally terrestrial) rosette herbs with a thickened, compact, corm-like axis; leaves linear with 4 large longitudinal air-chambers

Isoetaceae

Terrestrial or epiphytic herbs, never rosettes nor corm-like,
axis elongate and mostly branched; leaves short, without air-chambers

5 Heterosporous; leaves ligulate, 4-ranked, decussate but mostly dorsiventral with 2 larger rows beneath and 2 smaller rows above; stem often supported by stilt-like rhizophores at intervals...

Selaginellaceae

Homosporous; leaves eligulate, mostly arranged in close spirals, sometimes decussate, sometimes dorsiventrally flattened with all but 2 rows reduced; supported by clusters of roots

Lycopodiaceae

A fern is a vascular plant that differs from the more primitive lycophytes in having true leaves (megaphylls), and from the more advanced seed plants (gymnosperms and angiosperms) in lacking seeds.

Like all vascular plants, it has a life cycle, often referred to as alternation of generations, characterised by a diploid sporophytic and a haploid gametophytic phase. Unlike the gymnosperms and angiosperms, in ferns the gametophyte is a free-living organism. The life cycle of a typical fern is as follows:

1. A sporophyte (diploid) phase produces haploid spores by meiosis.
2. A spore grows by cell division into a gametophyte, which typically consists of a photosynthetic prothallus.
3. The gametophyte produces gametes (often both sperm and eggs on the same prothallus) by mitosis.
4. A mobile, flagellate sperm fertilizes an egg that remains attached to the prothallus.
5. The fertilized egg is now a diploid zygote and grows by mitosis into a sporophyte (the typical "fern" plant).

(b) Fern structure:

Like the sporophytes of seed plants, those of ferns consist of:

- **Stems**: Most often an underground creeping rhizome, but sometimes an above-ground creeping stolon (e.g., Polypodiaceae), or an above-ground erect semi-woody trunk (e.g., Cyatheaceae) reaching up to 20 m in a few species (e.g., *Cyathea brownii* on Norfolk Island and *Cyathea medullaris* in New Zealand).

- **Leaf**: The green, photosynthetic part of the plant. In ferns, it is often referred to as a frond, but this is because of the historical division between people who study ferns and people who study seed plants, rather than because of differences in structure. New leaves typically expand by the unrolling of a tight spiral called a crozier or fiddlehead. This uncurling of the leaf is termed circinate vernation. Leaves are divided into two types:
  - Trophophyll: A leaf that does not produce spores, instead only producing sugars by photosynthesis. Analogous to the typical green leaves of seed plants.
  - Sporophyll: A leaf that produces spores. These leaves are analogous to the scales of pine cones or to stamens and pistil in gymnosperms and angiosperms, respectively. Unlike the seed plants, however, the sporophylls of ferns are typically not very specialized, looking similar to trophophylls and producing sugars by photosynthesis as the trophophylls do.
• **Roots:** The underground non-photosynthetic structures that take up water and nutrients from soil. They are always fibrous and are structurally very similar to the roots of seed plants.

The gametophytes of ferns, however, are very different from those of seed plants. They typically consist of:

• **Prothallus:** A green, photosynthetic structure that is one cell thick, usually heart- or kidney-shaped, 3-10 mm long and 2-8 mm broad. The thallus produces gametes by means of:
  - **Antheridia:** Small spherical structures that produce flagellate sperm.
  - **Archegonia:** A flask-shaped structure that produces a single egg at the bottom, reached by the sperm by swimming down the neck.
  - **Rhizoids:** root-like structures (not true roots) that consist of single greatly-elongated cells, water and mineral salts are absorbed over the whole structure. Rhizoids anchor the prothallus to the soil.

(c) **Distribution:**

A large group of ancient or primitive land plants with worldwide distribution[^22-28], all continents except Antarctica and most islands, favoring moist temperate and tropical regions, they can be found in all but the most frigid and most arid environments. Free water is required for fertilization and the production of a new generation of plants. There are no marine species.

(d) **Reproduction and Life Cycle of Ferns and their Allies:**

The life cycle and reproduction of the pteridophytes or ferns and their allies is characterized by a complete absence of conspicuous flowers and fruit, the production of minute fine spores, often in copious quantities, and by alternating
generations of separate spore producing plants (sporophytes) and gamete producing plants (gametophytes)\textsuperscript{29}.

In these respects they are similar to the structurally less complex bryophytes or mosses and liverworts. In the bryophytes the gametophyte is the dominant more conspicuous and long-lived generation; the sporophyte is almost completely dependent on the gametophyte for support and nutrition for its entire lifespan. In the pteridophytes the sporophyte generation dwarfs and overtakes the gametophyte almost immediately and is totally independent of it for support and nutrition; it is the most commonly observed component that is recognized as a fern or fern ally.

The general cycle involves the production of asexual spores on the sporophyte which are shed and germinate to grow into gametophytes which produce male organs with motile sperm and female organs with non-motile egg cells. Fertilization follows and the egg cell develops into a new sporophyte to complete and continue the cycle.

Some pteridophytes dispense with the sexual aspect of the development cycle entirely in a process known as apogamy whereby a haploid sporophyte develops directly from the prothallus. It is not common but has been reported in a wide range of genera such as \textit{Asplenium}, \textit{Cheilanthes}, \textit{Pellaea}, \textit{Pteris}, \textit{Trichomanes}, \textit{Isoetes}. Sporelings develop directly from the prothallus without fertilization.

Given that pteridophytes produce so many easily dispersed spores, why do not all ferns and their allies occur everywhere and why aren't all land areas covered in them? The reproductive strategy of a pteridophyte is not particularly efficient or targeted and is very much a hit and miss affair. Although a mature plant may produce many millions of spores that may be dispersed hundreds of kilometers by favourable winds, a spore must land in a suitable microhabitat in order to germinate into a sexual prothallus. The viability of spores is anything from a few days to a few months; spores that are green and contain chlorophyll are short-
lived. The environmental conditions surrounding the prothallus must allow fertilization, and once fertilization is complete, conditions must allow development of the sporeling. As the sporeling grows out of the microhabitat of the prothallus, it must grow into an environment capable of supporting the mature plant, the requirements of which are quite different.

Figure 1: Life cycle of a typical fern; illustration by Murray Fagg.
**Gametophyte:**

The gametophyte generation, or prothallus, in ferns and their allies is nearly always short lived and inconspicuous. Germination from the spores, the prothallus is mostly only a few mm across, photosynthetic, generally simple in structure, without vascular tissue and often only one cell thick for the most part, tapering to many cells thick at the centre. They may be attached to the substrate by fine multicellular root hairs or rhizoids, and often resemble thallose liverworts. The prothallus has a single set of chromosomes (i.e. haploid) and is the sexual part of the life cycle. The sexual organs are microscopic or nearly so. The male organs or antheridia produce and shed numerous motile sperm or antherozoids. These antherozoids are armed with hair-like or whip-like cilia or flagellae and are able to swim through water; they do not travel great distances and are only released when free water is available. Only a continuous film of water is needed. They antherozoids are chemically attracted to the developing flask-like female organs or archegonia where fertilization of the single egg cell embedded in each archegonium takes place. On a given prothallus in some species the antheridia sometimes mature before the archegonia to reduce the risk of self fertilization. The fertilized egg cell or zygote contains a double set of chromosomes, one from the egg cell, one from the antherozoid, and is thus diploid. It repeatedly divides mitotically, becoming an embryo, developing in to a sporeling, then into juvenile plant and eventually into a mature diploid spore-bearing sporophyte. The embryo and sporeling is initially parasitic and dependent on the gametophyte, but rapidly develops roots of its own and becomes an independent photosynthetic plant as the prothallus withers and dies. Antheridia and archegonia may be borne on the same prothallus (monoecious), or in the case of heterosporus species, on separate male and female prothallia (dioecious).
Gametophytes have an ecology of their own which may be quite different to that of the sporophyte. They may be superficial or subterranean, and in some megasporous taxa, gametophyte development takes place within the spore wall. While the more robust sporophyte may be able to exist in a wider range of conditions, it may be the habitat requirements of the gametophyte that determines the distribution of a pteridophyte species. This is one of the reasons that ferns are most commonly found in moist shady places. Some ferns have adapted to arid environments, but they still need wet periods to produce gametophytes and complete fertilization.

**Sporophyte:**

The sporophyte generation in ferns and their allies is long-lived compared to the gametophyte. It is mostly larger and structurally more complex in that it has a vascular system of xylem and phloem, often associated structural supporting tissue and is mostly organized into highly specialized stems, leaves and roots. The aerial parts are protected by a waxy cuticle and are thus able to withstand greater degrees of exposure than the gametophyte; stomata are present allowing the exchange of gases between internal chambers and the external environment. The sporophyte has a double set of chromosomes (i.e. *diploid*) and is the asexual part of the life cycle.

At maturity the sporophyte develops specialized structures of varying complexity on the leaves called **sporangia** in which the **spores** are produced; a number of sporangia may be aggregated into structures called **sori**. Spores consist of a single cell surrounded by a durable cell wall; they are produced by meiotic division in which the number of chromosomes is halved and are hence haploid. Spores are minute, non-motile and often produced in large numbers; they are shed by rupturing of the sporangial wall and can be dispersed long distances by wind or water. When mature and under appropriate conditions the spores are dispersed
widely into the environment, and if they land in a suitable place germinate to create the next generation of gametophytes, completing the cycle.

Most pteridophytes produce spores of only one size (homosporous), but a significant number of species produce spores of two distinct sizes (heterosporous): minute microspores and relatively very large megaspores. Microspores produce the male antheridia and megaspores produce the female antheridia.

**Vegetative reproduction:**

In addition to the sexual gametophyte-sporophyte life cycle, some of pteridophytes have developed various vegetative means of propagation to increase the extent and number of their population. This is advantageous where seasons are unreliable or the environment is otherwise not conducive to gametophyte production, and in some cases it may just be advantageous to generate numerous genetically identical individuals.

A number of quite unrelated species produce vegetative buds or bulbils that are capable of producing roots and new plants. These bulbils are leaf derive structures and are produced in leaf axils or at various places on the leaf surface. Plants may chain along as successive bulbils take root, establish themselves and produce fronds and bulbils of their own. Proliferating bulbils can be found in *Asplenium*, *Camptosorus*, *Diplazium*, *Polystichum*, *Ampelopteris*, *Huperzia* and a number of other genera.

Some species with long creeping rhizomes can extend over large areas and it may not be obvious that plants on one side of a population were physically derived from plants on another. Large brakes or open areas can be covered by scrambling ferns with creeping underground rhizomes in this manner; Gleicheniaceae genera (*Gleichenia*, *Stichurus*, *Dicranopteris*) and bracken (*Pteridium*) are good
examples. Similarly populations of climbing epiphytes may be increased in extent and mass as rhizomes branch, break and regrow; examples include genera of Polypodiaceae (*Pyrrosia, Lemmaphyllum, Microsorum*). The floating ferns *Salvina* and *Azolla* are striking examples of propagation by stem fragmentation. Proliferation is possible from root stolons, tubers and similar structures. This occurs in some species of *Nephrolepis* and *Blechnum*.

More of a survival strategy than a means of propagation, some ferns of arid areas is able to dry out almost completely without actually dying. Crisp and brittle to touch, they resuscitate rapidly when rains come and continue their growth. A number of species of *Cheilanthes* behave these way commonly called resurrection ferns.

**(e) Cultivation and Propogation of Ferns**

Cultivation and propogation of ferns is an important field of interest for a pteridologist, especially when he is doing research on an endangered fern species. Various techniques involved are as under.

**i) Native Ferns for Cool Climates:**

There are a number of Australian native fern species, which are relatively easy to grow out door sin cool climate areas. Ferns require good drainage and it is important that some form of protection, such as overhanging trees, shrubs, a garden wall, the wall of a house or shade cloth, is provided. Where there are extremes of dry heat and cold, suitable growing conditions can be developed by enclosing areas sufficiently to keep the humidity high. When planning the site for a fern garden, easterly and southerly aspects are preferable. The majority of ferns grow best in filtered sunlight and although some will tolerate direct sunlight, they should not be subjected to it for long periods. Frost damage may occur to fronds, but it will not be as severe if regular watering is kept up throughout winter.
Damage to foliage may also occur during long periods of dry heat and wind. Damaged fronds will not recover and should be removed.

**ii) Preparation of Soil:**

Ferns generally grow best in slightly acid soils which contain plenty of organic matter. Good soil conditions can be created by digging to spade depth and incorporating liberal quantities of coarse peat moss, well-rotted leaf-mould or compost. The structure of heavy soil may be improved by the addition of gypsum and coarse sand.

**iii) Planting:**

Spring is the best time for planting, although a number of species do equally well if planted in early autumn. Mulching is important to keep weeds down and the soil moist. *Eucalyptus* leaf litter, hardwood shavings, weathered she oak (*Casuarina* and *Allocasuarina*) needles or oak leaf litter make good mulches. Mulches should be topped up regularly.

**iv) Fertilizing:**

Ferns are gross feeders and fertilizers are best applied during the warm months when plants are growing. Blood and bone or liquid organic fertilizers such as fish emulsion are suitable. Iron deficiency is common in ferns and can be corrected by applying iron chalets to the soil at the recommended rate. Typical symptoms are yellowing and bleaching of young fronds although, these symptoms may also occur in areas where there is high light intensity.
v) Maintenance:

Apart from the removal of dead fronds, ferns do not require a great deal of maintenance and, once established, the soil surface should be disturbed as little as possible. Daily watering is necessary while plants are young, even during winter. This should consist of deep soaking about once a week followed by a light daily watering. During the summer, watering needs to be consistent. Automatic sprinkler and drip irrigation systems are ideal for this purpose.

vi) Propagation:

Some ferns are readily propagated by means such as division of the rhizomes (under ground stems) or crowns, layering and plantlets. Division is best carried out just prior to the active growth period in spring. The rhizomes can be cut into lengths and those with a growing tip can be potted up. Fronds attached to the divisions may die but new fronds will grow. Some ferns, such as Asplenium bulbiferum and Polystichum species, produce small plantlets or bulbils along the stem or on, or near, the tip of the fronds. Plantlets can be pegged onto the ground and removed from the parent plant once roots are developed.

Ferns may also be propagated from spores.

vi) Pests:

Some common pests of ferns and the method of control are listed. Many ferns, especially fine-foliaged species, are sensitive to pesticides and care should be exercised in their application. When using pesticides, safety directions should be followed carefully. Caterpillars (late summer) can be are controlled by crushing the animals by hand, or, in cases of severe infestation, a spray such as Carbaryl
may be applied. Slugs and snails require regular baiting with snail bait, especially after wet weather.

Aphids may attack new fronds especially in spring and autumn and can be controlled with a contact spray such as those based on Pyrethrum extract. Scale insects are generally found on stems and on the undersurfaces of fronds. They may be brushed from the surface using a toothbrush or, in cases of severe infestation, white oil and 'Rogor 40' or 'Folimat' may be used. Foliage 'burn' may occur if these pesticides are used when temperatures exceed 25 °C.

(f) Growing ferns from spores

Ferns do not flower but reproduce sexually from spores. There are two distinct stages of the fern life cycle. Mature plants produce spores on the underside of the leaves. When these germinate they grow into small heart-shaped plants known as prothalli. Male and female cells are produced on these plants and after fertilization occurs the adult fern begins to develop.

i) Collecting spores:

To collect spores place a portion of mature frond on a piece of paper in a dry place. If spores are ripe they will be shed onto the paper and will appear as black, brown or yellow 'powder' which is a mixture of spores and fragments of the spore cases (sporangia).

ii) Sowing method:

It is important to sterilize the germination mixture before sowing the fern spores by pouring boiling water over it this kills the spores of fungi and other plants that may germinate and crowd out the developing fern prothalli. Spores should be sparsely sprinkled on a medium such as finely chopped tree fern fibre, peat moss or sphagnum moss. An equal part of loam, peat moss and finely crushed terracotta
spread to a depth of 2cm over a base of vermiculite also forms an excellent germination base. Once sown the containers should be covered with plastic or glass (allowing some airspace) and kept at around 20 degrees C in indirect light. Spores take from 2 to 6 weeks to germinate.

iii) Potting on:

After a few weeks the germinating spores appear as a mossy growth. When the prothalli are formed and well developed they may be picked off into a punnet containing a finely sifted soil mixture. The container should be covered with glass or plastic until the fronds appear. The developing ferns should not be exposed to direct light. Further potting up should be into a coarser textured mix with relatively high levels of organic matter. The mix should drain well. Materials such as Sharp River sand and gravel-sized charcoal pieces may be useful for this purpose.

(g) Economic uses of Ferns:

Ferns are not as important economically as seed plants but have considerable importance. Ferns of the genus Azolla are very small, floating plants that do not look like ferns. Called mosquito fern, they are used as a biological fertilizer in the rice paddies of Southeast Asia, taking advantage of their ability to fix nitrogen from the air into compounds that can then be used by other plants. A great many ferns are grown in horticulture as landscape plants, for cut foliage and as houseplants, especially the Boston fern (Nephrolepis exaltata). Several ferns are noxious weeds or invasive species, including Japanese climbing fern (Lygodium japonicum), mosquito fern and sensitive fern (Onoclea sensibilis). Giant water fern (Salvinia molesta) is one of the world’s worst aquatic weeds. The important fossil fuel coal consists of the remains of primitive plants, including ferns.
Other ferns with some economic significance include:

- *Dryopteris filix-mas* (male fern), used as a vermifuge.
- *Rumohra adiantoides* (floral fern), extensively used in the florist trade.
- *Osmunda regalis* (royal fern) and *Osmunda cinnamomea* (cinnamon fern), the root fiber being used horticulturally; the fiddleheads of *O. cinnamomea* are also used as a cooked vegetable.
- *Matteuccia struthiopteris* (ostrich fern), the fiddleheads used as a cooked vegetable in North America. *Pteridium aquilinum* (bracken), the fiddleheads used as a cooked vegetable in Japan and are believed to be responsible for the high rate of stomach cancer in Japan.
- *Diplazium esculentum* (vegetable fern), a source of food for some native societies.
- *Pteris vittata* (brake fern), used to absorb arsenic from the soil.
- *Polypodium glycyrrhiza* (licorice fern), roots chewed for their pleasant flavor.
- Tree ferns, used as building material in some tropical areas.
- Bracken fern often poisons cattle and horses.
- *Cyathea cooperi* (Australian tree fern), an important invasive species in Hawaii.
- *Dryopteris filix-mas*, this fern accidentally sprouting in a bottle resulted in Nathaniel Bagshaw Ward's 1829 invention of the terrarium or Wardian case.
- *Ceratopteris richardii*, a model plant for teaching and research, often called C-fern.
1.2.3 Introduction to genus *Actiniopteris*:

The term Actiniopteris has its origin from the Greek *aktis* (ray) and *pteris* (fern); refers to the *radiating leaf segments*. Genus Actiniopteris comes under the family Pteridaceae; subfamily Actiniopteridoideae. According to NCBI taxonomy, subfamilyActiniopteridoideae have four major species under it namely *Actiniopteris dimorpha* (which again have 2 subspecies namely A. dimorpha and A. diversiformis), *Actiniopteris paucibola, Actiniopteris radiata* and *Actiniopteris semifbellata*. Some other sources provide extended information on species and subspecific taxa of Actiniopteris by citing more species like *Actiniopteris kornasii* and *Actiniopteris australis*. There is also speculation that *Actiniopteris australis* is a variety under the species *Actiniopteris radiata*. Sometimes the term *Actiniopteris australis* is used as synonym for *Actiniopteris radiata*, but more authentic sources have identified them as a different variety due to the difference in the base sequence of some ribosomal protein genes (rps4, rbcL etc.). More elaborate research is due to define a non-controversial taxonomical position for *Actiniopteris australis*. 
1.2.4 Introduction to species:

a) *Actiniopteris australis*.

Vernacular names:

English: Peacock’s tail
Hindi: Mayursikha, Morpankhi
Sanskrit: Mayursikha

Other local names: Mapursika (Bombay)
Morpach (North-Western Provinces of India)
Nanmukhappullu (Kerala)
Nemali adugu (Andhra Pradesh)

**Geographical Distribution:**

It is a tiny terrestrial fern, found throughout India. It is also found in Burma, Sri Lanka, Afghanistan, Persia, Arabia, Yemen, South Eastern Egypt, Tropical Africa, Australia and Madagascar.

**Habit and habitat:**

*Actiniopteris australis* is a xerophytic species of the fern family Actiniopteridaceae. It is of limited distribution, and in areas where it occurs, is restricted to depleted walls and rocky crevices of steep slopes of exposed hilly areas, up to the altitude of 1200 m \(^{39}\). Black or reddish gravelly soil is best suited for its growth.

Like other plants in Pteridophyta, life cycle of *Actiniopteris australis* also shows alternation of generations of separate spore producing plants (sporophytes) and gamete producing plants (gametophytes). Sporophyte generation (the leafy plant) dominate the gametophyte generation (prothallus).

Sporophyte of *Actiniopteris australis* is a tiny terrestrial plant of 6-17 cm height, rooting in rock crevices in moist damp shade situation \(^{40}\). Rhizome of the
plant is short, erect to sub-erect, clothed with many smooth scales and having the
diameter of approximately 4 mm. Rhizome is dark to pale brown in colour. Stipes
are greenish to hay coloured, 5-15 cm long, fronds small, palm-shaped,
dicotomously dissected into fine segments: segments linear, fertile fronds larger
than sterile ones; sori sub-marginal, dark brown, covering the whole lower surface
of the fertile segments and are abundant in nature. These dichotomous segments
are having size approximately 2-3 x 1-2 cm; texture subcoraceous, edges refluxed
when dry, apex toothed, veins a few running sub-paralleled to inconspicuous
midrib. The spores are trilete with slightly convex sides and rounded corners. The
laesurae are long, crassi marginate with undulate surface, spore bear large
irregular closely-set verucae-like ridge with wavy margins. They are yellowish to
dark brown when mature. The average dimension of the spores are
49.39x54.83 µ. Normally the fern is triploid but occurrence of diploid, apogamous
specimens has also been reported.

Gametophyte of Actiniopteris australis has been studied by S. Bhambie
and C. X. George in 1972. On germination, the spores (from sporophyte) forms a
densely chlorophyllous germ-filament composed of 3-8 short barrel-shaped cells.
(figure-3)
The first rhizoid appears when it is in the form of filament. (figure-3) The anterior
cells of the germ-filaments including the terminal cells divide longitudinally
initiating the formation of a prothallial plate. (figure-4) In the beginning all the
cells except a few basal ones undergo division. The prothallial plate soon enlarges
and sometimes become cordate with apical notch. (figure-7,8) In other cases,
either it develops into a ribbon-shaped structure (figure-9) or into an asymmetrical
(figure-12, 13) or variously lobed thallus. (figure-10,11) The occurrence of
different shapes of prothalli within one species is an interesting and unique
feature. Another characteristic feature of most of the prothalli is the presence of
highly thick walled cells at the basal region from where the rhizoids arise. ((figure-12-16)

**Figure 2: stages in the development of prothallus of A. radiata**

In brief, prothalli of Actiniopteris shows a number of interesting features, viz. different shapes of mature prothalli, occurrence of thick walled cells at the basal region, procambial-like tissue in older prothalli and even the conversion of young detached leaf-tip into prothallus. These characters can be correlated with the xeric habitat of the plants as it has to face very adverse conditions during drought\textsuperscript{44, 45}.
b) *Caralluma adscendens* :

The genus *caralluma* (*asclepiadaceae*), which are comprises about 200 genera and 2500 species. The member of the genus is small plant, erect, fleshy. They have four grooved stems, round shape devoid of leaves and small flowers in several varieties of dark colors. The species of *Caralluma* found in India are edible and form part of the traditional medicine system of the country. *Caralluma fimbriata* is known for being an appetite suppressant, antidiabetic, antipyretic, and analgesic anti-inflammatory. *Caralluma attenuata* is eaten raw as a cure for diabetes and migraine along with black pepper. In addition to Caralluma species commonly used in treatment of rheumatism, diabetes, leprosy, antipyretic and anthelmintic, for tumor, fungal diseases, snake, scorpion bite and antinociceptive activity 46-48.

1.3 Introduction to Pain:

Pain is one of the most important human experiences, and also one of the most complexes. The international association for the study of pain (1986) has defined pain as;

“An unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage”**49**.

“Nociception can be defined as response specific to potentially tissue damage stimulation”. It is the mechanism whereby noxious peripheral stimuli are transmitted to the central nervous system. Pain is a subjective experience, not always associated with nociception.

Pain may be of acute or chronic type. Acute pain is generally well accounted for in terms of nociception giving rise to an intense and unpleasant sensation. In contrast, most chronic pain states are associated with aberrations of the normal physiological pathway, giving rise to hyperalgesia (an increased amount of pain associated with a mild noxious stimulus), allodynia (pain evoked by a non-noxious), or spontaneous spasms of pain with on precipitating Stimulus**50**.
The recent evidences suggest that pain may be postulated to exist in three different groups of processes, each predominating in different painful disorders. These involve,

1) Nociceptive pain
2) Neuropathic pain, and
3) Psychogenic pain.

Nociceptive pain may occur as a secondary phenomenon caused by a non-neurologic source of continuing noxious stimulation in the periphery.

Neuropathic pain may be generated directly by the disordered or damaged nervous system.

Neuropathic pain comprises of a number of very distinct subtypes, all of which are precipitated by neural injury. The important sub-types are:

1. Deafferentiation pain – that depend on the different function of the sympathetic nervous system, and
2. Sympathetic maintained pain – that is related to the ongoing activity in a peripheral focus of aberrant neural activity.

In case of psychogenic pain, the psychological factors play an important role in generating or magnifying pain even when other identifiable neurologic or peripheral causes exist.

1.4 Introduction to Inflammation:

Inflammation is fundamental pathophysiological response of the host involving a series of events to eliminate noxious stimulus, such as radiant, chemical, Physical infection and immune provocation.

Man owes to inflammation and repairs his ability to contain injuries and reconstitute defects. Inflammation is best defined as the local reaction of vascularized tissue to injury.

Cornelius Celsus, a Roman writer in 1st century A.D. described the four cardinal signs of inflammation: rubor, tumor, calor, and dolor (redness, swelling, heat and
pain). In 1793 the Scottish surgeon, John Hunter wrote that had a “Salutary” effect on its host. Julius Cohnhein (1839-1884) provided first microscopic descriptions of the inflammatory process. He observed injured blood vessels in the living state in thin, transparent membranes, such as the mesentery and tongue of frog.

**Types of Inflammation:**

Depending upon the cause and duration of response, inflammation can be classified as acute and chronic.

1. **Acute inflammation:** The inflammation is of relatively short duration, lasting for a few minutes, several hours, or fluid and plasma protein (edema) and the emigration of leukocytes, Predominantly neutrophils.

2. **Chronic inflammation:** It is generally of longer duration and is associated histologically with the presence of lymphocytes and macrophages and the proliferation of small blood vessels and fibroblasts.

**The Inflammatory Process:**

The character of the immediate inflammatory response is basically the same, regardless of the location or nature of the injurious agent. All the causes listed for cell injury may also provoke inflammation. When tissue injury is caused by physical agents such as trauma, burns, and radiation, both the inflammatory and reparative processes smoothly from injury to healing. When the injurious agent is bacteria or other living forms the ensuring inflammatory responses become much more complex and chronic because of sustained process of injury. Depending upon the severity of the injury and the adequacy of the defense, the inflammation may remain localized to its site of origin or may evoke systemic response.
Chemical Mediators of Inflammation:

Injury precipitates the inflammatory response, but released chemicals mediators can originate from plasma, cells or from damaged tissue. They can be divided into the following groups:
1) Vasoactive ammies: Histamine and serotonin
2) Plasma proteases:
   a) The Kinin system (bradykinin and kallikrein)
   b) The complement system (C₃ and C₅ Fragments; C₅₆₇.)
   c) The coagulation-fibrinolytic system (fibrinopetides, fibrin degradation products).
3) Prostaglandins
4) Neutrophil products: Cationic proteins, acid proteases, and neutral proteases.
5) Lymphocyte factors.
6) Other mediators of inflammation: SRS-A, endogenous, pyrogen, and substance P⁵³.

Prostaglandins play a major role in the entire inflammatory process. The arachidonic acid is metabolized by two pathways-cyclooxygenase and lipooxygenase pathway. The currently available non-steroidal anti-inflammatory drugs (NSAID’S) are potential inhibitors of this cyclooxygenase pathway of arachidonic acid metabolism. Aspirin-like drugs prevent prostaglandin production also reduce the signs and symptoms of inflammation.

As inflammatory conditions require prolonged treatment with anti-inflammatory drugs. The currently used NSAID’s have several toxic side effects which limit their use in long term therapy.

Thus, it can be mentioned that intensive research with indigenous drugs can definitely open up new vistas in inflammation therapy and purified natural compounds may serve as template for synthesis of a new generation of anti-
Inflammatory drugs which in turn may have low toxicity and better therapeutic index \(^5^4\).

**Introduction Rhematoid Arthritis:**

Rheumatoid arthritis is a chronic inflammatory disorder of the joints, which is characterized by potentially deforming poly arthritis and a wide spectrum of extra-articular manifestations.

It is the most common inflammatory arthritis and affects about 1\% of the adult population. Rheumatoid arthritis is more common in women than men and is an important cause of disability and morbidity. There is also significant mortality associated with the disorder, with a reduction in life expectancy of approximately your men and 10 years for women. The mortality from conditions including infection, cardiovascular disease, and gastrointestinal problems is also increased \(^5^5\).

**Aetiogy:**

The cause of RA is unknown It is possible that many different arthritogenic stimuli activate the immune response in the immunogenetically susceptible host. The incidence of seropositive RA is more common in people with HLA-DR4 antigens (60\%) than in the normal control population (15\%). There is no evidence that trauma, climate, diet stress, metabolic or endocrine factors are involved.

Epstein-Barr virus has been linked to RA for over 10 years of patients with RA, 80\% have circulating antibody directed against antigens specific for Epstein-Bar virus, and the antoanbody response in RA enhances the response to these antigens. Mycobacteria have also been linked to RA because these bacteria express heat shock proteins, which are the arthritogenic factors of adjeuvant arthritis in rats.
Pathogenesis:
In the normal joint, the articulating surfaces are covered with hyaline cartilage and the joint is enclosed within a capsule. The latter is lined with synovium, a specialised tissue that produces synovial fluid to lubricate the joint.

In early rheumatoid arthritis the synovium becomes inflamed (synovitis) and effusion into the joint space occurs which causes pain, stiffness and joint swelling. Once triggered, Synovitis becomes self-sustaining. During later disease, polymorphonuclear leucocytes, lysosomal enzymes and other enzymes which are contained in the inflammatory effusion actively degrade the cartilage. Sustained inflammation of the synovium also leads to the formation of panus, which is a granulating tissue. The tissue erodes the cartilage and eventually begins to erode the bone.

The combined effects of joint damage, muscle wasting, instability and continued use lead ultimately to joint deformity.

Pain Management:
Simple analgesics provide only limited relief from the pain, stiffness and inflammation associated with rheumatoid arthritis. E.g. Paracetomol, Paracetamol combinations and dihydrocodeine

The use of non-steroidal anti-inflammatory drug (NSAIDs). In single does, NSAIDs have analgesic activity but in full-regular dosage. They have both a long lasting analgesic and anti-inflammatory effect Example;

i) Ibuprofen, Ketoprofen, Naproxen (Propionic acid derivatives)
ii) Azapropazone, meloxicam, Piroxicam (Enolic acids)
iii) Diclofenac, indomethacin and Sulindac (Acetic acid derivatives)
NSAIDs work to suppress inflammation by preventing the production of prostaglandins through inhibition of the enzyme cyclo-oxygenase (COX) which is known to have two isoforms, COX-1 and COX-2. The anti-inflammatory actions of NSAIDs are due to COX-2 inhibition whereas the side effects are related to inhibition of COX-1. NSAIDs interfere with leukotriene synthesis, superoxide generation, lisosomal enzyme release, neutrophil function.\textsuperscript{56,57}

1.5.1 Analgesic Activity:

Several methods are available for testing central analgesic activity such as:

1) Haffner’s Tail Clip Method:

The method was described as early as 1929 by Haffner who observed the raised tail (Straub Phenomenon) in mice treated with morphine or similar opioid drugs and found the tail after drug treatment to be less sensitive to noxious stimuli.

In this method an artery clip is applied to the root of the tail to induce pain. The animal quickly response is measured by a stopwatch in 1/10 seconds increments.

2) Radiant Heat Method:

The method was developed by Schumacher et al.(1940), Wolff et al (1940). Mice are placed into cages leaving the tail exposed. A light beam is focused to the proximal third of the tail. Within a few seconds the animal flicks the tail aside or tries to escape. The time until this reaction occurs is measured.

3) Hot Plate Method:

The method was originally described by Woolfe and Mac Donald (1944) and has been modified by many investigators.
The hot plate consist of an electrically, heated surface. Mice or rats were placed on hot plate maintained at 55\(^{\circ}\)C and the time until either licking on jumping occurs is recorded by a stop-watch.

4) **Tail Immersion method:**

The method has been developed to be selective for morphine like compounds.

The procedure is based on the observation that morphine-like drugs are selectively capable of prolonging the reaction time of the typical tail-withdrawal reflex in rats induced by immersing the end of the tail in warm water of 55\(^{\circ}\)C\(^{58}\).

5) **Writting Induced by Chemicals:**

In the method as mentioned by Writhen *et. al.*, 1961, made mice are made to writhe by an intraperitoneal injection of 300 mg/kg of 3\% aqueous acetic acid. The test solution is given orally 15 minutes prior to acetic acid. Each mouse is then put into a liter beaker and total number of stretching episodes for 20 min is recorded.

6) **Rectodolorimeter:**

This method for measuring analgesia employs electric current as the noxious. This method was described by Charlier *et.al.* 1961.

A mouse cage is used in which the floor is made of metallic strips receiving an electric current of known voltage. Mouse is placed in the cage and minimum voltage for the current is determined that causes the animal to emit a cry. A substance having analgesic properties causes the voltage necessary for the production of cry to rise.

7) **Rectodolorimeter:**

A rat is placed in a small confining cage having a copper plate as the floor which is connected to an induction coil. The second pole of the coil, connected to a copper electrode is introduced into the rectum. The rectal electrode in fixed in
placed by attaching it to the tail, which is then extended out of the cage. The procedure and the computation are performed in the same way as for the pododolorimeter, described above.

### 1.5.2 Anti-Inflammatory Activity:

Anti-inflammatory activity of a compound is determined by observing specific suppression of signs of typical inflammatory reaction induced experimentally in laboratory animals.

Methods for testing acute, sub-acute and proliferative phase inflammation are:

1) **Rat Paw Edema method:**

The rats receive the test drug emulsion in the required volume. Thirty minutes later, 0.1 ml of 1% solution of carrageenan injected subcutaneously into the planar side of the left hind paw. The paw volume is measured immediately after injection, again 3 and 6 hours.

2) **Mouse ear edema method:**

According to the method of Brown and Robson (1964), the ear edema is produced in mice by the application of xylol to one of the ear keeping the other ear as control. The difference in weight between control and inflamed ear is the measure of the edema.

3) **Ultra-violet light induced erythema in skin:**

This test to measure anti-inflammatory activity against erythema is caused by radiation.

A small area on the back of guinea pig is exposed to ultra-violet radiation from lamp for 20 seconds. The animal is given degree of erythema estimated 120 minutes later.
4) The Granuloma Pouch Method:

Wistar rats, weighting between 150-200gm are used. Pneumoderma is made by injecting subcutaneously 25ml of air. 0.5ml of 1% solution of oil in sesame oil is injected avoiding any leakage of air.

On second day after formation of pouch air is removed by vacuum. On adhesions and a 4th day, pouch is opened and exudates fluid is aspirated to measure its volume.

5) Cotton-pellet Method:

The method was described by Meter et al (1950). an incision is made in the lumber region of rats. By forceps tunnels are formed and cotton pellet (sterilized) is placed on both sides in for 7 days subcutaneously or orally. Then animals sacrificed, the pellets prepared and dried until weight remains constant.

1.5.3 Antimicrobial activity:

Infective diseases are a critical problem for health and they are the main cause of death worldwide. Infection caused by pathogenic fungi is increasingly recognized as emerging threat to public health. Resistance of microbes to antibiotic sand toxicities produced by long term uses of antimicrobial compounds has initiated the search for safe antimicrobials, though different types of antimicrobial agents are available, there is an increase demand by people to use the natural products and also the researchers have identified a lot of plants with antimicrobial activity. Throughout the history of mankind, many infectious diseases have been treated with plant extracts.

Various strains of microorganisms, Different methods like cup-plate, agar diffusion and strike plate method are used for antimicrobial study.