CHAPTER 1

INTRODUCTION
1.1 INTRODUCTION

1.1.1 Medicinal plants in traditional healthcare system

Over the past twenty years, interest in medicinal plants has grown enormously from the use of herbal products as natural cosmetics and for self-medication by the general public to the scientific investigations of plants for their biological effects in human beings. Beyond this pharmaceutical approach to plants, there is a wide tendency to utilize herbal product to supplement the diet, mainly with the intention of improving the quality of life and preventing the diseases of elderly people. Study of Taylor et al showed that many medicinal plants are using as dietary supplements due to their nutrition value and demand of these plants is increasing day by day [1]. India has been identified as a major resourceful area in the traditional and alternative medicines globally. Medicinal plants constitute an important component of flora and these are wildly distributed in India. Recent estimate suggests that several thousands of plants have been known with medicinal application in various cultures [2]. Numerous studies have shown that aromatic and medicinal plants are sources of diverse nutrient and non – nutrient molecules, many of which display anti-oxidant and anti-microbial properties which can protect the human body against, both, cellular oxidation reaction and pathogens. Andrographis paniculata (Burm. f.) Nees is one of the wildly distributed medicinal plants in India and using since ancient times in traditional ayurvedic systems of medicines.

India is a known mega-diversity centre harboring a multitude of medicinal plant species each presumably studded with as yet unknown genetic and chemical variations of economic importance. Out of an estimated 17,000 higher plant species occurring in India, more than 1000 species are used over several centuries in the traditional systems of medicine viz. Ayurveda, Siddha and Unani. Andrographis paniculata (Burm. f.) Nees is one of the important herbs among them. The villagers and tribal folks spread across the length and breadth of the country make use of more than 7000 plant species through oral traditions. Nearly 3/4 of the herbal drugs and perfumery products used in the world are available naturally in India.

1.1.2 Variations

Although plants in general show habitat and distribution preferences but there are many species which are neutral and adapted to other environmental conditions. Much of the variations in
phenotype observed in natural populations of a species were earlier attributed to environmental influences [3]. Variations within and between populations of a species are not uncommon. Even within a medicinal plant species, sometimes one variety was preferred over others. It is also not surprising that curative properties of a plant species change according to seasons or developmental stages and hence “vaidyas” (Traditional medicines experts) prefer to collect required plants or their parts during certain periods only [4]. Thus individuals of a species adapted to a particular soil type and climatic zone and many botanists reasoned that distinct intraspecific variations of plants were merely due to habitat modifications and adaptation to environment.

The problem of variations is further compounded in medicinal plants apart from visible variations and that is synthesis and percentage of plant-specific chemicals. These compounds together called secondary metabolites and they are mostly high-value, low-volume compounds biosynthetically derived from primary metabolism and accumulated by certain plants or groups of plants in trace quantities. A study of variation in the active principles is often an important criteria in the investigation of variation in such plants. Although only limited number of individuals in a plant population were usually subjected to chemical scrutiny thus it became reasonable to assume that chemo types or chemical variants occurred in wild populations of medicinal plant species. A wide spectrum of simple and overlapping variations is now documented in plants. Therefore, the rich and varied plant diversity, especially the genetic diversity of medicinal and aromatic plants, is one of India's important strengths [5]-[11].

A study from China showed that not only plants growing in different geographical areas with different morphological characteristics could have different chemical constituents but also plants with similar morphological features and growing on the same site may have different contents of chemical constituents [12]. Phytochemical variation is also possible among populations, local races etc. and among different parts of the same plant. However, concerns on variation in content and chemical profile caused by plantation conditions (geologic area, fertility of soils, climate, season, etc.), plant breeding and age at harvest have usually been raised. Seasonal variation in chemical constituents present in plant is also an important issue because it directly affects the medicinal value. Therefore, in order to assure the effectiveness and the quality of the medicinal plants, it is necessary to monitor availability of the bioactive constituents both in terms of quantitative and qualitative aspects. Hence study of location variations of a particular species is an important issue with respect to chemical constituents present in finding pharmaceutical potential.
1.1.3 General description of *Andrographis paniculata* (Burm. f.) Nees

*Andrographis paniculata* (Burm. f.) Nees belongs to the family of Acanthaceae. It is available abundantly in south eastern Asia i.e., India, Sri Lanka, Pakistan and Indonesia. It is an annual herbaceous plant which is widely cultivated in Southern Asia, India, China and some parts of Europe. It is found in wild through out plains of India especially in Tamil Nadu, Karnataka, Maharashtra, Orissa, Uttar Pradesh and Uttarakhand. *Andrographis paniculata*, “Kalmegh” of Ayurveda is an erect annual herb extremely bitter in taste in each and every part of the plant body. The plant is known in north-eastern India as ‘Maha-tita’, literally ‘king of bitters’ and also known by various other vernacular names (Table 1.1). It is also known as ‘Bhui-neem’ since the plant, though much smaller in size and has bitter taste as that of Neem (*Azadirachta indica*).

Table 1.1: List of vernacular names of *Andrographis paniculata*

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Language</th>
<th>Common Name</th>
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<tbody>
<tr>
<td>1.</td>
<td>Arabic</td>
<td>Quassabhuva</td>
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<td>2.</td>
<td>Assamese</td>
<td>Chiorta</td>
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<td>3.</td>
<td>Bengali</td>
<td>Kalmegh</td>
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<td>4.</td>
<td>Chinese</td>
<td>Chuan Xin Lian</td>
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<td>5.</td>
<td>English</td>
<td>The Creat</td>
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<td>6.</td>
<td>Gujarati</td>
<td>Kariyatu</td>
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<td>7.</td>
<td>Hindi</td>
<td>Kalmegh, Kirayat</td>
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<tr>
<td>8.</td>
<td>Indonesian</td>
<td>Sambiloto</td>
</tr>
<tr>
<td>9.</td>
<td>Kannada</td>
<td>Nelaberu</td>
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<tr>
<td>10.</td>
<td>Malayalam</td>
<td>Nelavepu, Kiriyattu</td>
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<td>11.</td>
<td>Marathi</td>
<td>Oli-Kiryata</td>
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<td>12.</td>
<td>Oriya</td>
<td>Bhuinimba</td>
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<td>13.</td>
<td>Sanskrit</td>
<td>Kalmegha, Bhunimba</td>
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<td>14.</td>
<td>Tamil</td>
<td>Siriyanangai</td>
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<td>15.</td>
<td>Telugu</td>
<td>Nilavembu</td>
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<tr>
<td>16.</td>
<td>Thai</td>
<td>Fa-Talai-Jorn</td>
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</table>
The genus *Andrographis* consists of 28 species of small annual herbs essentially distributed in Tropical Asia. Only a few species are of medicinal value out of which *Andrographis paniculata* (*Burm. f.*) *Nees* is the most popular. It is generally found in all kinds of vegetative lands i.e., in pine, evergreen, deciduous areas, along roads and villages. It is easily cultivated from seeds on all types of soil. The plant is gregarious abundantly found in moist, shady waste grounds and sometimes in dry forests [13].

### 1.1.4 Morphology of *Andrographis paniculata* (*Burm. f.*) *Nees*

*Andrographis paniculata* is an erect annual herb, stem dark green, 0.3-1.0 m in height, 2-6 mm in diameter, quadrangular with longitudinal furrows and wings on the angles of the younger parts, slightly enlarged at the nodes. The macro and microscopic characteristics of the stem, leaves and floral parts of the plant have been studied and described in 1957 [14]. The stem is green, woody, annual and erect up to 1 m high, bearing numerous branches. The leaves are opposite having reticulate venation. The leaves possess a small winged petiole. Glandular and non-glandular hairs are present on both surfaces of the lamina. The mid-ribbed varies in outline at different parts of the leaves. Leaves are glabrous, up to 8.0 cm long and 2.5 cm broad, and are lanceolate, pinnate. Flowers are small, in lax spreading axillaries and terminal racemes or panicles. The different parts of the flower possess characteristic glandular and non glandular hairs. Flowers are white in colour having rose purple spots on the petals. The fruits are capsule, linearly-oblong, acute at both ends. They are around 2 cm long and a few millimeters wide. Fruit contain numerous seeds, which are sub quadrate and yellowish brown in colour. (Fig 1.1)
Fig. 1.1 *Andrographis paniculata* (Burm.f.) Nees plant.
1.1.5 Cultivation

The plant is cultivated as a Kharif season crop. It is helophyte and therefore prefers a sunny situation. Propagation is generally done through seeds; however, it can also be propagated by inducing rooting in cuttings. The seeds are sown during May-June. The seedlings are transplanted at a distance of 60 cm x 30 cm in the last week of July. Two or three irrigations may be required during the dry periods. It flowers during Aug – Nov. The plant is collected at maturity i.e. after complete flowering and fruiting. Depending upon area of cultivation harvesting is done in Oct - Nov. After harvesting, the whole plant is dried in shade by spreading on the floor for 7-8 days. During this period, it is protected from dew at nights.

The aerial parts of the plant in the whole form, fractured form and powdered form appear grayish black, whitish and greenish brown, respectively. Dried and powdered aerial part of the plant is sometimes sold under the name “chiretta” (Swertia) which possesses many medicinal properties similar to “Kalmegh” of Ayurveda and is much more expensive than “Kalmegh”, but both are different in morphology as well as in chemical constituents[15],[16].

1.1.6 Part used as crude drug

Use of *Andrographis paniculata* as a natural herb in India is very common. Crude drug consists of dried or fresh leaves or the aerial portion of the plant. Sometimes the whole plant including the roots is used. Panchang (stem, leaves, flowers, root and seeds) of the plant is being used in various formulation of Indian system of medicine for the treatment of many diseases. The drug normally should not contain more than 2 % of foreign organic matter [17].

1.1.7 Traditional medicinal use

Since ancient times *Andrographis paniculata* is used in traditional siddha and ayurvedic systems of medicines as well as in tribal medicine in India and some other countries for multiple clinical applications. The herb is the well-known drug Kalmegh or ‘green chiretta’ and forms ingredient of a reputed house hold medicine. Powdered plant mixed with mustard oil is using for the treatment of itching. The macerated leaves and juice together with certain spices prescribed for relief from gripe and other stomach ailments in infants and also used as domestic medicine for flatulence and
diarrhoea of children. It is used in torpidity of liver, neuralgia and convalescence after fever. A decoction of the plant is a blood purifier while an infusion is used in fever. A decoction or infusion of the leaves is useful in general debility and dyspepsia. The leaves and root are also used as febrifuge, tonic, stomachic, cholagogue and anthelmintic [17], [18].

*Andrographis paniculata* or Kalmegh is one of the most widely used plants in ayurvedic formulations. *Andrographis paniculata* was recommended in Charaka Samhita dating to 175 BC for treatment of jaundice along with other plants in multi plant preparations[19], [20]. It has also been used traditionally for sluggish liver as antidote in case of colic dysentery and dyspepsia[21]. It has been employed with benefit in case of general debility in convalescence after fever, disorders of liver and advanced stages of dysentery [22]. The juice of fresh leaves is a domestic remedy in the treatment of colic pain, loss of appetite, irregular stools and diarrhea [23]. Unlike other species of the genus, *Andrographis paniculata* is of common occurrence in most places in India, including the plains and hilly areas up to 500 m, which accounts for its wide use. Since time immemorial, village and ethnic communities in India have been using this herb for treating a variety of ailments. The demand of *Andrographis paniculata* is increasing day by day due to its importance in the treatment of different ailments.

### 1.1.8 Medicinal Formulations

It is found in the Indian Pharmacopoeia and is prominent in several Ayurvedic formulations. Andrographis 300 MG, andrographis standardized extract, andrographis 60V caps, andrographis 400MG, andrographis 200 MG, andrographis complex, Panchanga Churana, Kalmegharasa, Kalmeghasav, Kalmeghnayayas lauh, kalpataru ras, kalansunder ras, Liv 52, etc., are some of the herbal products in national and international markets having kalmegh as major ingredient

### 1.1.9 Phytochemistry

Andrographolide (C$_{20}$H$_{30}$O$_{5}$; MW 350.44) is the major constituent extracted from the leaves of the plant which is a bicyclic diterpenoid lactones. This Intensively bitter principle was isolated in pure form by Gorter in 1911 [24]. It has demonstrated a wide spectrum of pharmaco-biological activities. Some other activities as liver protection under various experimental conditions of treatment with galactosamine, paracetamol etc. are also attributed to Andrographolide.
The hepatoprotective action of Andrographolide is related to the activity of certain metabolic enzymes [25]-[32]. Some known constituents are mentioned in table 1.2.

Table 1.2: Phytochemical contents of plant of *Andrographis paniculata*:

- Andrographolide, Plant.
- NeoAndrographolide, Plant.
- 14-Deoxy-11-dehydroAndrographolide, Plant.
- 14-Deoxy-11-oxoAndrographolide, Plant.
- 5-Hydroxy-7,8,2’,3’-Tetramethoxyflavone, Plant.
- 5-Hydroxy-7,8,2’-Trimethoxyflavone, Tissue Culture.
- Andrographine, Root.
- Panicoline, Root.
- Paniculide-A, Plant.
- Paniculide-B, Plant.
- Paniculide-C, Plant.

1.2 Statement of the problems

Medicinal plants play a central role in medicines besides the use of animal and mineral materials. Medicinal plant resources are dwindling worldwide. Loss of medicinal plants is a serious problem for pharmaceutical industries. Finding of exact potential of plant is the need of today. Antimicrobial activity, phytochemical profile, antioxidant activity, pharmacological actions etc. are some important criteria in findings of pharmaceutical potential of any medicinal plant. The exact bioactivity should be identified in order to standardize traditional medicine. This study evaluates the pharmaceutical potential in terms of antioxidant activity, antimicrobial activity and phytochemical profile of extracts of *Andrographis paniculata* (Burm. f.) Nees and also helps to understand its biological activity against both Gram-negative and Gram-positive bacteria. Identification and isolation of main active constituents will be helpful in extracting maximum effectiveness of the plant by minimum use of its materials. Determination of the %w/w content of the active component (Andrographolide) in different parts of *Andrographis paniculata* will give the idea of active constituents enriched part of the plant. Study of seasonal variation of main active constituents of *Andrographis paniculata* will be helpful in harvesting of plants at perfect time so
that less quantity of drug can give high medicinal potential. Variation in yield percentage of the active constituents of *Andrographis paniculata* in different geographical sites of Uttarakhand will be helpful in selection of correct places for cultivation of *Andrographis paniculata* for the optimum use of natural resources available.

### 1.3 Aims of study

The indiscriminate use of antibiotics and their side effects has given rise to situation which is alarming in both developing as well as developed countries. Hence search for anti-microbial substance from other sources including plants is need of today. *Andrographis paniculata* is taken for chemical analysis to add to the present state of knowledge about different biological activities of the plant and its use for drug manufacture. The findings of the chemical analysis of the plant on the criterion of phyto-geography will definitely lead to its effective application in pharmaceutical industry in India and overseas.

The tropical forest of Uttarakhand in the Asia region remains a national heritage of bioprospecting avenues for the future developments of medicinal native plants. Medicinal plants of Uttarakhand available in abundance still need to be explored and subjected to investigation in order to ascertain the associated claims of medicinal uses and clinical applications. Traditional medicines need to be researched further for scientific proof of their efficacy and safety to ensure the rational and proper use. *Andrographis paniculata* (Burm. f.) Nees is one of the 28 species of *Andrographis* belonging to the family *Acanthaceae* One of the most widely used plants in Ayurvedic preparations in which twenty-six out of forty Indian polyplant formulations contain *Paniculata* species.

### 1.3.1 Method validation

Various methods have been suggested for quantitative estimation of Andrographolide in *Andrographis paniculata*. The gravimetric method described in the Indian pharmacopoeia was found to give high values. [33]. This is due to some yellow coloring substance other than Andrographolide which is also soluble in ethyl acetate. The spectrophotometric method proposed by Maiti et al., suffers from the disadvantage that the red colour formed with the addition of alcoholic potassium hydroxide to the solution of Andrographolide is unstable and fades away
quickly [34]. Spectrophotometric method used by Gained et.al, by extracting pure Andrographolide from kalmegh and measuring its absorbance at 226 nm was helpful in isolation of pure Andrographolide but the extraction process was very tedious [35]. In 1978 Bhat et al., suggested a chemical method involving a lactone titration but the method has been reported to be not suitable for detecting minute quantities [36]. Thin layer chromatographic methods were also described for estimation of Andrographolide in *Andrographis paniculata* extracts and also reported by using capillary electrophoresis chromatography [37]-[41]. The methods described above have several limitations like methods use for samples preparation for estimation of Andrographolide, low yield, time consuming, extraction process and in terms of authenticity . In the present study, accurate, simple, specific and reproducible HPLC and HPTLC methods have been developed and validated for the determination of Andrographolide in *Andrographis paniculata* herb and for different extracts at different stages of life cycle of plant, for different parts of plant (leaves, stems, flowers and whole plant etc ,and for the herb from different locations. All results also compared with standard biomarker of Andrographolide to increase the authenticity of results.

1.3.2 Pharmaceutical potential

This research topic “Chemical Study of *Andrographis paniculata* for pharmaceutical Potential” is envisaged to explore the medicinal plant *Andrographis paniculata* for pharmaceutical industries. Chromatographic and Spectroscopic studies of the constituents present in plant exhibited pharmaceutical Potential of *Andrographis paniculata*. The aims of this study are to investigate the antioxidant activity, phenol and flavonoids content, microbial activities and percentage of Andrographolide (Yield) of a potential medicinal herb *Andrographis paniculata* grown in natural environment and in cultivated environment. Study of different extracts of *Andrographis paniculata* will be helpful for bioactive. In the present study different extracts of *Andrographis paniculata* at different stages of the life cycle of crop have been screened and comparative evaluation with standard Andrographolide has been performed. Location variation and seasonal variation studies are also carried out to generate the data useful for resourceful application in pharmaceutical industries.
1.4 Objectives of the study

Little is known about the genetic diversity, phytochemical profile, pharmacological actions of the content of the active components of Andrographolide present in *Andrographis paniculata* (Burm. f.) Nees species grown in Uttarakhand, despite the long traditional use of *Andrographis paniculata* by the different communities. Therefore, such study will serve as the pioneering project to assess this medicinally important native plant and future research on other medicinal native plants of Uttarakhand based on the fact that plants from different sources may have different phytochemical profiles and pharmacological actions. This study used various interdisciplinary approaches to assess, phytochemical contents, and pharmacological actions to establish the profile of *Andrographis paniculata* (Burm. f.) Nees species grown in Uttarakhand.

The objective of this research are-
1. Fractionation of different parts of *Andrographis paniculata* using different solvents.
2. To make detailed study of Physico-chemical properties of *Andrographis paniculata*.
3. To investigate Physiological variation at different stages of life cycle of *Andrographis paniculata*.
4. To make study of the phytochemical profile and quantitative analysis of phytochemical constituents of *Andrographis paniculata* of Uttarakhand.
5. To analyze anti-oxidant activity of different extracts of different parts of *Andrographis paniculata*.
6. Screening of different extracts of *Andrographis paniculata* for anti-microbial activities.
7. To evaluate and investigate the bioactivities and the pharmaceutical potential of different parts of *Andrographis paniculata* at different stages of life cycle by HPTLC and HPLC analysis.
8. To determine the %w/w content of the active component Andrographolide in different parts of *Andrographis paniculata* including geographical variations.

The findings of this study may provide valuable information on the potential therapeutic use of *Andrographis paniculata* (Burm. f.) Nees of Uttarakhand as promising therapeutic drug.
1.5 Organization of thesis - Thesis comprises 5 Chapters namely:-

1. INTRODUCTION.
2. LITERATURE REVIEW.
3. MATERIALS AND METHODS.
4. RESULTS AND DISCUSSION.
5. CONCLUSION.

All chapters are separately discussed in the thesis.

1.5.1-Chapter 1 is the INTRODUCTION. Introduction includes the details of plant species *Andrographis paniculata* with respect to its biology and chemistry.

1.5.2-Chapter 2 is the LITERATURE REVIEW. Literature review describes the study done so far on biology and chemistry of the plant *Andrographis paniculata* in India and abroad in different organizations at different times. Literature review is very extensive and includes several references with respect to the research problem and results thereof.

1.5.3-Chapter 3 is MATERIALS AND METHODS. It gives the details of experiments conducted in laboratory and the use of different instruments applied for the purpose.

1.5.4-Chapter 4 contains RESULTS AND DISCUSSION. This chapter gives the details of experimental results and the results are discussed with respect to contemporary work in the field.

1.5.5-Chapter 5 is CONCLUSION. This concludes the resultant outcome of the research work done and scope for further work. Finally, References with respect to the research work are mentioned in the end of the thesis. Reference section includes all references of the plant introduction, research methodology, experimental work and the research results and discussion. The photographs and pictorial references are attached in relevance to the reported text.