Summary
The present research work entitled "Studies on diseases of certain herbaceous medicinal plants in some forests of Chikmagalur and Shimoga districts, Karnataka, India", was carried out during 2006-10. The results of the study are accommodated under the following sections - Introduction, Review of literature, Aims and objectives, Materials and methods, Results and discussion, General discussion, Summary and References.

The introductory section gives a brief account of the importance of medicinal plants in the treatment of ailments affecting humans, the international scenario, and diseases that affect medicinal plants in cultivation or wild. The knowledge of medicinal plants must have been accumulated over the centuries. The medicinal plants have curative properties due to the presence of various complex chemical substances of different composition, which are identified as secondary plant metabolites in one or more parts of plants. The allopathic modern medicines have wide acceptance but their toxic side-effects are forcing people to the use of alternate natural products. Consequently, many of the traditional medicines are being utilized along with the modern medicines. During the past decade, a dramatic increase in the export of medicinal plants attests to world-wide interest in these natural products, as well as in the traditional health system. Since much of the medicinal plants are harvested from the wild, hundreds of species are now threatened with extinction due to over-harvesting, destructive collection techniques and conversion of habitat to crop-based agriculture. While considerable reports are available on medicinal plants under cultivation, not much information is available on
diseases of certain uncultivated medicinal plants. In view of this, understanding of
diseases that affect uncultivated medicinal plants are essential for the sustainable
utilization and conservation of medicinal plant species.

The review of literature consists of information relating to medicinal plants and
their importance in therapeutics, the diseases that affect them and their management at
seed stage, and the effect of diseases on secondary metabolite content. Approximately,
one third of the crop losses are due to fungal, viral and bacterial pathogens and the
remainder to insects and nematodes. During the last 50 years, a lot of work has been done
on different diseases, which has generated a vast amount of knowledge on
symptomatology, disease cycle, and epidemiological parameters in the development of
disease, host-parasite relationship, resistance-virulence relationship and existence of
different biotypes in the pathogen. The study of medicinal plant diseases is of immense
academic, practical and economic importance, with findings potentially having
implications for the pharmacological industry, world-wide. Less than 20 species of plants
are under commercial cultivation as compared to over 400 uncultivated species used for
herbal drug production by industries. Over 95% of medicinal plants used by Indian
industries today are collected from the wild. The cultivated medicinal plants are reported
to be infected by a variety of pathogens that cause disease(s) at different growth stages
and seasons. Diseases of culinary medicinal plants have been studied extensively; for
example, species of *Zingiber officinale*, *Piper nigrum*, *Curcuma longa*, *Coriandrum
sativum*, *Allium cepa* and *Capsicum annuum* and others. Some of the diseases of
medicinal herbs reported in the literature are, leaf spot disease of *Cassia angustifolia
(Khan et al., 2001), seedling rot, leaf blight, and die-back of ashwagandha (Pandey et al.,
1985) and rust, powdery mildew, and leaf blight and stolon rot of Mentha arvensis, and foot rot of betel vine (Dastur, 1927; Ganguly, 1962; Dasgupta, 2005). The survey of literature also indicated that uncultivated medicinal plants are affected by a few diseases. For example, leaf spot in Acalypha indica (Hasija et al., 1989), leaf necrosis in Polygonum (Xinog et al., 2012), septoria leaf spot of Lantana camera (Eduardo et al., 1995), and leaf spot of Calotropis gigantea (Sain et al., 2009). Spoilage of seed in storage and mortality of seedlings by seedborne pathogens is a serious problem, which could be effectively controlled through integrated control involving measures like quarantine and seed certification, suitable seed production site, proper seed beds, proper storage and potential fungicide treatment (Mehrotra and Mehrotra, 2000). A few reports also suggested that therapeutic values were altered due to the infection of medicinal plants by pathogens. The plant disease adversely affected the production of secondary metabolites (Mona, 2005; Achar and Shivanna, 2013; Parashurama et al., 2013) as well as the primary metabolites (Pandey et al., 1993; Tripathi et al., 2003).

Information on diseases of cultivated medicinal plants are limited only to commercial species. The lack of information of sound cultivation and disease management practices for medicinal plants has resulted in the gradual depletion of raw materials from wild sources. In view of this, understanding of diseases that affect medicinal plants and their management is essential for the sustainable utilization of medicinal plants. Hence, the following objectives were taken up for the study.

1. To isolate and characterize causal organism of foliar diseases, seedborne nature and transmission of pathogens that cause foliar disease in C. asiatica, E. scaber, P. zeylanica, R. serpentina, R. cordifolia and W. somnifera.
Summary

2. To determine the incidence and severity of foliar diseases for three years period and to prepare disease calendars for each pathogen, and disease map of study area.

3. To analyze samples of selected medicinal plants for seed borne mycoflora and their management.

4. To determine secondary metabolites qualitatively and quantitatively in healthy and diseased materials of selected medicinal plants.

The materials and methods section consists of detailed experimental methodologies related to the objectives of the study. The study area selected is certain forests located in the Chikmagalur and Shimoga districts of Karnataka, India, which are situated within Bhadra Wildlife sanctuary. The sanctuary consisted of four ranges - Lakkavalli, Tanigebylu, Muthodi and Hebbe, which were in turn divided into 12 state forest regions such as Aldara, Gangegiri, Hebbegiri, Kakanahosudi, Kagemanegiri, Kemmannugundi, Lakkavalli, Muthodi, Madla Madhuguni, Singanamane and Thammadihally. Thirty-six study sites were established within these state forests, with three study sites identified randomly in each state forest region. Each study site consisted of three replicates and in each replicate, three quadrates were established.

Surveys were conducted to document the medicinal herbs growing in each study site, in all three seasons. Six plant species - *Centella asiatica*, *Elephantopus scaber*, *Plumbago zeylanica*, *Rauwolfia serpentina*, *Rubia cordifolia* and *Withania somnifera*, out of several medicinal herbs in the sanctuary, were selected for study. The plant species were studied for diseases caused in leaves and stems and other parts, which were collected in moistened polypropylene covers and brought to the laboratory for further studies. The diseased plant materials were segmented, surface disinfected (NaOCl, 0.2%,...
2 min) and incubated on blotters and PDA. The incubated samples were studied under stereo-binocular microscope and fungal colonies were identified based on the morphological characteristics by using the standard identification manuals (Barnett et al., 1998; Sivanesan, 1983 and Subramaniyan, 1983; Booth, 1977). The fungal isolates associated with diseased plant materials were cultured on PDA medium and tested for pathogenicity by leaf bioassay in their respective hosts (Shivanna and Mallikarjunaswamy, 2009) and symptoms of disease produced were compared with that produced in the study area. The incidence and severity of disease (Shivanna and Mallikarjunaswamy, 2009) in six selected medicinal plants were studied at an interval of 30 days for a period of 36 months from August 2006 to July 2009 in all the 36 study sites. The mean variance of disease incidence was used to determine the spatial distribution of disease in each study site by using the modified Taylor’s power law (Taylor, 1984; Madden et al., 1995). The data of weather parameters of study area were collected and disease incidence and severity were subjected to Pearson’s correlation.

Seeds of five medicinal plants- E. scaber, P. zeylanica, R. serpentina, R. cordifolia and W. somnifera were collected from the study area and pooled species-wise to obtain seed samples. The seeds were subjected to standard blotter method for determining the seed mycoflora (Anon., 2003). The data on the occurrence of fungal species in seed samples and seed germination were recorded. Seed component plating method (Maden et al., 1975) was employed to determine the location of the pathogen in the seed. Seed transmission of the disease causing organism was determined by the sand method in the greenhouse (Shivanna and Shetty, 1991). Data of pre and post-emergence seedling mortalities and disease occurrence were recorded. The data of seedling mortality and
disease incidence and severity were subjected ANOVA. Seed samples of medicinal plants were subjected seed treatment by dusting with four individual fungicides- Bavistin, Hyzeb M-45, Antracol, or Captra @ 2% (mg g⁻¹) for controlling the fungal incidence in seed samples.

Four secondary metabolites viz., alkaloids, flavonoids, phenols and steroids present in the selected medicinal plants with or without fungal infection were determined by standard methods (Folin and Denis, 1939; Swain and Hillis, 1959; Sanchez et al., 1972; Ikan, 1969). The alkaloid quality of one of the medicinal plants, W. somnifera was determined in healthy and diseased leaves by HPTLC.

A brief account of the Results and Discussion section is detailed below. Medicinal plants- C. asiatica, E. scaber, P. zeylanica, R. serpentina, R. cordifolia and W. somnifera were distributed unevenly in 12 state forest regions. They were affected with major diseases like leaf spot and leaf blight. The diseased leaf samples from the respective plant species upon incubation produced one dominant fungal species and many associated fungi. The dominant fungal species was found to cause disease when tested by leaf bioassay. The following plant pathogens were established to cause disease - C. centellae caused leaf spot disease in C. asiatica, C. dematium leaf spot disease in E. scaber, F. chlamydosporum in P. zeylanica, C. rauwolfiae in R. serpentina, C. cladosporioides in R. cordifolia and A. alternata in W. somnifera. Among the above, C. centellae, C. rauwolfiae and A. alternata have been previously reported (Manoharachary et al., 2003; Ganguly and Pandotra, 1962; Pandey and Nigam, 1985). However, all other diseases caused in E. scaber, P. zeylanica and R. cordifolia are newly described in the present study. Species of Colletotrichum, Alternaria and Fusarium are causing foliar
diseases and considerable damage in food and horticultural crops (Farr and Rossman,
2011; Prajapati et al., 2003).

Frequent field visits were taken up to collect data of the incidence and severity of
disease for 36 months during Aug 2006-July 2009. All the foliar diseases caused by
 pathogens such as C. dematium, F. chlamydosporum, C. cladosporioides and A. alternata
caused leaf spot disease in E. scaber, P. zeylanica, R. cordifolia and W. somnifera,
respectively, were initiated during the early rainy season that reached a peak during the
winter season and decreased during summer. Shivanna and Shetty (1991) reported that
disease due to Alternaria cyamopsidis and C. dematium were initiated during March-
April, peaked during July-September and declined during December-January. In the other
hand, species of Cercospora were recorded as major pathogens in C. centellae and
R. serpentina, which were high during September-November. Ijaz et al. (2011) also
observed similar correlation on Cercospora leaf spot disease development in peanut
plants. Maximum temperature and maximum RH positively correlated ($p<0.05$) with the
development of disease caused by most fungal pathogens. This suggested their
importance in disease establishment and spread. The power law analysis based on the
data of foliar disease incidence caused by C. centellae in C. asiatica, C. dematium in
E. scaber, F. chlamydosporum in P. zeylanica, C. cladosporioides and A. alternata in
W. somnifera indicated the homogeneous distribution of the above diseases in the forest
regions of the sanctuary, as well as in the entire sanctuary. There was no considerable
variation in the disease distribution in the sanctuary area. Siddiqui and Shaukat (2002)
analyzed the spatial pattern of root rot-root knot disease in egg plants and reported that
the disease is heterogeneously distributed. Similarly, Achar and Shivanna (2013) showed
that Colletotrichum leaf spot disease in Clitoria ternatea in Kemmannugundi region of the above sanctuary is heterogeneously distributed, while that in Lakkavalli region, it was homogeneously distributed. A disease calendar was designed which indicated the course of disease due to C. centellae, C. dematium, F. chlamydosporum, C. rauwolfiae, C. cladosporioides and A. alternata in C. asiatica, E. scaber, P. zeylanica, R. serpentina, R. cordifolia and W. somnifera, respectively. Based on the data of disease incidence and severity and spatial distribution of disease in the sanctuary, disease hot-spots in Bhadra wildlife sanctuary were identified. The disease map was essentially based on the occurrence of more than 5% of severity of foliar disease in the study area.

Seed samples of five plant species- E. scaber, P. zeylanica, R. serpentina, R. cordifolia and W. somnifera were colonized by several seedborne fungal species. The seeds of E. scaber were colonized to the maximum extent with C. dematium, while the seed sample of P. zeylanica was colonized by F. chlamydosporum. The minor foliar pathogen of R. serpentina- Alternaria alternata occurred in high incidence in the seed sample. In case of R. cordifolia, seeds were extensively colonized by C. cladosporioides and the seed sample of W. somnifera by A. alternata and F. oxysporum. Species of Alternaria and Fusarium are destructive pathogens and reported to be seedborne in many crops (Magarey et al., 2005; Patil et al., 2000).

The seed components such as cotyledons and the embryonic axis were frequently colonized by C. dematium, A. alternata and C. cladosporioides in their respective host plants such as E. scaber, R. serpentina and R. cordifolia. Fusarium chlamydosporum and A. alternata were localized in the seed coat as well as in the cotyledon, and to some extent in the embryo axis in seeds of P. zeylanica and W. somnifera. Further, the seed
infection due to above pathogens resulted in the expression of seedling disease, suggesting its seed to seedling transmission. *Alternaria alternata* inoculum in *R. serpentina* and *W. somnifera* seeds was transmitted to seedlings. The high recovery of *C. cladosporioides* from different seed parts of *R. cordifolia* suggested its seedborne nature and transmission to seedlings. Among the fungicides used for seed treatment, Bavistin caused significant reduction in the seedborne occurrence of *C. dematium*, *F. chlamydosporum* and *F. oxysporum* in *E. scaber*, *P. zeylanica* and *R. serpentina* respectively, and other seedborne fungal species, as well. The seed treatment with Bavistin also caused improvement in seed germination ability which corroborated with the findings of Singh *et al.* (2003). Captra was also an effective fungicide which helped in the management of seedborne infection by *C. dematium* and *A. alternata* in other crop plants. Hyzeb is also effective against *A. niger*, *P. macrotrichia*, *A. versicolor* and *R. stolonifer* and *A. alternata* incidence. Antracol was effective in reducing *C. cladosporioides* incidence.

The alkaloids, one of the secondary metabolites, decreased in both partially infected and completely diseased foliar samples of *C. asiatica*, *P. zeylanica* and *R. serpentina*. However, the alkaloid content increased in both partially infected and completely diseased foliar samples of *E. scaber*. In case of *R. cordifolia*, alkaloids increased significantly in partially infected but decreased in completely diseased samples, as compared to the healthy leaf samples. The flavonoid content also increased both in the partially infected and completely infected leaf samples of *C. asiatica*, *E. scaber*, *P. zeylanica*, *R. serpentina* *R. cordifolia* and *W. somnifera*. As for as the total phenol is concerned, its increase over control was observed in partially infected and completely
infected leaf samples of all the five plant species except *W. somnifera*. In the latter species, phenolic compounds decreased in the partially infected samples, while it increased in completely diseased sample, as compared to the healthy sample. However, the steroid content was shown to decrease in infected leaf samples of *C. asiatica, E. scaber, P. zeylanica, R. serpentina, R. cordifolia* and *W. somnifera*. The HPTLC analysis suggested that fungal infection of *W. somnifera* foliages caused transformation of alkaloids into unknown altered compounds, as a result of the possible biochemical interaction between the host and pathogen. The decrease or increase in secondary metabolite content following infection was probably associated with their conversion into simpler compounds by biotransformation or degradation (Huszcza and Dmochowska-Gladysz, 2003; Vanitha and Balagurunathan, 2008).

In conclusion, results of present study indicated that the foliar diseases in six medicinal plants caused considerable damage to foliages through different seasons in different forest regions of the sanctuary. Most fungal pathogens are seedborne and seed transmitted to seedlings. The seedborne inocula of pathogens could be effectively managed by seed dressing with potential fungicides. Foliar infection affected the contents of secondary metabolites such as alkaloids, steroids, phenolics and flavonoids. The altered components of secondary metabolites were possibly changed in respect of its quality; this could be a cause of concern since some of them are therapeutically important compounds used in treating various diseases and disorders in humans. The study helped in the generation of baseline data, which could be used while designing the package of practices for intensive cultivation of these medicinal plants and in pharmaceutical industries.