Chapter 1:

Introduction

The ancient Indian subcontinent is known for different ancient civilizations. This part of the world has been very popular for its ancient practices of science, architecture, philosophy and medicine therapy. Traditional medicinal treatments like Ayurveda, Siddha, Unani, have been mentioned in different Indian sculptures and has been well documented in ancient Indian literatures like the Vedas (Subhose *et al*., 2005). 'Ayurveda' means the 'Science of Life', or the science for longevity. The ancient Indian health care system was more directed to the well-being of human life through preventive measures than the illness related curative remedies. Healthy life-style practices are thought to be the reasons of longer life-span in such ancient systems. The concept of Ayurveda emphasizing 'everything can be a drug' is deeply rooted in Indian culture. Ancient Ayurveda practitioners did extensive documentations of herbal remedies over time and currently, around 70% of the Indians rely on Indian herbal medicines for their primary health care (Vaidya and Devasagayam, 2007). Ayurvedic remedies, their resources and uses have been well documented in the Sanskrit. *Rigveda*, an ancient sacred collection of Sanskrit hymns from Vedic period (1500- 500 B.C.E.) and the *Atharvaveda*, the last of the four Vedas (600 B.C.E.) are the earliest documentations of different herbal products, minerals, animal products with their medicinal purposes (Sen and Chakraborty, 2020). *Charaka Samhita* and *Shushruta Samhita* (400 B.C.E. to 200 B.C.E.) provides documentation of 526 and 516 Indian herbal medicines (Pan *et al*., 2014).

Indian subcontinent is rich in biodiversity, includes almost 16 agro-climatic zones and more than 400 biomes. India possesses almost 8% of the estimated biodiversity of the world (Pan *et al*., 2014). The western part of the Himalaya possesses about 80% of the herbal drugs of Ayurveda origin, 46% of the Unani and 33% of the allopathy system (Baragi *et al*., 2008). The usage of the Indian herbal resources in different Indian medicinal systems has been briefed in the **Fig. 1.1**. The majority of the Indian herbal medicines are derived from either the whole plant or from their specific parts like root, bark, stem, flower, seed or leaf. Some drugs are also prepared or extracted from the excretory end products of those plants including gums, resins or latex. An account of 28 chronic human diseases treated with the help of the herbs and herbal formulae has been documented by Sharma and coworkers (Sharma *et al*., 2007).

India also plays a great role as the supplier of the herbal products at the domestic and overseas markets. Among the exported products, 60% are processed plant materials unique to India, 30% are plant extracts and 10% are Ayurvedic formulations (Pan *et al*., 2014). Indian herbal medicine research has been a successful attempt to innovate drug discovery (Patwardhan and Mashelkar, 2009). In 2008, India had herbal medicine-based market of approximately 70-75 Billion Rupees with export of over 36 Billion Indian Rupees (Sharma *et al*., 2008) which was projected to be doubled by 2015, i.e. 150 billion rupees with a growth rate of 15% (Saini *et al*., 2011). According to a latest study conducted by Confederation of India Industry (CII) in 2016, the total estimated market size of Indian Ayurveda industry is USD 3 billion (220 billion Indian Rupees approximately) including ethical, classical, personal care, beauty and health products which is expected to grow up to USD 9 billion by the year 2022 (CII, 2017). The sector is rapidly growing in India as well as globally.

The herbal medicines come with the benefit of low cost and usually, lack of side-effects. The usage and practices are generally simple and easy to understand by the laymen. A strong evidence-based usage of these medications in the form of the complementary and alternative medicine (CAM) has increased with time and showed positive influence in well-being of mankind. Newer scientific approaches have been used to determine the potency of the documented herbal medications in the last few decades. Isolation and identification of pure compounds and identification of most efficient bioactive compounds has been done from different plants and plant-based resources.

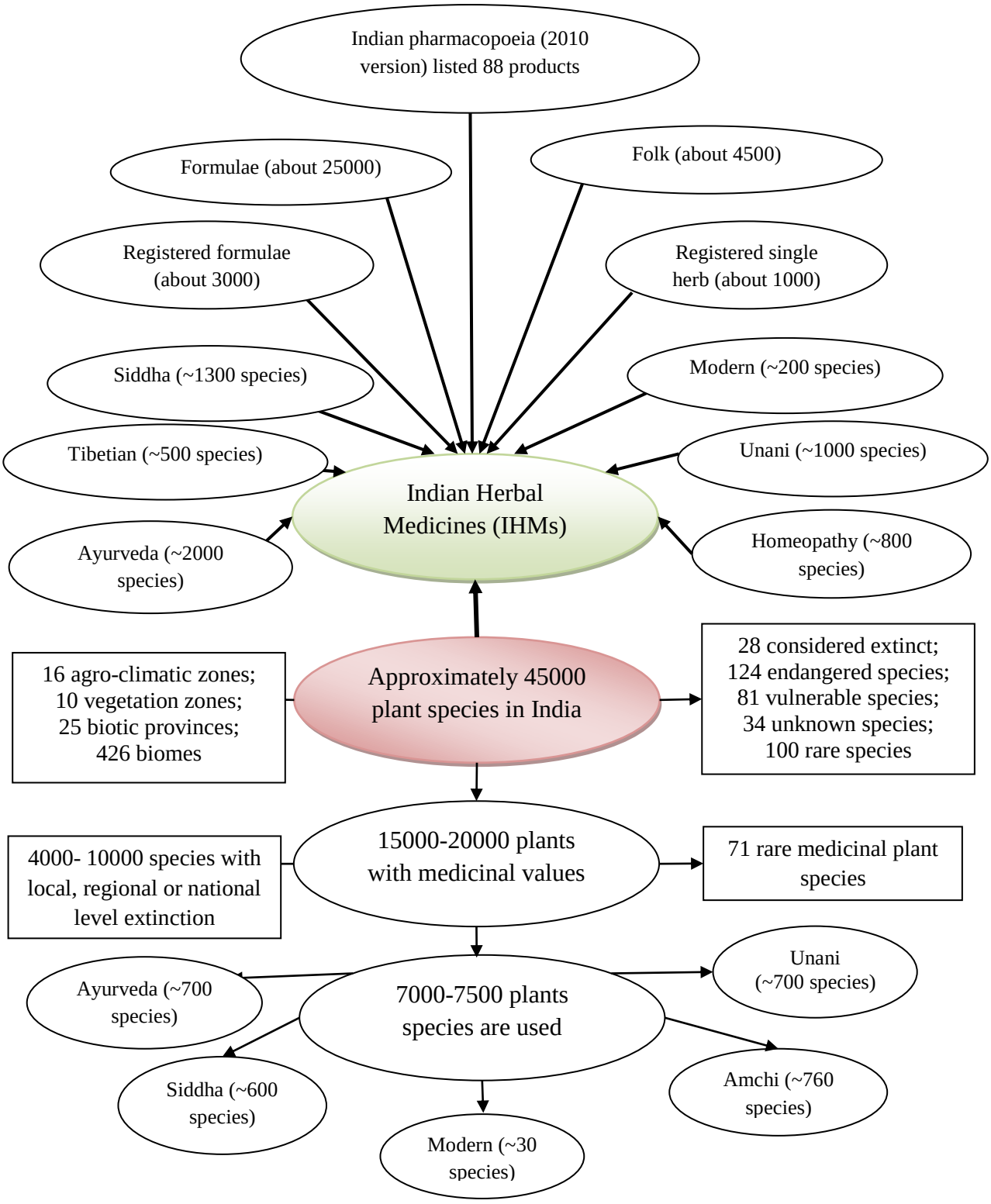

Figure 1.1. Plant species in India and Indian herbal medicine. (modified from Pan *et al*., 2014)

The arthritic diseases are one of the major diseases which are commonly found globally. One of the major arthritic types, the rheumatoid arthritis (RA), is an auto-immune multifactorial inflammatory bone-joint disease affecting at least 0.5–1% of the population in the industrialized world. It commonly leads to significant physical disability and restricted physical movement (Gabriel, 2001). It is 2 to 3 times more frequent in women than in men and can initiate at any age, with a peak incidence between the age of 35-55. Its prevalence in India ranges from 0.28% in the urban population to 0.55% in the rural population (Chopra and Ahmed, 2008). However, the actual number of affected population may vary from 2-5% in the country as the cases from rural area are not registered in many occasions.

Commonly used drugs against RA including NSAID and DMARD are limited by a low (<70%) response rate and induce serious side-effects (>30%). This has been cited as one of the major reasons for increased use of CAM by the patients suffering from RA. The usage of CAM has been complemented along with conventional drugs or has been used as the sole remedy to fight RA. About 60% to 90% of patient populations have been reported to be using CAM from different countries (Efthimiou *et al*., 2010).

As defined by the National Centre for Complementary and Alternative Medicine (NCCAM), "Complementary and alternative medicine is a group of diverse medical and health care systems, practices, and products that are not presently considered to be part of conventional medicine" (WHO, 2000). These practices or therapies included within the scope of complementary medicine field can be broadly categorized into three categories:

1. Treatments in which people can administer themselves the remedies orally or topically (e. g., botanical herbs, nutritional supplements, health food, meditation, and magnetic therapy).

2. Treatments which are administered by specialist therapy providers including acupuncture, chiropractic, massage, reflexology, and osteopathic manipulations etc.

3. Treatments which can be introduced by the patient under the periodic supervision of a therapy provider or doctor (e. g., Tai Chi, Yoga, Homeopathy, and Ayurveda).

The world health organization (WHO) reports that about 80% of the world population primarily depends on animal and plant-based medicines. Out of the 252 essential chemicals

that have been selected by the WHO, about 11.1% come from plants and 8.7% have animal based origin (WHO, 2000). So there is an increasing need for research in this field.

In the Indian perspective, its rich biodiversity has diverse array of medicinal plants. Different medicinal plants have been explored for their anti-arthritic properties. *Camellia sinensis var. assamica* (J. W. Hart.) Kitam (Assam tea) (Family Theaceae) is a well-cultivated plant species from India which showed inhibition of the matrix-metalloproteinase (MMP) activity *in vitro* and in different arthritic models (Ahmed *et al*., 2005; Rathore *et al*., 2007). *Curcuma longa* L. (Turmeric) (Family Zingiberaceae) is known to down-regulate different molecules contributing to RA pathogenesis including NF-κB, Cox-2 and Lox; down-regulate IL-1β-induced MMP-3 synthesis and increases IL-1 β induced type II collagen synthesis (Liacini *et al*., 2002; Schulze-tanzil *et al*., 2004; Kukur, 2010). *Semecarpus anacardium* L. f. (Nut milk extract) (Family Anacardiaceae) has shown effectivity against adjuvant-induced arthritic models (Vijayalakshmi *et al*., 1996; Ramprasath *et al*., 2006). *Saraca asoca* (Roxb.) Willd. (Ashoka) (Family Fabaceae) has shown significant anti-inflammatory activities in arthritic animal models. It also reduced the pro-inflammatory cytokine expressions in animal models (Saravanan *et al*., 2011; Gupta *et al*., 2014). *Cannabis sativa* L. (Ganja) (Family Cannabaceae) contains cannabinoid and cannabidiol as their prime phytocompounds, works against a large array of immune-mediated diseases including RA through its interaction with cannabinoid receptor 2 (CB 2) (Reider *et al*., 2010). It also shows reduction of acute-inflammation and inhibits Cox-2 (Costa *et al*., 2004; Takeda *et al*., 2008). *Ocimum tenuiflorum* L. Syn. *Ocimum sanctum* L. (Tulsi) (Family Lamiaceae) showed potent anti-arthritic as well as anti-inflammatory activity on different arthritic models; the plant contains eugenol as its prime active constituent (Singh and Majumder, 1996). *Withania somnifera* (L.) Dunal *(*Ashwagandha) (Family Solanaceae) showed significant normalization of arthritic parameters in arthritic animal models (Rasool *et al*., 2007). It also reduced the expression of pro-inflammatory cytokines *in vitro* (Singh *et al*., 2007). *Premna serratifolia* L. (Agnimantha) (Family Lamiaceae) has shown efficient amelioration of arthritic conditions in Freund's complete adjuvant (FCA)-induced arthritic rats (Rajendran and Krishnakumar, 2010; Kavitha *et al*., 2011). Among the other plants found in India, *Nyctanthes arbor-tristis* (Shiuli) (Family Oleaceae) (Paul and Saxena, 1997), *Swertia chirayita* (Roxb.) Buch-Ham. Ex C. B. Clarke (Chirata) (Family Gentianaceae)

(Kumar *et al*., 2003), *Crocus sativus* L. (Saffron) (Family Iridaceae) (Hossein and Younesi, 2002), *Strobilanthus callosus* Nees. (Karvi) (Family Acanthaceae) (Agarwal and Rangari, 2003), *Acacia farnesiana* (L.) Willd. (Hukkeri *et al*., 2002), *Aegle marmelos* (L.) Correa (Bel) (Gurulingappa and Halur, 2002), *Anacardium occidentale* L. (Cashew) (Mota and Thomas, 2002), *Azadirachta indica* A. Juss. (Neem) (Chattopadhyay, 1998), *Cedrus deodara* (Roxb. ex D. Don) G. Don (Deodar) (Shinde and Phadke, 1999), *Morus indica* L. (Mulberry) (Balasubramanian and Ramalingam, 2005), *Emilia sonchifolia* (L.) DC. Ex DC. (Shylesh and Padikkala, 1999) are some other promising plants having potential anti-inflammatory activities. These plants can be further used to investigate their role in rheumatoid arthritis.

Aloe vera (L.) Burm. f. (Ghritokumari) (Family Xanthorrhoeaceae) is a very well-known plant in the Indian traditional medicinal system. In this regard, studies on compound isolation have been done which revealed the presence of potent anti-inflammatory bio-molecules in the plant (Grindley and Reynolds, 1986). The plant is well distributed in India and in neighboring countries. Anti-inflammatory activity of this plant was screened preliminarily by different authors which has been discussed in the *Review of Literature* (Section 3.4). Different brief experiments and reports prove that the plant has some strong protective role against inflammation and rheumatism. However, some more precise methodologies are needed to establish its role in such disease. Elaborate experimentation on animal models and molecular approaches regarding the anti-inflammatory character of this plant has not been done so far.

The present thesis portrays the results of a detailed exploration of the anti-arthritic and anti-inflammatory property of this plant through *in vitro* and *in vivo* experiments. This study explored the efficacy of the *Aloe vera* gel in amelioration of inflammatory condition in animal models. The study also investigates the synergistic role of the phyto-compounds present in the plant leaf-gel in inflammation and anti-inflammatory arthritis. The orally consumable plant gel has been used in a simple aqueous homogenate form, as it is used in ethno-botany. The simple homogenization also preserves the natural proportions of the phyto-compounds present in the plant. To our knowledge, this is the first detailed report on the efficacy of such naturally harvested unprocessed *Aloe vera* leaf gel against inflammation in appropriate models. The study also represents toxicity and consumable dose of *Aloe vera* gel in *in vivo* and *in silico* model systems.