CHAPTER 1

INTRODUCTION

The traditional medicinal herbs used by the Darjeeling Himalayan region people of India to treat diabetes were the subject of ethnobotanical, chemical, and biological investigations for this PhD project. This chapter gives an overview of the history of using plants as medicine and the significance of plant-based natural products in contemporary medicine and medication development. There is also an outline of the thesis objectives.

1. Introduction

1.1 Natural Products: A continuing source for drug discovery

Nature has been the most reliable source of medicinal products from the beginning of time. Plant-based medicines have revolutionized the traditional medicine system [1], indicating that herbal preparation is the oldest remedy for humanity throughout history, and slowly, it has become an integral part of civilization's development [1]. In the last 30 years, approximately 50% of new chemical entities approved by the US Food and Drug Administration are obtained directly from nature or inspired by nature [2]. In Western countries, more than 30% of the total community has started taking herbal drugs. With every passing day, herbal drug's usage are increasing extensively for their better effectiveness with fewer side effects and reasonable price [3]. Drugs discovered from plants can treat various chronic diseases based on their diverse chemical entities. It is much better than conventional therapy due to its targeting potential and mechanism [4]. About 165 herbal products are exported from India [3]. A recent study revealed a notable change in the scenario of the global herbal market, and it is expected to reach a market value near $111 billion with a peak position by 2025 [5]. This increasing demand for medicinal plants worldwide for products such as medicines, phytochemicals, nutraceuticals, cosmetics, and other items presents a potential for Indian trade and commerce [6]. Using a safe reverse pharmacology approach based on a traditional knowledge database, herbal medications that are technologically standardized and scientifically validated can be produced. This may be essential for addressing Western prejudice towards Ayurveda and drug research, discovery, and treatments [7]. Herbal drug technology encompasses all the processes involved in

turning plant resources into pharmaceuticals; quality assurance and standardization, along with the appropriate fusion of contemporary scientific methods and traditional wisdom, will continue to be crucial. Unlike conventional pharmaceutical medicines, which are usually created from synthetic, chemically pure components using repeatable production techniques and procedures, herbal therapeutic items might vary in composition and quality. Thus, accurate starting material identification and quality control testing are important preconditions for guaranteeing the reproducible quality of herbal medicine, which enhances its safety and effectiveness [8, 9].

Many home remedies and collective practices associated with traditional and folklore medicine are carried forward from one generation to other generation. Recently, developed nations have been resorting to traditional therapeutic systems, including herbal cures and pharmaceuticals. A recent assessment conducted in the Member States of the European Union found that almost 1400 herbal remedies are used widely [10].

1.2 Herbal Medicine derived from Natural products

In the botanical literature, "herb" refers to non-woody vascular plants lacking persistent woody stems, such as annual, biennial, and some perennial crops [11]. But in pharmacology, the phrase refers to all herbs, shrubs, and trees, much like a synonym for "plant." Herbal medicine, botanical medicine, or phytomedicine refers to any medicine made from plants, plant parts, or plant products and requires little to no chemical processing [12]. Herbal medicines encompass a more comprehensive range of products, including herbal preparations, plants, plant parts, and other materials derived from plants, or combinations of these, as their primary ingredients. This also includes herbal materials such as leaves, stems, woods, bark, flowers, roots, rhizomes, fruits, seeds or other plant parts and active ingredients [13].

The synthesis of primary metabolites directly supports the growth, development, and reproduction of plants. These metabolites are subsequently transformed by a variety of enzyme reactions and molecular modifications into an enormous array of secondary metabolites, or natural products [14]. Secondary metabolites are divided into three categories based on their metabolic pathways: alkaloids, phenols, and terpenes. Flavonoids,

which include a variety of compounds like flavonols, flavones, flavanones, flavan-3ols (catechins), isoflavonoids, and anthocyanidins, are the most significant component group of phenols (also known as phenolics), which are defined as hydroxyl groups (-OH) directly bonded to aromatic hydrocarbon groups [15]. They are typically further classified based on the carbon's number. Terpenes are a broad class of chemical compounds with solid smells produced biosynthetically from isoprene units. They result in the chemical modification of terpenoids (or isoprenoids). Another group of natural chemicals called alkaloids primarily comprises basic nitrogen atoms [16]. Amino acids, such as ornithine, lysine, tyrosine, phenylalanine, tryptophan, histidine, aspartic acid, and anthranilic acid, are the biological precursors of the majority of alkaloids. Secondary metabolites include 33000 terpenoids, 16000 alkaloids, and 8182 flavonoids according to the Dictionary of Natural Products. These are essential to primary metabolism and play an ecological role in protecting plants from biotic and abiotic stressors [17, 18].

1.3 Medicinal Plants: Future Source of New Drug

Herbal medicine has become a global trend, with 4 billion people using it for primary healthcare. The World Health Organization (WHO) reports that about 11% of 252 drugs are derived from plants [19]. Plants have been used for food, medicine, clothing, and shelter since ancient times. Vegetarian foods contain antioxidants, phytochemicals, and micronutrients that promote health and protect against diseases. Plants also have antioxidant properties, which may mitigate oxidative stress and reduce the risk of various diseases. Many herbal medicines are derived from plants either directly or indirectly [20]. Medicinal plants are used as raw materials to make pharmaceuticals that provide people with reasonable and adequate healthcare. All plants, however, can produce phytochemicals, which are advantageous to human health because the human body cannot produce them [20, 21]. In addition, plants provide a rich nutritional supply of macromolecules, vitamins, and minerals essential for preserving bodily health. Many plants have been found to have pharmacological effects due to their metabolites. Plant metabolites are organic substances that fall into mainly two types, i.e, Primary metabolites and Secondary metabolites. The Primary metabolites are mainly organic substances that are good for the

overall growth and progress of the human body. These molecules include glucose, starch, polysaccharides, protein, lipids, and nucleic acid. Plant metabolites include tannins, alkaloids, flavonoids, saponins, terpenoids, steroids, glycosides, and volatile oils. These secondary metabolites give plants medicinal efficacy in treating various illnesses. Pharmacologically active substances are known as phytochemicals. These include alkaloids, which have diuretic, analgesic, antispasmodic, antimalarial, and antiviral properties; terpenoids, which have antiviral, anthelmintic, antibacterial, anticancer, and antibacterial properties; glycosides, which have antifungal and antibacterial properties; phenols and flavonoids, which have antioxidant, antiallergic, and antibacterial properties; and saponins, which have anti-inflammatory, antiviral etc [17, 20, 22, 23].

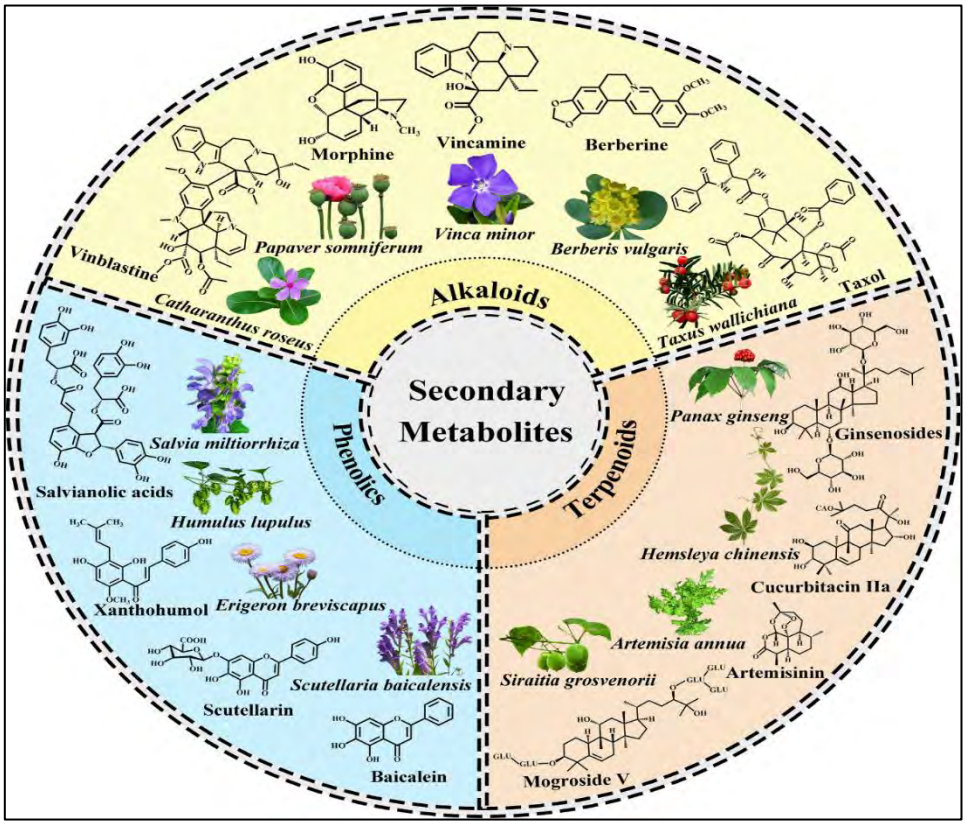


Figure 1.1. Examples of secondary metabolites produced by medicinal plants and their chemical structures [24]

Table 1.1. List of medicinal plants based on secondary metabolites[25]

Secondary Metabolites	Class	Medicinal Plants	Medical Properties	Example
Alkaloids	Opium (Phenanthrene Alkaloid)	*Papavera somniferous* (Poppy)	Analgesic, Narcotics	Morphine, Heroin
	Belladonna (Tropane Alkaloid)	*Datura metel, Atropa belladonna, Hyoscyamus niger*	Anticholinergic, Local anesthetics, stimulants	Atropine, scopolamine, hyoscyamine
	Cinchona (Quinoline Alkaloids)	*Cinchona officinalis*	Anti-malarial, anti-arrhythmic	Quinine, Quinidine
	Rauwolfia (Reserpine Alkaloids)	*Rauwolfia serpentine*	Anti-hypertensive	Reserpine
	Vinca Alkaloids (Indole Alkaloids)	*Catharanthus roseus*	Anti-cancer, Anti-tumour	Vincristine, Vinblastine
Flavonoids	Flavanone	Citrus fruits (Oranges, Lemons etc)	Anti-inflammatory	Hesperitine, Naringenin
	Flavones	Labiatae and Umbelliferae family	Antitumor	Apigenin, luteolin
	Flavanols	Green tea leaves and Angiosperms	Anti-oxidants and microbial enzyme inhibitors	Myricetin, Kaempferol, Quercetin
	Anthocyanidins	Fruits(grapes, raspberry), vegetables	Anti-hepatoxic, anti-lipolytic vasodilators	Malvidin, cyanidin
	Isoflavonoids	Legumes, cereals	Anticancer, treatment of heart disease	Daidzein, genistein
Terpenes	Monoterpenes	Pinus species	Analgesic, Anti-inflammatory	Camphor
	Sesquiterpenes	Essential oils	Anti-bacterial, anti-fungal	Santonin
	Diterpenes	Taxus sp	Anti-cancer, anti-hypertensive	Taxol
	Triterpenes	Betula alba	Anti-inflammatory, homolytic	Betuline, β-sitosterol,
	Tetraterpenes	Carrot, pumpkin	Antioxidants	β-Carotene

1.4 Ethnomedicinal Plants from Darjeeling Himalayan Region

Numerous plants with critical medical qualities previously unknown to science have been discovered by ethnobotanical research [26]. Ethnobotanical research is one of India's most prosperous, oldest, and most varied traditional medical systems. The livelihoods of local rural populations depend heavily on medicinal plants gathered from Himalayan woods, which are also gaining international recognition. Indians are among the first to use plant resources for their daily needs and nutrition. The usefulness of wild edible vegetables in food security has yet to receive enough attention in India, even though plants have been used for food, fodder, shelter, clothing, medicine, and various other essential commodities since ancient times [26, 27]. The magnificent Himalayan range contributes to the rich diversity of healing plants in the eastern foothills. Due to human activity, many of the foothills' medicinal plants are rapidly going extinct or facing serious threats [28-30]. In the Himalayan region, the value of medicinal plants is becoming more widely acknowledged, especially when considering it from an ecological, social, and economic standpoint [26, 31].The Lepcha, Bhutia, and Nepalese are the main ethnic groups of the Kalimpong Hills, and their descendants are still found in the village regions. Every one of these groups has a unique work ethic, culture, language, and customs. These tribes' ethnic knowledge has allowed them to preserve various plant species. Besides, the use of plant species for therapeutic purposes has been passed down orally from generation to generation, as a result of ongoing socio-economic changes and a shift in attitude, younger generations have begun to disregard this knowledge of therapeutic plants, leading to its decline and eventual obsolescence [32].

Table 1.2: List of medicinal plants from Darjeeling Himalayan region and their ethnomedicinal uses [26]

Sr No.	Scientific Name	Ethno-medicinal uses	Traditional medicine preparation
1.	*Acorus calamus*	Skin diseases, fever, distressing cough	Rhizome paste is externally applied to the affected area to treat skin conditions and the forehead when a fever occurs. A tiny piece of dried rhizome is eaten to treat a cough.

Sr No.	Scientific Name	Ethno-medicinal uses	Traditional medicine preparation
2.	*Ageratina adenophora L.*	Cures cuts, wounds, jaundice, rheumatism, and dysentery	Tender leaves steeped in water and juice are given orally to treat jaundice and diarrhea, and leaf paste is applied to wounds.
3.	*Aloe barbadensis Mill.*	Liverdisorders, spleen disorders, cough, skin diseases, dyspepsia, and burns.	A drink made from leaves steeped in water to relieve spleen and liver disorders. Burns were treated immediately with succulent leaves to provide relief.
4.	*Artemisia vulgaris L.*	Sinusitis, nausea, menstrual disorder, rheumatism, bio-pesticide, and ethical value.	Crushed leaves are consumed.
5.	*Ageratum conyzoides Linn.*	Antiseptic	Crushed leaves are applied topically to wounds.
6.	*Cannabis sativa L.*	Body pain, cattle in flatulence, and an ethical value.	When experiencing extreme bodily discomfort, seeds are ground into a powder, combined with water, and taken in tiny amounts.
7.	*Curcuma Longa L.*	Pain relief, blood cell levels, cough cure, asthma treatment, hiccup cure, and poison antidote all come from this remedy.	The rhizome is consumed orally.
8.	*Cynodon dactylon (linn.)*	Burning urination, liver cirrhosis, and treatment of piles	Ground, combined with water and ingested.
9.	*Datura stramonium L.*	To treat bronchitis or asthma, regulate oral salivation	Patients with asthma can find comfort by inhaling smoke produced from burning leaves. Seeds are pasted and administered externally to treat neuralgia and rheumatoid arthritis.
10.	*Oroxylum indicum L.*	Treats irritable bowel syndrome, pneumonia, bronchitis, bronchial	After being ground, seeds are eaten with water. Flowers are

Sr No.	Scientific Name	Ethno-medicinal uses	Traditional medicine preparation
		asthma, cough, and throat irritations	utilized in food preparation.
11.	*Thysanolaena latifolia*	Beneficial for abortion, boils, and tonsillitis.	Boiling fresh roots yields an extract that can be used for burns and boils, or it can be combined with water and ingested to cure tonsillitis and induce abortions.
12.	*Laportea terminals*	Wight Diuretic: used to lower blood pressure and fight dandruff in hair.	Inflorescence is cooked and consumed as a curry to lower blood pressure, improve heart health, or stimulate urine.
13.	*Vitex negundo L.*	Beneficial for body edoema, bone fractures, and jaundice	Juice from fresh leaves is eaten.
14.	*Zingiber officinale L.*	Treat rheumatism, bronchitis, asthma, throat issues, coughing, fever, and coughing up mucus.	Rhizome taken orally
15.	*Juglans regia, Linn*	In addition to being considered antitoxic, it enhances cognitive and intestinal health. Along with improving complexion, it strengthens and intensifies physical strength and reduces discomfort.	Nuts and fruits are eaten raw, or their paste is applied topically.

1.5 Drug Development from medicinal Plants: Phytochemical and Phyto Therapeutic Approaches

Two approaches to developing drugs from medicinal plants are Phytochemical and phytotherapeutic. The former focuses on pure phytochemicals as drugs, while the latter uses crude plant preparations like extracts, active fractions, or mixtures. Developing a chemical entity from a plant as a drug can be expensive and time-consuming. The Phytotherapeutic approach is cost-effective and incorporates the experiential philosophy and traditional wisdom of holistic approaches. This approach addresses the multifactorial pathogenicity of chronic diseases by utilizing the entire plant, resulting in synergism and therapeutic benefits without toxic manifestation [33, 34].

1.5.1 Phytochemical approach to drug research

There is growing recognition of phytochemicals or substances derived from plants as potentially effective alternative therapies for various diseases. Several phytochemicals are being intensively investigated for their potential application. Rapid extraction of bioactive compounds from plants is facilitated by combinatorial chemistry, high throughput screening, and bioassay-guided isolation. Through computational molecular modeling for pharmacological evaluation, the investigation of structural and chemical databases in combination with target gene and protein databases will enable the creation of new chemical entities [35-37].

1.5.2 Phyto therapeutic approaches

According to Calixto et al. [38], Standardised herbal remedies consisting of complex mixtures of one or more plants are called phytotherapeutic agents, the active ingredients of which are unknown but which give rise to pharmaceutical effects. The lack of a rapid or potent pharmacological activity is a fundamental feature of phyto-therapeutic drugs. According to Akerele et al. [39], phototherapeutic substances are not employed for acute medical conditions. Standardization of extract, safety, and quality are the key factors that phytotherapy considers. Regulation bodies such as the United States Food and Drug Administration (US FDA) or authorities guaranteeing Good Manufacturing Practices (GMP) should be in charge of checking quality [19, 40]. Methods such as gas chromatography, high-performance liquid chromatography, thin-layer chromatography, Liquid chromatography-mass spectroscopy, and ultraviolet/visible spectroscopy are employed to quantify bacteriological contamination, identify species, evaluate potency, and finally provide certificates of analysis for the plant material. One significant factor in extracts is safety assessment. To guarantee a drug's safety profile, toxicity studies are crucial. Standardization in herbal medicine refers to providing processed plant extracts with a given marker ingredient concentration. The issue is that plant constituents work in concert, so even amounts of active ingredients could be deceptive indicators of efficacy if cofactors are missing. As a result, chromatographic fingerprinting occasionally contributes to standardization techniques [41, 42].

1.6 Antioxidant, antidiabetic, anti-inflammatory details

- It is firmly established that oxidative stress is a major pathogenetic element in diabetic complications.
- Consequence of an overload of glucose in blood creates oxidative phosphorylation and enhance generation of ROS which is reactive oxygen species.
- In this situation, damage indirectly caused by ROS generation in body, can trigger an inflammatory response [43].

1.7 Related to Research work in this thesis

The abovementioned discussions substantiated the global scenario of plant drug research, its utilizations, and its beneficial impact on human society. In this thesis, I have worked on two medicinal plants: *Asparagus racemosus willd* aerial part, *Duabanga grandiflora* leaves, and flower. Both plants are edible and consumed by local tribes of the Darjeeling Himalayan region. These two plants were explored scientifically under modern pharmacognostic, phytochemical, and pharmacological schemes, and the results could serve as future benchmarks.

1.8 Aim objectives

The overall objective of this PhD project was to undertake chemical and biological investigations on the medicinal plant's named *Asparagus racemosus* willd and *Duabanga grandiflora*. This project involved conducting a inclusive literature evaluation on these two plants for managing diabetes and related inflammation. The work would exploit Indigenous resources to develop the scaffold(s) to be helpful in cost-effective, target-specific, and less toxic diabetes management.

Keeping the aim in mind, the objectives are-

i) To evaluate the pharmacognostical and phytochemical profile of these selected plants
ii) To identify bioactive fractions by bioassay-guided isolation
iii) To investigate the lead compounds responsible for bioactivity
iv) To assure pharmacological activity by different assays like *in vitro* and *in vivo*, particularly against metabolic disorders and inflammation.

1.9 References

1. Chandira, M. and B. Jayakar, *Formulation and evaluation of herbal tablets containing Ipomoea digitata Linn. extract.* Extraction, 2010. 3(1): p. 022.
2. Katz, L. and R.H. Baltz, *Natural product discovery: past, present, and future.* Journal of industrial microbiology & biotechnology, 2016. 43(2-3): p. 155-176.
3. Ali, M., *Present status of herbal medicines in India.* Journal of herbal medicine and toxicology, 2009. 3(2): p. 1-7.
4. Pelkonen, O., Q. Xu, and T.-P. Fan, *Why is research on herbal medicinal products important and how can we improve its quality?* Journal of traditional and complementary medicine, 2014. 4(1): p. 1-7.
5. Organization, W.H., *National policy on traditional medicine and regulation of herbal medicines: Report of a WHO global survey.* 2005.
6. Singh, J., A. Singh, and S. Khanuja, *Medicinal plants: India's opportunities.* Pharma Bioworld, 2003. 1: p. 59-66.
7. Patwardhan, B., A.D. Vaidya, and M. Chorghade, *Ayurveda and natural products drug discovery.* Current science, 2004: p. 789-799.
8. Straus, S.E., *Herbal medicines—what's in the bottle?* New England Journal of Medicine, 2002. 347(25): p. 1997-1998.
9. De Smet, P.A., *Health risks of herbal remedies.* Drug safety, 1995. 13: p. 81-93.
10. Hoareau, L. and E.J. DaSilva, *Medicinal plants: a re-emerging health aid.* Electronic Journal of biotechnology, 1999. 2(2): p. 3-4.
11. Parveen, B., et al., *Challenges and opportunities for traditional herbal medicine today, with special reference to its status in India.* Ann Phytomed, 2020. 9(2): p. 97-112.
12. Iqbal, M., et al., *Establishing the botanical identity of plant drugs based on their active ingredients under diverse growth conditions.* Journal of Environmental Biology, 2018. 39(1): p. 123-136.
13. Organization, W.H., *WHO global report on traditional and complementary medicine 2019*. 2019: World Health Organization.

14. Anjitha, K., P. Sameena, and J.T. Puthur, *Functional aspects of plant secondary metabolites in metal stress tolerance and their importance in pharmacology.* Plant Stress, 2021. 2: p. 100038.
15. Costa, T., et al., *Secondary metabolites.* 2012.
16. Pagare, S., et al., *Secondary metabolites of plants and their role: Overview.* Current trends in biotechnology and pharmacy, 2015. 9(3): p. 293-304.
17. Jain, C., S. Khatana, and R. Vijayvergia, *Bioactivity of secondary metabolites of various plants: a review.* Int. J. Pharm. Sci. Res, 2019. 10(2): p. 494-504.
18. Crozier, A., M.N. Clifford, and H. Ashihara, *Plant secondary metabolites.* Occurrence, Structure and Role in the Human Diet, 2006: p. Blackwell Publishers.
19. WHO, G., *WHO monographs on selected medicinal plants.* 1999.
20. Shakya, A.K., *Medicinal plants: Future source of new drugs.* International journal of herbal medicine, 2016. 4(4): p. 59-64.
21. Martinez, M.J.A., et al., *Anti-infectious activity in the anthemideae tribe.* Studies in Natural Products Chemistry, 2008. 35: p. 445-516.
22. Maurya, R., G. Singh, and P.P. Yadav, *Antiosteoporotic agents from natural sources.* Studies in natural products chemistry, 2008. 35: p. 517-548.
23. Chopra, A. and V.V. Doiphode, *Ayurvedic medicine: core concept, therapeutic principles, and current relevance.* Medical Clinics, 2002. 86(1): p. 75-89.
24. Zhao, Y., et al., *Multilayered regulation of secondary metabolism in medicinal plants.* Molecular Horticulture, 2023. 3(1): p. 11.
25. Haddad-Kashani, H., et al., *Pharmacological properties of medicinal herbs by focus on secondary metabolites.* Life Science Journal, 2012. 9(1): p. 509-520.
26. Biplov, C.S., et al., *Documentation and utilization pattern of ethno-medicinal plants in Darjeeling Himalayas, India.* Int J Usuf Mngt, 2015. 16(2): p. 3-11.
27. Reddy, K., et al., *Traditional knowledge on wild food plants in Andhra Pradesh.* 2007.
28. Pala, N., et al., *Medicinal plant resources in sacred forests of Garhwal Himalaya.* 2012.

29. Larsen, H.O., C.S. Olsen, and T.E. Boon, *The non-timber forest policy process in Nepal: actors, objectives and power.* Forest Policy and Economics, 2000. 1(3-4): p. 267-281.
30. Olsen, C.S., *Valuation of commercial central Himalayan medicinal plants.* Ambio: A Journal of the Human Environment, 2005. 34(8): p. 607-610.
31. Negi, V.S., R. Maikhuri, and L. Rawat, *Non-timber forest products (NTFPs): a viable option for biodiversity conservation and livelihood enhancement in central Himalaya.* Biodiversity and Conservation, 2011. 20(3): p. 545-559.
32. Kala, C.P., *Status and conservation of rare and endangered medicinal plants in the Indian trans-Himalaya.* Biological conservation, 2000. 93(3): p. 371-379.
33. Subramoniam, A., *The problems and prospects of plant drug research in India: pharmacological evaluation of ecotypes in herbal drug development*. 2001, Medknow. p. 145-146.
34. Tiwari, A.K. and J.M. Rao, *Diabetes mellitus and multiple therapeutic approaches of phytochemicals: Present status and future prospects.* Current science, 2002: p. 30-38.
35. David, F., et al., *A perspective on synthetic biology in drug discovery and development—current impact and future opportunities.* SLAS DISCOVERY: Advancing the Science of Drug Discovery, 2021. 26(5): p. 581-603.
36. Yin, S.-Y., N.-S. Yang, and T.-J. Lin, *Phytochemicals approach for developing cancer immunotherapeutics.* Frontiers in pharmacology, 2017. 8: p. 274931.
37. Egbuna, C., et al., *Phytochemicals as lead compounds for new drug discovery*. 2019: Elsevier.
38. Calixto, J., *Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (phytotherapeutic agents).* Brazilian Journal of medical and Biological research, 2000. 33: p. 179-189.
39. Akerele, O., *WHO guidelines for the assessment of herbal medicines.* 1992.
40. Li, W., *Botanical drugs: a future for herbal medicines.* J. Contemp. Health L. & Pol'y, 2002. 19: p. 117.

41. Rather, M.A., B.A. Bhat, and M.A. Qurishi, *Multicomponent phytotherapeutic approach gaining momentum: Is the "one drug to fit all" model breaking down?* Phytomedicine, 2013. 21(1): p. 1-14.
42. Das, D.N., et al., *Phytotherapeutic approach: a new hope for polycyclic aromatic hydrocarbons induced cellular disorders, autophagic and apoptotic cell death.* Toxicology mechanisms and methods, 2017. 27(1): p. 1-17.