Chapter 10

Chemical Profiling of *Nothapodytes nimmoniana* for Camptothecin, an Important Anticancer Alkaloid: Towards the Development of a Sustainable Production System

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Abstract Camptothecin (CPT), a pyrrolo quinoline alkaloid, is one of the most promising anticancer drugs of the 21st century. The compound was first isolated from the Chinese deciduous tree, *Camptotheca acuminata*. CPT exhibits a broad spectrum of antitumor activity both under *in vitro* and *in vivo* conditions. Irinotecan (CPT11) and Topotecan (TPT), two water-soluble derivatives of CPT, have been approved by the United States Food and Drug Administration for treating colorectal and ovarian cancers as well as against several types of brain tumor in children. Although CPT has been reported to exist in several species, the highest concentration (about 0.3%) to date has been realized from *Nothapodytes nimmoniana*. The tree commonly referred to as "stinking tree" is native to warmer regions of South India. In the last few decades, driven by the enormous demand for CPT, there has been a decline of at least 20% in the population, leading to red listing of the species. In recent years, efforts have been initiated in India to identify high-yielding individuals and populations of *N. nimmoniana* in its natural distribution range with the ultimate aim of using these lines to develop clonal orchards, as well as in developing *in vitro* produc-

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tion systems. In this chapter, we briefly review the overall status of *N. nimmoniana* as a source of CPT. Drawing upon existing literature as well as ongoing work at our laboratory, we discuss the basic patterns of accumulation of CPT in *N. nimmoniana*. We review the population variability for CPT accumulation along the distributional range of the species in the Western Ghats, India. Using a relatively new tool, namely the ecological niche model, we predict the chemical hot-spots of the species in the Western Ghats and offer a test of this prediction. Finally, we discuss strategies for a sustainable model of extraction of CPT from *N. nimmoniana*.

Keywords Camptothecin, Chemical profiling, HPLC, DIVA-GIS, *Nothapodytes nimmoniana*, Western Ghats

Abbreviations

CPT	Camptothecin
FDA	United States Food and Drug Administration
GIS	Geographic Information System

10.1 Introduction

Nature has been recognized as a rich source of medicinal compounds for hundreds of years. Today, a vast range of drugs that represent the cornerstones of modern pharmaceutical care are either natural products or have been derived from them [1]. It is estimated that over 50% of all drugs (and their derivatives and analogs) in clinical use are higher-plant-derived, natural products [2]. According to the World Health Organization, about 80% of the people in developing countries still rely on traditional medicine for their primary health care, and about 85% of such medicines involve the use of plant extracts. In other words, an incredibly large number of people (about 3.5–4 billion) in the world rely on plants as source of drugs [1, 3].

In recent years, with the advent of newer tools, including high-throughput screening for bioactive molecules, there is a resurgence of interest in mining higher plants for a variety of metabolites. In fact, nowhere has the effort been more pronounced than in the National Cancer Institute (USA), which has screened over 435,000 plants for antineoplastic effects [4]. Plant-based natural products have played a significant role in the development of contemporary cancer chemotherapy. Several novel antitumor compounds, including taxols, camptothecin (CPT) and its derivatives, maytansine, tripdiolide, homoharringtonine, vinblastine, vincristine, indicine-N-Oxide, baccharin, podophyllotoxin derivatives, and etoposide, are being extracted from plant sources [5,

6]. Considering the enormous significance that these compounds hold, several laboratories worldwide have been striving to intensively mine such compounds and standardize methodologies for their large-scale production.

Among the plant-derived compounds, CPT, a pyrrolo quinoline alkaloid, has been used extensively as a novel antitumor compound. CPT is lauded as one of the most promising anticancer drugs of the 21st century [7]. CPT exhibits a broad spectrum of antitumor activities under both *in vitro* and *in vivo* conditions [8]. CPT and its analogs in the presence of topoisomerase-I produce DNA damage by binding to and stabilizing a covalent DNA-topoisomerase-I complex in which one strand of DNA is broken [5–7, 9–11].

Irinotecan (CPT11) and Topotecan (TPT), two water-soluble derivatives of CPT, have been approved by the United States Food and Drug Administration (FDA) for treating small-cell lung cancer, colorectal cancer, and ovarian cancer [11–17]. They have also been approved by the FDA for the treatment of acquired immune deficiency syndrome [18].

CPT was first discovered in the Chinese deciduous tree, *Camptotheca acuminata* (Nyssaceae) [19]. The other plant species from which CPT is isolated are *Merriliodendron megacarpum* [20] and *Nothapodytes nimmoniana* Graham [21], both belonging to the family Icacinaceae, *Ophirrohiza mugos* [22] and *O. pumila* [23] from the family Rubiaceae, *Eravatamia heyneana* [24], belonging to Apocynaceae, and *Mostuea brunonis* [25], belonging to the family Loganiaceae (Table 10.1). However to date, the highest content of CPT has been realized from *N. nimmoniana* (about 0.3% on a dry weight basis) [21].

The market demand for Irinotecan and Topotecan has been ever increasing and has currently reached approximately US$ 1000,000,000, which represents approximately 1 tonne of CPT in terms of natural material [1, 26]. Most of this demand is currently met from plantations of *C. acuminata* that have been established extensively in China. In India, however, *N. nimmoniana* remains the main source of CPT. Based on current market price, it is estimated that the *N. nimmoniana* available along the northern part of the Western Ghats *per se* is worth over US$ 350,000,000 (Ganeshaiah and Uma Shaanker, unpublished data). While official records are not available, it is reliably learnt that the tree is extensively harvested from the Western Ghats and the billets exported for commercial extraction of CPT. In fact, it is estimated that in the last decade alone, there has been at least a 20% decline in the population, leading to the red listing of the species [27, 28]. Indiscriminate felling of trees for short-term gains could perhaps lead to the loss of elite individuals and populations that could otherwise potentially serve as sources of high CPT.

With no synthetic source of this alkaloid and with an increasing global demand, it has become imperative that the demand for CPT is met from a sustainable supply rather than the current destructive harvesting. Among the various approaches, prospecting for populations and/or individuals of the species for higher yields of the alkaloid could potentially help in establishing high-yielding clonal orchards and in developing *in vitro* production systems, thereby relieving the pressure on natural populations. Toward this end, in recent years,

Table 10.1 Camptothecin (*CPT*; % dry weight) content in different plant species and tissues (Adapted from Ramesha et al., unpublished data). *HPLC* High-performance liquid chromatography, *DAD* diode array detection, *ESI* electrospray ionization, *UV* ultraviolet, *IR* infrared, *NMR* nuclear magnetic resonance, *MS* mass spectrometry

Plant species	Tissue analyzed	CPT (% dry weight)	References	Chromatographic analysis
Camptotheca acuminata	Young leaves	0.4–0.5	[51]	HPLC
	Seeds	0.30	[51]	HPLC
	Bark	0.18–0.2	[51]	HPLC
	Young leaves	0.24–0.30	[52]	HPLC
	Hairy roots	0.1	[11]	HPLC
	Callus	0.20–0.23	[53]	HPLC
Camptotheca lowreyana	Young leaves	0.39–0.55	[52]	HPLC
	Old leaves	0.09–0.11	[52]	HPLC
Camptotheca yunnanensis	Young leaves	0.25–0.44	[52]	HPLC
	Old leaves	0.059	[52]	
Ervatamia heyneana	Wood and stem bark	0.13	[24]	HPLC
Merriliodendron megacarpum	Leaves and stem	0.053	[20]	HPLC
Ophiorrhiza pumila	Young roots	0.1	[54]	HPLC
	Hairy roots	0.1	[54]	
Ophiorrhiza mungos	Whole plant	0.0012	[22]	HPLC
Ophiorrhiza rugosa	Albino plants	0.1	[55]	HPLC
	Normal plant grown *in vitro*	0.03	[55]	HPLC
Mostuea brunonis	Whole plant	0.01	[25]	HPLC
Pyrenacantha klaineana	Stems	0.0048	[56]	HPLC
Nothapodytes foetida	Stem wood	0.14–0.24	[57]	HPLC
	Shoot	0.075	[34]	HPLC
	Plant	0.048	[58]	HPLC-DAD-ESI
Nothapodytes nimmoniana	Stem bark	0.3	[21]	UV, IR, NMR and MS
	Leaves	0.081	[33]	HPLC
	Stem bark	0.236	[33]	HPLC
	Root bark	0.333–0.775	[33]	HPLC
	Stem wood	0.14	[33]	HPLC
	Root wood	0.18	[33]	HPLC

attempts have been made to chemically characterize populations of *N. nimmoniana* along the distributional range of the species in the Western Ghats, India, with the ultimate aim of identifying populations/individuals with high CPT yields. High-yielding sources can be used to produce material for clonal multiplication and to develop cell lines with a high CPT yield. Attempts have also been made to identify the ecological correlates of CPT accumulation and identify the ecological niche of *N. nimmoniana* in Western Ghats and identify the "hot-spots" of CPT accumulation. The latter can guide collection of accessions for conservation as well as for use in sustainable models of extraction of CPT.

In this chapter, we briefly review the overall status of *N. nimmoniana* as a source of CPT. We draw upon existing literature as well as ongoing work in our laboratory to review the population variability for CPT along the distributional range of the species in the Western Ghats, India. We examine whether the population variability reflects intrinsic genetic variations or whether they are related to the ecological correlate of the populations. Using a relatively new Geographic Information System (GIS) technique, namely the ecological niche modeling tool, we predict the chemical hot spots of the species in the Western Ghats and offer a test for this prediction. Finally, we discuss a possible sustainable model of extraction of CPT from *N. nimmoniana*.

10.2 *N. nimmoniana:* Ecology and Distribution

N. nimmoniana, Graham, formerly known as *N. foetida* Sleumer and *Mappia foetida* Meirs, is a small tree belonging to the family Icacinaceae (Fig. 10.1). The genus *Nothapodytes* includes *N. obtusifolia*, found in China, *N. montana*, distributed in Thailand, north-eastern Sumatra, western Java, and western Sumbava, and *N. pittosporoides*, distributed in China and Indonesia [29]. It

Fig. 10.1 *Nothapodytes nimmoniana* (Photograph courtesy of Dr. G. Ravikanth)

has also been reported in Taiwan [30]. *N. nimmoniana*, commonly referred to as "Stinking Tree," is native to warmer regions of South India. It is reported in the western parts of the Deccan peninsula, North Bengal, and Assam [31] (Fig. 10.2). The tree is distributed in the shola forests in Nilgiris and present in both the Western Ghats and the Eastern plateau. It is also distributed in Sri Lanka, Myanmar, Indonesia, and Thailand [27].

The species exhibits a wide array of breeding systems including male, female, hermaphrodite, monoecious, andromonoecious, gynomonoecious, and trimonoecious individuals [27]. The trees flower during July–August, and most of the early flowering trees are dioecious, whereas late flowering trees are monoecious, hermaphrodite, and a mixture of other breeding types [27]. The fruits ripen during November–December and germinate during May–June after the onset of monsoon rainfall.

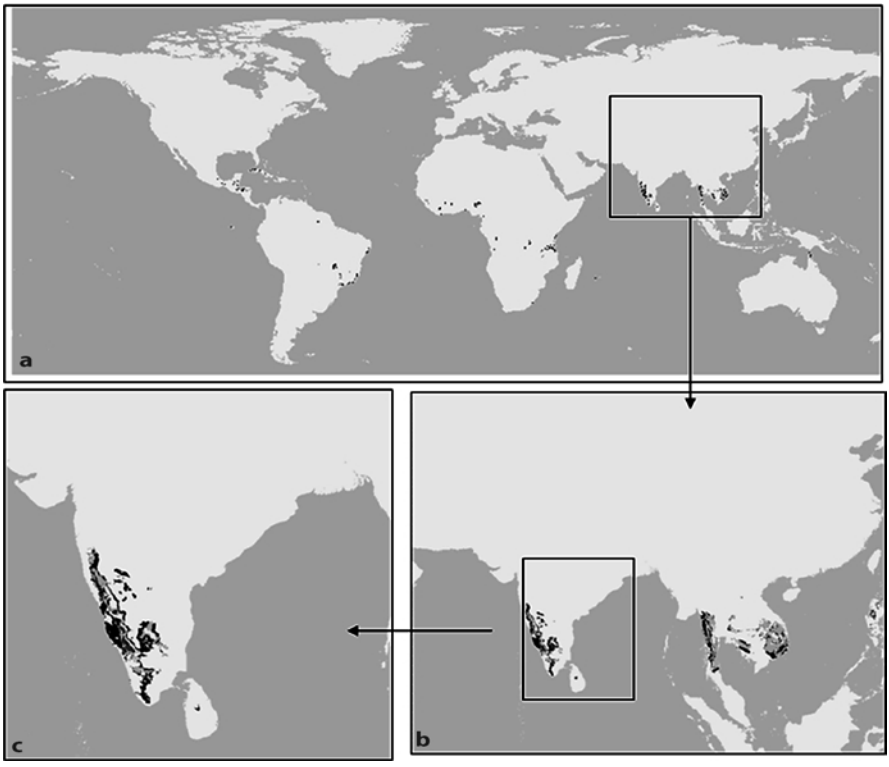

Fig. 10.2 Predicted distribtution of *N.Nimmoniana* in (**a**) the world (**b**) south-east Asia and (**c**) in the Western Ghat region of South India

10.3 Basic Patterns of Accumulation of CPT in *N. nimmoniana*

Although CPT has been reported in over nine species, the basic patterns of accumulation of CPT have been well documented only in *C. acuminata* and, to a lesser extent, in *N. nimmoniana*. For example, Yan et al. [6] reported the highest levels of CPT in leaves of *Camptotheca. acuminata.* CPT content was at least tenfold higher in younger than older leaves [6]. In fact, the high concentration of CPT in leaves has reportedly led to the poisoning of goats that browse on the leaves and even the honey bees foraging on the floral rewards [6]. Although the precise mechanism of transport and storage of CPT is not yet fully understood, it is conjectured that CPT is synthesized in the leaves and sequestered in old and dead tissues [6]. At the cellular level, CPT is localized in mesophyll and subpalisade layers of young leaves [6]. It has also been reported to be localized in vacuoles of young and older leaves [32].

The basic patterns of accumulation of CPT in *N. nimmoniana* have been characterized with respect to the age and sex of the plant and plant parts [33]. Among the various plant parts, the inner root bark is reported to yield the highest CPT content, followed by the inner stem bark. The average CPT content in the inner root bark is about $0.33 \pm 0.21\%$, compared to $0.23 \pm 0.15\%$ in inner stem bark (Fig. 10.3). The CPT content in the root and stem wood is significantly lower than that of the respective inner bark tissue. While the root wood contained $0.18 \pm 0.09\%$, the stem wood contained only $0.14 \pm 0.12\%$ CPT. Seeds on an average contained only about 0.17% CPT [33]. The CPT content in 2-year-old seedlings was highest in the root tips (0.4%), followed by leaves and stem (0.2%). The CPT content did not differ between the old and the young leaves. There was no difference in the CPT content between the sexes [33]. These studies reaffirm the earlier findings of Govindachari and Vishwanathan [21], who reported the highest yields of CPT from roots of *N. nimmoniana*. In

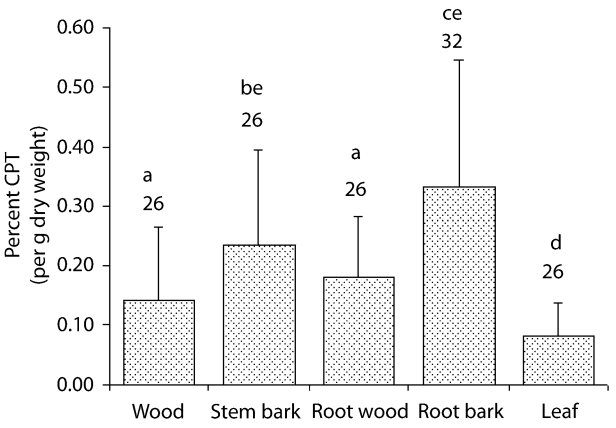

Fig. 10.3 Mean percent camptothecin (CPT) per gram dry weight in different tissues of *N.nimmoniana*. The numbers on the histograms indicate number of trees used in the analysis. Respective histograms with dissimilar letters indicate a significant difference in CPT content (*t*-test $p<0.05$; redrawn from Padmanabha et al. [3])

fact traditionally, CPT has been extracted from root, root bark, and fruits [34]. Fairly good amounts (0.10%) of the alkaloid have also been reported from seeds [34]. Quite obviously, because of the relatively low levels of CPT in leaves, extraction of CPT from these trees has been mostly destructive, involving the felling of the trees.

10.4 Chemical Profiling of Populations of *N. nimmoniana* for CPT

While *N. nimmoniana* forms one of the richest sources of CPT, commercial production of the alkaloid is still limited for want of high-yielding lines. Prospecting for high-yielding individuals or populations across the distributional range of the species could help in using the identified high-yielding lines for clonal multiplication and commercial production of CPT. Toward this end, recently, Suhas et al. [35] chemically profiled populations of *N. nimmoniana* along the Western Ghats, a mountain chain running parallel to the west coast of south India, and considered as one of the 34 mega-biodiversity hot spots of the world [36]. Based on primary and secondary data sources, the occurrence of the species in the Western Ghats was digitized on a GIS platform. While the species occurs along the length of the Western Ghats, it is clear that the distribution is not uniform; certain parts, namely the southern and central Western Ghats have a greater density of records of distribution. Based on the relative distribution, Suhas et al. [35] analyzed 11 populations from 8° to 15° N latitude (Fig. 10.4). For each of the 11 populations, 10–15 trees were sampled randomly and the CPT estimated in the inner stem and root bark tissues, respectively.

Significant variation exists among populations with respect to their mean CPT content, both in stem bark (one-way ANOVA, $P<0.004$) and root bark ($P<0.001$). The levels of CPT in stem bark ranged from as low as 0.03% to as high as 2.7%, with an overall mean of 0.7%. The mean CPT content in the root bark ranged from 0.003 to 1.41%, with an overall mean of 0.48%. The northern Kerala populations had the highest CPT content both in their stem bark (1.10±0.462%) and root bark (0.93±0.359%). The CPT content of stem bark was significantly positively correlated with that of the respective root bark ($n=126$; $r=0.320$, $P<0.05$). Finally, the frequency distribution of CPT content over all populations was highly positively skewed (Fig. 10.5).

Suhas et al. [35] found no clear relationship between CPT content and the girth size of trees. In 7 of the 11 populations, there was no relationship; however, in 3 of the remaining 4 there was a significant positive relationship ($r=0.678$, $r=0.762$, $r=0.728$; all $P<0.05$), and in 1 it was negatively related ($r=-0.728$; $P<0.05$). Thus, the differences in CPT content among populations and individuals could not be attributed to age or size class differences. Suhas et al. [35] also showed that even after normalizing for girth differences among the trees, if any, the CPT content expressed as CPT/girth was significantly different among the populations ($P=0.0016$). The mean CPT content of populations was not correlated with latitude, longitude or altitude of their occurrence and collection.

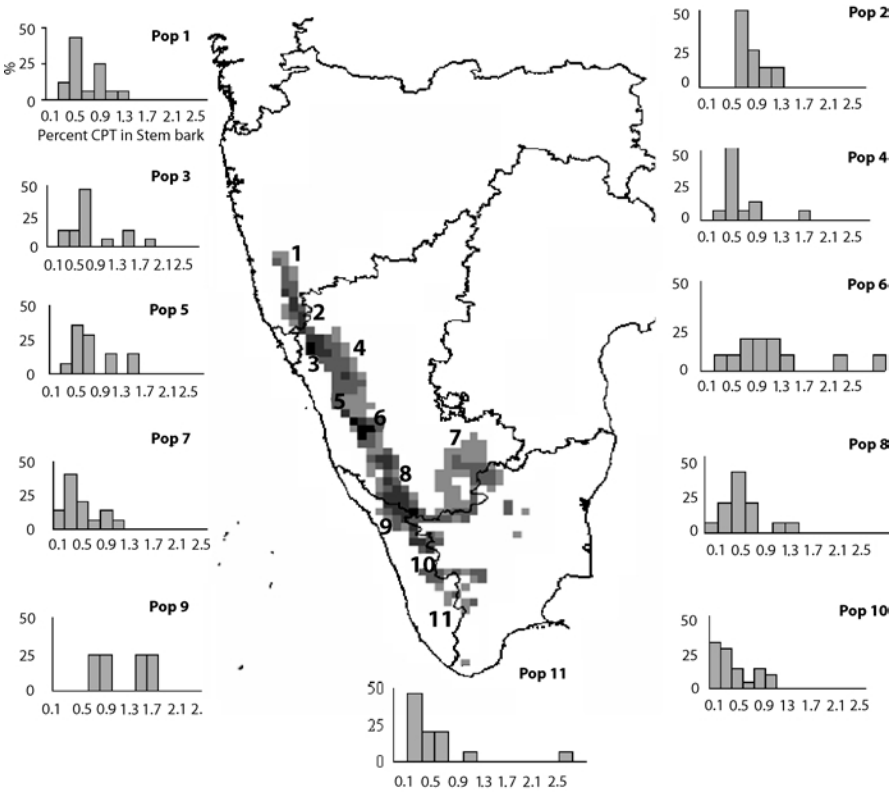

Fig. 10.4 Density distribution map and frequency distribution of percent CPT in the stem bark of *N. nimmoniana* in 11 different populations in the Western Ghats, India. The density distribution map was developed based on 64 points of occurrence of the species using a Geographic Information System (GIS) platform. The different shades of gray indicate the relative concentration of records of the species in the Western Ghats (light to dark indicating increasing concentration). The classification of the latitudinal gradient of the species into the different zones is purely for the purpose of discussion in the text (*x*-axis: percent CPT; *y*-axis: frequency of individuals; adapted from Suhas et al. [35])

CPT content in *C. acuminata* was found to vary significantly across latitude [37, 38]. In a more recent analysis, Ramesha et al. (unpublished data) conducted a forward, step-wise regression for CPT content using 19 climatic variables averaged over 30 years for each of the collection sites. Only two variables, namely mean temperature of the driest and wettest quarters of the year, significantly explained the differences in stem bark CPT among the populations; for root bark CPT, only one variable, namely the mean monthly temperature, was significant. Similar studies conducted in *C. acuminata* showed that CPT content varied significantly with several environmental variables such as temperature, evaporation capacity, and precipitation. Low temperature and precipitation was found to increase the CPT content [39].

The studies of Suhas et al. [35] provide one of the most exhaustive chemical screening of *N. nimmoniana* for CPT. The study assumes significance in

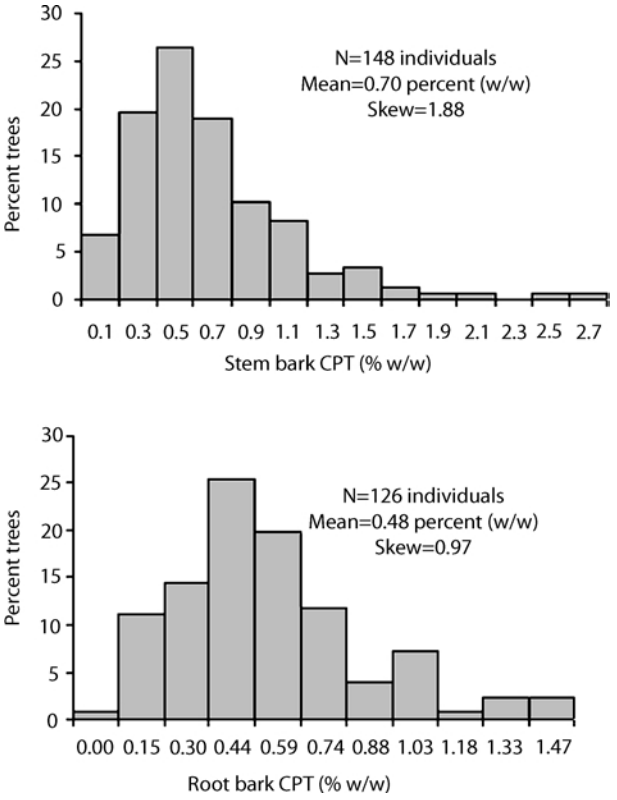

Fig. 10.5 Frequency distribution of CPT (%, w/w) in stem bark (3a) and root bark (3b) of *Nothapodytes nimmoniana* (Adapted from Suhas et al. [35])

that it is perhaps the first to report at least five- to eightfold more CPT in *N. nimmoniana* than has hitherto been reported. Of the 148 individuals assayed, 23 yielded more than 1% CPT. These estimates are nearly three- to eightfold more than what has been reported hitherto in the literature [21]. The study has demonstrated a significant population level variation in CPT content – a tool kit that can be exploited for developing clonally multiplied material from the identified high-yielding populations. While it will be important to examine whether these differences reflect the intrinsic genetic predisposition of populations to synthesize and accumulate CPT, preliminary analyses do indicate a genetic basis. Clearly, more studies will be required to examine this issue critically. Populations in the northern Western Ghats had the highest mean CPT and least intrapopulation variation, based on the analysis of both the stem and root bark. These populations could be important source material for developing high-yielding clonal materials. Furthermore, it will be important to study the heritability of the accumulation patterns across generations by analyzing the parent–offspring regression in the accumulation of CPT. It would be interesting to investigate the proximate/ultimate reasons for the enormously high levels of CPT produced by these trees, as a first step towards domesticating the species for obtaining high CPT yields.

10.5 Modeling Habitat Suitability for CPT Production

One of the key challenges in prospecting for high-yielding sources of specific plant metabolites is to develop algorithms or approaches that can help predict hot spots of distribution of the metabolite. Prediction of hot spots and its subsequent validation not only helps to focus efforts in collecting material from such sites, but also serves to prioritize sites at which plants can be domesticated or conserved. Unfortunately, few studies have seriously modeled the conditions that might help predict the spatial distribution of metabolites.

Recently, a GIS-based approach called the ecological niche model has been used to model the spatial distribution of a given species and offer predictions on the habitat suitability of the species. Using specific algorithms, the model iteratively identifies habitats over a landscape that match best the climatic variables corresponding to sites of known occurrence of the species. Accordingly, habitats are classified from those that are highly suitable (highest match) to those that are not suitable (least match) for the potential occurrence or invasion of the species [40, 41]. The ecological niche models have been used successfully in a variety of scenarios, including in locating rare and threatened species, and in rationalizing the choice of habitats for species reintroduction [42, 43].

At our laboratory, attempts have been made to extend the use of ecological niche modeling tools to offer predictions on the spatial distribution of plant metabolites. An underlying assumption of this application is that, plants would be selected to accumulate secondary metabolites at sites predicted to be highly suitable for the given species compared to sites that are predicted to be unsuitable. Thus, one would expect that a phytochemical such as santalols is best produced in sites suitable for the growth of sandal trees and not in those that are predicted to be unsuitable. Recently, Prakash Kumar [44] modeled the distribution of *Withania somnifera* in south India and showed that individuals in sites predicted to be highly suitable accumulated higher levels of withaferin-A and withanolide-A compared to individuals in sites that were predicted to be unsuitable or poorly suitable.

Figure 10.6 shows the predicted habitat suitability for *N. nimmoniana* in the Western Ghats. It is evident that not all regions in the Western Ghats are uniformly suitable for the species. In fact, within the Western Ghats, certain areas (Fig. 10.6, dark areas) are highly suitable and others (Fig. 10.6, grey areas) are unsuitable. In fact, two distinct sites in the central and northern Western Ghats are predicted to be excellent in their match to the habitat requirements of the species. Analysis of the CPT content of individuals occurring in the different habitat suitability areas indicated that individuals in highly suitable areas accumulated significantly higher levels of CPT compared to those that occurred in unsuitable or poorly suitable areas (Fig. 10.7). Furthermore, over 60% of the trees that accumulated greater that 1% CPT were all from regions predicted to be highly suitable (Fig. 10.8). In summary, these results have demonstrated for the first time the utility of the ecological niche models in predicting the spatial richness of plant metabolites, and hold several important implications.

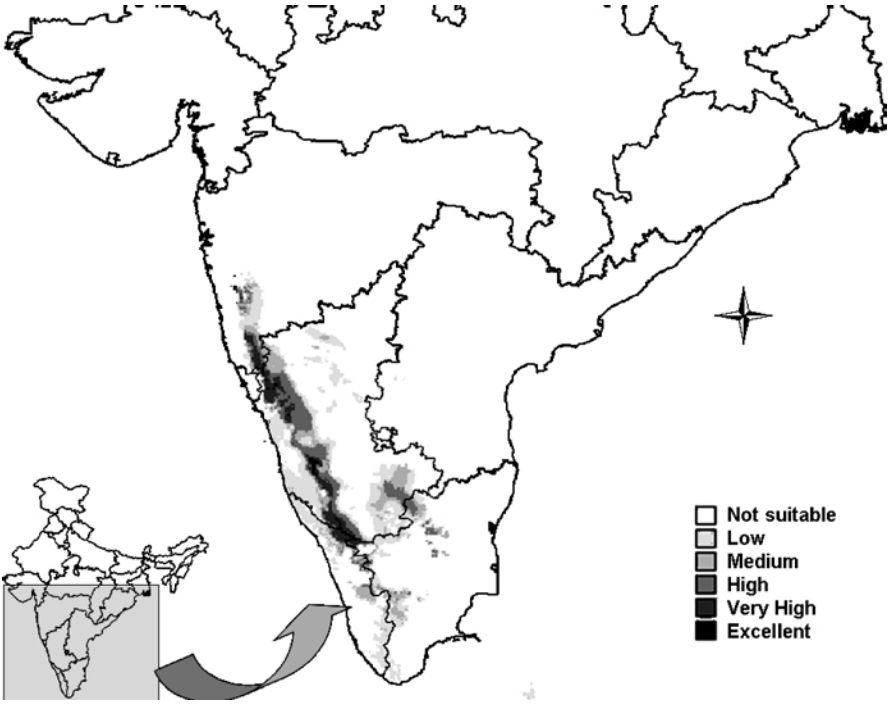

Fig. 10.6 Predicted habitat suitability map of *Nothapodytes nimmoniana* in Western Ghats, India. The different shades of grey indicate different habitat suitability categories as given in the legend

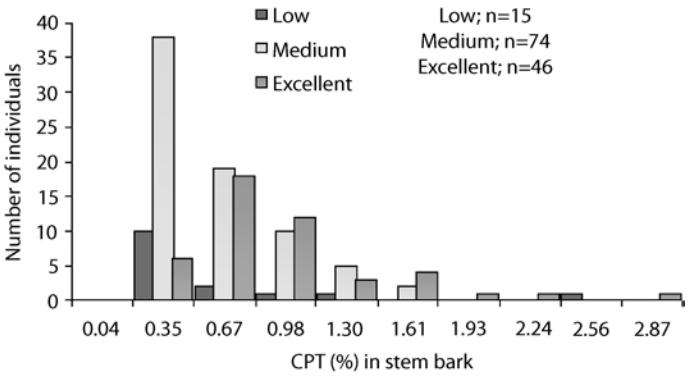

Fig. 10.7 Frequency distribution of CPT (%, w/w) in the stem bark of *Nothapodytes nimmoniana* individuals from different habitat suitability categories (Kolmogorov-Smirnov test low vs. excellent, D_{max}=0.53, p=0.001)

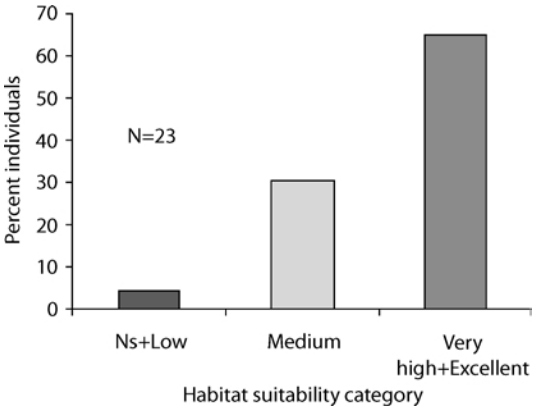

Fig. 10.8 Graph showing percentage of individuals of *Nothapodytes nimmoniana* (with CPT more than 1% in the stem bark) grouped into different habitat suitability categories (NS = not suitable)

For instance, the results raise interesting prospects for further research on how the habitat suitability or otherwise can influence the accumulation of a secondary metabolite. Do ecologically good habitats serve as areas in which the species are genetically predisposed to synthesizing secondary metabolites and other defense compounds that can lead to a potentially higher fitness of the populations? The outputs of the ecological niche model provide a powerful handle and direction to further explore newer populations of *N. nimmoniana* in areas/regions that have not yet been sampled from in search for higher CPT yields. The results have important implications for intelligent prospecting for economically important secondary metabolites such as CPT.

10.6 Development of a Sustainable Extraction Approach

Realizing the ever increasing demand for CPT, plantations of *C. acuminata* have been established in China since 1993 to supply material for CPT extraction [37]. In India, the major source of CPT continues to be *N. nimmoniana*. However, since there are no commercial plantations, all of the demand is sourced from the trees extracted destructively from the natural populations of *N. nimmoniana*. This of course is not sustainable in the long run because of loss of standing populations of the trees in the distributional range of the species. While no detailed inventory of the distribution and abundance of the tree is available in the Western Ghats, it is conjectured that the estimated demand may not be met solely by sourcing trees from their natural populations. Clearly, strategies need to be developed that can ensure a sustained supply of CPT from *N. nimmoniana*.

Several approaches could be deployed to ensure the sustainable extraction of *N. nimmoniana*. For example, establishment of captive plantations using clonally multiplied material from high-yielding lines could greatly contribute to the

rising demand for the compound without jeopardizing the naturally occurring populations. In fact, towards this end, as mentioned elsewhere in this chapter, efforts are being made to identify high-yielding lines and populations from the distributional range of the species in India. The recent discovery of population variability for CPT accumulation in *N. nimmoniana* holds immense promise in developing high-yielding clonal orchards and other captive plantations [35].

Extraction of renewable plant parts such as leaves and fruits instead of bark could be one of the possible approaches to sustain the extraction. However, because of the extremely low levels of CPT in the leaves and fruits of trees, this is not economically attractive. However, more recently, Santosh et al. (unpublished data) demonstrated that the CPT content of leaves could be strongly related to the age of the plant, just as was shown for *C. acuminata* [37]. Thus, the leaves of seedlings could accumulate relatively higher levels of CPT than those of juveniles and adults. It is estimated that 1-year-old seedlings producing about 15–20 g of leaf biomass can easily yield about 50 mg of CPT (Santosh et al., unpublished data). Although the economics of extraction need to be analyzed further, it appears that by simple ratooning of the seedling crop once a year, CPT could be extracted on a sustainable basis.

Sustainable extraction of CPT could also be arrived by exploring several *in vitro* production systems. For example, stabilization of cell cultures from high-yielding individuals could help develop *in vitro* production systems. However the economic viability of this approach will depend upon optimizing several protocols, including the sustained growth of the cell culture, *in vitro* elicitation of CPT, and CPT yields. A more recent and exciting possibility has emerged from the discovery that CPT is also produced by an endophytic fungus, *Entero-phospora infrequens*, which is associated with *N. nimmoniana* [45]. Cultivation of the fungus and optimizing production of CPT in a liquid culture system could potentially lead to an economically viable and ecologically sustainable model of supply of CPT.

10.7 Conclusions

Plants have been a major source of pharmaceutically important compounds worldwide. It is estimated that even today, 11 of the top 20 best-selling drugs are being derived from plants. In most cases, and particularly in the biodiversity rich regions of the world, the plants are nearly entirely sourced from the wild. For example, in India, of about 880 medicinal plants that are traded for various uses, 538 (61%) are sourced from the wild only [46]. Indiscriminate harvesting of these species has already led to a serious threat to these species. It is estimated that about 100 species (58 of which are globally threatened) of medicinal plants in the Western Ghats, a mega-biodiversity center in south India, might already be highly threatened due to excessive harvesting [28]. Thus, unless alternative approaches are developed, the extraction of most of the medicinal plants to meet global demands will be unsustainable. The extraction of CPT

from natural populations of *N. nimmoniana* is a case in point. As mentioned elsewhere, due to increasing harvesting pressures from the wild, this species is already listed as endangered [28]. If global demands for CPT are to be met, it is essential to develop sustainable models of extraction, including developing clonal orchards for high-yielding elite lines, using alternate but renewable biomass resources such as leaves and fruits, and finally in developing *in vitro* production systems. Toward this end, as described in this chapter, several initiatives could be taken that explore all possibilities to meet the global demand sustainably. With the identification of high-yielding populations of *N. nimmoniana* in the Western Ghats, it is possible to develop high-yielding clonal orchards and establish captive plantations to meet the demand outside of the natural populations. These "elite" trees could also be focused toward deriving tissue material for *in vitro* production systems, as was done for several other systems, for example taxane from *Taxus wallichiana* [47, 48] and for podophyllotoxin from *Podophyllum peltatum* [49, 50].

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References

1. Raskin I, Ribnicky DM, Momarnytsky S, Ilic N, Poulev A, Borisjuk N, Brinker A, Moreno DA, Ripoll C, Yakoby N, O'Neal JM, Cornwell T, Pastor I, Fridlender B, (2002) Trends in Biotechnology 20:522
2. Farnsworth NR, Morris RW (1976) Am J Pharm Educ 148:46
3. Fransworth NR (1988) Biodiversity Wilson 24:83
4. Daniel SF, Fransworth NR (2001) Environmental Health Perspective 109:69
5. Nalawade SM, Abhay PS, Chen YL, Chao LK, Hsin ST (2003) Bot Bull Acad Sin 44:79
6. Yan XF, Yang W, Yu T, Zhang Y, Dai (2003) Bot Bull Acad Sin 44:99
7. Li SY, Adair KT (1994) *Camptotheca acuminata* Decaisne Xi Shu (Chinese Happy Tree) – A Promising Anti-tumour and Antiviral Tree for the 21st Century (ed. Stephen, FA, Henry M) Rockwell Monograph, Austin State University, Texas, 1994
8. Vladu B, Woynarowski J, Manikumar G, Mansukhlal C, Wani MEW, Daniel D, Von Hoff, Wadkins RM (2000) Molecular Pharmacology 57:243
9. Wall ME, Wani MC, Cook CE, Palmer KH, McPhail AT, Sim GA (1966) J Am Chem Soc 88:3888
10. Cragg GM, Boyd MR, Khanna RR, Kneller R, Mays TD, Mazan KD, Newman DJ, Sausville EA (1999) Pure Appl Chem 71:1619
11. Lorence A, Craig LN (2004) Phytochemistry 65:2731
12. Masuda N, Fukuoka M, Kusunoki Y (1992) J Clin Oncol 10:1225

13. Abigers D, Catimel G, Kozimor R (1995) Ann Oncol 6:141
14. Lilenbaum RC, Ratain MJ, Miller AA, Hargis JB, Hollis DR, Rosner GL, O'Brien SM, Brewster L, Green MR, Schilsk RL (1995) J Clin Oncol 13:2230
15. Romanelli SP, Perego G, Pratesi N, Carenini M, Tortoreto ZF (1998) Cancer Chemother Pharmacol 41:385
16. Clements MK, Jones CB, Cumming M, Doud SS (1999) Cancer Chemother Pharmacol 44:411
17. Giovanella BC, Steblin JS, Wall ME, Wani MC, Nicholas AW, Liu LF, Siber R, Potmesil M (1989) Science 246:1046
18. Priel E, Showalter SD, Blair DG (1991) AIDS Res. Hum. Retroviruses 7:65
19. Wall ME, Wani MC (1968) J Org Chem 34:1364
20. Arisawa M, Gunasekera SP, Cordell GA, Farnsworth NR (1981) Planta Med 43:404
21. Govindachari TR, Viwanathan N (1972) Phytochemistry 11:3529
22. Tafur S, Nelson JD, DeLong DC, Svoboda GH (1976) Lloydia 39:261
23. Aimi, Hoshino NH, Nishimura M, Sakai S, Haginiwa J (1990) Tetrahedron Lett 31:5169
24. Gunashekera SP, Badawi MM, Cordell GA, Farnsworth NR, Chitnis M (1979) J Nat Prod 42:475
25. Dai JR, Cardellina JH, Boyd MR (1999) J Nat Prod 62:1427
26. Watase I, Hiroshi S, Mami Y, Kazuki S (2004) Plant Biotechnology 21:337
27. Hombegowda HC, Vasudeva R, Georgi PM, Uma Shaanker R, Ganeshaiah KN (2002) Curr Sci 83:1077
28. Ravi Kumar, Ved DK (2000) (eds) 100 Red listed Medicinal Plants of Conservation Concern in Southern India, Foundation for revitalization of local Health Traditions, Bangalore
29. Ku KY, Tang TC (1980) Zhing Cao Yao 11:476
30. Wu TS, Leu YL, Hsu HC, Ou LF, Chen CC, Chen C, Ou JC, Wu YC (1995) Phytochemistry 39:383
31. Biswanath D, Padma SR, Kashinatham A, Srinivas KVN (1997) Indian Journal of Chemistry 36:208
32. Nolte BA (1999) M.S. Thesis, Texas A&M University, College Station, TX
33. Padmanabha BV, Chandrasekar M, Ramesha BT, Hombegowda HC, Gunaga R, Suhas S, Vasudeva R, Ganeshaiah KN, Uma Shaanker R (2006) Curr Sci 90:95
34. Roja G, Heble MR (1994) Phytochemistry 36:65
35. Suhas S, Ramesha BT, Ravikanth G, Rajesh PG, Vasudeva R, Ganeshaiah KN, Uma Shaanker R (2007) Curr Sci 92:1142
36. Myers N, Russell AM, Cristina GM, Gustavo ABF, Jennifer K (2000) Nature 403 :853
37. Liu Z, John CA, Viator HP, Roysell J, Constantin J, Stanley BC (1999) Physiol Plant 105:402
38. Liu Z, Stanley BC, Wayne JB, Yu Y, Raysell JC, Mathew JF, John CA(1998) Bot Bull Acad Sin 44:265
39. Yan XF, Wang Y, Yu T, Zhang YH, Yin LJ (2002) J Instu Anal 21:15
40. Ganeshaiah KN, Narayani B, Nilima N, Chandrasekara K, Swamy M, Uma Shaanker, R (2003) Curr Sci 85:1526
41. Hijmans RJ, Guarino L, Mathur P, Jarvis A (2004), DIVA-GIS. Free GIS for biodiversity research. http://www.diva- gis.org/
42. Hijmans RJ, Cruz E, Rojas L, Guarino L (2001) Manual. International Potato Center and International Plant Genetic Resources Institute, Lima, Peru
43. Hijmans RJ, David MS (2001) American Journal of Botany. 88:2101
44. Prakash Kumar UM (2007) M.Sc Thesis University of Agricultural Sciences, Bangalore
45. Puri SC, Handa G, Gupta RK, Srivastava TN, Somal P, Sharma SN (1999) J. Indian Chem. Soc 76:370

46. Ved DK (2003) Medicinal plants trade in India- An overview. In: Ankila JH, Gladwin CJ, Uma Shaanker R (eds) Proceedings of the conference on Policies, management, Utilization and conservation of non-timber forest products (NTFP's) in the South Asia region. Ashoka Trust for Research in Ecology and Environment, Bangalore and Forestry Research support Program for Asia and the Pacific, Food and Agriculture Organisation, Bangkok
47. Swapna M, Biswajit G, Timir BJ, Sumita J (2002) Planta Med 68:757
48. Poupat C, Ingrid H, Francoise G, Alain A, Daniel G, Pierre P (2000) Planta Med 66:580
49. Rita MM, Bedir E, Barrett H, Burandt JC, Canel C, Khan IA (2002) Planta Med 68:341
50. Rita MM, Hemanth L, Ebru B, Muhammed M, Kent C (2001) Trends New Crops New Uses 36:527
51. Lopez-Meyer M, Nessler CL, McKnight TD (1994) Planta Med 60:558
52. Li S, Yi Y, Wang Y, Zhang Z, Beasley RS (2002) Planta Med 68:1010
53. Wiedenfeld H, Furmanowa M, Roeder E, Guzewska J, Gustowski W (1997) Plant Cell Tiss Org Cult 49:213
54. Saito K, Sudo H, Yamazaki M, Koseki-Nakamura M, Kitajima M, Yamazaki M, Takayama H, Aimi N (2001) Plant Cell Rep 20:267
55. Vineesh VR, Fijesh PV, Jelly Louis, Jaimsha VK, Jose Padikkala (2007) Curr Sci 92:1216
56. Zhou BN, Hoch JM, Johnson RK, Mattern MR, Eng WK, Ma J, Hecht SM, Newman DJ, Kingston DGI (2000) J Nat Prod 63:1273
57. Aiyama R, Nagai H, Nokata K, Shinohara C, Sawada S (1988) Phytochemistry 27:3663
58. Yamazaki Y, Urano A, Sudo H, Kitajima M, Takayama H, Yamazaki M, Aimi N, Saito K (2003) Phytochemistry 62:461