19. Phillips, D. A. and Torrey, J. G., Cytokinin production by *Rhizobium japonicum*. *Physiol. Plant.*, 1970, **23**, 1057–1063.
20. Janzen, R. A., Rood, S. B., Dormaar, J. F. and McGill, W. B., *Azospirillum brasilense* produces gibberellin in pure culture on chemically-defined medium and in co-culture on straw. *Soil Biol. Biochem.*, 1992, **24**, 1061–1064.
21. Abd-Alla, M. H., Solubilization of rock phosphate by *Rhizobium* and *Bradyrhizobium*. *Folio Microbiol. (Prague)*, 1994, **39**, 53–56.
22. Molla, A. H., Shamsuddin, Z. H., Halimi, M. S., Morziah, M. and Puteh, A. B., Potential for enhancement of root growth and nodulation of soybean co-inoculated with *Azospirillum* and *Bradyrhizobium* in laboratory systems. *Soil Biol. Biochem.*, 2001, **33**, 457–463.

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Chemical profiling of *Nothapodytes nimmoniana* populations in the Western Ghats, India for anti-cancer compound, camptothecin

S. Suhas[1,2], B. T. Ramesha[1,2], G. Ravikanth[2,3], Rajesh P. Gunaga[4], R. Vasudeva[3,4], K. N. Ganeshaiah[2,3,5,6] and R. Uma Shaanker[1,2,3,6,*]

[1]Department of Crop Physiology; [2]School of Ecology and Conservation; [5]Department of Genetics and Plant Breeding, University of Agricultural Sciences, GKVK Campus, Bangalore 560 065, India
[3]Ashoka Trust for Research in Ecology and the Environment, #659, 5th A Main, Hebbal, Bangalore 560 024, India
[4]Department of Forest Biology, College of Forestry, Sirsi 581 401, India
[6]Jawaharlal Nehru Centre for Advanced Scientific Research, Jakkur, Bangalore 560 065, India

Camptothecin (CPT) is a potent anti-tumour isoquinoline alkaloid used extensively in the treatment of several cancers. Among several plant species known to contain the compound, by far the highest concentration of about 0.3% (w/w) has been reported from *Nothapodytes nimmoniana*, a small tree distributed in the Western Ghats, South India. With no synthetic sources of this alkaloid and with an increasing global demand, there has been a heavy dependence on naturally existing populations of *N. nimmoniana*. 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 and thereby relieving the pressure on natural populations.

Towards this end, we have chemically profiled 148 individuals from 11 populations of *N. nimmoniana* in the Western Ghats, a mega-diversity hotspot in South India. CPT was estimated in stem and root bark of individual trees. There was significant variation in CPT content, both in stem and root bark samples, among the populations. Differences in CPT content among individuals did not seem to be related to either their size (age) or their geographical origin. Of the 148 individuals assayed, 23 yielded more than 1% CPT. These estimates are nearly three to eight-fold more than what has been reported hitherto in the literature. Subject to further confirmation, these 'elite' trees could be focused for conservation and judicious utilization through clonal multiplication, as also for deriving tissue material for *in vitro* production systems.

Keywords: Camptothecin, chemical profiling, conservation, Icacinaceae, *Nothapodytes nimmoniana*, Western Ghats.

CAMPTOTHECIN (CPT), isoquinoline alkaloid is one of the most promising anti-cancer drugs of the twenty-first century[1–6]. Several water-soluble derivatives of CPT are currently being used for treating colorectal and ovarian cancer[7–9]. The projected global demand for CPT in 2002 was valued at US$ 4045 million[10]. CPT was first discovered in the Chinese deciduous tree, *Camptotheca acuminata* (Nyssaceae)[11]. Later, the alkaloid has been reported from several plant species, with by far the highest concentration (about 0.3% on a dry weight basis) from *Nothapodytes nimmoniana*[12]. *N. nimmoniana*, formerly known as *Nothapodytes foetida* Sleumer and *Mappia foetida* Meirs, is a small tree, naturally distributed in many parts of the Western Ghats, South India, some parts of Assam, the Himalayan foothills, Sri Lanka, Myanmar and Thailand.

In the absence of synthetic sources, the global demand for this alkaloid is being met by the extraction of naturally existing populations of *N. nimmoniana* from the Western Ghats, India. Consequently in the last decade alone, over 20% of the population of the species has been lost from the Western Ghats[13,14]. In fact due to the extremely high pressure, the species has been declared as endangered[15]. In recent years, several independent groups have addressed the need to conserve the species and to explore the possibilities of identifying high-yielding individuals or populations for the development of *in vitro* production systems[16–22]. Padmanabha *et al.*[23] reported patterns of accumulation of the alkaloid in *N. nimmoniana* with respect to age, sex and seasonality. They found significant variation in CPT content among individuals and emphasized the need for chemically screening more populations in order to identify high-yielding sources of the alkaloid. In this study, we present

*For correspondence. (e-mail: rus@vsnl.com)

results on chemical profiling of 11 populations of *N. nimmoniana* from the Western Ghats for CPT content. We show that there is significant population-level variation of CPT content that could be effectively employed in developing clonal banks of high-yielding individuals as well as in planning a rationale conservation strategy for the species. We also report almost three to eight-fold higher CPT content in individuals than hitherto reported. We discuss the possibilities of using these individuals as source materials for either establishing high-yielding clonal populations or for developing suitable *in vitro* production systems.

The study was conducted on *N. nimmoniana* Graham. (Icacinaceae). The species is polygamous in nature. It shows a wide array of breeding types with male, female, hermaphrodite, monoecious, andromonoecious, gynomonoecious, trimonoecious at individual level, while at population level it shows androdioecious and gynodioecious types[13]. The basic patterns of accumulation of CPT in this species have been reported by Padmanabha *et al.*[23]. They found significant variation in CPT content among individuals and across sites of collection. There was no difference in CPT content between male and female trees. There was a fairly reliable degree of consistency in CPT content across independent samplings over two seasons. Finally, CPT yields were largely unaffected by the size class of stems (age) beyond 16 cm gbh. As a first step in chemically profiling the species, we developed a spatially explicit distribution map of the species in the Western Ghats. Information about the distribution of *N. nimmoniana* was obtained from secondary sources, namely floras, herbaria, books and other published sources, including Forest Department records and from Sasya Sahayadri, the digital plant database of the Western Ghats[24]. For each of the sites of occurrence recorded, the geographical coordinates (latitude and longitude) were obtained by accessing the geographical coordinate database[25]. Besides, we undertook primary surveys in the major forest divisions of the Western Ghats in Kerala, Tamil Nadu, Karnataka and Maharashtra. The geographical coordinates of the sites of occurrence were obtained directly using a Global Positioning System (Garmin 4.5 version).

Using both primary and secondary datasets, we developed a spatially explicit density distribution map of the species on a GIS platform[26] (Figure 1). Based on the distribution map, we selected 11 representative sites from 8 to 15°N latitude for chemically profiling the species for CPT. At each of the chosen sites, individuals were sampled from an area of at least 5 ha. Based on population size (number of individuals/site), on an average about 10 to 15 trees were sampled randomly from the respective sites (Table 1). Each tree was given a unique identification number and details of collection (tissue collected), name of the site, and latitude and longitude of collection were recorded in a registry maintained at the School of Ecology and Conservation, University of Agricultural Sciences, GKVK, Bangalore, India. For each tree, girth at breast height was recorded and the stem and root bark collected. For stem bark collections, the outer bark at breast height was scrapped using a knife and a section of the inner bark (5 cm × 5 cm) was collected into a plastic bag and sealed. Similarly, for the

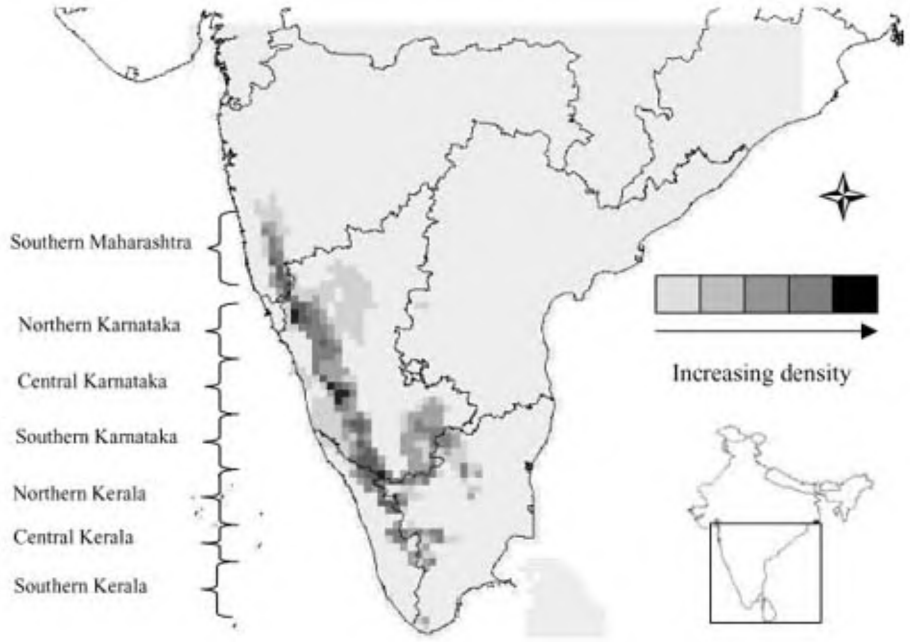

Figure 1. Density distribution map of *Nothapodytes nimmoniana* in the Western Ghats, India. The density distribution map was developed based on 64 points of occurrence of the species using a GIS platform. Different shades of grey indicate the relative concentration of records of the species in the Western Ghats (light to dark indicating increasing concentration). Classification of the latitudinal gradient of the species into different zones is purely for the purpose of discussion in the text.

Table 1. Sampling sites of *Nothapodytes nimmoniana* in the Western Ghats, India

Population ID	Location	Number of individuals sampled	Tissues sampled for CPT analysis
Pop 1	Southern Maharashtra	15	SB and RB
Pop 2	Northern Karnataka	8	SB and RB
Pop 3	Northern Karnataka	15	SB and RB
Pop 4	Northern Karnataka	15	SB and RB
Pop 5	Central Karnataka	14	SB and RB
Pop 6	Central Karnataka	11	SB and RB
Pop 7	Southern Karnataka	15	SB and RB
Pop 8	Southern Karnataka	15	SB and RB
Pop 9	Northern Kerala	4	SB and RB
Pop 10	Central Kerala	20	SB
Pop 11	Southern Kerala	15	SB and RB

SB, Stem bark; RB, Root bark.

root bark samples, exposed (surface) roots were scrapped and the inner bark was collected into a separate plastic bag and sealed.

All samples were dried to constant moisture content at 60°C for 96 h in a hot-air oven. Dried samples were ground to fine powder using a pestle and mortar. 0.1 g of fine tissue powder of each sample was extracted in 10 ml of 61% ethanol at 60°C for 90 min in a shaking water bath[23]. After cooling to room temperature, 1 ml of the extract was centrifuged at 10,000 rpm for 10 min at 10°C (Eppendorf 5415R, Germany)[27]. The supernatant was passed through 0.2 µm filter (Tarsons, India) and analysed for CPT content using a HPLC. CPT accumulation was determined for both the stem and root bark.

CPT was analysed by reverse phase HPLC (Supelco 516, LC-10AS, Shimadzu, Japan) on a C_{18} column (250 × 4.6 mm, 5 µm). The HPLC conditions were: 254 nm as the detector wavelength, 1.6 ml/min flow rate and 10 µl sample loop. The mobile phase was adjusted as follows: 40% acetonitrile and 60% water + 0.1% trifluroacetic acid (TFA) in an isocratic mode[5,11]. A CPT (95% HPLC-purified) standard sample was procured from Sigma Aldrich, USA. The standard was prepared using DMSO and methanol in 1 : 50 (v/v) ratio respectively. The retention time of CPT was 3.5 min; for every five runs, the HPLC was re-standardized using the CPT standard. On an average, the coefficient of variation (CV) for the peak area for five consecutive runs of standard CPT was 0.55%. Data were subjected to relevant statistical treatment using the Statistica version 4.0 software package[28].

In all, 148 individuals from 11 populations in the Western Ghats were chemically profiled for CPT. Per cent CPT in stem bark ranged from as low as 0.03 to as high as 2.7, with an overall mean of 0.7. Populations from northern Kerala had the highest CPT content (1.10 ± 0.462%) followed by that in Central Karnataka (1.02 ± 0.755%); CPT content was least in the Central Kerala population (0.35 ± 0.331%; Figure 2). Frequency distribution of CPT content over all the populations was highly positively skewed (Figure 3 *a*; skew = 1.88). There was significant variation among populations in their mean CPT content (one-way ANOVA, $P < 0.004$, Table 2).

CPT content in the root bark ranged from 0.003 to 1.41%, with an overall mean of 0.48% (Figure 3 *b*; skew = 0.97). Mean CPT content in root bark was highest in northern Kerala population (0.93 ± 0.359%), followed by southern Kerala (0.58 ± 0.269%) and southern Karnataka populations (0.23 ± 0.144%). There was significant variation in root CPT content among the populations ($P < 0.001$; Table 2). CPT content of stem bark was significantly positively correlated with that in the respective root bark ($n = 126$; $r = 0.320$, $P < 0.05$).

We analysed the correlation between CPT content and size class (girth at breast height) of the individual trees for each of the 11 populations. In seven of the 11 populations, there was no relation; however of the remaining four, in three there was significant positive relation ($r = 0.678$, $r = 0.762$, $r = 728$; all $P < 0.05$), while in one it was negatively related ($r = -0.728$; $P < 0.05$). Over all populations, mean CPT content was not significantly correlated with mean girth of trees ($n = 11$; $r = 0.39$, $P > 0.05$).

Nevertheless, to remove the effect of girth size, if at all any, we normalized the CPT content of each tree with its respective girth and re-analysed the CPT content of the populations. A one-way ANOVA indicated that there were no significant differences in girth among the populations (Table 3; $P = 0.062$). Further and even after normalizing for differences in the girth, there was significant variation among the populations in the CPT content (Table 3; $P = 0.0016$).

Mean per cent CPT content of populations was not correlated with either the latitude or longitude of their occurrence and collection ($r = 0.243$, $P = 0.441$). However, the coefficient of variation for per cent CPT content was significantly positively correlated with longitude and negatively with latitude of occurrence of the population (data not shown). In other words, within population, individual variations in CPT content were relatively lesser at higher latitudes and lower longitudes. Populations in the northern Western Ghats had a relatively higher consistency in per

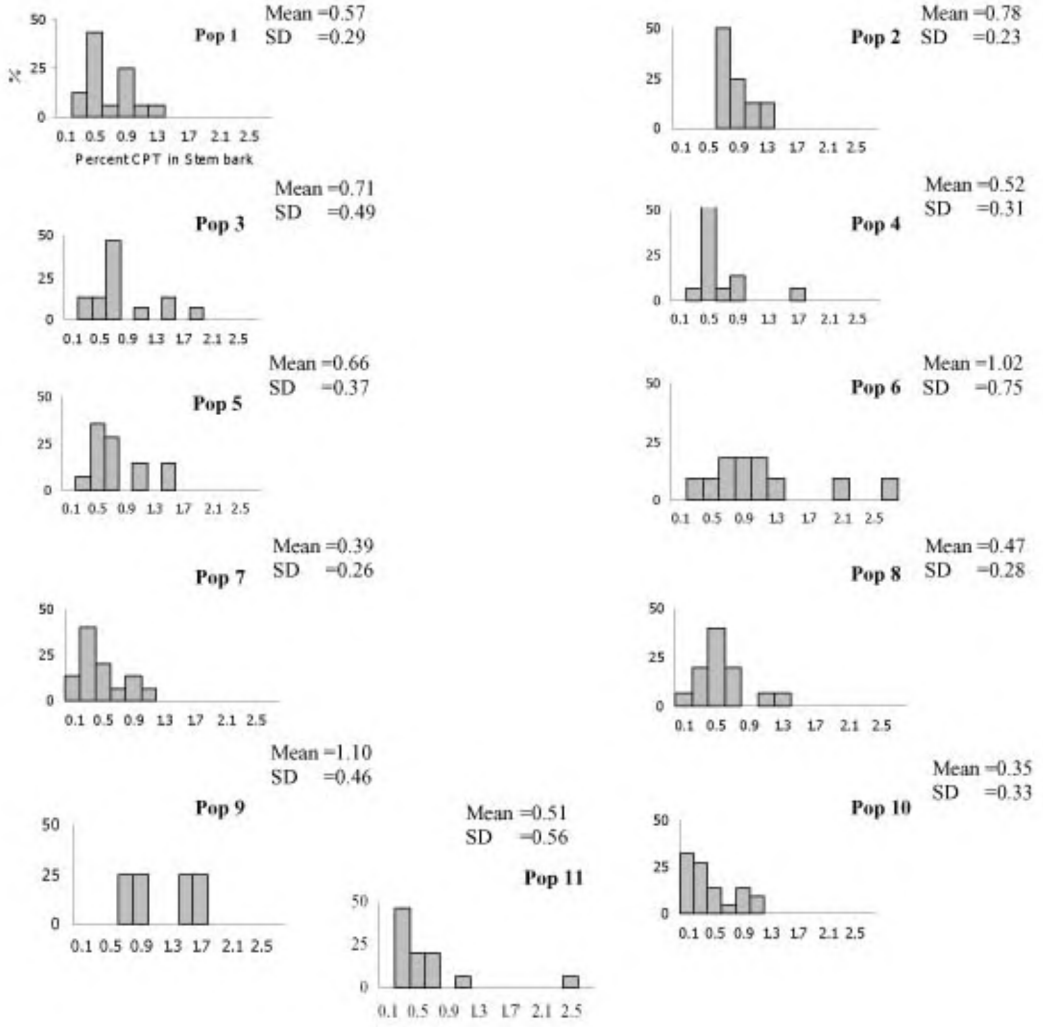

Figure 2. Frequency distribution of per cent CPT in stem bark of *N. nimmoniana* from 11 different populations in the Western Ghats, India. Population IDs conform to that presented in Table 1. x-axis, Per cent CPT; y-axis, Frequency of individuals; and SD, Standard deviation.

Table 2. One-way ANOVA for per cent CPT (% w/w) in stem and root bark of *N. nimmoniana* populations

Tissue	Source of variation	*df*	MSS	*F*	*P*-value
Stem bark	Among populations	10	0.595	3.491	0.0004
	Within population	138	0.170		
	Total	148			
Root bark	Among populations	9	0.281	3.521	0.001
	Within population	116	0.080		
	Total	125			

df, degrees of freedom; MSS, Mean sum of squares.

cent CPT across individuals sampled compared to those in the southern Western Ghats. The significance of this association is not immediately understood.

To reconfirm the high CPT estimates obtained in certain samples, individuals with CPT above 1% were subjected to re-extraction from fresh tissues and re-analysed. There was a significant positive correlation of per cent CPT between the first and second analysis (Figure 4; $r = 0.71$, $P < 0.01$), indicating that the estimates derived from HPLC analysis are consistent.

Finally, in order to identify populations that are characterized by a high mean CPT yield but with low CV (consistent yielders), we computed individual population mean CPT and SD for each population and compared it

Table 3. One-way ANOVA for girth and per cent CPT/girth in nine populations of *N. nimmoniana* in the Western Ghats

Trait	Source of variation	*df*	MSS	*F*	*P*-value
Girth (cm)	Among populations	8	695.1	1.928	0.062
	Within population	116	360.6		
	Total	124			
CPT/girth (%/cm)	Among populations	8	0.0005	3.3743	0.0016
	Within population	118	0.0001		
	Total	126			

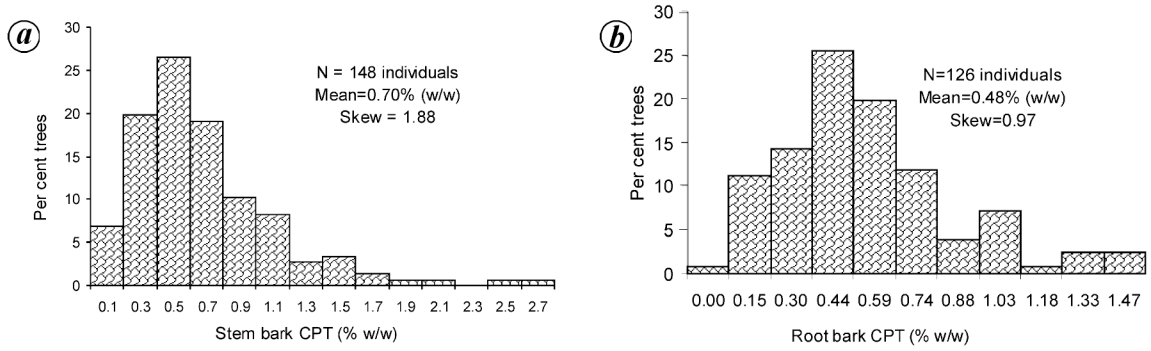

Figure 3. Frequency distribution of CPT (% w/w) in stem bark (*a*) and root bark (*b*) of *N. nimmoniana*.

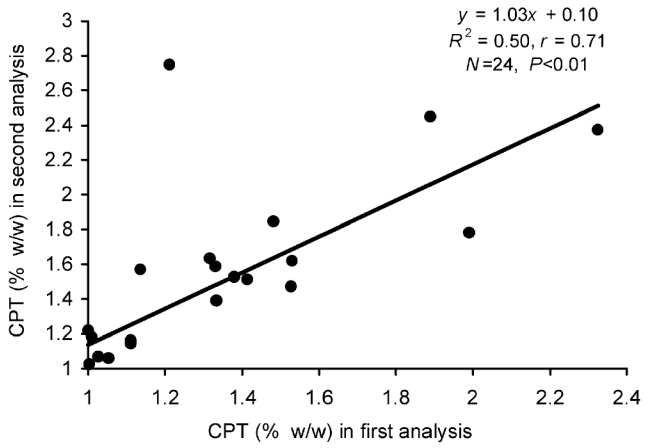

Figure 4. Correlation of CPT (% w/w) in stem bark between first and second analysis.

with the overall mean CPT and standard deviation (SD) (overall mean = 0.57% and SD = 0.46%) computed over the 11 populations. Populations with mean CPT higher than the overall mean but with a CV lesser than the overall CV were regarded as consistent high yielders. Population #2 from North Karnataka had the highest mean CPT and least SD both based on the stem and root bark analysis (Figure 2). This population could be an important source of material for developing high-yielding clonal materials.

The results presented here form one of the most exhaustive chemical screening of *N. nimmoniana* for CPT. 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 if these differences reflect intrinsic genetic predispositions of populations to synthesize and accumulate CPT, preliminary analyses do indicate a genetic basis. We failed to obtain any significant association of the levels of CPT accumulated with either the latitude or longitude of the places of collection of the populations. Neither was the variation explicable due to ontogenetic effects. There was no clear relation between the accumulation pattern and size class of individuals (though as mentioned earlier, in four of the 11 populations there did appear a size-class effect). Clearly, more studies will be required to critically examine this issue. Especially it will be important to study the heritability of the accumulation patterns across generations by analysing 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[29].

Of the 148 individuals assayed, 23 yielded more than 1% CPT. These estimates are nearly three to eight fold more than what has been reported hitherto in the literature[6,12,23,30]. Re-analysis of these samples indicated a high level of consistency in the estimates (see Figure 4; $r = 0.71$). The incredibly high yields of these individuals from several populations could not be attributed to their girth; the frequency distribution of the girth size of these individuals

(with >1% CPT yields) was generally indistinguishable from that of individuals with <1% CPT and from those of the entire population. Subject to further confirmation, these 'elite' trees could be focused for conservation and judicious utilization through clonal multiplication as also for deriving tissue material for *in vitro* production systems, as was done for several other systems such as taxane from *Taxus wallichiana*[31,32] and for podophyllotoxin from *Podophyllum peltatum*[33,34]. Clonally multiplied elite lines could be deployed as a profitable perennial tree component in suitable agroforestry systems from which CPT could be extracted on sustainable basis[29].

1. Li, S. Y. and Adair, K. T., *Camptotheca acuminata Decaisne Xi Shu (Chinese Happy Tree) – A Promising Anti-tumour and Anti-viral Tree for the 21st Century* (ed. Stephen, F.), A Henry M. Rockwell Monograph, Austin State University, Texas, 1994.
2. Wall, M. E., Wani, M. C., Cook, C. E., Palmer, K. H., McPhail, A. T. and Sim, G. A., Plant antitumour agents-I. The isolation and structure of camptothecin, a novel alkaloidal leukemia and tumour inhibitor from *Camptotheca acuminata*. *J. Am. Chem. Soc.*, 1966, **88**, 3888–3890.
3. Cragg, G. M. *et al.*, International collaboration in drug discovery and development: the NCI experience. *Pure Appl. Chem.*, 1999, **71**, 1619–1633.
4. Nalawade, S. M., Abhay, P. S., Chen-Yue Lee, Chao-Lin Kao and Hsin-Sheng Tsay, Studies on tissue culture of Chinese medicinal plant resources in Taiwan and their sustainable utilization. *Bot. Bull. Acad. Sin.*, 2003, **44**, 79–98.
5. Yan, Xiu-Feng, Yang Wang, Tao Yu, Yu-Hong, Zhang and Shao-Jun Dai, Variation in camptothecin content in *Camptotheca acuminata* leaves. *Bot. Bull. Acad. Sin.*, 2003, **44**, 99–105.
6. Lorence, A. and Craig, L. N., Camptothecin, over four decades of surprising findings. *Phytochemistry*, 2004, **65**, 2731–2841.
7. Lilenbaum, R. C. *et al.*, Phase I study of paclitaxel and topotecan in patients with advanced tumours: A cancer and leukemia group B study. *J. Clin. Oncol.*, 1995, **13**, 2230–2237.
8. Romanelli, S. P., Perego, G., Pratesi, N., Carenini, M. and Tortoreto Zunino, F., *In vitro* and *in vivo* interaction between cisplatin and topotecan in ovarian carcinoma systems. *Cancer Chemother. Pharmacol.*, 1998, **41**, 385–390.
9. Vladu, B. *et al.*, 7- and 10-Substituted camptothecins: Dependence of topoisomerase I-DNA cleavable complex formation and stability on the 7- and 10-substituents. *Mol. Pharmacol.*, 2000, **57**, 243–251.
10. Raskin, I. *et al.*, Plants and human health in the twenty-first century. *Trends Biotechnol.*, 2002, **12**, 522–531.
11. Wall, M. E. and Wani, M. C., Plant antitumour agents II: The structure of two new alkaloids from *Camptotheca acuminata*. *J. Org. Chem.*, 1968, **34**, 1364–1367.
12. Govindachari, T. R. and Viswanathan, N., Alkaloids of *Mappia foetida*. *Phytochemistry*, 1972, **11**, 3529–3531.
13. Hombe Gowda, H. C., Vasudeva, R., Mathachen, G. P., Uma Shaanker, R. and Ganeshaiah, K. N., Breeding types in *Nothapodytes nimmoniana* Graham. *Curr. Sci.*, 2002, **83**, 1077–1078.
14. Ravi Kumar and Ved, D. K., 100 Red listed medicinal plants of conservation concern in southern India. Foundation for Revitalization of Local Health Traditions, Bangalore, 2000, pp. 261–263.
15. Ved, D. K., Trade in medicinal plants – the state of our ignorance. *Amruth*, 1997, **1**, 2–8.
16. Ciddi, V. and Shuler, M. L., Camptothecin from callus cultures of *Nothapodytes foetida*. *Biotech. Lett.*, 2000, **22**, 129–132.
17. Thengane, S. R., Kulkarni, D. K., Shrikhande, V. A. and Krishnamurthy, K. V., Effect of thidiazuron on adventitious shoot regeneration from seedling explants of *Nothapodytes foetida*. *In Vitro Cell Dev. Biol. (Plant)*, 2001, **37**, 206–210.
18. Fulzele, D. P., Satdive, R. K. and Pol, B. B., Growth and production of camptothecin by cell suspension cultures of *Nothapodytes foetida*. *Planta Med.*, 2001, **67**, 150–152.
19. Sundervelan, R. B., Desireddy and Ciddi, V., Camptothecine – A novel anti-cancer agent from tissue cultures of *Nothapodytes foetida*. *Indian J. Pharm. Sci.*, 2002, **65**, 101–105.
20. Fulzele, D. P. and Satdive, R. K., Somatic embryogenesis, plant regeneration and the evaluation of the camptothecin content in *Nothapodytes foetida*. *Soc. In Vitro Biol.*, 2003, **39**, 212–216.
21. Thengane, S. R., Kulkarni, D. K., Shrikhande, V. A., Joshi, S. P., Sonawane, K. B. and Krishnamurthy, K. V., Influence of medium composition on callus induction and camptothecin(s) accumulation in *Nothapodytes foetida*. *Plant Cell Tissue Organ Cult.*, 2003, **72**, 247–251.
22. Roja, G. and Heble, M. R., The quinoline alkaloids camptothecin and 9-methoxycamptothecin from tissue cultures and mature trees of *Nothapodytes nimmoniana*. *Phytochemistry*, 1994, **36**, 65–66.
23. Padmanabha, B. V. *et al.*, Patterns of accumulation of camptothecin, an anti-cancer alkaloid in *Nothapodytes nimmoniana* Graham., in the Western Ghats, India: Implications for identifying high-yielding sources of the alkaloid. *Curr. Sci.*, 2006, **90**, 95–100.
24. Ganeshaiah, K. N., Digital database of plant resources of Western Ghats, India. University of Agricultural Sciences, Bangalore, 2002.
25. Geographical coordinate database; http://www.mapsofindia.com/lat_long/index.html.
26. Mapinfo Professional Version 7.0, Mapinfo Corporation, 1985–2002.
27. Gunasekera, S. P., Badawi, M. M., Cordell, G. A., Farnsworth, N. R. and Chitnis, M., Plant anticancer agents X. Isolation of camptothecin and 9-methoxycamptothecin from *Ervatamia heyneana*. *J. Nat. Prod.*, 1979, **42**, 475–477.
28. Statistica ver 4.5. Statsoft, Inc., USA, 1993.
29. Vasudeva, R., Hombe Gowda, H. C. and Raghu, H. B., Suitability of *Nothapodytes nimmoniana* as a profitable medicinal tree component for agroforestry systems of hill and transition zones of Karnataka. *My Forest*, 2004, **40**, 39–44.
30. Puri, S. C., Handa, G., Gupta, R. K., Srivastava, T. N., Somal, P. and Sharma, S. N., Quantitation of camptothecin in *Nothapodytes foetida*. *J. Indian Chem. Soc.*, 1999, **76**, 370–371.
31. Swapna, M., Biswajit, G., Timir, B. J. and Sumita, J., Variation in content of taxol and related taxanes in Eastern Himalayan population of *Taxol wallichiana*. *Planta Med.*, 2002, **68**, 757–759.
32. Poupat, C., Ingrid, H., Francoise, G., Alain, A., Daniel, G. and Pierre, P., Neutral and basic taxoids in the needles of *Taxus* species. *Planta Med.*, 2000, **66**, 580–584.
33. Rita, M. M., Bedir, E., Barrett, H., Burandt Jr C., Canel, C. and Khan, I. A., Evaluation of *Podophyllum peltatum* accessions for podophyllotoxin production. *Planta Med.*, 2002, **68**, 341–344.
34. Rita, M. M., Hemanth, L., Ebru, B., Muhammed, M. and Kent, C., The American May apple and its potential for podophyllotoxin production. *Trends New Crops New Uses*, 2001, **36**, 527–632.

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