

REVIEW ARTICLE

Phytochemical and pharmacological aspects of *Nothapodytes nimmoniana*. An overview

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Summary

Nothapodytes nimmoniana (J. Graham) (Icacinaceae), commonly known as Amruta is found in India particularly in Maharashtra, Goa, Kerala, Assam, Jammu and Kashmir as well as Tamilnadu areas. It is an important medicinal plant, the major source of a potent alkaloid, namely camptothecin, of a wide spectrum of pharmacological activities like anti-cancer, anti-HIV, antimalarial, antibacterial, anti-oxidant, anti-inflammatory, anti-fungal and also applied in the treatment of anaemia. Camptothecin is still not synthesized, therefore, its production entirely depends on natural sources. *N. nimmoniana* is one such plant which yields contain camptothecin in significantly high amount. The plant is gaining international recognition due to its diversified medicinal uses. It is subjected to excessive harvest. It has been categorized as a vulnerable and endangered plant. The present review encompasses the phytochemical, analytical, pharmacological, biotechnological, and other specific aspects of *N. nimmoniana*.

Key words: *Nothapodytes nimmoniana*, camptothecin, pharmacological activities, phytochemistry

INTRODUCTION

India's rich repository of medicinal plant species (1/4 of the world) fulfills health care needs of more than 80% of the population of the country [1]. Out of these plants, 25% are found at Western ghats, a mega biodiversity hot-spot, important site of collection [2]. India ranks second in terms of the volume and value of exported medicinal plants [3]. Several tree species from the western ghats are gaining international recognition due to their newly and recently identified pharmacological and curative properties. This has led to their indiscriminate harvesting and hence their very existence is under severe threat. One of them is *N. nimmoniana* [formerly *Mappia foetida* Miers], a rich source of the potent alkaloids such as camptothecin (CPT) [(S)-4-ethyl-4-hydroxy-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinoline-3,14-(4H,12H)-dione], 9-methoxy camptothecin and mappicine [4, 5]. Leaves, stem wood, stem bark, root bark, root wood and seeds are, in general, used to isolate CPT. For the first time, CPT was isolated from Chinese tree *Camp-tothecia acuminata* [6], then from a variety of plant species including *Merriliodendron megacarpum* [7, 8], *N. nimmoniana* [9], *Ophiorrhiza mungos* [10], *O. pumila* [11] (*Rubiaceae*), *Eravatamia heyneana* (*Apocynaceae*) and *Mostuea brunonis* (*Loganiaceae*) [7]. Among these species, the highest concentration of CPT (0.3% w/w) has been reported in *N. nimmoniana* [12].

CPT and 9-methoxy-CPT were also isolated as major constituents from *Mappia foetida* by Professor Govindachari from *Mapia foetida* leaves and their structures were established from their spectral data as well as by direct comparison with authentic compounds [13]. CPT is an inhibitor of the DNA-replicating enzyme topoisomerase I and is believed to act by stabilizing a strand break in the phosphodiester backbone of DNA. CPT binds reversibly to a Topo-1-DNA cleavable complex to form a stable ternary complex [14]. Numerous analogs of CPT have been synthesized and proved as potential therapeutic agents [15]. Irinotecan [16] and topotecan [17] are two water-soluble derivatives of CPT, which have been approved by the Food and Drug Administration (FDA) of the United States of America for treating colorectal and ovarian cancer [18].

N. NIMMONIANA: A BRIEF ACCOUNT

This plant is known by different names: [2] of *N. nimmoniana*, durvasanemara, kodsas, hedare (Kannada), gheenera (Hindi), amruta, narkya, kalgur, kalagaura (Marathi), arali, choral, perum pulagi, kal kurinj (Tamil).

The plant is native for Indomalaysia and Indochina. Occurs in Western ghats (Shimoga, Sirsi, Ulvi, Hassan, Joida, and B R Hill), South India, Central and South Maharashtra (Pune – Maval taluk, Purandar taluk, Raigad taluk, Sawantwadi taluk 1&2), Sahyadris, Munnar, Goa [19].

It is a small tree, 3-8 m high. Bark smooth, grey, wrinkled, about 5 mm thick. Branchlets slightly angled, corky, with prominent leaf scars. Leaves alternate, slightly leathery, broadly egg-shaped to elliptic-oblong, 1–25 x 4–12 cm, base often unequal, apex acute to acuminate, margin entire, hairless above, thinly hairy beneath, crowded at the ends of branchlets, lateral nerves 8-10 pairs, leaf stalks 3–6 cm long. Flower in cymes, creamy yellowish, foul smelling (strongly foetid), about 5 mm across, in terminal corymbose cymes, petals hairy inside. Drupes ovoid, 1.25–1.9 cm long smooth, purplish black when ripe. Seed 1 or 2, albuminous. The fruit resembles jamun or jambul fruit in taste and appearance [20].

Since there is no convenient synthetic source for CPT, we depend on raw material from natural populations. *Camptotheca acuminata* (tree of Chinese origin) and *N. nimmoniana* are the only convenient sources for large scale extraction and purification of CPT [21]. As CPT accumulates in stem and root of *N. nimmoniana*, whole tree is cut to generate biomass for extraction. In Indian market, the current demand for its biomass is 500–700 metric tons a year. In Maharashtra, overexploitation and habitat destruction for raw material has led to population decline by 50–80% in last decade. Total loss has been recorded from certain areas. Currently, the species population density is as low as 1-2 individuals/hectare in some areas. However, it extends up to 30–40 individuals/hectare at some localities such as forest of Mahabaleshwar, Satara where populations of *N. nimmoniana* survive against the severe threat of destruction [22].

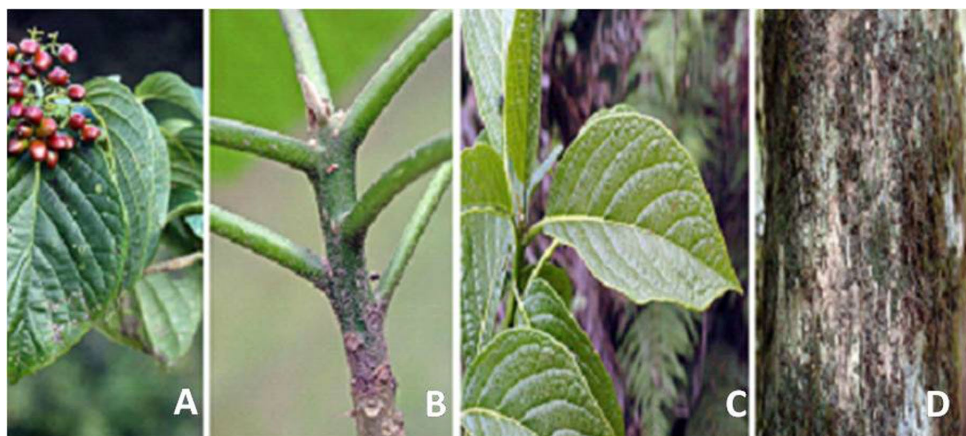


Figure 1.

Different parts of *N. nimmoniana* tree A) mature leaves with fruits, B) stems, C) immature leaves, D) mature stem

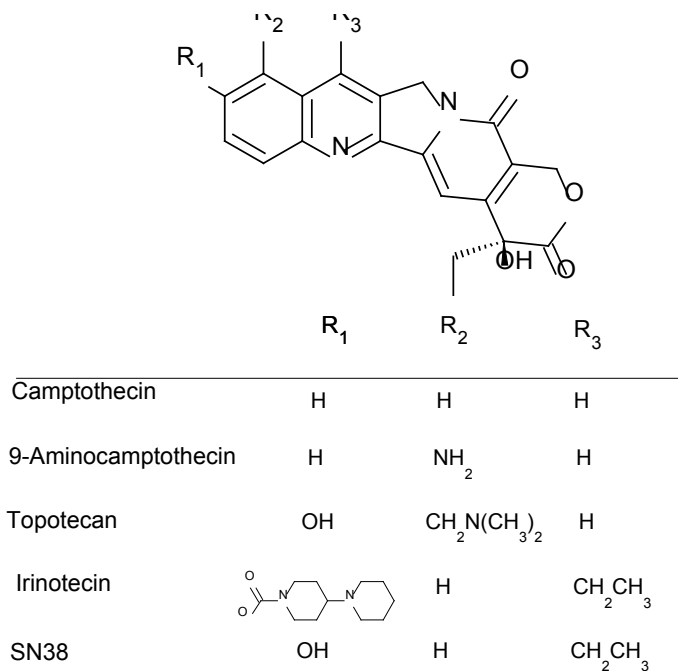


Figure 2.

Major chemical constituents reported from *N. nimmoniana*

N. nimmoniana trade and importance in traditional medicine

In India, research on clinical trials of CPT is conducted only at laboratory scale. Countries like Japan, USA and Spain are into the commercialization of CPT as a drug. These countries import dried raw material from India which is now one of the leading exporters worldwide. According to State Forest Department records, the annual demand from Japan for dried stem of *N. nimmoniana* was 200–300 tons in 1994 [22]. Since then, there has been an increasing trend. The trade volume increased to 1600 tons in 2002. In 2006–2008, the reported trade in the volumes has exceeded 1000 tons, whereas unreported trade is thought to be at least twice the reported one. The ever-increasing worldwide market of Irinotecan and Topotecan (semi synthetic CPT analogues) has currently reached 1000 million US dollars, which represents approximately 1 ton of CPT raw material [23]. To meet this ever increasing demand for CPT related drugs from all over the world, more and more plants are being cut, dried and exported. This export business is completely managed by private sector. The collectors have trained local tribal and rural laborers in cutting and drying processes. They are paid Rs. 10-15/ kg of dried stems and exported at the price of 1500 US dollars per kg (meaning Rs. 60,000 i.e. 1000 times higher price) [24].

Traditionally, the aqueous extract of *N. nimmoniana* has been used as anti cancer [25]. It's medicinal use has not been reported in any codified systems of Indian medicine. In fact, CPT is regarded as one of the most promising anticancer drug. In recent years, CPT has also emerged as a promising drug to be used in AIDS chemotherapy. The anti HIV activity of CPT is due to the inhibition of Tat-mediated transcription from the viral promoter. It is also active against parasitic trypanosomes and leishmania. CPT is also active against the malaria, antibacterial activity and anti-inflammatory activity from the leaves of *N. nimmoniana* Miers.

***N. nimmoniana*: cultivation and collection**

N. nimmoniana is cultivated in moist, deciduous place. It requires average annual rainfall of approximately 4000-7000 mm with 8–9 months of dry period. In India, it is grown under a wide range of conditions from the coastal areas up to altitude of 2300 m. In general, the crop is raised in south India's warm climate. Well drained, aerated fertile soils with a pH value ranging from 5.0 to 6.0 are the most favorable conditions for its cultivation. Seeds are used for cultivation. The seeds are shown on the seed beds in winter and early spring, the optimum temperature for germination is 12–35°C. Percentage seed germination depends on seed weight. If seed weight is high, per cent germination will be higher. *N. nimmoniana*, a tree crop, has got a 7–8-year-long gestation period. CPT yield depends on the age of plant. If the plant age is high, then per cent yield will also be higher. Highest percentage of CPT is produced by root wood, and minimum by the leaves. Collection in summer is best for obtaining CPT.

***N. NIMMONIANA*: VERSATILE SOURCE OF CHEMICAL CONSTITUENTS**

N. nimmoniana is a rich source of the potent alkaloid CPT and 9-methoxy camptothecin. It also contains 3-ketooctadec-cis-15-enoic acid (16.0%), palmitic acid (12.3%), stearic acid (4.2%), oleic acid (16.2%), linoleic acid (11.6%) and linolenic acid (39.7%). Other chemical constituents isolated from this plant are acetylcampthotecin, (+)-1-hydroxypinoresinol, Ω -hydroxypropioquaiacone, p-hydroxybenzaldehyde, scopoletin, uracil, thymine, sitosterol, sitosteryl- β -D-glucoside, 3- β -hydroxystigmast-5-en-7-one, stigmast-5-en-3- β , 7- α -diol, 6- β -hydroxystigmast-4-en-3-one, sitost-4-en-3-one, linoleic acid, trigonelline, and pumiloside isolated from the stem of *N. nimmoniana* and characterized [26].

Topotecan is 4-ethyl-4,9-dihydroxy-10-[(dimethylamino)methyl]-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinoline-3,14(4H,12H)-dione; irinotecan is S)-4,11-diethyl-3,4,12,14-tetrahydro-4-hydroxy-3,14-dioxo1H-pyrano[3',4':6,7]-indolizino[1,2-b]quinolin-9-yl-[1,4'bipiperidine]-1'-carboxylate, SN-38 is 7-ethyl-10-hydroxycampthotecin.

Methods of CPT extraction and isolation

Fulzele and Satdive reported the comparison of techniques for the extraction of the CPT from *N. nimmoniana*. Extraction methods using stirring extraction, soxhlet extraction, ultrasonic extraction and microwave-assisted extraction (MAE) were evaluated for the percentage extraction of CPT and 9-methoxycamptothecin (9-Me-CPT) from *Nothapodytes foetida*. The extracts were analyzed by HPLC. Methanol (90%, v/v) extracted high percentage extraction of CPT and 9-Me-CPT as compared to ethanol (90%, v/v). The result showed that the percentage extraction of CPT and 9-Me-CPT from *N. nimmoniana* by MAE was more efficient followed by soxhlet extraction, ultrasonic and stirring extraction methods. Maximum percentage extraction of CPT was obtained by MAE technique [27].

Hsiao *et al.* determined camptothecins in DMSO extracts of *N. nimmoniana* by direct injection capillary electrophoresis. The hydrophobic compound was extracted from plant tissue with a water-miscible organic solvent, DMSO, at the elevated temperature (60°C). The extract was directly injected into the separation capillary (untreated fused silica, 34 cm in length, 75 micro m i.d.) and analyzed in MEKC mode (369 nm). Within 5 minutes of migration, camptothecins were successfully separated and quantified by adding organic modifiers to the running buffer (20% DMSO, 90 mM SDS in 10 mM borate buffer, pH 8.60). This method had been proved to be very suitable for monitoring of the amount of camptothecins during the cultivation of the medicinal plant [28].

Yamazaki *et al.* reported isolation of camptothecin-related alkaloids from the methanolic extracts of *Ophiorrhiza pumila*, *Camptotheca acuminata* and *N. foetida*. Plants were profiled and identified using a reverse-phase HPLC coupled with on-line photodiode array detection and electrospray-ionization ion-trap mass spectrometry [29].

Puri *et al.* reported the separation of 9-methoxycamptothecin and CPT from *N. nimmoniana* by semi preparative HPLC. The purity of the isolates was determined by physicochemical data and liquid chromatography-mass spectrometry [30].

In the US Patent 6893668, an improved, and economical process for the isolation of CPT from the twigs and stem of *N. nimmoniana* was described which comprised of drying, grinding and hot defatting of *N. nimmoniana* twigs and stems with light petroleum fraction followed by successive sequential hot extraction with two solvents selected from CH_2Cl_2 , CHCl_3 , EtOAc, ether, acetone, MeOH, EtOH and CH_3CN ; removal of solvents under vacuum at a temperature in the range of 35-40°C, precipitation and filtration of crude extracts gave CPT with up to 0.15% yield [31]

Quantification

Li *et al.* carried out the quantitative analysis of CPT derivatives in *N. nimmoniana* using $^1\text{H-NMR}$ method. In the region of $\Delta 9.5-5.5$, the signals of H-7 of CPT, H-10 of 9-methoxycamptothecin, H-19 of pumiloside and H-2 of trigonelline, were well

separated one from another in DMSO. These results were compared with those obtained using conventional HPLC method. The advantages of the method over the conventional HPLC method included the absence of reference compound for calibration curves, realization of quantification on a crude extract and achievement of a very significant time-gain [32].

Fulzele and Satdive determined the distribution of CPT in *N. nimmoniana*. The bark contained 0.27% dry weight of CPT and 0.11% dry weight of 9-methoxycamptothecin followed by the root, stem and leaves. Immature seeds contained higher concentrations of CPT (0.32% of dry weight) and 9-methoxycamptothecin (0.16% of dry weight) compared to mature seeds. Zygotic embryos of immature seeds contained 0.11% of dry weight of CPT and 0.04% of dry weight of 9-methoxycamptothecin. The highest concentration of CPT (0.42% of dry weight) and 9-methoxycamptothecin (0.18% of dry weight) were accumulated in the cotyledons of immature seeds [33].

Padmanabha *et al.* reported the patterns of accumulation of the alkaloid in *N. nimmoniana* with respect to age, gender and seasonality. Individual trees with as high as 100 per cent greater camptothecin content than hitherto were reported. 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 [21].

Suhas *et al.* reported that CPT had highest concentration of about 0.3% w/w in *N. nimmoniana*. 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. These estimates were nearly three to eight - fold more than what had been reported hitherto in the literature [12].

Vidya Dighe *et al.* reported a sensitive and reliable high-performance thin layer chromatographic method developed for quantification of CPT in the dry stem powder of *N. foetida*. A methanolic extract of dry powder was chromatographed on silica gel 60F₂₅₄ plates with toluene – acetonitrile – glacial acetic acid, 6.5+3.5+0.1 (v/v), as a mobile phase. Detection and quantification were performed by densitometric scanning in fluorescence mode at $\lambda=366$ nm, using a mercury lamp. The accuracy of the method was checked by determination of recovery, using the standard-addition method. Recovery was 99.49%. The average CPT content of the powder was 0.059%. The method was rapid, simple, and precise [34].

Ramesha *et al.* revealed that the highest levels of CPT (approximately 0.3% w/w) were reported from *N. nimmoniana*. In this study both HPLC and LC-MS techniques were used. They showed for the first time the production of a few minor camptothecines, including 10-hydroxy camptothecin, in the stem and root extracts of the tree [35].

Namdeo *et al.* conducted a research study. They concluded that geographical factors play important role in CPT content in *N. nimmoniana*. They reported that maximum concentration of CPT was found in root (2.62%) collected from Mahabaleshwar, Patan (1.21%) and Sirsi (0.88%) regions followed by stem collected from

Patan (1.45%), Sirsi (0.70%) and Mahabaleshwar (0.43%) regions. The lowest concentration of CPT was found in leaves collected from Sirsi (0.29) region, followed by Patan (0.37) and Mahabaleshwar (0.70%) region. Fruits collected from Mahabaleshwar region contained maximum concentration of camptothecin (0.63%), whereas fruits from Patan region contain minimum concentration of camptothecin (0.36%). CPT quantification was carried out by HPLC. There was a two-fold higher concentration of camptothecin observed in roots from Mahabaleshwar region than roots from Sirsi and Patan regions. It is evident that geographical and climatic conditions remarkably influence the content of camptothecin in *N. nimmoniana* [36].

Namdeo *et al.* developed the method of validation and quantification of CPT by high performance thin layer chromatography. Chloroform-ethyl acetate-methanol (4 : 5 : 0.5 v/v) was used as a mobile phase. The method was validated for linearity, precision (interday and intraday), repeatability, limit of detection (LOD), limit of quantitation (LOQ) and accuracy. The relationship between the concentration of standard solutions and the peak response was linear within the range of 80 to 480 ng/spot with a correlation coefficient of 0.998 ± 0.020 . Instrumental precision was evaluated as 0.54 (% CV). Repeatability of sample and standard were estimated to be 1.08 and 1.01 (% CV), and LOD and LOQ were found to be 40 and 80 ng/spot, respectively. The accuracy of the method was checked out by a recovery study and the average percentage recovery was calculated as being 99.13 % [37].

Nazeerullah *et al.* have reported a simple, precise and accurate, high performance thin layer chromatographic method and validated it for the determination of CPT in *N. nimmoniana* collected from different geographical sources. The estimation was carried out using Stationary phase Silica gel 60F₂₅₄ pre coated plates (20 cm × 10 cm). The mobile phase used was a mixture of ethyl acetate: acetone (4:1 v/v). The detection of spot was carried out at 363 nm. The calibration curve was found to be linear between 0.3 and 3 µg/ml with a good correlation coefficient of 0.982 for CPT. The proposed method can be used to determine the best CPT content in different geographical sources [38].

Roja reported comparative studies on the CPT from the indigenous plants namely *N. nimmoniana*, *Ophiorrhiza mungos* and *Ophiorrhiza rugosa*. The study indicated highest yields of CPT and 9-methoxy camptothecin in *N. nimmoniana* [39].

N.NIMMONIANA: PHARMACOLOGICAL STUDIES

Antimicrobial activity

Kumar *et al.* successfully determined petroleum ether, chloroform and methanol extracts of *N. nimmoniana*. Leaves and stems were tested for their antibacterial activity. The methanol fractions were found to be most effective against the entire tested organism [40].

Antimalarial activity

Bodley *et al.* determined the effects of CPT, a potent and specific topoisomerase I inhibitor, on erythrocytic malaria parasites *in vitro*. In *Plasmodium falciparum*, camptothecin trapped protein-DNA complexes, inhibited nucleic acid biosynthesis and was cytotoxic. These results provided the proof for the concept that topoisomerase I was a vulnerable target for new antimalarial drug development [41].

Anti-inflammatory activity

Sheeja *et al.* reported the anti-inflammatory activity of the *N. nimmoniana* by carrageenan-induced hind paw edema method in rats. The activities of the extracts were compared with control and standard ibuprofen. All the drugs were administered orally. When compared with petroleum ether extract, the anti-inflammatory activity of ethanolic extract was found to be more effective and 200 mg/kg dose of ethanolic extract significantly (p less than 0.01) reduced the inflammation, which was comparable with that of the standard, ibuprofen [42].

Immunomodulatory activity

Puri *et al.* reported immunomodulatory activity of an extract of the novel fungal endophyte *Entrophospora infrequens* isolated from *N. nimmoniana*. The study evaluated the bioactivities of chloroform and methanolic extracts of *Entrophospora infrequens* with respect to their immunomodulatory potential *in vitro* and *in vivo* (in Balb/c mice). The endophyte *E. infrequens* was found to synthesize CPT, which was positively tested in chloroform. This showed for the first time the immunomodulatory potential of this neoteric CPT-producing endophyte from *N. nimmoniana* [43].

Antitumor/cytotoxic activity

Luo *et al.* reported potent antitumor activity of 10-methoxy-9-nitrocamptothecin. The high cytotoxic potency of 10-methoxy-9-nitrocamptothecin was paralleled with its ability to increase the cellular accumulation of DNA damage. These results suggested that cell cycle regulation might contribute to the anticancer properties of 10-methoxy-9-nitrocamptothecin and strongly supported further anticancer development of 10-methoxy-9-nitro-camptothecin [44].

Huang *et al.* reported that CPT activated S or G2-M arrest and the homologous recombination repair pathway in tumor cells [45].

Cuong *et al.* demonstrated that the plant alkaloid CPT caused DNA damage by specifically targeting DNA topoisomerase, effectively devastating a broad spectrum of tumors [46].

Wu *et al.* reported a new naturally occurring alkaloid, acetylcamptothecin, along with 17 known compounds. Among these, scopoletin, camptothecin, 9-O-methoxycamptothecin and O-acetylcamptothecin showed significant cytotoxic activity [26].

Rehman *et al.* reported the *in vitro* cytotoxicity of an endophytic fungus isolated from *N. nimmoniana*. The *in vitro* cytotoxicity of fractions/extracts from endophyte was carried out while ethyl acetate fraction and it showed sufficient growth inhibition against all cell lines [47].

N. NIMMONIANA: BIOTECHNOLOGICAL WORK

Rehman *et al.* investigated an endophytic *Neurospora* sp. from *N. nimmoniana* producing camptothecin. Endophyte was isolated from the seed of *N. nimmoniana* and was examined as a potential source of anticancer drug lead compound i.e. camptothecin, when grown in Sabouraud liquid culture media under shake flask conditions. The presence of anticancer compound CPT in this fungus was confirmed by chromatographic and spectroscopic methods in comparison with authentic camptothecin [48].

Amna *et al.* conducted the bioreactor studies on the endophytic fungus *Entrophospora infrequens* for the production of CPT. Twigs (young and old) from *N. nimmoniana* growing in the Jammu and Mahabaleshwar regions in India were used for the isolation of 52 strains of endophytic fungi and were tested for their ability to produce the CPT [49].

Sirikantaramas *et al.* investigated on CPT and revealed that it was produced in many plants belonging to unrelated orders of angiosperms. CPT was reported to be produced from the endophytic fungus isolated from the inner bark of *N. nimmoniana*. These reports suggested the possibility to develop large-scale production of CPT [50].

Karadi *et al.* reported the assessment of callus in different genotypes of *N. nimmoniana*. The CPT content was analyzed [51]. Ravishankar reported rapid clonal propagation of *N. nimmoniana* [52]. Satheeshkumar and Seeni reported the *in vitro* multiplication of *N. nimmoniana* (through seedling explant cultures [53]. Fulzele and Satdive 2001 reported the growth and production of camptothecin by cell suspension cultures of *N. nimmoniana* [54].

CONCLUSION

An exploration of the phytochemical aspects and therapeutic potential of *N. nimmoniana* reveals that it is a natural source of CPT possessing a wide spectrum of pharmacological properties such as anti-cancer, anti-AIDS, anti-malarial, anti-inflammatory, anti-oxidant, anti-bacterial, anti-fungal, anti-anaemic etc. What is

noteworthy, CPT is still not available synthetically and plant like *N. nimmoniana* is the prime source of CPT in high amounts. Other phytochemical constituents are acetyl CPT, methoxy CPT, hydroxy CPT, scopoletin, β -sitosterol, sitostery l- β -D- glucoside, trigonelline and pumiloside. As it is a vulnerable species, biotechnological approaches like plant tissue culture will be a better option for enhancing secondary metabolite production. Thereby, conserving this significant species using techniques like micro propagation and maintaining genetic uniformity of species.

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PROFIL FITOCHEMICZNY I FARMAKOLOGICZNY *NOTHAPODYTES NIMMONIANA*. PRZEGLĄD

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Streszczenie

Nothapodytes nimmoniana (J. Graham) (*Icacinaceae*), powszechnie znana jako Amruta, rośnie w Indiach, szczególnie w prowincjach Maharashtra, Goa, Kerala, Assam, Dżammu i Kaszmir, a także na niektórych obszarach Tamilnadu. Jest jedną z ważnych roślin leczniczych, głównym źródłem silnego alkaloidu, kamptotecyny, mającej szerokie spektrum działania: przeciwnowotworowe, anty-HIV, antymalaryczne, antibakteryjne, przeciwutleniające, przeciwzapalne, przeciwgrzybicze. Znajduje również zastosowanie w leczeniu anemii. Jak dotąd kamptotecyna nie została zsyntetyzowana, dlatego jej produkcja całkowicie zależy od źródeł naturalnych. *N. nimmoniana* jest jedną z roślin zawierających duże ilości kamptotecyny. Zyskała ona międzynarodowe uznanie dzięki szerokiemu spektrum zastosowań, co powoduje zwiększenie jej eksploatacji. Z tego powodu została uznana za gatunek zagrożony. Poniższy przegląd obejmuje badania fitochemiczne, analityczne, farmakologiczne, biotechnologiczne i inne prowadzone nad gatunkiem *N. nimmoniana*.

Key words: *Nothapodytes nimmoniana*, kamptotecyna, działanie farmakologiczne, fitochemia