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IMPACT OF ENVIRONMENTAL FACTORS ON CAMPTOTHECIN CONTENT OF *NOTHAPODYTES NIMMONIANA* GROWING IN WESTERN GHAT

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ABSTRACT

Herbal products have served as a major source of drugs for centuries, and about half of the pharmaceuticals in use today are derived from them. About WHO, 80% of the population of developing countries rely on traditional medicines, mostly plant drugs, for their primary health care needs. Even, modern pharmacopoeias still contain at least 25-30% drugs derived from plants. Secondary metabolites synthesized in plants are unique sources for pharmaceuticals and their accumulation as well as synthesis in plants subjected to stresses including various elicitors or signal molecules. These metabolites play a major role in the adaptation of plants to their environment and in overcoming stress conditions. Physiological-biochemical reactions are the result of plants adaption to environmental condition.The growth, yield, primary and secondary metabolites as well as metabolic pathways in the medicinal plants are significantly influenced by regional and seasonal variations such as light, temperature, humidity, precipitation, water availability, soil, geographical position of their habitat. Hence present study was investigated to investigate the environmental response of Nothapodytes nimmoniana, which are abundant at Amboli (Sindhudurg) and Patgaon (Kolhapur) in Western Ghats.The results revealed that maximum content of camptothecin was reported in root sample (0.59%) collected from Amboli, followed by Patgaon region (0.54%) in summer season. The lowest content of camptothecin was recorded in leaves (0.0097%) in Patgaon region as compared to stem and root samples. The stem samples collected from Amboli region also showed highest content of camptothecin in summer (0.39%) season which was followed by winter (0.25%) and rainy (0.18%) season. It is indicated that geographical and climatic conditions have remarkable influence in the content of camptothecin in N.nimmoniana.

KEYWORDS : *Nothapodytes nimmoniana,* camptothecin LC/MS, seasonal, regional variation

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INTRODUCTION

The Western Ghats are well known for their rich and unique assemblage of flora and fauna. The medicinal plants' diversity is also very rich in it. It is the treasure house of variety of medicinal plants, which are supplying crude drugs for years together to cure several human diseases. *Nothapodytes nimmoniana* (J.Graham) Mabb (syn *Mappia foetida* Meirs or *Nothapodytes foetida* Sleumer) belongs to family Icacinaceae, which is known as locally Amruta, Ghanera or Kalgur. It is shrubby small tree up to 8m high, with broad dark green leaves and flowers distributed naturally in Western Ghats of India. *N.nimmoniana* is a rich source of potent alkaloid camptothecin, 9-methoxy camptothecin and mappicine.[1, 2]

The active principle of the wood- camptothecin (CPT) is known as a potent drug that breaks single-strand DNA in the mammalian systems and is found to be useful in the treatment of tumours. CPT is also known to inhibit retroviruses such as human immunodeficiency virus (HIV) and equine infectious anaemia virus. It is believed that CPT is the third most important alkaloid sought after by the pharmaceutical companies around the world. The molecular target of camptothecin is inhibiting the nuclear enzymes topoisomerase IDNA complex.[3]Camptothecin inhibits Tat-mediated transactivation of human immunodeficiency virus type 1 (HIV-1) LTR and this important result offered a potential target for therapy of HIV- 1 infection.[4,5,6]

MATERIALS AND METHODS:

Plant material:

Plant material of *N.nimmoniana* was collected from Amboli and Patgaon region in summer, rainy and winter season from randomly selected plants of same age.

Chemicals:

All chemicals used were analytical grade. Reference standard of CPT (purity 95% w/w) was purchased from Hi Media (Mumbai, India).

Preparation of standard solution of camptothecin:

The reference standard was prepared by dissolving 5mg of standard in 5ml pure methanol.

Calibration curve for CPT:

Quantization of CPT in the extracts prepared from different stem, leaves and root barks was done on the basis of the calibration curves established by injecting seven concentrations of the CPT standard in the concentration range of 10 ng/ml to 1000 ng/ml each time before sample analysis. Calibration curves for CPT were prepared by plotting peak area vs concentration with correlation coefficient r =0.9971 (y=176x+187).(Figure 1)

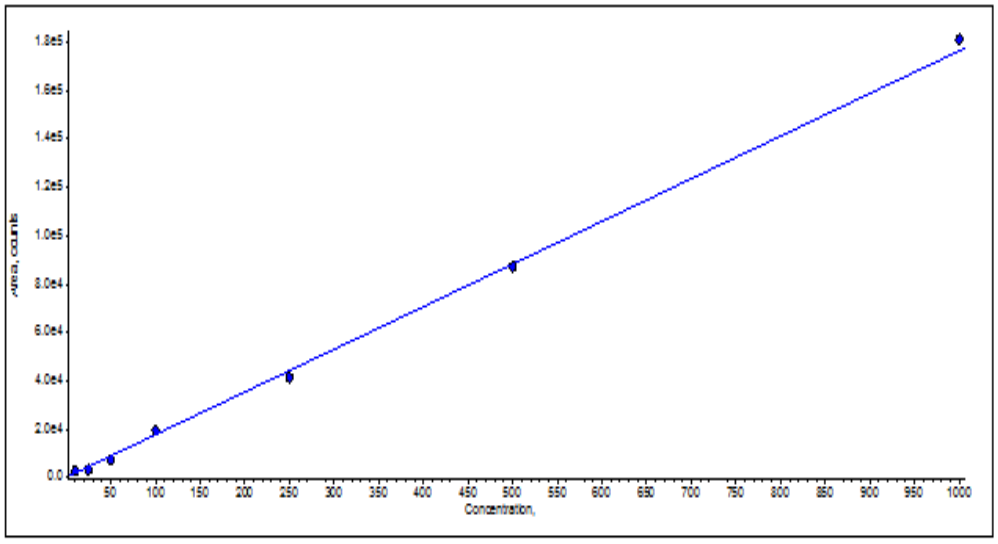

Figure 1: Calibration curve for CPT

Extraction of drug material and Preparation of sample solutions:

Composite samples of leaves, stem and root of *N. nimmoniana* were collected, freshly washed and dried in an air dryer for 48 h. Dried material was powdered and stored in sealed plastic bags at room temperature. Dried powdered material (2g) was taken in 50 ml volumetric flask, percolated with 50 ml of methanol and vortexed for 2 min followed by sonication (33 MHz, Roop Telesonic, India) at room temperature for 15 min. The process was repeated twice for complete extraction. After sonication, the methanolic extract was evaporated to dryness at 40^0C in vacuo using a Buchi Rotavapour (RE111,Switzerland).The dried extracts were transferred to volumetric flask and the volume was made up to 50 ml with methanol to furnish the final concentration of 100µg/ml.

Mobile phase:

A- 5mM ammonium formate in Water: Methanol (80:20),

B- 5mM ammonium formate in water: methanol (10:90).

LCMS/MS analysis:

The LC-MS/MS analysis was done with HPLC (PE 200 series) hyphenated to API 2000mass spectrometer (AB Sciex) equipped with electrospray ionization (ESI+) probe. Atalantis dC18 (150 x 2 mm x 5µm, Waters India Pvt. Ltd, Bangalore). The mobile phase flow rate 0.4mL/min with gradient profile was 0-1 min 85% A, 1-8 min 85-2% A, 8-12min 2% A,12-13 min 2-85% A and 13-18 min 85% A phase. Oven temperature was 35ºC and injection volume 10 µl. Mass parameters: curtain gas 25 psi, nebulizer gas (GS_1) 30 psi, heater gas (GS_2) 60psi, ion spray voltage 5500 V and temperature 500°C chromatographic peaks were identified by comparing their retention time and double transition with those of the pure standards.

Statistical analysis:

The data were presented as arithmetic means of three replicates with standard deviation. The significance of the mean differences was calculated through one-way ANOVA statistics followed by DMRT (Duncan's multiple range tests) at p=0.05 as a post hoc test. SPSS for Windows ver.20 and Microsoft Excel 2007 were used for statistical analyses and graphical presentations.

RESULTS AND DISCUSSION:

The LCMS/MS analysis resulted in a sharp peak of authentic CPT at the retention time of 8.53 (Figure 2 and 3). The contents of CPT in various part of *N. nimmoniana* collected from Amboli and Patgaon during summer, rainy and winter season showed similar peaks at retation time 8.53, corresponding to camptothecin is shown in figure 3.

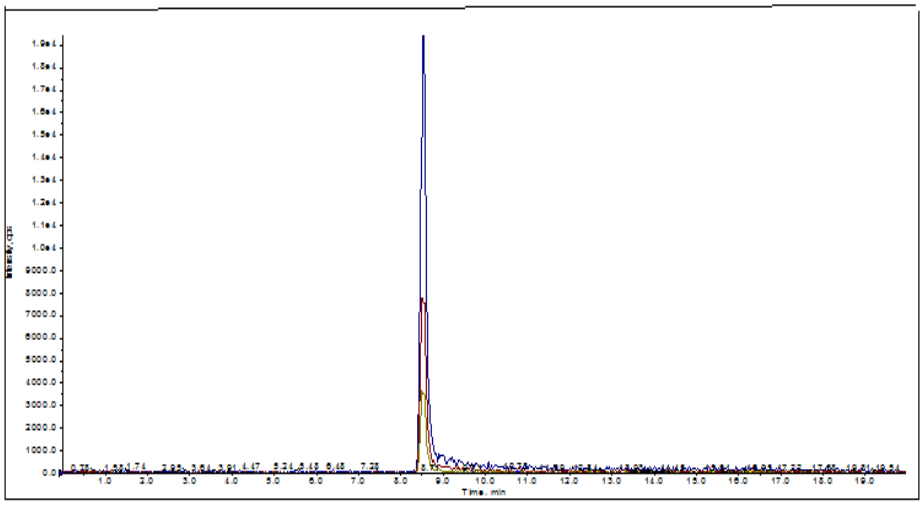

Figure 2: LCMS chromatogram authentic CPT.

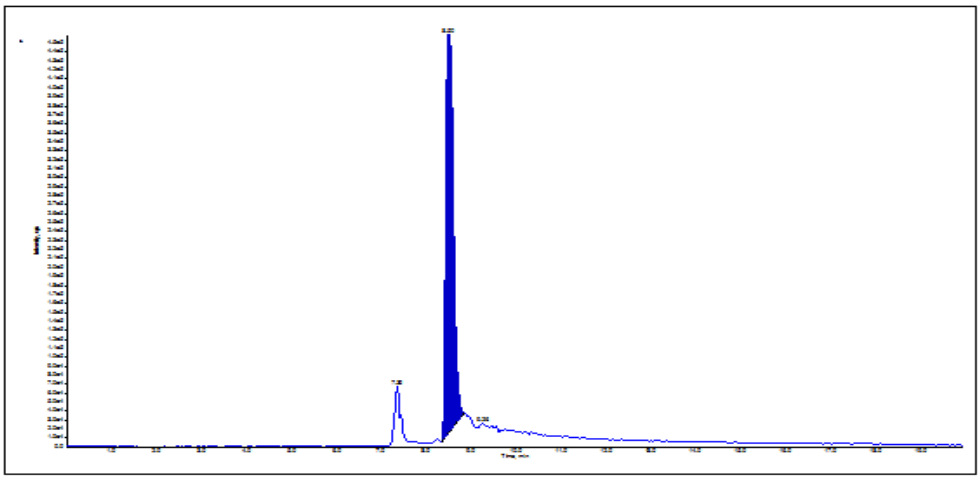

Figure 3: LCMS chromatogram of root sample of N.nimmoniana spiked with CPT.

CPT content was maximum in root sample as compared to stem and leaf. The maximum concentration of CPT was obtained in root collected in summer season as Amboli (0.59%) and Patgaon (0.54%) region. At Amboli the accumulation of camptothecin was in roots during summer over rainy (13.68%) and winter (6.41%).The accumulation of camptothecin was in roots during summer over rainy (8%) and winter (4%) at Patgaon. The stem samples collected from Amboli region also showed highest content of camptothecin in summer over rainy (44.75 %) and winter (35%) season. The minimum concentration of camptothecin was found in leaves (0.0097%) collected from Patgaon region.(Figure 4).

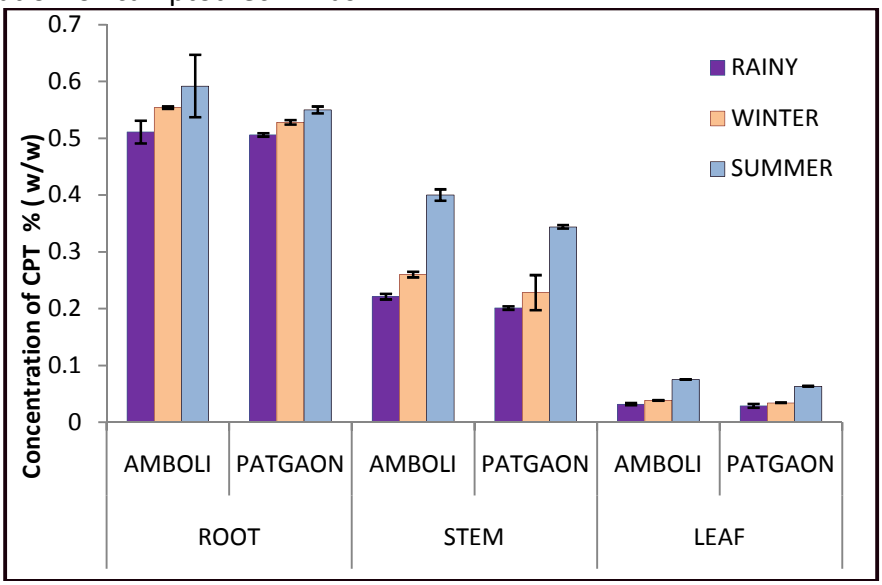

Figure 4. LCMS analysis of camptothecin in root, stem and leaf of *N. nimmoniana* in Amboli and Patgaon region during different seasons.

The trend of accumulation of camptothecin was summer > winter > rainy in both the region. The root, stem and leaf sample collected from Amboli region showed highest CPT content as compared to the material collected from Patgaon region. This variation may be due to geographical differences of Amboli and Patgaon. Environmental/ecological factors playing key role in growth, yield and production of secondary metabolites in medicinal plants. The physiological and biochemical changes are synergistic effects of different biotic and abiotic parameters. The study area Amboli having high altitude, very high rainfall, low temperature range and red laterite soil was highly favorable for growth, yield and contents of

therapeutic principles like camptothecin in *N.nimmoniana* as compared to Patgaon.

Our results corroborate with Namdeo and Sharma (2012)[7] and Patwardhan, (2006)[8] showed that root sample had highest CPT content as compared to stem and leaf. Concentration of the active constituent present in the herbals may vary due to many factors like, time and period of collection, geographical and climatic conditions.[9] Camptothecin content was highest in summer season in *Camptotheca acuminate.* They also showed that camptothecin content variation with season[10].Camptothecin content in fruit sample of ***Camptotheca acuminate*** varies with geographical position and seasonal change[11]**.**Camptothecin content in *N.nimmoniana* varies with different geographical sources. Collection in summer was best for obtaining camptothecin. Better season for collect leaves during dry days to increase CPT yield. [12, 13] Water stress led to higher CPT content in *C.acuminata* leaves, otherwise abundant rainfall favours the growth of *C.acuminata* rather than CPT accumulation. Azadirachtin content in the seeds of *Azadirachta indica* varied from geographical location[14].The results of the present study showed that concentration of camptothecin was affected by geographical origin and climatic conditions.

REFERENCES

1) Shuhas S, Ramesha BT, Ravikanth G, Rajesh P, Vasudeva R and Umashankar R. Camptothecin accumulation in *Ophiorrhiza rugosa* var. *prostrata* from northern Western Ghats. Current science, 2007; 92-8.
2) Pai SR, Nimbalkar MS, Pawar NV, Patil RP and Dixit GB. Seasonal discrepancy in phenolic content and antioxidant properties from bark of *Nothapodytes nimmoniana* (Grah).Mabb. International journal of pharma and biosciences.1(3): 2010; 1-17.
3) Govindachari TR. and Viswanathan N. Quinoline alkaloids Camptothecin and 9-metoxy camptothecin. Phytochemistry. 36, 1994; 65-66.
4) Priel E, Showalter SD and Blair DG. The topoisomerase I inhibitor, camptothecin, inhibits equine infectious anemia virus replication in chronically infected CF2Th cells. AIDS Research and human Retroviruses.7, 1991; 65-72.
5) Sheeja E, Edwin E, Dhanbad SP and Suresh B. Antiinflammatory activity of the leaves of Nothapodyts foetida,Miers. Indian journal of pharmaceutical sciences, 67(2): 2005; 251-3.
6) Khan N. Kumar S, Singh RP and Dhankhar N. Anthelmintic activity in root of Nothapodytes nimmoniana in different extracts. International journal of research in pharmaceutical and biomedical sciences. 3(1): 2012; 31-33.
7) Namdeo AG and Sharma A. HPLC analysis of camptothecin content in various parts of *Nothapodytes foetida* collected on different periods. Asian pacific journal of tropical Biomedicine. 2012; 389 -393.
8) Patwardhan A. Domestication of *Nothapodytes nimmoniana* (grah) Mabb. An endangered medicinal tree from Western Ghats of India. The rufford small grants foundation, UK 2006, 7-15.

   Namdeo AG,Sharma A, Fulzele DP and Mahadik KR. Influence of Geographical and Climatic Conditions on Camptothecin Content of *Nothapodytes nimmoniana.* Records of natural products. 4(1): 2010; 64-71.
9) Yong G, Yun LG, Xia SY, Yong WX and Xing SD. Studies on the seasonal changes of camptothecin content in the leaves of *Camptotheca acuminata*. Southwest China journal of agricultural sciences. 119 (16): 2006; 143-145.
10) Zhang ZU and Yuan G. Study on camptothecin content in leaves of *camptothecia acuminate* from different regions and seasons. Chinese bulletin of botany.20 (5): 2003; 572-575.
11) Padmanabha BV, Chandrashekar M, Ramesha BT, Hombe Gowda HC, Gunaga RP and Suhas S. 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. Current science. 90 (1):2006; 95-100.

12) Khan N, Kumar S, Singh RP and Dhankhar N. Evaluation of *Nothapodytes nimmoniana* leaves stem and root collected from different geographical region. Research journal of pharmaceutical, biological and chemical sciences. 3(1): 2012; 363.

13) Kaushik N, Singh G, Tomar UK, Naik SN, Vir S, Bisla SS, Sharma KK, Banerjee SK and Thakkar P.Regional and habitat variability in azadirachtin content of Indian neem (*Azadirachta indica*). Current science. **92,** 2007; 1400-1406.

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