VARIATION IN CONTENT OF CAMPTOTHECIN, AN ANTI-CANCER AGENT, WITH GROWTH STAGE OF *Nothapodytes nimmoniana* OF SRI LANKA

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Introduction

Camptothecin (CPT), a pentacyclic quinoline alkaloid, is regarded as one of the most promising anticancer agents that inhibit DNA topoisomerase I in eukaryotic cells. CPT is mainly extracted from *Camptotheca acuminata* (in China) and *Nothapodytes nimmoniana* (in India) which have been reported as primary CPT producing plants. CPT is the starting material for clinical CPT derived drugs, topotecan and irinotecan. Distribution of CPT in various tissues of *N. nimmoniana* of Sri Lankan origin and suitable tissue-specific manner of extraction methods for maximum yields were previously reported. The objective of the present work was to investigate the effect of growth stage/age in the content of CPT in same plants of *N. nimmoniana* surveyed in previous study.

Methodology

N. nimmoniana plant samples (leaf, bark, stem and root) from 5 plants were collected from Hakgala in September, 2012 in the previous study. Tissue samples were collected from the same plants once again in May 2013 and January 2014. CPT was extracted using tissue-specific extraction method validated in our previous study. For barks, air-dried plant tissue ground to a powder was extracted in chloroform: methanol (4:1, v: v) at the ratio of 0.1:10 (w: v). Extract was centrifuged at 10,000 rpm for 10 min. at 10 °C. For roots, dried plant tissue powder was extracted with 90% methanol (methanol/water) at the ratio of 0.1:10 (w: v) with sonication (40 kHz, Elma T 490DH) for 15 min. at room temperature (RT). The extract was centrifuged at 10,000 rpm for 10 min. at 10 °C. For stems and leaves, dried plant tissue powder was extracted in 61% ethanol (ethanol/water) at the ratio of 0.1:10 (w: v) at 60 °C for 90 min. in a shaking water bath. Extract was cooled to RT and centrifuged at 10,000 rpm for 10 min. at 10 °C. The supernatants collected in all three extraction methods were evaporated at room temperature to dryness and the concentrate was re-dissolved in methanol (HPLC grade) and filtered by 0.45 µm filters before injecting to the HPLC column.

HPLC analysis of CPT:

Ten microlitres of filtered samples were analyzed in an Agilent 1200 series HPLC system comprising of a binary HPLC pump, a diode array detector, an auto sampler, an online degasser and a thermostatic column oven. Separations were carried out using 150 mm x 4.6 mm, 5 µm Ascentis® C_{18} column (SUPELCO, Sigma USA) (Mobile phase- Acetonitrile: water 45:55 (v: v), flow rate 0.5 mL/min). The eluent was monitored at 254 nm. The identity of the peak was confirmed by comparing with authentic sample of CPT (95% purity, Sigma USA).

LC-MS (Liquid Chromatography-Mass Spectrometry) analysis of CPT: This test was carried out (outsourced) at Waters India Pvt. Ltd., Bangalore, India using Tandem Quadrupole MS, ESI (electrospray ionization) positive mode with RADAR functionality.

Results and Discussion

The retention time of authentic CPT was 4.9 minutes. Presence of CPT in various tissues of *N. nimmoniana* was confirmed *by* HPLC. The RADAR Scan of the CPT standard (Figure 1) and plant extract (Figure 2) with dual detection mode with photodiode array detection (PDA) showed similar chromatographic elution and MS Spectral pattern. The results (Figure 3) indicated that CPT contents in root tissue were varied in the following years from 2 mg/g dry weight (in September 2012) to 0.80 mg/g dry weight (in May 2013) and 0.83 mg/g dry weight (in January 2014). There was a two and a half fold higher CPT content in root collected in September 2012 than that collected from same plants in May 2013 and January 2014.

Similarly, a decreasing tendency in the CPT content with time was also observed in leaf tissues. Leaves contained four-fold and two and a half- fold higher CPT content in September 2012 compared that in those collected in May 2013 and January 2014 respectively. Leaf sample collected during May 2013 contained the lowest amount of CPT (0.02 mg/g dry weight). In contrast, CPT contents in stems were increased with time/age. There was a two-fold higher CPT content in stem collected in May 2013 and January, 2014 than in those collected in September 2012 from same plants. Bark tissue underwent the widest yield fluctuation during this period of study. It was increased from 1.2 mg/g (September 2012) to 1.7 mg/g (May 2013) and then decreased by two-fold in January 2014. The distribution pattern of CPT in tissues during the three collection period in decreasing order was as follows.

First collection in September 2012	: Root > Bark > Stem > Leaf
Second collection in May 2013	: Bark > Root > Stem > Leaf
Third collection in January 2014	: Bark > Root > Stem > Leaf

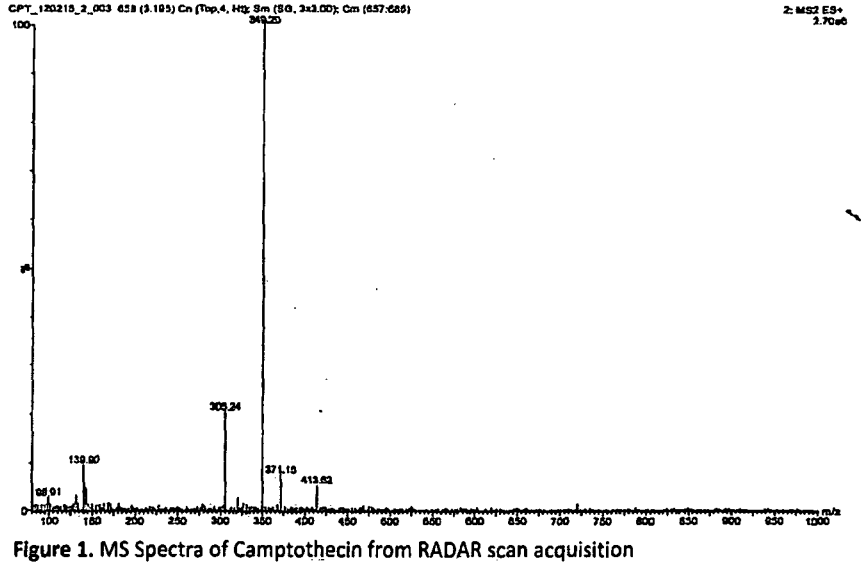

Figure 1. MS Spectra of Camptothecin from RADAR scan acquisition

CPT is extracted from bark tissues of *N. nimmoniana* for commercial purpose. Current study indicated that it is the bark tissues of *N. nimmoniana* undergoes a significant fluctuation in CPT content, indicating the importance of the proper time to harvest the bark tissue for maximum yield of this pre-drug. This study further indicated that there is no correlation between CPT content and age of the plant. Therefore, the periodical pattern of variation in CPT observed may be due to environmental changes like temperature, relative humidity, light and soil water and nutrient availability.

The highest yield (root 1.9 mg/g) of CPT of Hakgala population was much lower compared that of Indian plants (bark 13.37 mg/g). However, significant differences in the CPT content were reported among individuals and among sites of collection in Indian plant population.

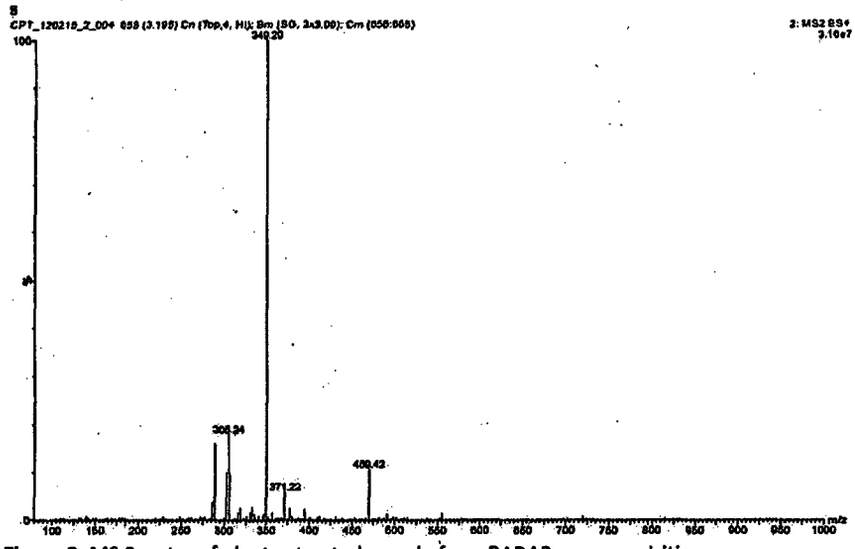

Figure 2. MS Spectra of plant extracted sample from RADAR scan acquisition

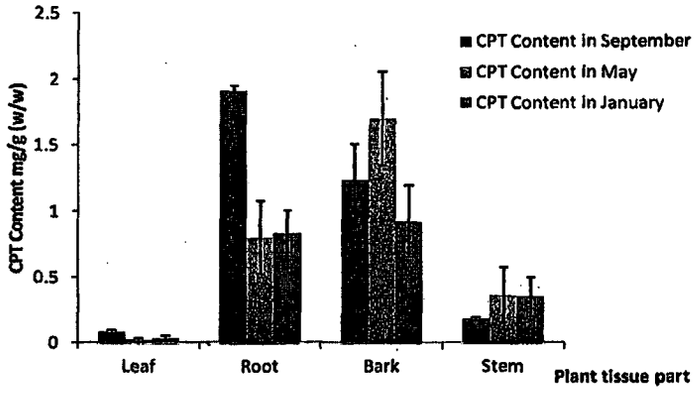

Figure 3. Content of camptothecin (mg/g) (w/w) from leaf, root, bark and stem extracts of *N. nimmoniana* collected during September 2012, May 2013 and January 2014. Values expressed as mean ± S.E (n=5).

Conclusions and Recommendations

These findings revealed that the content of CPT in *N. nimmoniana* varied with seasons. However, the variation pattern is not the same for different tissues. Further studies are required to identify seasonal variation of CPT content in different tissues of this plant. Root sample collected during September 2012 yielded the highest CPT content and the lowest CPT content was found in leaf sample collected during May 2013 (0.02 mg/g dry weight). This study indicates the potentiality of further screening of *N. nimmoniana* plants to identify high-yielding individuals which would be important in developing cell lines and other plant tissue culture systems for production of CPT.

Acknowledgement

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