Summary

Wall and Wanni first extracted Camptothecin (CPT) from the stem wood of C. acuminata. CPT and its analogues are naturally occurring group of quinoline alkaloid having profound cytotoxic activity. Until now, camptothecin and its analogues are the only plant secondary metabolite known to inhibit eukaryotic topo-1 and thus appear to be the prototype of a new class of cancer chemotherapeutic agents. At present, two semi synthetic camptothecin, Topotican and irinotecan are also clinically used as antitumor agents. Several other analogues such as 10-hydroxy CPT, 9-nitro CPT, rubitican and lurotecan are currently under clinical development at various stages.

However CPT obtained from natural plant sources are attaining importance. Ophiorrhiza stands first as a viable source. Production in nature, however, can be limited by several facts of instability and over exploitation. Moreover, Production of CPT from in vivo plant is largely affected by complicated extraction procedures, tissue age etc. The rapid pharmaceutical market and economic value of these alkaloids have prompted efforts to produce them with plant cell tissue cultures, but until now, only a few studies addressing this possibility have been carried out. Thus the present study envisaged with the idea of exploiting the techniques of plant tissue culture to improve the camptothecin content of the selected plant in in vitro condition, with particular emphasis on the improvement of the alkaloids content and its possible commercial exploitation.
The study was conducted in three separate steps and is presented here in three separate parts for clarity and easy understanding. The first phase involved screening the available species of Ophiorrhiza to select the high yielding species for further studies. The six species selected for the study were:

1. *O. mungos*
2. *O. rugosa*
3. *O. pectinata*
4. *O. barberi*
5. *O. munnaensis*
6. *O. caudata*

Four organs – stem, leaf, root and flower – were collected and the dry powder was analyzed for their camptothecin content using widely accepted HPLC method.

In this study three species – *O. mungos*, *O. rugosa* and *O. barberi* emerged as promising sources of camptothecin, warranting further investigation. Among them *O. mungos* have been pointed out as a source worth investigating in previous studies as they have appreciable quantities of camptothecin. *O. barberi* is a new entrant and is not exploited in terms of camptothecin content even at *in vitro* level.

The highest quantities of camptothecin in four out of six species were detected in the flower, contrary to the widely reported occurrence of highest content in roots. In the present study, the degree of camptothecin accumulation was in the order flower > root > leaf > stem. The range of value obtained for camptothecin in different parts is as follows:
Flower : 0.007 % - 0.26%
Root : 0.009 % - 0.1 %
Leaf : 0.08 % - 0.082 %
Stem : 0.0013 % - 0.074 %

Based on the overall performance of the plants in the initial screening and especially the camptothecin content in leaves and root, *O.mungos*, *O.rugosa* and *O.barberi* were selected for further investigation in *in vitro* conditions.

The next step, which comprises the second step of the study, was to develop protocols for the establishment of different *in vitro* systems (callus, multiple shoots and root cultures) using leaf as the explant. For callus induction from *O.mungos* leaves, culture on MS medium supplemented with 2 mg/l NAA and 0.2 mg/l BAP was found most effective. For *O.rugosa* 3 mg/l NAA and 0.2 mg/l BAP and for *O.barberi* 4 mg/l NAA and 0.2 mg/BAP was found to be ideal to produce continuously proliferating calli.

Multiple shoots were induced from young leaves in media supplemented with varying concentration of BAP. Multiple shoots were developed from leaves of *O.mungos* and *O.rugosa* in media supplemented with 2 mg/l BAP and 4 mg/L BAP respectively. BAP at a concentration of 0.2 mg/l was found to be optimum for continuous growth of multiple shoots. Twenty to thirty percent coconut water as medium supplement showed active growth and proliferation in multiple shoot culture.

For initiating root culture, *in vitro* adventitious roots were established from in vitro raised leaf explants which were then transferred to liquid media. For *O.mungos*, medium supplemented with 4 mg/l NAA under dark condition was
selected. For *O. rugosa* and *O. barberi* 0.5 mg/l NAA and 2 mg/l NAA respectively was selected as the root inducing medium. To select the suitable medium for root culture, the nutritional and hormonal influences affecting the twin aspects of differentiation (new roots) and growth were considered. Based on the overall promotion of these parameters, half strength MS medium containing 1 mg/l NAA and 2 mg/l NAA was selected for *O. mungos* and *O. barberi* respectively. For *O. rugosa* root culture half strength MS media devoid of any auxins was selected.

The experiments to achieve the primary objective of the study, i.e., the augmentation of camptothecin content were carried out (the third phase of the study) on the *in vitro* material generated in the optimized media for each system. Production of camptothecin in undifferentiated callus was found to be very low compared to other differentiated culture systems like multiple shoot and root culture.

In the present study, multiple shoot culture emerged as a good source of camptothecin production. Camptothecin content in multiple shoot cultures of *O. mungos* and *O. rugosa* considerably increased in dark condition. Further, these cultures have responded positively to changes in medium parameters such as high sucrose concentration and half strength MS medium. *O. barberi* multiple shoot cultures did not show presence of camptothecin even in minute quantity.

Untransformed root cultures responded well in terms of camptothecin production. They produce camptothecin almost reaching levels similar to those found in intact plants or sometimes even higher. The growth of roots and camptothecin content in them were found to be critically affected by medium parameters such as low sucrose concentration, media strength and hormone concentration.
A comparison of the three *in vitro* systems showed the superiority of multiple shoot cultures of *O.mungos* and *O.rugosa* synthesizing and accumulating camptothecin in appreciable quantities.

Continuous production of camptothecin was achieved by liquid root culture and subsequent harvesting of camptothecin from the medium using adsorbent resin. This is the first report on adventitious root culture of *O.rugosa* and *O.barberi* and the extraction of camptothecin in such large quantities.

In conclusion, the present study proved that the aim of increasing camptothecin level in *in vitro* conditions for commercial exploitation is easily achieved with careful and systematic selection of the system of culture and the manipulation of medium. Differentiated culture system definitely rewarding results than undifferentiated ones. *O.mungos* and *O.rugosa*, in keeping with the high camptothecin content shown in *in vivo* conditions, showed better adaptability and responsiveness to manipulations in *in vitro* conditions than *O.barberi*. 