MEDICINAL CHEMISTRY | RESEARCH ARTICLE

HPTLC-based quantification of camptothecin in Ophiorrhiza species of the southern Western Ghats in India

Renjith Rajan, Sibi Chirakkadamoolayil Varghese, Rajani Kurup, Roja Gopalakrishnan, Ramaswamy Venkataraman, Krishnan Satheeshkumar and Sabulal Baby

Cogent Chemistry (2016), 2: 1275408
HPTLC-based quantification of camptothecin in \textit{Ophiorrhiza} species of the southern Western Ghats in India

Renjith Rajan$^1$, Sibi Chirakkadamoolayil Varghese$^2$, Rajani Kurup$^1$, Roja Gopalakrishnan$^3$, Ramaswamy Venkataraman$^4$, Krishnan Satheeshkumar$^2$ and Sabulal Baby$^1$*

**Abstract:** Camptothecin (CPT), a modified monoterpene indole alkaloid, is a potential anticancer drug, and due to high demand, search for its new plant-based sources is a priority. Genus \textit{Ophiorrhiza} is a candidate group in the search for new resources of CPT. Here, CPT contents in 38 \textit{Ophiorrhiza} accessions, belonging to 11 species and 3 varieties, collected from the southern Western Ghats region in India were quantified by HPTLC-densitometry. \textit{Ophiorrhiza mungos} (396.54 μg/g, dr. wt.) and \textit{O. mungos} var. \textit{angustifolia} (373.19 μg/g, dr. wt.) were the two best CPT sources among the screened species/varieties. \textit{O. rugosa} var. \textit{decumbens} (18.55 μg/g, dr. wt.) and \textit{O. hirsutula} (17.14 μg/g, dr. wt.) showed moderate contents of CPT. This is the first systematic CPT screening of \textit{O. hirsutula}, \textit{O. barnesii}, \textit{O. incarnata}, \textit{O. radicans} and \textit{O. villosa}. This study shows the significance of choosing high CPT-yielding ecotypes/chemotypes of \textit{Ophiorrhiza} species or varieties for commercial purposes.

**Subjects:** Bioscience; Pharmaceutical Science; Pharmacology; Biology

**Keywords:** \textit{Ophiorrhiza} species; camptothecin; ecotypes/chemotypes; HPTLC-densitometry

1. Introduction
Camptothecin (CPT) (Figure 1), a topoisomerase inhibitor, was first isolated from \textit{Camptotheca acuminata} in 1958 (Wall et al., 1966), and it became a potential anticancer drug through a series of

**ABOUT THE AUTHOR**
Dr Sabulal Baby is Principal Scientist and Head at the Phytochemistry and Phytopharmacology Division of Jawaharlal Nehru Tropical Botanic Garden and Research Institute at Thiruvananthapuram in India. Dr Sabulal received his doctorate in Chemistry from the Indian Institute of Technology Bombay. He did postdoctoral research at the University of Pennsylvania, USA and University of Toronto, Canada. His research areas are phytochemistry and biochemistry. He published over fifty peer reviewed papers in these research areas.

**PUBLIC INTEREST STATEMENT**
Camptothecin (CPT), a modified monoterpene indole alkaloid, is a potential anticancer drug. High demand of CPT as a drug necessitates its supply demand equilibrium, and prompts to search for its new natural sources. Genus \textit{Ophiorrhiza} is a candidate group in the search for new resources of CPT. A total of 82 accessions (phase I 44, phase II 38) of 14 species and 3 varieties in genus \textit{Ophiorrhiza} collected from the southern Western Ghats region in India were screened for CPT using HPTLC-densitometry. We found \textit{O. mungos} and \textit{O. mungos} var. \textit{angustifolia} as the two best sources of CPT in \textit{Ophiorrhiza} species/variants in genus \textit{Ophiorrhiza}. Some of the screened \textit{Ophiorrhiza} species/varieties showed zero or non-detectable levels of CPT. This study shows the significance of choosing high CPT-yielding ecotypes/chemotypes of \textit{Ophiorrhiza} species or varieties for commercial purposes.
clinical trials (Hsiang, Hertzberg, Hecht, & Liu, 1985; Li, Zu, Shi, & Yao, 2006; Liu et al., 2000; Pommier, 2006). However, low aqueous solubility and toxicity of CPT led to its temporary withdrawal from the market, and semisynthetic analogs replaced it (Khazir, Mir, Pilcher, & Riley, 2014; van Hattum et al., 2002). Newer semisynthetic CPT analogs such as Karenitecin, Diflomotecan, and Gimatecan are in various stages of clinical trials against advanced stages of solid tumors (Garcia-Carbonero & Supko, 2002; Zunino & Pratesi, 2004). Recent clinical studies revealed two other CPT analogs Elomotecan and DRF-1042 as potent drugs against various tumors (Khazir et al., 2014). Natural CPT derivatives 9-methoxy CPT and 10-hydroxy-CPT were also isolated (Lorence & Nessler, 2004), and both them are water soluble with antitumor potentials.

Recent advances in therapeutic strategies using target oriented drug delivery systems made CPT a major candidate in the treatment of multi-drug resistant cancers (Porta et al., 2013; Tang, Cao, & Cheng, 2014). Newer drug delivery with liposomal and copolymer vehicle mediated systems drastically improved the safety and efficacy of CPT (Bissett et al., 2004; Khazir et al., 2014; Wachters et al., 2004). CPT release mediated by pH-sensitive block copolymers was also reported recently (Luo, Yang, Xu, Chen, & Zhao, 2014). Stem cell-based research in apoptogenic signaling of CPT is also in progress (García et al., 2014). This could stimulate CPT-based applications in regenerative medicine. In addition to its basic anticancer properties, CPT is also used for other pharmacological activities (López-Meyer, Nessler, & McKnight, 1994).

CPT has been isolated from various plants such as C. acuminata, C. lowreyana, C. yunnanensis, Nothapodytes nimmoniana, Pyrenacantha klaineana, Merrilliodendron megacarpum,Evtamia heyneana, Mostuea brunonis, O. mungos, O. pumila, and O.filistipula (Lorence & Nessler, 2004). Due to its demand, the search for alternative, viable plant-based sources of CPT is a priority. Cultivable and tissue culture multipliable plants are the priority sources of CPT. Ophiiorrhiza is a candidate group in this resource search for CPT (Renjith et al., 2013). Genus Ophiiorrhiza belongs to the family Rubiaceae and is represented by 47 species and 9 varieties in the Indian subcontinent (Deb & Mondal, 2001). Sixteen species and 3 varieties are reported from the southern Western Ghats region in India (Joseph & Joseph, 2009; Sasidharan, 2004). In the first phase of this work, we reported CPT contents in 44 accessions belonging to 9 Ophiiorrhiza species, and 3 varieties (Renjith et al., 2013). HPTLC–densitometry is one of the techniques useful in chemical profiling of plant extracts and quantification of the bioactive molecules in them (Haridas, Rajani, Mathew, & Sabulal, 2015; Renjith et al., 2013; Thomas et al., 2010). CPT contents in plant sources (C. acuminata) and in their tissue cultured systems were also analyzed by HPLC-based techniques (López-Meyer et al., 1994; Sankar-Thomas & Lieberei, 2011). Here, in the second phase of this screening program, we report the quantification of CPT contents in 38 accessions of Ophiiorrhiza, collected from southern Western Ghats, belonging to 11 species and 3 varieties by HPTLC-densitometry.

2. Results and discussion
CPT peaks (Rf 0.42 ± 0.01, n = 20) in Ophiiorrhiza extracts were well resolved from other signals (Figure 2). CPT contents (Table 1) in Ophiiorrhiza extracts were determined by means of the calibration plot, \( y = (2 \times 10^6 + 06) \times + 67.12 \), \( R^2 = 0.999 \), and linearity of the calibration curve in the range
0.0001–0.004 μg was ensured. On repeated measurements, mean percentage recovery observed for CPT was 100.10 ± 0.72 (Table 2). % Residual Standard Deviations (RSD) (for 20 accessions, n = 6) were determined as 1.02. Limit of detection (LOD, 25 accessions, n = 6) and limit of quantification (LOQ, 25 accessions, n = 6) were determined for CPT as 0.000085 and 0.000258 μg, respectively.

CPT contents (μg/g, dr. wt.) in 38 accessions of 11 species and 3 varieties of *Ophiorrhiza* collected from various locations in Western Ghats are given in Table 1. *O. mungos* (396.54 μg/g, dr. wt.) and *O. mungos* var. *angustifolia* (373.19 μg/g, dr. wt.) were the two best sources of CPT among the species/varieties screened. *O. mungos* (O. mungos var. mungos) is a herb of 8–100 cm height. *O. mungos* var. *angustifolia* is a smaller herb of 20–35 cm height, and it is a better candidate for cultivation and tissue culture based multiplication (Renjith et al., 2013). *O. rugosa* var. *decumbens* (18.55 μg/g, dr. wt.) and *O. hirsutula* (17.14 μg/g, dr. wt.) showed moderate CPT contents. Various *Ophiorrhiza* species (or varieties) showed significant intra and inter species variations in their CPT contents. The present data clearly support our previous report of CPT contents in species/varieties.
Table 1. Estimation of CPT in Ophiorrhiza species/varieties by HPTLC-densitometry

| Accession No. | Species                      | Location         | District      | CPT ± SD (μg/g, dr. wt.)^a |
|---------------|------------------------------|------------------|---------------|-----------------------------|
| 69998         | Ophiorrhiza barnesii         | Kadalar          | Idukki        | 0.00 ± 0.00                 |
| 52740         | O. caudata                  | Thattekadu       | Ernakulam     | 0.00 ± 0.00                 |
| 52722         | O. eriantha                 | Chemmunji        | Thiruvananthapuram | 0.00 ± 0.00 |
| 69943         | O. eriantha                 | Attayar          | Thiruvananthapuram | 0.00 ± 0.00 |
| 69982         | O. eriantha                 | Pandimotta       | Kollam        | 0.06 ± 0.02                 |
| 52720         | O. grandiflora              | Chummunji        | Thiruvananthapuram | 0.00 ± 0.00 |
| 52732         | O. hirsutula                | Periya           | Wayanadu      | 17.14 ± 4.42                |
| 52733         | O. incarnata                | Periya           | Wayanadu      | 0.00 ± 0.00                 |
| 52727         | O. mungos                   | Silent Valley    | Palakkad      | 396.54 ± 0.81               |
| 52744         | O. mungos                   | Pooyamkutty     | Ernakulam     | 354.55 ± 12.95              |
| 52748         | O. mungos                   | Brymore          | Thiruvananthapuram | 303.32 ± 6.35 |
| 52750         | O. mungos                   | Idinjar          | Thiruvananthapuram | 276.66 ± 4.53 |
| 52751         | O. mungos                   | Palode           | Thiruvananthapuram | 227.67 ± 6.29 |
| 52753         | O. mungos                   | Aluvamkudi       | Pathanamthitta | 168.33 ± 3.20               |
| 52755         | O. mungos                   | Konni            | Pathanamthitta | 215.37 ± 6.99               |
| 69923         | O. mungos                   | Kunthirikkopalam | Idukki        | 339.98 ± 28.65              |
| 52734         | O. mungos var. angustifolia | Periya           | Wayanadu      | 373.19 ± 0.04               |
| 52746         | O. mungos var. angustifolia | Pooyamkutty     | Ernakulam     | 211.07 ± 14.50              |
| 69918         | O. mungos var. angustifolia | Bonacaud        | Thiruvananthapuram | 214.05 ± 5.84 |
| 69961         | O. mungos var. angustifolia | Plappally        | Pathanamthitta | 110.13 ± 7.98               |
| 52745         | O. pectinata                | Pooyamkutty     | Ernakulam     | 0.09 ± 0.01                 |
| 52752         | O. pectinata                | Aluvamkudi       | Pathanamthitta | 0.04 ± 0.01                 |
| 69926         | O. pectinata                | Thekkadi         | Idukki        | 2.84 ± 0.02                 |
| 69935         | O. pectinata                | Anakulam         | Idukki        | 0.26 ± 0.01                 |
| 69936         | O. pectinata                | Anakulam         | Ernakulam     | 0.24 ± 0.016                |
| 69956         | O. pectinata                | Angamaoozhi      | Pathanamthitta | 0.67 ± 0.02                 |
| 52736         | O. radicans                 | Rosemala         | Thiruvananthapuram | 0.00 ± 0.00 |
| 52724         | O. rugosa var. decumbens    | Silent Valley    | Pathanamthitta | 18.55 ± 0.02 |
| 52725         | O. rugosa var. prostrata    | Silent Valley    | Pathanamthitta | 1.51 ± 0.00                 |
| 52735         | O. rugosa var. prostrata    | Rosemala         | Thiruvananthapuram | 0.00 ± 0.00 |
| 52747         | O. rugosa var. prostrata    | Pooyamkutty     | Ernakulam     | 0.47 ± 0.01                 |
| 52754         | O. rugosa var. prostrata    | Aluvamkudi       | Pathanamthitta | 0.59 ± 0.01                 |
| 52749         | O. rugosa var. prostrata    | Brymore          | Thiruvananthapuram | 0.24 ± 0.02 |
| 69917         | O. rugosa var. prostrata    | Bonacaud         | Thiruvananthapuram | 0.00 ± 0.00 |
| 52737         | O. trichocarpon             | Rosemala         | Thiruvananthapuram | 0.00 ± 0.00 |
| 69959         | O. trichocarpon             | Plappally        | Pathanamthitta | 0.32 ± 0.01                 |
| 69965         | O. trichocarpon             | Pampa            | Pathanamthitta | 0.97 ± 0.02                 |
| 52721         | O. villosa                  | Chemmunji        | Thiruvananthapuram | 0.00 ± 0.00 |

^aEach CPT ± SD data is an average of six values.

^bOphiorrhiza mungos var. mungos (Deb & Mondal, 2001) is commonly termed as Ophiorrhiza mungos (Lorence & Nessler, 2004; Rehman et al., 2009), and thus counted as a species (not as a variety) in our data (Renjith et al., 2013).
such as *O. mungos*, *O. mungos* var. *angustifolia*, *O. eriantha*, *O. grandiflora*, *O. pectinata*, *O. trichocarpum*, and *O. caudata* (Renjith et al., 2013). This study is the first systematic CPT screening in *O. barnesii*, *O. hirsutula*, *O. incarnata*, *O. radicans*, and *O. villosa*. The four first-time screened *Ophiorrhiza* species (*O. barnesii*, *O. incarnata*, *O. radicans*, *O. villosa*) and two other species (*O. caudata*, *O. grandiflora*) showed zero or non-detectable levels of CPT (Table 1). *Ophiorrhiza* species with zero or near-zero levels of CPT can be ruled out as its primary sources.

The integrity of the analyte (CPT) (Figure 1) was confirmed by its isolation from the MeOH extract of *O. mungos* var. *angustifolia* and spectral characterization. A pale yellow powder, IR (λ\text{max}, cm\textsuperscript{-1}): 3430, 1737, 1650, 1600, 1579, 1437, 1156, 1040; 1H NMR (δ): 5.28 (H-5), 8.66 (H-7), 8.10 (d, H-9, J = 8.0), 7.70 (t, H-10, J = 7.2), 7.85 (t, H-11, J = 7.6), 8.16 (d, H-12, J = 8.4), 7.37 (s, H-14), 5.41 (dd, H-17 J = 16.4, 23.4), 0.90 (t, H-18, J = 7.2), 1.89 (H-19), 6.30 (H-10); 13C NMR (δ): 152.6 (C2), 145.5 (C3), 50.1 (C5), 129.7 (C6), 131.5 (C7), 128.0 (C8), 128.4 (C9), 127.5 (C10), 130.26 (C11), 129.0 (C12), 148.1 (C13), 96.7 (C14), 150.0 (C15), 119.1 (C16), 156.8 (C16a), 65.3 (C17), 7.7 (C18), 30.7 (C19), 72.4 (C20), 172.2 (C21); LC-ESI-MS, m/z: 349.52 (M + H +). These spectral data are in good agreement with literature reports (Krishnan, Dileepkumar, Nair, & Oommen, 2014; Rehman et al., 2009). CPT standard and isolated CPT showed exactly similar Rf values on HPTLC. On relative distribution analysis, root (403.64 μg/g, dr. wt.) of *O. mungos* var. *angustifolia* showed highest CPT content compared to its stem (216.35 μg/g, dr. wt.) and leaves (192.47 μg/g, dr. wt.) (Table 3). But in *C. acuminata* highest CPT content was found in its leaves (0.4–0.5%, dr. wt.) compared to its seeds and bark (López-Meyer et al., 1994).

### 3. Experimental

#### 3.1. Plant materials

Thirty-eight accessions of 11 *Ophiorrhiza* species viz., *Ophiorrhiza barnesii* C.E.C. Fisch. (1 accession), *O. caudata* C.E.C. Fisch (1), *O. eriantha* Wight (3), *O. grandiflora* Wight (1), *O. hirsutula* Wight ex Hook.f. (1), *O. incarnata* C.E.C. Fisch. (1), *O. mungos* L. (8), *O. pectinata* Arn. (6), *O. radicans* Gardn. (1), *O. trichocarpum* Blume (3), *O. villosa* Roxb. (1), and three varieties viz., *O. mungos* L. var. *angustifolia*
3.2. Extraction
Shade dried, powdered plant materials (whole plants, 38 accessions, 10 g each) were Soxhlet extracted (separately) using 200 ml MeOH for 6 h. In order to find the relative CPT distribution, O. mungos var. angustifolia root, stem and leaves (52734; 10 g each) were Soxhlet extracted (separately) with MeOH (200 ml, 6 h). After extraction, solvents were fully removed using a rotary evaporator (Buchi, Switzerland) and yields of extracts (w/w) were recorded.

3.3. HPTLC analysis
Quantification of CPT in Ophiorrhiza (whole plant) extracts was carried out using an HPTLC (CAMAG, Switzerland) made up of Linomat V sample applicator, twin trough plate development chamber, TLC Scanner 3, and WinCATS Software 4.03 (Renjith et al., 2013). Briefly, Ophiorrhiza extracts dissolved in MeOH were filtered through nylon 0.45 μ membrane filters (PALL Gelman Laboratory, India). Filtered extracts of O. mungos, O. mungos var. angustifolia, O. mungos var. angustifolia (52734, root, stem and leaf extracts) (5 μl or 0.5 μg each), O. trichocarpon (10 μl or 10 μg, 20 μl or 200 μg) and other Ophiorrhiza species (20 μl or 200 μg each) were applied onto silica gel HPTLC plates (60F-254, E. Merck, Germany, 20 × 10 cm, 0.2 mm thickness) as 6 mm wide bands with the automatic Linomat V sample applicator fitted with a micro syringe in N2 flow (application rate—150 nl/s, space between two bands—11 mm, slit dimension—6 × 0.45 mm, scanning speed—20 mm/s). CPT standard (Sigma Aldrich, India) was also applied along with the Ophiorrhiza extracts. Plates were developed up to 80 mm in 20 ml of EtOAc:CHCl3:MeOH (5:4.5:0.5, v/v) mobile phase in the twin trough glass chamber, under saturated conditions (30 min). These plates were scanned densitometrically at 366 nm (tungsten lamp) using TLC Scanner 3 and the data were analyzed using WinCATS Software 4.03 (Table 1).

3.4. Data analysis, validation
HPTLC method was validated in terms of accuracy, precision, repeatability, and linearity (Fischedick, Glas, Hazekamp, & Verpoorte, 2009; Reich & Schibli, 2007; Renjith et al., 2013; Thomas et al., 2010). CPT contents (Table 1) in Ophiorrhiza extracts were determined by means of the calibration plot, $y = (2 \times 10^6 + 06) x + 67.12$, $R^2 = 0.999$, made of standard CPT (at 0.001 μg/μl in MeOH). Linearity of CPT calibration curve in the range 0.0001–0.004 μg was ensured. Specificity was tested by repeated application of standard CPT. RF values of CPT were reproducible and found to be same as the values observed for the CPT peak in Ophiorrhiza extracts (0.42 ± 0.01, n = 20) (Figure 2). Integrity and purity of CPT peak were also tested by spectral analysis. Recovery studies were carried out by the addition of three varying concentrations of CPT to pre-analyzed Ophiorrhiza extracts (O. mungos 52753, 52755) and they were analyzed under similar conditions. Repeatability of sample application (instrumental precision) was assessed by applying an Ophiorrhiza extract on the HPTLC plate, developed under similar conditions, the same spot was scanned six times, and the % coefficient of variation was acceptable (Table 4). Robustness of the method was checked by slightly altering the mobile phase composition and plate developing distance. Two different mobile phases viz., 20 ml each of EtOAc:CHCl3:MeOH (5:4.5:0.5, v/v) and EtOAc:CHCl3:MeOH (4.5:5:0.05, v/v), were used and
developing distances were varied to 75, 85 and 90 cm. No considerable effect on the data (Rf values of CPT) was found. % RSD (SD of CPT area/average CPT area) × 100 (for 20 accessions, n = 6) were determined. Limit of detection (LOD, average of 3.3 × SD of CPT area/slope of calibration curve for 25 accessions, n = 6) and limit of quantification (LOQ, average of 10 × SD of CPT area/slope of calibration curve for 25 accessions, n = 6) were determined for CPT (Haridas et al., 2015; Renjith et al., 2013; Thomas et al., 2010). HPTLC analyses were carried out at laboratory conditions, 21 ± 0.5°C and 40% relative humidity (Barigo Humidity Meter, Germany).

3.5. Isolation, characterization of CPT

CPT (Figure 1) was isolated from the MeOH extract of O. mungos var. angustifolia (whole plant) by repeated column chromatography using hexane:ethyl acetate and characterized using FT-IR (PerkinElmer, USA), 1H-, 13C-, 2D-NMR (Bruker, Germany), and LC-MS (Thermo Finnigan, USA).

4. Conclusions

High demand of CPT as a drug necessitates a supply-demand equilibrium and prompts to search for its new natural sources. A total of 82 accessions (phase I 44, phase II 38) of 14 species and 3 varieties in genus Ophiorrhiza collected from the southern Western Ghats region in India were screened for CPT (Renjith et al., 2013). O. mungos and O. mungos var. angustifolia are the two best sources of CPT in genus Ophiorrhiza. In this study, four (O. barnesii, O. incarnata, O. radicans, O. villosa) of the five first-time screened species showed zero or non-detectable levels of CPT. These screening data can be utilized to choose elite eco-/chemotypes of Ophiorrhiza species/varieties for multiplication, and drug development.

Acknowledgments

We acknowledge Kerala Forest Department for permitting us to collect Ophiorrhiza specimens from protected forest areas. R.R. and S.C.V. are DAE-BRNS/MS University research fellows.

Funding

This work was supported by Board of Research in Nuclear Sciences, Department of Atomic Energy, Government of India [grant number 2010/35/4/BRNS/1278] dated 11/08/2010.

Author details

Renjith Rajan1
E-mail: renjith08@gmail.com
Sibi Chirakkadamooyal Varghese2
E-mail: cbsivamente@gmail.com
Rajani Kurup3
E-mail: rajanikurupsr@gmail.com
Roja Gopalakrishnan3
E-mail: groja@barc.gov.in
Ramswamy Venkataraman4
E-mail: rvraman3@rediffmail.com
Krishnan Satheeshkumar4
E-mail: kumarkrishnan59@gmail.com
Sabulal Baby5
E-mail: sabulal@sabulal.com, sabulal@jntbgri.res.in

1 Phytochemistry and Phytopharmacology Division, Jawaharlal Nehru Tropical Botanic Garden and Research Institute, Pocha-Palode, Thiruvananthapuram 695 562, Kerala, India.
2 Biotechnology and Bioinformatics Division, Jawaharlal Nehru Tropical Botanic Garden and Research Institute, Pocha-Palode, Thiruvananthapuram 695 562, Kerala, India.
3 Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre, Mumbai 400 085, Maharashtra, India.
4 Department of Chemistry, Sri Paramakalyani College, Manoranjan Sundararajan University, Alwarkunchi, Tirunelveli 627 412, Tamil Nadu, India.
5 Department of Chemistry, Sri Paramakalyani College, Manoranjan Sundararajan University, Alwarkunchi, Tirunelveli 627 412, Tamil Nadu, India.

Citation information

Cite this article as: HPTLC-based quantification of camptothecin in Ophiorrhiza species of the southern Western Ghats in India, Renjith Rajan, Sibi Chirakkadamooyal Varghese, Rajani Kurup, Roja Gopalakrishnan, Ramswamy Venkataraman, Krishnan Satheeshkumar & Sabulal Baby, Cogent Chemistry (2016), 2: 1275408.

Cover image

Source: Authors.

References

Bissett, D., Cassidy, J., de Bono, J. S., Muirhead, F., Main, M., Robson, L., … Twelves, C. (2004). Phase I and pharmacokinetic (PK) study of MAG-CPT (PNU 166148): A polymeric derivative of camptothecin (CPT). British Journal of Cancer, 91, 50–55. http://dx.doi.org/10.1038/sj.bjc.6601922

Deb, D. B., & Mondal, D. C. (2001). Taxonomic revision of the genus Ophiorrhiza L. (Rubiaceae) in Indian subcontinent. Bulletin of the Botanical Survey of India, 39, 1–148.

Fischbedick, J. T., Glas, R., Hazekamp, A., & Verpoorte, R. (2009). A qualitative and quantitative HPTLC densitometry method for the analysis of cannabinoids in Cannabis sativa L. Phytochemical Analysis, 20, 421–426. http://dx.doi.org/10.1002/pca.v20:5

Garcia, C. P., Videla Richardson, G. A., Romorini, L., Miriuka, S. G., Sevlever, G. E., & Scassa, M. E. (2014). Topoisomerase I inhibitor, camptothecin, induces apoptogenic signaling in human embryonic stem cells. Stem Cell Research, 12, 400–414. http://dx.doi.org/10.1016/j.scr.2013.12.002

Garcia-Carbonero, R., & Supko, J. G. (2002). Current perspectives on the clinical experience, pharmacology, and continued development of the camptothesins. Clinical Cancer Research, 8, 641–661.

Haridas, P., Rajani, K., Mathew, P. J., & Sabulal, B. (2015). Levodopa in Mucuna pruriens and its degradation. Scientific reports, 5, 11078.
Hsiang, Y. H., Hertzberg, R., Hecht, S., & Liu, L. F. (1985). Camptothecin induces protein-linked DNA breaks via mammalian DNA topoisomerase I. Journal of Biological Chemistry, 260, 14873–14878.

Joseph, G., & Joseph, J. P. (2009). Rediscovery of Ophiorrhiza caudata (Rubiaceae) from the Western Ghats of Kerala. Rheedia, 19, 45–46.

Khazir, J., Mir, B. A., Pilcher, L., & Riley, D. L. (2014). Role of plants in anticancer drug discovery. Phytochemistry Letters, 7, 173–181.

Krishnan, S. A., Dileepkumar, R., Noor, A. S., & Oommen, O. V. (2014). Studies on neutralizing effect of Ophiorrhiza mungos root extract against Daboia russelli venom. Journal of Ethnopharmacology, 151, 543–547.

Li, Q. Y., Zu, Y. G., Shi, R. Z., & Yao, L. P. (2006). Review on the accumulation of the antitumor alkaloid camptothecin in Camptotheca acuminate. Planta Medica, 70, 558–560.

Luo, Y. L., Yang, X.-L., Xu, F., Chen, Y.-S., & Zhao, X. (2014). pH-triggered PMMA-b-HTPB-b-PMMA copolymer micelles: Physicochemical characterization and camptothecin release. Colloid and Polymer Science, 292, 1061–1072.

Luo, Y. L., Yang, X.-L., Xu, F., Chen, Y.-S., & Zhao, X. (2014). pH-triggered PMMA-b-HTPB-b-PMMA copolymer micelles: Physicochemical characterization and camptothecin release. Colloid and Polymer Science, 292, 1061–1072.

Luo, Y. L., Yang, X.-L., Xu, F., Chen, Y.-S., & Zhao, X. (2014). pH-triggered PMMA-b-HTPB-b-PMMA copolymer micelles: Physicochemical characterization and camptothecin release. Colloid and Polymer Science, 292, 1061–1072.

Luo, Y. L., Yang, X.-L., Xu, F., Chen, Y.-S., & Zhao, X. (2014). pH-triggered PMMA-b-HTPB-b-PMMA copolymer micelles: Physicochemical characterization and camptothecin release. Colloid and Polymer Science, 292, 1061–1072.

Luo, Y. L., Yang, X.-L., Xu, F., Chen, Y.-S., & Zhao, X. (2014). pH-triggered PMMA-b-HTPB-b-PMMA copolymer micelles: Physicochemical characterization and camptothecin release. Colloid and Polymer Science, 292, 1061–1072.

Luo, Y. L., Yang, X.-L., Xu, F., Chen, Y.-S., & Zhao, X. (2014). pH-triggered PMMA-b-HTPB-b-PMMA copolymer micelles: Physicochemical characterization and camptothecin release. Colloid and Polymer Science, 292, 1061–1072.