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Phytochemical analysis of *Ophiorrhiza pectinata* ARN. (*Rubiaceae*) a potential anticancer plant

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Abstract
Ophiorrhiza pectinata (whole plant) solvent extracts (chloroform, methanol and water) were prepared by Soxhlet extraction method and these extracts were subjected to thin layer chromatography. The mobile phase used was chloroform: methanol (24: 1 ratio). Camptothecin (authentic standard) served as the reference. Retention factor value (0.6) was observed in the extracts of chloroform and ethyl acetate, corresponding to the similar band observed from reference standard (Camptothecin). Camptothecin appeared blue fluorescence under UV light and turned orange red colour while spraying dragondorff's reagent. Camptothecin was quantified using High performance liquid chromatography, A standard graph of Camptothecin was made using Camptothecin and the Camptothecin content of whole plant (field grown plant) was quantified by calibration with this standard graph. Presence of Camptothecin was confirmed, the yield of Camptothecin (field grown plant) was 250.54 µg g^{-1} d.wt.

Keywords: Camptothecin, suspension culture, *Rubiaceae*, high performance liquid chromatography.

Introduction
The genus *Ophiorrhiza* L. (*Rubiaceae*) with about 400 species is distributed from Eastern India to the West Pacific and from South China to Northern Australia [1]. The genus *Ophiorrhiza* consists of 49 species in India [2, 3]. Different species of the genus have been used in traditional medicines against snake bite, ulcers and wound healing, ulcers, helminthiasis, poisonous wounds, gastropathy, leprosy and hydrophobia [4]. Most species of *Ophiorrhiza* are characterized by the presence of a cytotoxic alkaloid, camptothecin, which is the only naturally occurring topoisomerase-1inhibitor [5, 6]. The presence of camptothecin gives great importance to *Ophiorrhiza* species in cancer research and hence *Ophiorrhiza* species are economically very important due to the presence of CPT and other valuable alkaloids. *Ophiorrhiza pectinata* Arn. is a herbaceous plant and is distributed over peninsular India and Sri Lanka. The main objective of the present study is to evaluate *Ophiorrhiza pectinata* and quantify camptothecin using HPLC

Materials and Methods
Plants were harvested, washed and air dried. Dried samples were powdered and 5 g was extracted with chloroform at room temperature under constant stirring. The first extraction was carried out for 6h. The extracts were separated through Whatman No. 1 filter paper and the residues were again extracted at 5h interval till the extract became colourless. The extract was concentrated under vacuum in Rotavapor (Superfit, India).

Thin Layer Chromatography (TLC)
Glass plates (5 mm thick) of dimension 5 x 20 and 10 x 20 cm and rectangular glass chromatography chambers (30 x 20 x 25 cm, Borosil) were cleaned thoroughly and rinsed with 95% acetone and dried. TLC plates were prepared using silica gel as the adsorbent. Cleaned glass plates were kept flat closely side by side over a uniform flat surface of the TLC plating device. A slurry of silica gel was prepared by mixing silica gel with distilled water 1:2 (w/v) ratio and spread on the glass plates with the help of a TLC applicator which was adjusted to the thickness of 1.0 mm. The plates were dried at room temperature and activated at 110^0C for 30 minutes in a thermo-regulated hot air oven before use. Standard sample, 1mg camptothecin (zigma chemicals, USA) dissolved in 10 ml chloroform and concentrated samples from cultures dissolved in chloroform were spotted on TLC plates using capillaries or micro syringe about 2.0 cm from the lower end and allowed to dry the spot in air. The plates were then placed in the chromatography chambers containing 40 ml of solvent system such as chloroform: Methanol (24:1). Which was closed with lid and kept for 10 minutes to provide saturated atmosphere. The plates were kept slanting on the wall of the chamber and its mouth was sealed tightly with the lid. The chromatographic run was carried out at room temperature.

The solvent migrated up and when it reached three-fourth of the total length of the plates, they were taken out, the solvent front was marked and allowed to dry in air. The plates were viewed under the UV chamber and the fluorescent spots were marked. The plates were sprayed with dragondorff's reagent and the coloured spot appeared were marked.

Preparation of Dragendorff's Reagent [7]

Solution a: 0.85 grams of basic bismuth nitrate was dissolved in a mixture of 10 ml acetic acid and 40 ml water.

Solution b: A solution was made of 8 grams of potassium iodide in 20 ml water.

Stock Solution: Equal volumes of solution a and solution b were mixed (stored in dark coloured vessels)

Spray Reagent: 1 ml of stock solution was mixed with 2 ml acetic acid and 10 ml of water before use.

Comparative Phytochemical Profile

Samples of (5 g each) of field plants were extracted with different solvent (50 ml each) systems viz. water, chloroform, methanol, petroleum ether, ethyl acetate and the extracts were spotted on TLC plates using capillary tube and were co-chromatographed using authentic sample camptothecin. The fluorescent blue spot which appeared on UV chamber corresponding to the Rf value of standard sample was marked.

Quantification of Camptothecin

The field grown plants were air dried and extracted with chloroform and the extracts were dried under vacuum. The residues obtained were re dissolved separately in known volume of acetonitrile: water (24:1). CPT was analyzed using an HPLC system [8].

High performance Liquid Chromatography (HPLC)

Waters 600 series Pump, Waters C18 Symmetry column (4.6 x 250 mm), Waters 717 plus Auto sampler, Mobile phase-Acetonitrile/Water-60/40, Flow Rate – 1.0 ml/min. Waters 2487 UV Detector – 256nm.

Results and Discussion

Thin Layer Chromatography

Extracts, that was obtained from chloroform, methanol, water, ethyl acetate and petroleum ether were subjected to thin layer chromatography. The mobile phase used was chloroform: methanol (24:1 ratio). Camptothecin (authentic standard) served as the reference. Rf value (0.6) was observed in the extracts of chloroform and ethyl acetate, corresponding to the similar band observed from reference standard (CPT). Camptothecin appeared blue fluorescence under UV light and turned orange red colour while spraying dragondorff's reagent.

Quantification of CPT in samples by HPLC

A standard graph of CPT was made using CPT (sigma) and the CPT content of each sample was quantified by calibration with this standard graph. Presence of CPT in the sample was confirmed by HPLC. The CPT standard gave a retention time of 3.052 min. (fig. 1). The yield of CPT from field grown plant was 250.54 $\mu_g g^{-1}$ d.w. (fig. 2).

In *O. pectinata*, we can detect the compound using thin layer chromatography from a trace amount of extract and visualize the presence of compound in UV-chamber. Similar results was also obtained from *Camptotheca acuminata* that using thin layer chromatography in conjunction with fluorescence imaging to obtain reproducible measurements in the nanogram range [9].

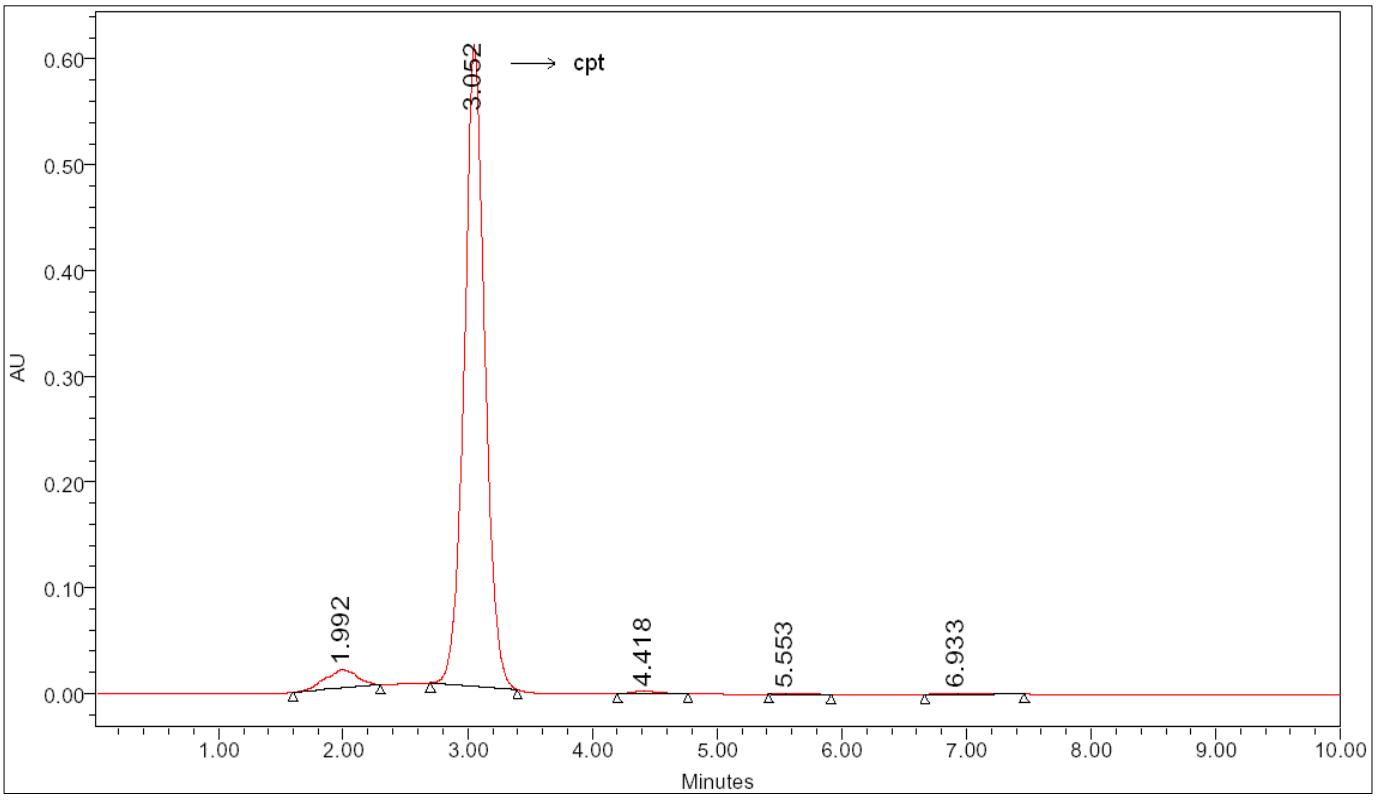

Fig 1: HPLC of standard camptothecin.

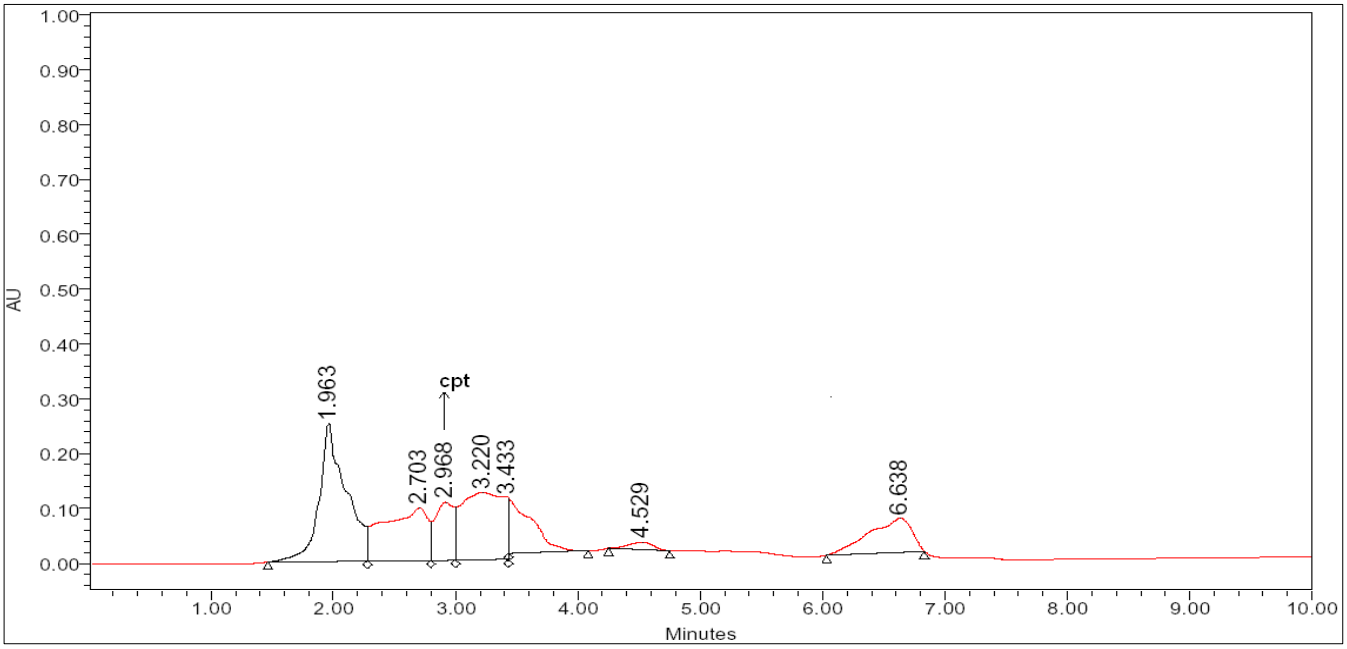

Fig 2: HPLC of whole plant (field grown) of *Ophiorrhiza pectinata.*

Conclusion

Samples of field plants were extracted with different solvent systems *viz.*, chloroform/methanol/ water/ ethyl acetate /petroleum ether and were subjected to thin layer chromatography. The mobile phase used was chloroform: methanol (24:1 ratio). Camptothecin (authentic standard) served as the reference. Rf value (0.6) was observed in the extracts of chloroform and ethyl acetate, corresponding to the similar band observed from reference standard (CPT). Camptothecin appeared blue fluorescence under UV light and turned orange red colour while spraying dragondorff's reagent.

CPT was quantified using HPLC and was found in field grown plant

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References

1. Sibi CV, Dintu KP, Renjith R, Krishnaraj MV, Roja G, Satheesh kumar K. A New Record of *Ophiorrhiza trichocarpon* Blume (*Rubiaceae*: *Ophiorrhizeae*) from Western Ghats, India: Another Source Plant of Camptothecin. J Sci. Res. 2012; 4:529-532.
2. Deb DB, Mondal DC. Taxonomic revision of the genus *Ophiorrhiza* L. (*Rubiaceae*) in Indian subcontinent. Bull. Bot. Surv. India. 1997; 39:99-100.
3. Mabberley DJ. Mabberley's Plant Book: A portable dictionary of plants, their classification and uses. Third Edition. Cambridge University Press, Cambridge, UK, 2008.
4. Kirtikar KR, Basu BD. Indian Medicinal plants ed. M/S Bishen Singh Mahendrapal Singh, New Delhi, India. 1975; 2:1268-1269.
5. Martin KP, Shahanaz Beegum, Zhang CI, Slater A, Madusoodanan PV. *In vitro* propagation of *Ophiorrhiza prostata* through somatic embryogenesis. *Biologia plantatum.* 2007; 51:769-772.
6. Yamazaki Y, Akiko Urano, Hiroshi Sudo, Mariko Kitajima, Hiromitsu Takayama, Mami Yamazaki *et al*. Metabolite profiling of alkaloids and strictosidine synthase activity in camptothecin producing plants. Phytochemistry. 2003; 62:461-470.
7. Stahl E. Thin layer chromatography. A laboratory hand book. George Allen and Unwin Limited. London, 1969.
8. Vineesh VR, Jelly CL, Fijesh PV, Jaimsha VK, Jose padikkala. Effect of N^6- Benzyl amino purine and Naphthalene acetic acid on camptothecin production through *in vitro* propagation of *Ophiorrhiza rugosa* wall. Var. decumbens (Gardn. Ex Thw.) Deb and Mondal. Natural product Radiance. 2007; 6:405-409.
9. Beth Nolte A, Daniel R, Lineberger, David W. Reed, Mary E. Rumpho Rapid Micro- assay of Camptothecin in *Camptotheca acuminata*. Planta Med. 2001; 67:376-378.