

Original Research Article

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Optimization of TLC Solvent Systems for Camptothecin Analysis in *Pyrenacantha volubilis* Hook and *Sarcostigma kleinii* Wight & Arn

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ABSTRACT

Keywords

Pyrenacantha volubilis, *Sarcostigma kleinii*, camptothecin, alkaloids, phytochemical screening, methanolic extract, thin-layer chromatography, TLC solvent system, Icacinaceae.

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India possesses a rich diversity of medicinal plants that are widely used in traditional medicine and the herbal industry (Gupta *et al.*, 1998; Ravikumar and Ved, 2000). Camptothecin (CPT) is a naturally occurring alkaloid with potent anticancer activity and has attracted considerable attention due to its therapeutic potential (Lorence and Nessler, 2004). In the present study, *Pyrenacantha volubilis* Hook. and *Sarcostigma kleinii* Wight & Arn., belonging to the family Icacinaceae, were investigated for the preliminary detection of alkaloids and the presence of camptothecin using thin-layer chromatography (TLC). Methanolic extracts of the leaves and stems of both plants were subjected to preliminary phytochemical screening using Mayer's, Wagner's, Hager's and Dragendorff's reagents. Alkaloids were detected in the extracts, with prominent reactions observed with Wagner's and Hager's reagents. For TLC analysis, different solvent systems comprising chloroform, methanol and ethyl acetate in varying proportions were evaluated using authentic camptothecin as the reference standard. The solvent systems chloroform (1:1), chloroform (2:1), chloroform (24:1), chloroform acetate (1:1) and chloroform acetate (2:1) produced R_f values of 0.78, 0.87, 0.83, 0.46 and 0.50, respectively. Among the solvent systems tested, chloroform acetate (2:1) provided a suitable chromatographic separation with an R_f value of 0.50 and was selected for further analysis. The TLC profiles of the methanolic leaf and stem extracts of *P. volubilis* and *S. kleinii* showed spots corresponding to the authentic camptothecin standard under UV illumination. The findings indicate that TLC using chloroform acetate (2:1) is a simple and suitable method for the preliminary detection of camptothecin in these two Icacinaceae members.

Introduction

Medicinal Plants and Bioactive Secondary Metabolites

Medicinal plants are important sources of biologically active natural products and have contributed significantly to traditional healthcare systems as well as modern drug discovery. India possesses a rich diversity of medicinal plant species, many of which are traditionally used for the treatment and management of various diseases. The Western Ghats is recognized as one of the major centres of plant diversity and is particularly rich in medicinal plant resources (Myers *et al.*, 2000). A wide variety of secondary metabolites, including alkaloids, flavonoids, coumarins, tannins and phenolic compounds, are responsible for several of the pharmacological properties associated with medicinal plants.

The chemical composition of medicinal plants is influenced by several factors, including geographical origin, soil conditions, temperature, rainfall, cultivation practices, stage of harvesting, drying and storage. Such variations can affect the quality, efficacy and reproducibility of herbal preparations. Therefore, appropriate methods for the identification, characterization and quality assessment of medicinal plant materials are essential (WHO, 1998). Chromatographic fingerprinting has become an important approach for the qualitative assessment of complex plant extracts because it enables the detection and comparison of characteristic phytochemical constituents.

Camptothecin: A Bioactive Anticancer Alkaloid

Among the diverse alkaloids produced by plants, camptothecin (CPT) is a highly important natural product because of its potent anticancer activity. Camptothecin is a monoterpenoid indole alkaloid originally isolated from *Camptotheca acuminata* Decne. and has subsequently been reported from several other plant species, including members of the family Icacinaceae (Lorence and Nessler, 2004). It acts primarily by inhibiting DNA topoisomerase I, thereby interfering with DNA replication and causing cytotoxic effects in rapidly dividing cells.

The pharmacological importance of CPT has led to the development of clinically useful semisynthetic derivatives, particularly topotecan and irinotecan (Lorence and Nessler, 2004). However, the natural

compound has limitations such as poor water solubility and toxicity, which have stimulated continued research into its derivatives and alternative plant sources. The occurrence of CPT in unrelated plant taxa also indicates the importance of exploring additional natural sources of this valuable alkaloid (Lorence and Nessler, 2004).

Camptothecin-Producing Plants and Alternative Sources

The increasing pharmaceutical importance of CPT has encouraged the investigation of alternative plant sources capable of producing this alkaloid. Several members of the family Icacinaceae have been reported to contain camptothecin and related compounds (Lorence and Nessler, 2004).

Earlier investigations reported camptothecin and 9-methoxycamptothecin from *Nothapodytes foetida* through studies involving tissue cultures and mature trees (Roja and Heble, 1994).

Pyrenacantha volubilis Hook. is an important medicinal climber belonging to the family Icacinaceae and occurs in the dry scrub and deciduous forests of southern India. The species has attracted attention as a potential natural source of camptothecin.

Studies have reported the occurrence of CPT and related derivatives in different parts of *P. volubilis*, highlighting its potential as an alternative source of this anticancer alkaloid.

Sarcostigma kleinii Wight & Arn. is another woody climber belonging to the Icacinaceae and is distributed in parts of southern India. Its occurrence in natural and semi-evergreen habitats and its phytochemical importance make it a relevant species for investigation of bioactive constituents. However, compared with well-studied CPT-producing species, comparatively limited information is available on the chromatographic detection of CPT in *S. kleinii*. Therefore, investigation of both *P. volubilis* and *S. kleinii* may contribute to the identification of potentially useful plant sources of this important alkaloid.

Thin-Layer Chromatography in Phytochemical Analysis

Plant extracts generally contain complex mixtures of

primary and secondary metabolites. Separation and preliminary identification of individual constituents are therefore important steps in phytochemical investigation. Several chromatographic techniques, including thin-layer chromatography (TLC), high-performance thin-layer chromatography (HPTLC) and high-performance liquid chromatography (HPLC), are used for the analysis and quality assessment of medicinal plants (Schulz *et al.*, 2001; Li *et al.*, 2004; Ong, 2004).

Among these techniques, TLC is a simple, rapid, economical and convenient method for the preliminary analysis of plant extracts. It permits simultaneous analysis of several samples along with an authentic reference standard and requires relatively small quantities of sample and solvent (Sherma, 2000; Poole, 2003). TLC has been widely used for the separation, detection and preliminary identification of phytoconstituents in medicinal plants (Wagner and Bladt, 1996).

The chromatographic behaviour of a compound is strongly influenced by the composition and proportion of the mobile phase. The retention factor (R_f) of a compound, together with its colour and fluorescence characteristics after suitable visualization, can be compared with those of an authentic standard. Consequently, selection of an appropriate solvent system is an important step in developing a reliable TLC method for the preliminary detection of a target compound.

Rationale and Objective of the Present Study

Although camptothecin has been reported from several plant species, there is a need to explore additional plant materials and establish simple preliminary methods for its detection. In particular, comparative evaluation of TLC solvent systems can help identify a suitable mobile phase for the separation and detection of CPT in crude plant extracts.

The present study was undertaken to investigate the occurrence of alkaloids and the preliminary detection of camptothecin in methanolic leaf and stem extracts of *Pyrenacantha volubilis* Hook. and *Sarcostigma kleinii* Wight & Arn. Preliminary phytochemical screening was performed using standard alkaloid reagents, followed by TLC analysis using authentic camptothecin as the reference standard. Different solvent systems containing chloroform, methanol and ethyl acetate were evaluated based on the migration behaviour of the standard and extracts. The study aimed to identify a suitable TLC

solvent system for the preliminary chromatographic detection of camptothecin in the selected plant species.

Materials and Methods

Study Area and Plant Materials

The present investigation was carried out to evaluate the preliminary occurrence of camptothecin (CPT) in the leaf and stem extracts of *Pyrenacantha volubilis* Hook. and *Sarcostigma kleinii* Wight & Arn. and to identify a suitable solvent system for their thin-layer chromatographic (TLC) analysis.

Collection and Authentication of Plant Material

The aerial parts, comprising leaves and stems, of *Pyrenacantha volubilis* Hook. were collected from the sacred grove of Villukatti Ayyanar Kovil, Varakalpattu, Cuddalore District, Tamil Nadu. The leaves and stems of *Sarcostigma kleinii* Wight & Arn. were collected from Pala, Kottayam District, Kerala.

The collected plant materials were examined for their morphological characteristics and identified using relevant taxonomic literature. The plant materials were subsequently processed for phytochemical investigation. General laboratory procedures and techniques were followed according to Purvis *et al.*, (1966) and Dodds and Roberts (1984).

Preparation of Plant Material

The collected leaves and stems were thoroughly washed with distilled water to remove adhering soil and other extraneous materials. The plant materials were then shade-dried at room temperature for approximately two weeks. The dried materials were separately powdered using a suitable mechanical grinder and stored in clean, dry containers until extraction.

Preparation of Methanolic Extracts

The powdered leaves and stems of both plant species were separately extracted with methanol by the cold-percolation method. The extracts were filtered and concentrated by evaporation of the solvent under controlled conditions.

The resulting concentrated extracts were obtained as

sticky masses and stored at 10°C until further phytochemical and chromatographic analysis.

Preliminary Phytochemical Screening

Crude methanolic extracts of leaf and stem of *P. volubilis* and *S. kleinii* were subjected to preliminary phytochemical screening of Alkaloid tests of i) Mayer's reagent, ii) Hager's reagent, iii) Wagner's reagent and iv) Dragendorff's reagent. Phytochemical Tests.

Test for Alkaloid

Solvent free extract, 50 mg was stirred with few ml of dilute hydrochloric acid and filtered. The filtrate was tested carefully with various alkaloidal reagents as follows.

(i) Mayer's Test: To a few ml of filtrate, a drop or two of Mayer's reagent were added by the side of the test tube. A white or creamy precipitate indicated the test as positive.

Mayer's Reagent: Mercuric chloride (1-35 g) was dissolved in 60 ml of water and potassium iodide (5.0 g) was dissolved in 10 ml of water. The two solutions were mixed and made up to 100 ml with water.

(ii) Wagner's test: To a few ml of filtrate, few drops of Wagner's reagent were added by the side of the test tube. A reddish-brown precipitate confirmed the test as positive. Wagner's reagent: Iodine (1.27 g) and potassium iodide (2 g) was dissolved in 5 ml of water and made up to 100 mL with distilled water.

(iii) Hager's test: To a few ml of filtrate 1 or 2 ml of Hager's reagent (saturated aqueous solution of picric acid) were added. A prominent yellow precipitate indicated the test as positive.

(iv) Dragendorff's test: To a few ml of filtrate, 12 ml of Dragendorff's reagent were added. A prominent yellow precipitate indicated the test as positive.

Thin-Layer Chromatographic Analysis

TLC analysis was carried out following the general procedure described by Fried, with modifications appropriate to the present study.

Pre-coated silica gel 60 F254 aluminium-backed TLC plates (10 × 15 cm; layer thickness 0.2 mm) were used for chromatographic separation. The plates were handled carefully to avoid contamination and mechanical damage.

A starting line was marked approximately 1.0 cm from the lower edge of the plate, while the solvent-development limit was marked approximately 1.5 cm below the upper edge. The plant extracts and authentic camptothecin standard were dissolved separately in methanol prior to application.

Preparation and Application of Camptothecin Standard

Authentic camptothecin (CPT; 95% HPLC grade, Sigma-Aldrich, USA) was used as the reference standard. A standard solution was prepared in methanol at a concentration of 1 mg/mL.

The standard solution and the methanolic extracts of the leaves and stems of *P. volubilis* and *S. kleinii* were applied separately onto the silica gel F254 plates using fine microcapillaries.

Approximately 50 µL of the standard solution and 50 µL of each plant extract were applied as discrete spots along the marked application line.

Evaluation of TLC Solvent Systems

Different solvent systems were evaluated to determine a suitable mobile phase for the preliminary chromatographic detection of camptothecin. The solvent systems investigated were:

The developing chamber was charged with the respective mobile phase and allowed to equilibrate before development. The TLC plates were developed in a vertical glass chamber under saturated conditions, following the general chromatographic procedure described by Strobel *et al.*, (1992).

The solvent was allowed to migrate up the plate until the predetermined solvent-development limit was reached. The plates were then removed from the chamber and allowed to dry before visualization.

Visualization and Detection of Camptothecin

The developed TLC plates were examined under short-wave (254 nm) and long-wave (365 nm) ultraviolet illumination. The chromatographic behaviour of the plant extracts was compared with that of the authentic camptothecin standard. The plates were subsequently sprayed with 10% sulphuric acid in methanol and examined for the development of characteristic colour reactions and fluorescence. The position and appearance of the spots in the plant extracts were compared with those of the authentic CPT standard.

A spot exhibiting chromatographic behaviour comparable to that of the authentic standard was considered evidence for the preliminary presence of camptothecin in the respective plant extract.

Determination of Retention Factor (Rf)

The retention factor (Rf) of the standard and sample spots was calculated using the following equation:

$$R_f = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent front}}$$

The Rf values obtained with the different mobile phases were compared to assess their suitability for the chromatographic separation and preliminary detection of camptothecin. The solvent system producing a distinct and suitable CPT-associated spot with an appropriate Rf value was selected for further analysis.

Results and Discussion

The present investigation was carried out to explore the potential anti-cancer active compound camptothecin (CPT) in the family Icacinaceae, among the different genus in Icacinaceae, we have selected two plants namely i) *Pyrenacantha volubilis* Hook, and ii) *Sarcostigma kleinii* Wight and Arn., for qualitative analysis of alkaloid test and thin layer chromatographic analysis with authentic compound camptothecin (CPT - 95% HPLC graded) purchased from Sigma-Aldrich.

Collection of plant material and sample preparation

Pyrenacantha volubilis is a small climber, collected from the scared grove of Villukatti Ayyanar Kovil,

Varakalpattu, Cuddalore district, Tamil Nadu. *Sarcostigma kleinii* Wight and Arn., is a woody climber were collected from the place Pala, Kottayam district, Kerala for present investigation. The aerial part leaves with stem of both the plant were collected, cleaned thoroughly with distilled water and the desired plant parts were dried under shade for two weeks. The shade dried aerial parts were pulverized in a mechanical grinder to obtain coarse powder.

Preparation of extracts and Phytochemical analysis for alkaloid test

The powdered plants were subjected to methanolic extraction by cold percolation method. Evaporation of solvent from the extract was done by oven dry method. A sticky mass was obtained after evaporation of the solvent. The samples were stored at 10°C till further use of phytochemical analysis and TLC analysis.

Preliminary Phytochemical Screening

The methanolic extracts of the leaves and stems of *Pyrenacantha volubilis* Hook. and *Sarcostigma kleinii* Wight & Arn. were subjected to preliminary phytochemical screening for alkaloids using Mayer's, Wagner's, Hager's and Dragendorff's reagents.

The results indicated the presence of alkaloids in both plant species. In the leaf extracts, moderate reactions were observed with Wagner's and Hager's reagents, while the stem extracts showed comparatively stronger reactions. The observations are presented in Table 1.

The alkaloid screening showed a similar qualitative pattern in both species, with the stem extracts exhibiting stronger reactions than the corresponding leaf extracts, particularly with Wagner's and Hager's reagents.

TLC Solvent-System Screening

Different solvent systems were evaluated for the TLC separation of camptothecin. The migration behaviour of the compounds was assessed based on their retention factor (Rf) values.

The solvent systems chloroform (1:1), chloroform (2:1), chloroform (24:1), chloroform acetate (1:1) and chloroform acetate (2:1) produced Rf values of 0.78, 0.87, 0.83, 0.46 and 0.50, respectively (Table 2).

Table.A Evaluation of TLC Solvent Systems

S. No.	Mobile phase	Ratio (v/v)
1	Chloroform : Methanol	1:1
2	Chloroform : Methanol	2:1
3	Chloroform : Methanol	24:1
4	Chloroform : Ethyl acetate	1:1
5	Chloroform : Ethyl acetate	2:1

Table.1 Preliminary screening of alkaloids in methanolic extracts of *P. volubilis* and *S. kleinii*

Plant species	Plant part	Mayer's	Wagner's	Hager's	Dragendorff's
<i>P. volubilis</i>	Leaf	—	++	++	+
	Stem	+	+++	+++	++
<i>S. kleinii</i>	Leaf	—	++	++	+
	Stem	+	+++	+++	++

+++ = appreciable reaction; ++ = moderate reaction; + = mild reaction; — = absent.

Table.2 R_f values obtained with different TLC solvent systems

S. No.	Solvent system	Ratio	R _f value
1	Chloroform : Methanol	1:1	0.78
2	Chloroform : Methanol	2:1	0.87
3	Chloroform : Methanol	24:1	0.83
4	Chloroform : Ethyl acetate	1:1	0.46
5	Chloroform : Ethyl acetate	2:1	0.50

Among the solvent systems tested, chloroform acetate (2:1) produced a suitable chromatographic migration of the camptothecin standard, with a R_f value of 0.50. Therefore, this solvent system was selected for further TLC analysis of the plant extracts.

TLC Detection of Camptothecin in *Pyrenacantha volubilis*

The methanolic leaf and stem extracts of *P. volubilis* were subjected to TLC using chloroform acetate (2:1) as the mobile phase, along with authentic camptothecin as the reference standard.

The developed chromatograms were examined under UV illumination at 254 nm and 365 nm. The leaf and stem extracts showed dark blue spots at positions corresponding to the camptothecin standard. Following spraying with 10% sulphuric acid in methanol, the corresponding spots showed characteristic colour development and fluorescence.

The chromatographic behaviour of the extracts was compared with that of the authentic CPT standard, indicating the preliminary presence of camptothecin in the tested extracts of *P. volubilis*.

TLC Detection of Camptothecin in *Sarcostigma kleinii*

The methanolic leaf and stem extracts of *S. kleinii* were similarly analysed by TLC using chloroform acetate (2:1) as the mobile phase and authentic camptothecin as the reference standard.

The chromatograms showed dark blue spots corresponding to the position of the authentic camptothecin standard under UV illumination at 254 nm and 365 nm. Treatment with 10% sulphuric acid in methanol produced characteristic colour development and fluorescence of the corresponding spots.

The present study investigated the preliminary occurrence of alkaloids and camptothecin (CPT) in the methanolic leaf and stem extracts of *Pyrenacantha volubilis* Hook. and *Sarcostigma kleinii* Wight & Arn., two members of the family Icacinaceae.

Preliminary phytochemical screening revealed the presence of alkaloids in both species, with comparatively stronger reactions observed in the stem extracts, particularly with Wagner's and Hager's reagents.

Camptothecin is an important natural alkaloid with potent anticancer activity and has been reported from several plant species, including members of the Icacinaceae (Lorence and Nessler, 2004).

Previous studies have reported camptothecin and related compounds from *Nothapodytes* species (Roja and Heble, 1994), supporting the importance of investigating alternative plant sources.

In the present study, five solvent systems were evaluated for TLC analysis. Chloroform (1:1), (2:1) and (24:1) produced R_f values of 0.78, 0.87 and 0.83, respectively, while chloroform acetate (1:1) and (2:1) produced R_f values of 0.46 and 0.50, respectively. Among the solvent systems tested, chloroform acetate (2:1) was selected for further analysis based on the suitable migration of the authentic CPT standard.

Using the selected solvent system, the leaf and stem extracts of both plants showed spots corresponding to the authentic CPT standard under UV illumination at 254 and 365 nm. Characteristic colour development was also observed after spraying with 10% sulphuric acid in methanol. These findings provide preliminary evidence for the presence of CPT in the investigated extracts.

TLC is a simple, rapid and economical technique for preliminary phytochemical analysis and comparison with authentic standards. Further studies using HPLC or LC-MS are required for definitive confirmation and quantitative estimation of CPT in these plant extracts.

The current study aimed to investigate the potential anti-cancer compound camptothecin (CPT) within the Icacinaceae family. Two plants were chosen for this research: i) *Pyrenacantha volubilis* Hook, and ii) *Sarcostigma kleinii* Wight and Arn. *Pyrenacantha volubilis* was gathered from the sacred grove of

Villukatti Ayyar Koil, Varakalpattu, in the Cuddalore district of Tamil Nadu. *Sarcostigma kleinii* Wight and Arn was collected from Pala in the Kottayam district of Kerala. The aerial parts, including leaves and stems, of both plants were harvested, thoroughly washed with distilled water, and then dried in the shade for two weeks. Once dried, the aerial parts were ground into a coarse powder using a mechanical grinder. This powdered material underwent methanolic extraction through the cold percolation method. The solvent was removed from the extract using the oven dry method, resulting in a sticky residue after evaporation. The methanolic crude extracts from the leaves and stems of *P.volubilis* and *S.kleinii* underwent initial phytochemical analysis using Alkaloid tests, including i) Mayer's reagent, ii) Wagner's reagent, iii) Hager's reagent, and iv) Dragendroff's reagent. Of the reagents tested, Wagner's reagent resulted in a reddish-brown precipitate, while Hager's reagent produced a yellow solution, confirming the presence of alkaloids in the leaf and stem extracts of *P.volubilis* and *S.kleinii*. For Chromatogram standardization, various mobile phases were evaluated, including chloroform (CHCl₃), methanol (MeOH), and ethyl acetate (EtOAc). The combinations tested were CHCl₃:MeOH (1:1), CHCl₃:MeOH (2:1), CHCl₃:MeOH (24:1), CHCl₃:EtOAc (1:1), and EtOAc (2:1), yielding different R_f values of 0.78, 0.87, 0.83, 0.46, and 0.50, respectively. Among these, the chloroform: ethyl acetate (2:1) mixture resulted in an R_f value of 0.50. The extract of *P. volubilis* and *S. kleinii* further screened using this ratio alongside the standard compound camptothecin. On a TLC plate, the *P. volubilis* extracts displayed CPT as dark blue spots visible at 254nm and 365nm, and fluorescent spots appeared when sprayed with a 10% H₂SO₄ in methanol reagent. For *S. kleinii*, the methanolic crude extracts from the leaf and stem, along with CPT, were applied to a TLC plate. Using a mobile phase of chloroform and ethyl acetate, CPT appeared as dark blue spots at 254nm and 365nm. The developed plates, when treated with a 10% H₂SO₄ in methanol reagent, revealed fluorescent spots under normal light, facilitating the detection of camptothecin and its comparison with the standards.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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