

RESEARCH

Open Access



Design and characterization of camptothecin gel for treatment of epidermoid carcinoma

Sunil T. Galatage^{1*}, Aditya S. Hebalkar¹, Raviraj V. Gote¹, Omkar R. Mali¹, Suresh G. Killedar¹, Durgacharan A. Bhagwat² and Vijay M. Kumbhar³

Abstract

Background: The objective of present research work is to design and characterize camptothecin gel using Carbopol-934 for the treatment of epidermoid carcinoma. Optimized herbal gel formulations were evaluated for homogeneity and appearance, viscosity, extrudability, spreadability, drug content, drug release, pH, and in vitro skin cancer activity on A431 cell lines.

Results: Mass and Infrared Spectra respectively conforms molecular weight and functional groups present in camptothecin. All the formulations F1 to F5 showed good homogeneity, pH from 6.68 to 6.90, spreadability in the range of 15.81–23.37 gm.cm/s, extrudability 85.51–90.45% w/w, drug content 89.12–96.64%, and in vitro diffusion 88.36–98.40%, respectively. The drug release study showed that all the formulations followed a diffusion-controlled, zero-order release mechanism. Anticancer activity results indicate that camptothecin gel induce cell death in A-439 cells having IC₅₀ 48.03 µg and % apoptosis 54.67 ± 4.58.

Conclusion: Topical delivery of camptothecin alleviates the side effects caused by systemic chemotherapy; hence, the developed herbal gel formulation can be effectively useful to deliver camptothecin in the treatment of epidermoid carcinoma on A-431 cells.

Keywords: Skin cancer, Camptothecin, Diffusion, Herbal gel, Drug content, Cell line-A431

Background

Nowadays' burden of growing global cancer was drastically raised, and patients suffering from it deeply required an ideal therapy that completely cures the cancer [1]. Current treatments of cancer include chemotherapy, surgery, and radiation therapy, having hazardous adverse effects. From the ancient era, various types of cancer were cured and prevented by using natural and herbal drugs [2]. Epidermoid carcinoma, also known as skin cancer, is a more versatile and common cancer in human being. Ultraviolet radiations which are present in sunlight are prominent source of skin cancer. Change in appearance of

the skin and a sore that does not heal within 2 weeks are primary indicators and signs of skin cancer. It has been estimated that 2–3 million new cases occur annually and the number is increasing each year. It is estimated that in the northern United States, almost half of the people who live up to 65 years will develop skin cancer once. This represents growing public concern [3]. In recent years, utilization of herbal medicines along with its active constituents to treat cancer tremendously increased due to lesser side effects [4]. Phytoconstituents which are present in herbal drugs were prominently utilized in cancer treatment due to its multimolecular target action. Herbal drugs contain various types of phytoconstituents having versatile pharmacological actions like alkaloids, glycosides, and tannins. Along with the active ingredients, plants contain vitamins, minerals, proteins, etc. [5]. *Nothapodytes*

* Correspondence: gsunil201288@gmail.com

¹Sant Gajanan Maharaj College of Pharmacy, Mahagaon, Site: Chinchewadi, Kolhapur, Maharashtra 416502, India
Full list of author information is available at the end of the article

nimmoniana (Icacinaeae) is mainly tropical and subtropical species which mainly occur in the Western Ghats of Maharashtra, India. Camptothecin (CPT) is a prime phytoconstituent of *Nothapodytes nimmoniana* which is reported for various pharmacological activities such as antiviral [6], HIV [7], antibacterial, antifungal [8], colorectal cancer, malignancies, ovarian cancer, recurrent small cell lung cancer [9–11], and breast cancer [12]. Camptothecin demonstrates a broad-spectrum anticancer activity. Their molecular target has been firmly established to be the human DNA topoisomerase-I. CPT inhibits topoisomerase-I by blocking the rejoining step of the cleavage reaction of topoisomerase-I, resulting in accumulation of a covalent reaction intermediate, the cleavable complex. The primary mechanism of cell killing by CPT is the S-phase-specific killing through potentially lethal collisions between advancing replication forks and topoisomerase-I cleavable complexes. Collisions with the transcription machinery have also been shown to trigger the formation of long-lived covalent topoisomerase-I DNA complexes, which contribute to CPT cytotoxicity which is significantly responsible for skin cancer activity [13, 14]. However, no studies have undertaken in the quest of anticancer activity of camptothecin on skin cancer. Side effects that occurred during conventional treatment of melanoma patients treated with chemo and immunotherapy who prominently suffer from anorexia, nausea, fatigue, vomiting, renal toxicity, abdominal pain, dermatitis, hepatitis, and infection among others are avoided by topical herbal gel formulations [15, 16]. As skin cancer can be better treated by the use of topical gel formulations as these are painless and can be applied at the targeted site, many skin cancers were treated by gel formulation, and there are herbal gel formulations with other aids that have shown better penetration [17]. Hence, the present investigation is aimed to utilize camptothecin isolated from *Nothapodytes nimmoniana* in treatment of skin cancer by developing a camptothecin herbal gel formulation.

Materials

Carbopol-934, sodium CMC-HiMedia Labs Pvt. Ltd., Mumbai, Glycerine-Loba Chemie Pvt. Ltd., Mumbai, propylene glycol, propylparaben, methylparaben-Nice Chemicals Pvt. Ltd., Mumbai, Tri-ethanolamine-Ozone Int. Chemicals, Mumbai. Double-distilled water procured from Unique Chemical, Kolhapur. All chemicals and reagents used in present research work were of analytical grade.

Methods

Preparation of *Nothapodytes nimmoniana* extract

Camptothecin was extracted by using microwave irradiation technique from *N. nimmoniana* with the help of

surfactant solution (Emulgen). Five grams dried *N. nimmoniana* plant material was kept in 200 ml of 0.2% w/v surfactant solution of pH 8 in 250 ml Erlenmeyer flask. Suspension of *N. nimmoniana*-containing surfactant was kept in microwave for irradiation for 1 min at 350 W. Microwave irradiation was stopped after 1 min and cooled for 2 min. Same procedure was repeated for 3 min. The filtrate of extraction was acidified with 2% w/w sulfuric acid solution until pH comes to 3–4. Mayer's reagent was added to precipitate alkaloids. The precipitated alkaloids were dissolved in 5% alkaline solution of Na_2CO_3 . The organic layer was neutralized by washing with distilled water until it has neutral pH and was dried and concentrated to get (camptothecin) alkaloids [18, 19].

Identification of camptothecin

Fourier transform infrared spectroscopy (FTIR)

FTIR spectroscopy is utilized to analyze characteristic moieties present in developed formulation. The chemical composition of the camptothecin gel and excipients were studied. Functional moieties present in camptothecin gel were determined and confirmed by FTIR (PerkinElmer FTIR Series model-1615 spectrophotometer), and the spectra was scanned in the range of 4000 to 400 cm^{-1} range at a resolution of 4 cm^{-1} [20].

Differential scanning calorimetry (DSC)

DSC (Mettler Toledo, Stare SW 12.10) equipped with intracooler and refrigerated cooling system was used to obtain the thermogram at a heating rate of 10 °C per minute over a temperature range of 0–300 °C. Inert condition was maintained by purging nitrogen at 10 ml/min rate. In an aluminum crucible, the sample was sealed hermetically [21].

Mass spectroscopy

An equine LC ion trap system was used for mass spectrometric detection for positive and negative ion mode mass and MS spectra. The capillary voltage was set to –3800 V and at the end 500 V plate in +Ve ion mode. The sample was infused directly via a syringe pump, and nebulizer gases were reduced to 5 l/min and 5 psi, respectively. For precision mass detection, a micro TOF-Q equipped with the Apollo ESI ion source was utilized. Capillary voltage was maintained at 4500 V and end plate voltage to –500 V in negative ion mode. The molecular formula was generated by matching high mass accuracy and isotopic pattern [22].

Compatibility study

The development of formulation drug and polymer are in close contact with each other, and the stability of the developed formulations depends on these interactions. For the determination of compatibility of CPT and

excipients, modern analytical techniques such as FTIR and DSC were used to confirm compatibility.

Formulation of camptothecin gel

With continuous vigorous stirring, Carbopol-934 as a gelling agent and sodium carboxyl methyl cellulose as a thickening agent were dissolved in 50 ml double-distilled water. Preservative solution was prepared by adding required quantities of preservatives in 5 ml double distilled water on heating water bath. Preservative solution was cooled, and glycerin was added in it. Then, it was mixed with the first resultant solution. Predetermined quantity of camptothecin was added to this solution and mixed homogeneously. Finally, this whole mixture was mixed properly and the pH was adjusted to skin pH (6.8–7); triethanolamine was added drop by drop with continuous stirring to obtain a gel of predetermined viscosity and consistency [23] (Table 1).

Evaluation parameters

Percentage yield

The % practical yield was calculated from the weight of camptothecin gel recovered from each batch in comparison to total starting weight of raw materials [24]. The % yield was calculated using the following formula.

$$\% \text{yield} = \frac{\text{Practical mass}}{\text{Theoretical mass}} \times 100$$

Measurement of pH

About 20 mg of the camptothecin gel was taken within 24 h of manufacture in a beaker, and the pH was measured with the help of a digital pH meter (Hitech Lab India) [25].

Spreadability

The apparatus consists of a wooden block having a pulley at one end. The drag characteristic of the gel was

used to measure the spreadability. About 2 g of the gel sample was placed on a ground slide, and the gel was sandwiched between ground slides which were fixed and having a hook. Weight of 1 kg was placed on top of slides for 5 minutes due to which formation of uniform gel film between slides. The top plate is subjected to pull 80 g by using a string attached to the hook; meanwhile, the time required by the top slide to cover the distance of 7.5 cm was recorded in seconds [26]. Spreadability was calculated by using the following formula:

$$S = M \times L/T$$

where S is the spreadability, M is the weight in pan, L is the length moved by the glass slide, and T is the time taken to separate the slide completely from each other.

Extrudability

Camptothecin gel was incorporated in aluminum collapsible tube which was sealed at one end. The tubes' weights were recorded. The tubes were clamped by placing between 2 glasses. The cap was removed by placing 500 g camptothecin gel over the slides. An extruded amount of gel was weighted after collection, and % of extrusion was calculated [27].

Drug content

Accurately weighted 1 g camptothecin gel was placed in 100 ml phosphate buffer pH 5 as solvent. The resultant solution was continuously shaken until complete dissolution of gel formulation in solvent. The resultant solution was filtered by Whatman filter paper, and drug content was measured with the help of UV visible spectrophotometer (Shimadzu UV-1900) at 225 nm with the help of calibration curve [28].

$$\text{Drug content} = \text{Actual yield/Theoretical yield} \times 100$$

Viscosity

Brookfield DV-E viscometer (Middleborough, MA, USA) having spindle #64 was used to measure viscosity of gel formulation at 10 to 100 rpm. Viscosity of gel formulations was automatically recorded and displayed on digital screen. Once measurement was over, the spindle was lowered. The digital readings were multiplied by the factor mentioned in the catalog [29].

Diffusion studies

Diffusion study was carried out with the help of Franz diffusion cell (Logan Instruments) having a capacity of 16 ml equipped with cellophane membrane. Accurately weighted 01 g camptothecin gel was placed over cellophane membrane in donor compartment and receptor compartment filled with pH 6.8 phosphate buffer. With

Table 1 Formulation table of camptothecin herbal gel

Ingredients	Quantity				
	F1	F2	F3	F4	F5
Camptothecin (mg)	100	100	100	100	100
Carbopol-940 (g)	1	2	1	1	2
Sodium CMC (g)	1	1	2	3	1
Glycerin (ml) (g)	2	2	2	2	2
Methylparaben (0.5%) (ml)	0.2	0.2	0.2	0.2	0.2
Propylparaben (0.2%) (ml)	5	5	5	5	5
Triethanolamine (ml)	q. s.	q. s.	q. s.	q. s.	q. s.
Purified water (ml)	100	100	100	100	100

constant stirring, the resultant assembly was placed on a magnetic stirrer at 50 rpm. And temperature was kept constant at $37\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$ by continuous flow of water through jacket. Each time, 1 ml sample was withdrawn at every 1 h up to 12 h and same time equals the amount of phosphate buffer was placed in the receptor compartment. Withdrawn samples were analyzed for drug content at 225 nm against blank by UV-visible spectrophotometer (Shimadzu UV-1900) [27].

Anticancer activity

Cell culture

Cell line was procured from the NCCS (National Centre for Cell Sciences), Pune. The cell line were cultured in DMEM medium which was supplemented with 10% heat-inactivated fetal calf serum (FBS) and 1% antibiotic-antimycotic 100 \times solution and incubated in CO₂ incubator (Eppendorf, New Brunswick, Galaxy 170R, Germany) maintained at 37 $^{\circ}\text{C}$, 5% CO₂ with 95% humidity until the completion of experiments.

Cytotoxicity

The cells were seeded at a density of approximately 5×10^3 cells/well in a 96-well flat-bottom micro plate and maintained at 37 $^{\circ}\text{C}$ in 95% humidity and 5% CO₂ for overnight. Different concentration (60, 50, 40, 30, 20, 10 $\mu\text{g/ml}$) of samples was treated. For another 48 h, the cells were incubated. The wells were washed with phosphate buffer solution twice and 20 μl of the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) staining solution (5 mg/ml in phosphate buffer solution) was added to each well, and the plate was incubated at 37 $^{\circ}\text{C}$. After 4 h, 100 μl of dimethyl sulfoxide (DMSO) was added to each well to dissolve the formazan crystals, and absorbance was recorded with 570 nm using a microplate reader [30].

Formula: Surviving cells (%) = Mean OD of test compound / Mean OD of negative control \times 100

Using graph Pad Prism Version 5.1, we calculate the IC₅₀ of compounds

Surviving cells (%)—cell viability

Mean OD of test compound—mean optical density of test compound

Mean OD of Negative control—mean optical density of Negative control

OD—optical density

DAPI (4',6-diamidino-2-phenylindole)

The cells were seeded at a density of approximately 1×10^4 cells/well in a 24-well flat-bottom microplate containing cover slips and maintained at 37 $^{\circ}\text{C}$ in CO₂ incubator for overnight. Less than the 50 $\mu\text{g/ml}$ of compounds was treated at 48 h. After the

incubation, the cells were washed with PBS and fixed with 4% paraformaldehyde for 30 min. Twenty microliters of DAPI (0.1 $\mu\text{g/ml}$) was incubated for 5 min at room temperature in the dark and examined under fluorescent microscope, randomly selecting the fields in the microscope and counted the number of cells undergone apoptosis and then calculated the percentage of apoptotic cells [31].

Results and discussion

Identification of camptothecin

FTIR spectroscopy

The use of FTIR technique allowed pointing out the implication of the different functional groups of guest and host molecules by analyzing the significant changes in the size and position of the absorbance bands. The principal absorption peak of standard camptothecin which showed OH stretching at 3437.29 cm^{-1} , ester stretching at 1741.78 cm^{-1} , C=O stretching at 1642.28 cm^{-1} , C=C at 1621.28 cm^{-1} , C=N at 1438.94 cm^{-1} , C–O at 1033.88 cm^{-1} , and peak at 775.41 cm^{-1} appears to be a contribution of four adjacent hydrogen bonds on the heteroaromatic nucleus. The values are near or equal to the values mentioned in the standard structure of camptothecin [32] (Fig. 1).

Differential scanning calorimetry (DSC)

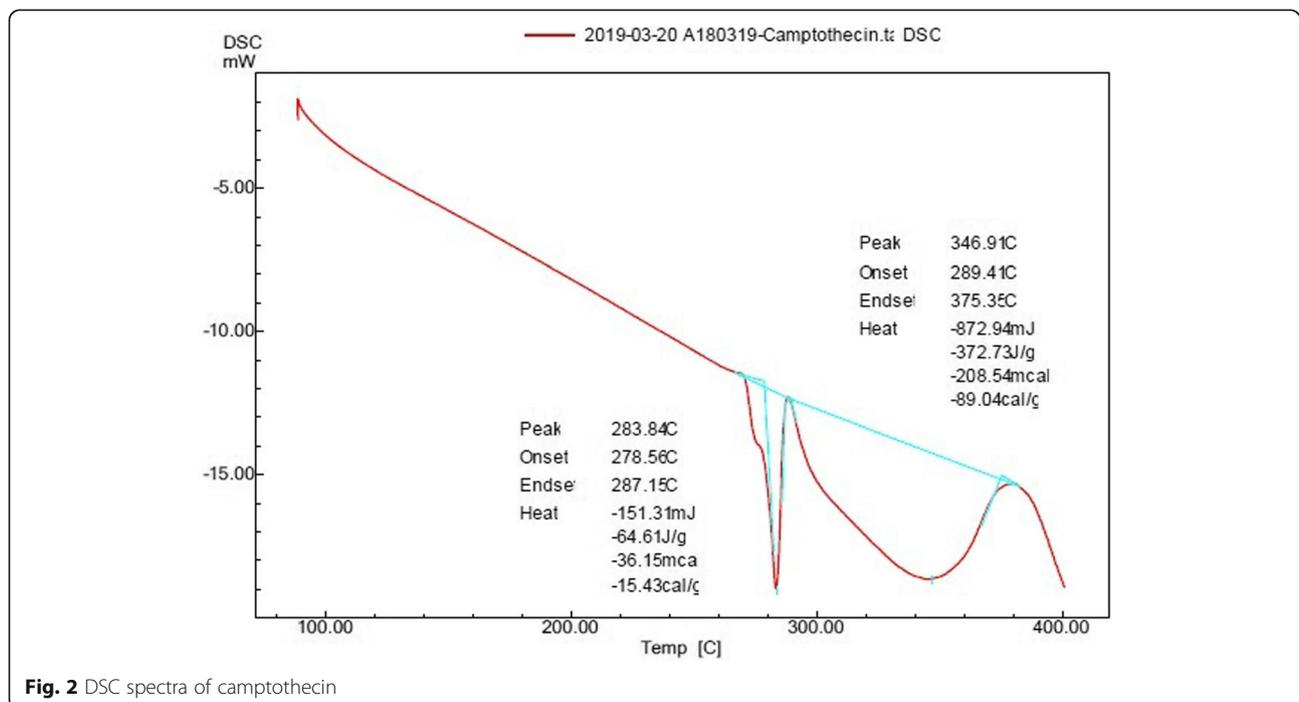
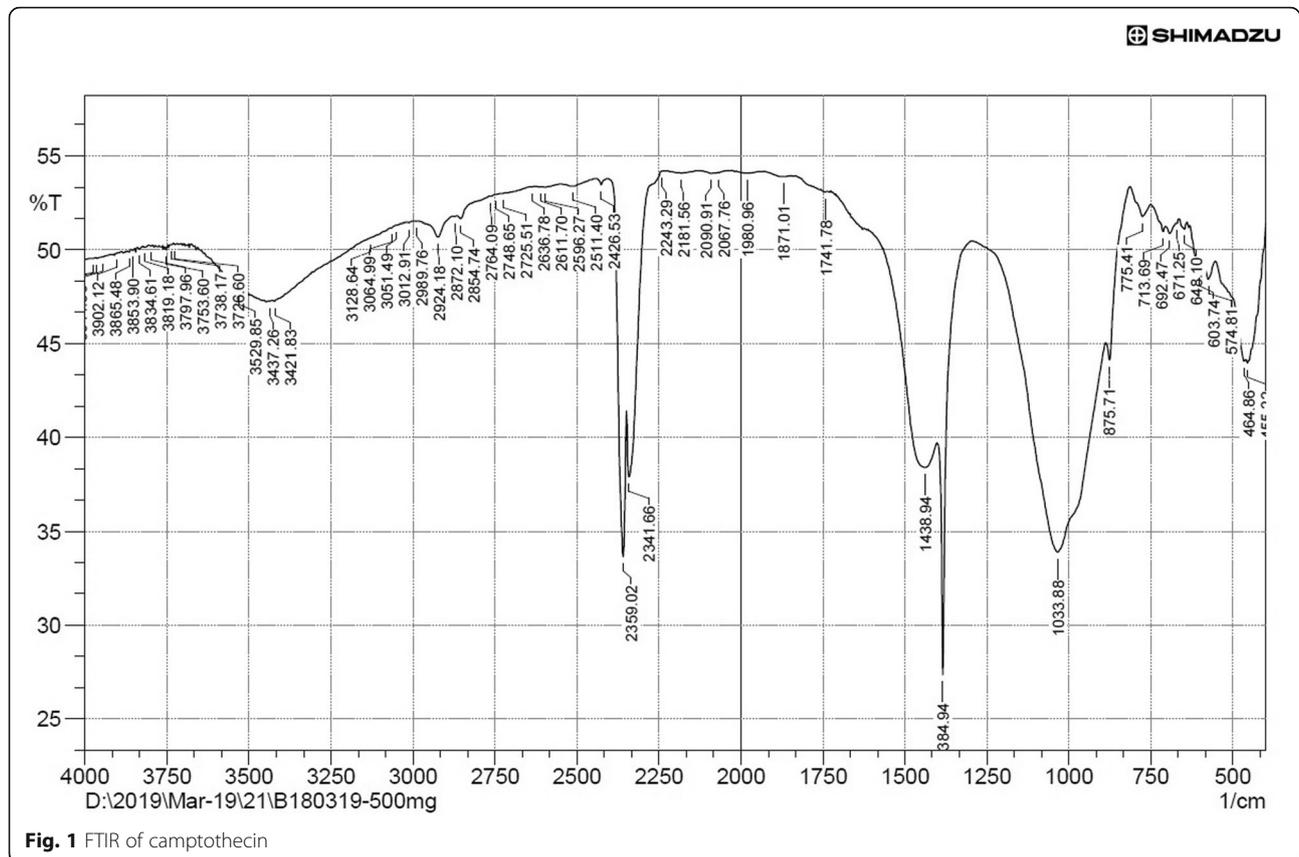
DSC gives information about the transition temperature by comparing resulted thermal transition which includes melting decomposition and outgassing for change in the heat capacity. DSC spectra shows sharp exothermic and endothermic melting temperatures at 278.56 $^{\circ}\text{C}$ and 346.91 $^{\circ}\text{C}$, respectively, which confirms the purity of isolated moiety by its melting point [33] (Fig. 2).

Mass spectroscopy

Mass spectroscopy gives information about the molecular weight of the specific compound which is the most important confirmatory test for the identification of isolated moieties which exactly matches with the standard molecular weight of standard camptothecin. The mass spectra of camptothecin give precursor m/z peak at 349.2 [M+H]⁺ in plain, and the mass spectra match with the peak for standard camptothecin [34–36]. Results were shown in Fig. 3.

Compatibility studies

Compatibility between camptothecin and other excipients was confirmed by FTIR study of camptothecin herbal gel formulation and was recorded and compared. The FTIR spectrum of camptothecin herbal gel formulation shows characteristic peaks of functional group at C=C Str. (Ar. Ring)-1652.46, C–H Str. (Alkyl)-2938.89, C–H det-1364.88, C=O Str-1704.47,



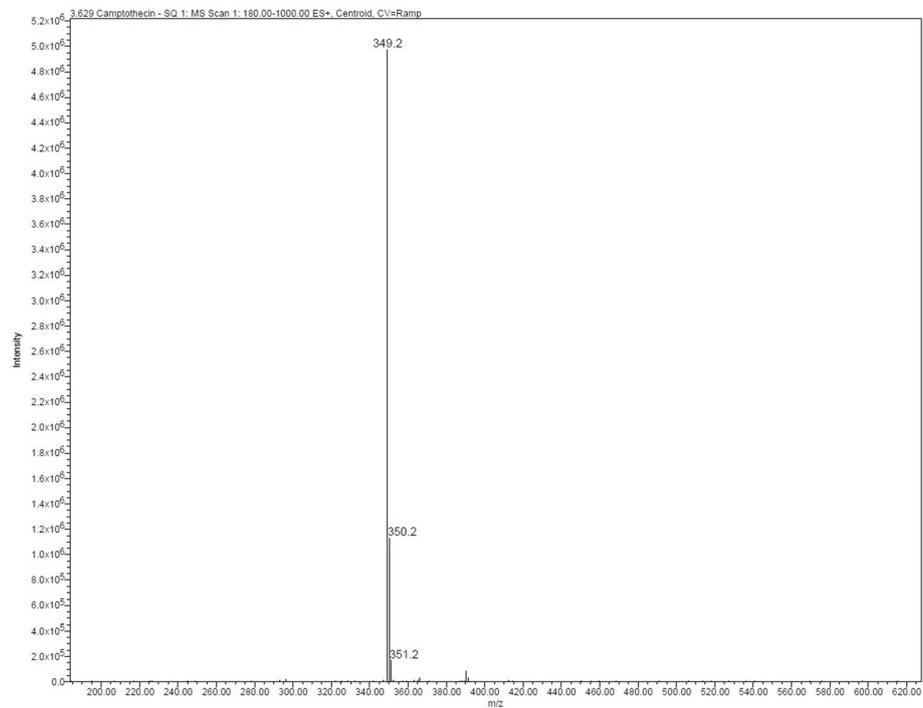


Fig. 3 Mass spectra of camptothecin

and C–N Str. (Aryl N)-1450.23. FTIR results clearly indicate compatibility among camptothecin, excipients, and processing condition and the integrity of camptothecin was unaffected after conversion into herbal gel. Results were shown in Fig. 4.

Differential scanning calorimetry (DSC)

The results of DSC analysis showed that the exothermic melting temperature for camptothecin was at 278.56 °C. The integrity of the camptothecin remained unaffected after formulation into herbal gel; this is confirmed by

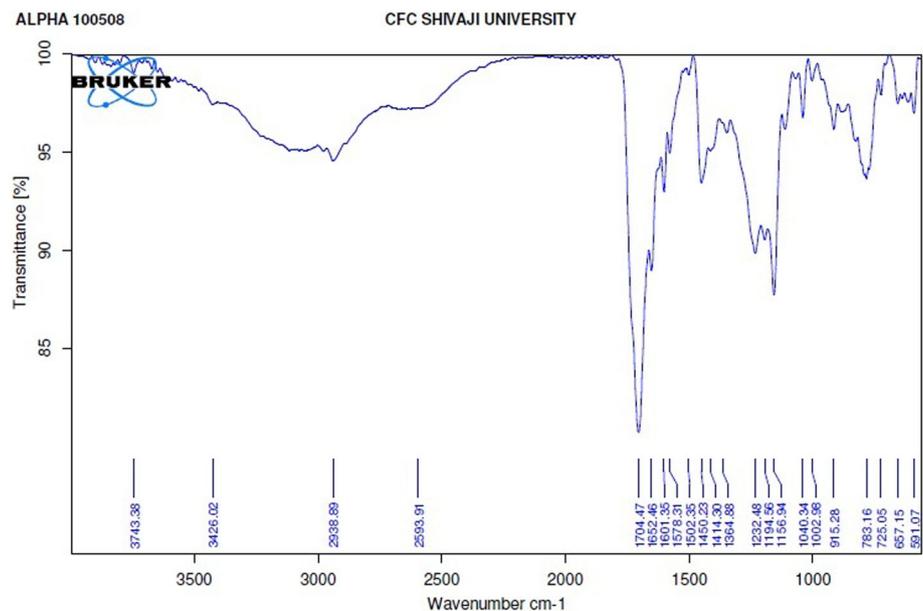


Fig. 4 FTIR of camptothecin herbal gel formulation

the DSC of formulation where the composite melting peaks of camptothecin was found to be at 245.24 °C, indicating compatibility between camptothecin, polymer, and processing conditions. The results of DSC thermograms are shown in Fig. 5.

Evaluation of herbal gel of camptothecin

Camptothecin gel formulations were prepared by using various concentrations of Carbopol-934 as gelling agent and sodium CMC polymer, keeping the concentration of all excipients including that of camptothecin constant, so as to check the effect of concentration of the gelling agent and the polymer. The formed herbal gels were inspected physically and were found to be brownish in color and smooth.

Percentage yield

Percentage yields of all the five formulations were very high and were affected by the concentration of the polymer; the increase in polymer concentration leads to an increase in percentage yield. The percentage yield of all herbal gels was found to be in the range of 93.78 to 97.41%. Results were shown in Table 2.

pH measurement

Camptothecin gel formulations' pH was measured by a digital pH meter. The pH of all herbal gel formulations was in between 6.7 and 6.9 range. The pH of all formulations is shown in Table 2.

Spreadability study

All the prepared camptothecin gel formulations were evaluated for spreadability. Spreadability of all herbal gels was in between 15.81 g-cm/s to 23.27 g-cm/s range. As the concentration of sodium CMC increases, the spreadability of formulation increases. The spreadability of all formulations was shown in Table 2.

Extrudability study

All the prepared herbal gels were evaluated for extrudability. The extrudability of all herbal gel formulations was in between 85.51 and 90.45 % w/w range indicating good extrudability. The extrudability of all formulations is shown in Table 2.

Drug content

The drug content of herbal gel formulations was found to be in the range of 89.12–96.64%. Formulation F3 showed high drug content (96.64%). This indicates that the drug is uniformly distributed throughout the system. The analyzed drug content displayed in Table 2.

Viscosity analysis

The viscosity of herbal gel formulations was primarily depending on the polymeric content of the formulation. The viscosity of the formulation is dependent on the polymer; the viscosity increases as the ratio of the polymer increases. Results are shown in Fig. 6.

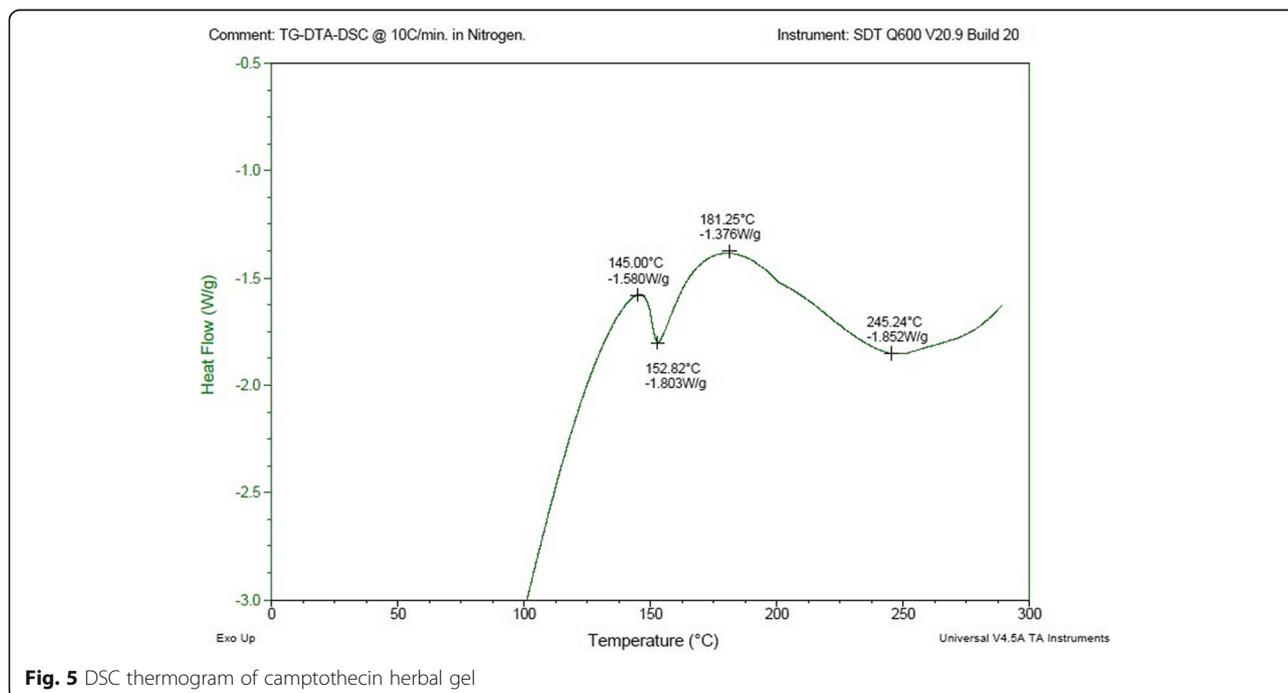


Fig. 5 DSC thermogram of camptothecin herbal gel

Table 2 Evaluation parameters of camptothecin gel formulation

Parameter	Formulation code				
	F1	F2	F3	F4	F5
pH*	6.71 ± 0.03	6.83 ± 0.05	6.68 ± 0.02	6.81 ± 0.04	6.90 ± 0.03
Spreadability* (g-cm/s)	15.81 ± 0.42	17.93 ± 0.35	20.04 ± 0.53	22.16 ± 0.50	23.27 ± 0.31
Extrudability* (% w/w)	86.32 ± 0.10	85.51 ± 0.12	90.45 ± 0.11	89.64 ± 0.11	88.57 ± 0.13
Drug content (% w/w)	89.12 ± 1.3	90.61 ± 1.1	96.64 ± 1.2	94.31 ± 0.8	92.76 ± 1.0
% Yield	93.78	95.85	96.33	96.72	97.41

*Each value represents mean ± S.D. of three observations

Diffusion study

Diffusion study of camptothecin herbal gel formulation was performed by Franz diffusion cell using phosphate buffer as solvent for 8 h. All formulations F1 to F5 containing Carbopol-934 and sodium CMC in different ratio has shown the drug release of 88.36%, 93.42%, 97.84%, 98.40%, and 89.40%, respectively, after 8 h as shown in Fig. 7. Results' regression coefficient values from different kinetic models are shown in Table 3.

Anticancer activity

The cytotoxicity of viability of the camptothecin gel was evaluated using MTT assay. The IC_{50} value of camptothecin gel was found to be 48.03 $\mu\text{g/ml}$. As the concentration of gel increases, the cell viability decreases, which shows a dose-dependent cytotoxicity. When compared to untreated cells, camptothecin gel-treated cells showed significant cell death ($P < 0.01$) as shown in Fig. 8.

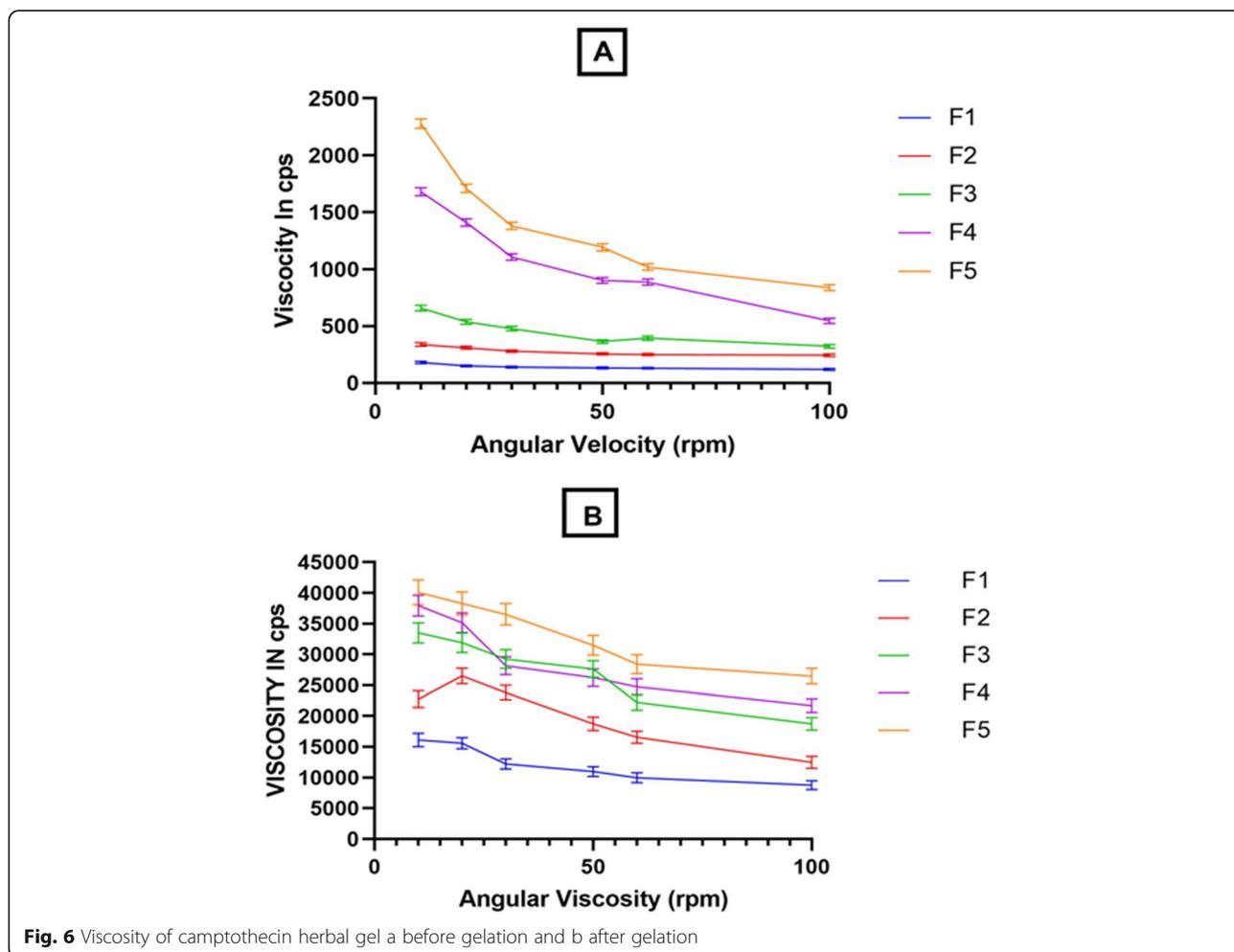
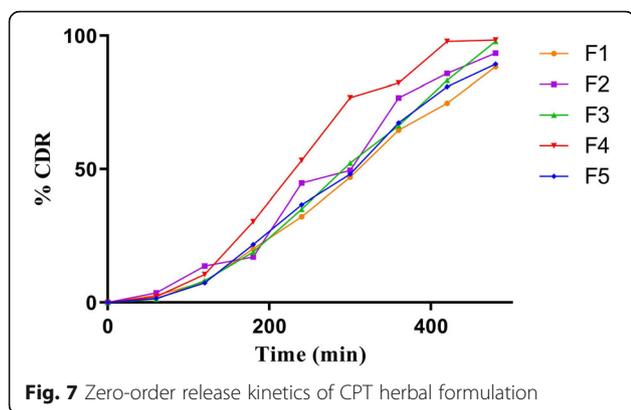


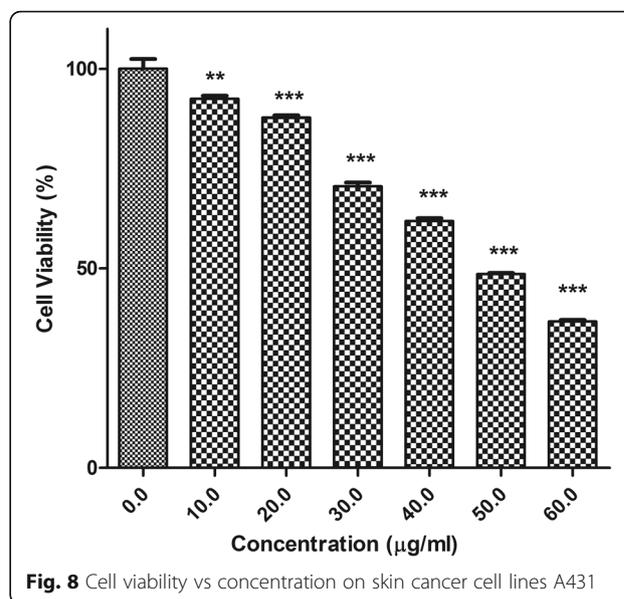
Fig. 6 Viscosity of camptothecin herbal gel a before gelation and b after gelation



DAPI is a dye used to stain nucleus to characterize the stages of apoptosis. The apoptotic nuclear morphology changes in A-439 cells following the treatment with camptothecin gel for 48 h were observed. The fluorescence microscopic photographs of untreated cells showed round intact nucleus, whereas camptothecin gel-treated cells showed condensed nucleus and fragmentation of DNA occurs as shown in the Fig. 9. The apoptosis (%) for control and camptothecin gel is 3.25 ± 1.11 and 54.67 ± 4.58 , respectively.

Discussion

Chemical fingerprinting of isolated camptothecin was performed by FTIR, DSC, and mass spectroscopy. FTIR spectra values are near or equal to the values mentioned in the standard structure of camptothecin. Compatibility study of camptothecin excipients and processing conditions was performed comparing FTIR and DSC spectra of camptothecin; its formulation confirms that the peaks of the characteristic functional group of camptothecin are retained in the formulation. DSC is a tool which provides qualitative physiochemical status of camptothecin and by comparing DSC spectra of camptothecin and its formulation for possible interactions, it was confirmed that composite melting peaks of camptothecin was retained in the formulation which is an indication of no effect of processing parameters of potency of camptothecin. Optimized camptothecin gel was evaluated for its



pH, spreadability, extrudability, and drug content. All parameters were found to be satisfactory. Viscosity study clearly indicates that as the polymer concentration increases, the viscosity of the gel also increases after and before gelation. Kinetics modeling and regression values further demonstrate that the release kinetic study of all formulations followed a zero order and diffusion-controlled order. To evaluate the cytotoxicity of the developed camptothecin gel for their possible use as an anticancer agent has its own vital importance. The in vitro cytotoxicity of CPT gel was investigated against A-439 cell line by MTT assay. Figure 8 showed the percentage viability of CPT gel for 48 h. The viability of A-439 cells treated with concentrations (1–60 µg/ml) exhibited concentration-dependent cytotoxicity. The untreated cells (Fig. 9a) displayed normal intact nuclei with weak homogenous blue staining, whereas in the groups treated with CPT gel (Fig. 9b) showed small nuclei with bright chromatin condensation, nuclear fragmentation, and apoptotic body (small spherical fragments) formation. The results indicate that camptothecin induce apoptosis in A-431 cell fragmentation of nuclei and uneven edges

Table 3 Kinetic modeling of drug release from herbal gel formulations

Formulation code	Zero order		First order		Higuchi's equation		Peppas's equation	
	R ²	Slope	R ²	Slope	R ²	Slope	R ²	Slope
F1	0.9738	0.1972	0.8688	0.0018	0.8109	4.251	0.8033	1.479
F2	0.9639	0.2164	0.8677	0.0023	0.8170	4.705	0.8483	1.504
F3	0.9700	0.2186	0.7228	0.0028	0.7989	4.686	0.7207	1.541
F4	0.9617	0.2419	0.8489	0.0039	0.8494	5.368	0.7891	1.596
F5	0.9753	0.2060	0.8811	0.0019	0.8173	4.455	0.7781	1.508

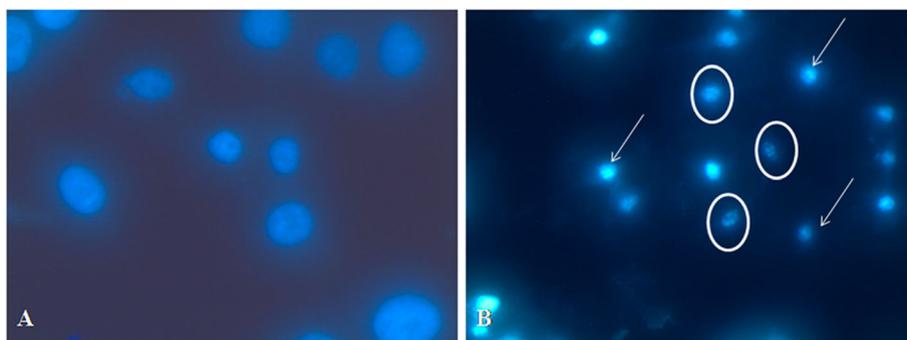


Fig. 9 a Negative control activity on skin cancer cell lines A431. b Anticancer activity of camptothecin gel on skin cancer cell lines A431

nearly the nuclei in cells treated with camptothecin gel which are an indication of cell undergoing apoptosis.

Conclusion

From the results, it can be concluded that camptothecin isolated from *Nothapodytes nimmoniana* can be delivered in the form of herbal gel formulation by transdermal route with the help of Carbopol-934 and sodium CMC as the polymer. The controlled release can be achieved for a longer period of time by using these herbal gel formulations; hence, the developed herbal gel formulation can be effectively useful to deliver camptothecin in the treatment of skin cancer which is proved by anticancer activity on the epidermoid carcinoma cell line-A431 having an IC_{50} value of $48.03 \mu\text{g/ml}$ and % apoptosis of 54.67 ± 4.58 .

Abbreviations

A-431 cells: Human epidermoid carcinoma cells; CPT: Camptothecin; Sodium CMC: Sodium carboxyl methyl cellulose; FTIR: Fourier transform infrared spectroscopy; pH: Hydrogen ion concentration; UV: Ultraviolet; DSC: Differential scanning calorimetry; F: Formulation code for herbal gel formulations; CDR: Cumulative drug release; SQRT: Square root of time; SD: Standard deviation; OD: Optical density

Acknowledgments

The authors are thankful to the Department of Pharmaceutics SGMCP, Mahagaon, and the Trustees of Sant Gajanan Maharaj College of Pharmacy, Mahagaon, for providing the required facilities, guidance, and support to complete the present research work. The authors are also thankful to NCBI, Pune, for providing the skin cancer cell lines A431 and Maratha Mandal Dental College and the research center Belagavi for the anticancer activity.

Plant source

The whole plant of *Nothapodytes nimmoniana* family (Icacinaceae) was collected from the Ajara Forest Region and identified and authenticated by Dr. Suresh.G. Killedar, principal of SGMCP Mahagaon, Maharashtra, India, and the voucher specimen No.SGMCP/PH.COG/HERB/01-2019 herbarium was deposited in the pharmacognosy department of SGMCP, Mahagaon, Shivaji University, Kolhapur.

Authors' contributions

SG, AH, RG, and OM are the researchers who carried out the isolation and all the research activities related to camptothecin; SG has a major contribution in writing the manuscript; SK and DA are the supervisors who guided in the design and characterization of camptothecin gel; VK has a major contribution in monitoring the anticancer studies and in writing the discussion. All authors read and approved the final manuscript.

Funding

No funding was obtained for this study.

Availability of data and materials

The datasets used and/or analyzed during the current research work are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they don't have any competing interests.

Author details

¹Sant Gajanan Maharaj College of Pharmacy, Mahagaon, Site: Chinchewadi, Kolhapur, Maharashtra 416502, India. ²Bharati Vidyapeeth College of Pharmacy, Near Chitranagari, Morewadi, Kolhapur, Maharashtra 416013, India. ³Central Research Laboratory, Maratha Mandal's Nathajirao G. Halgekar Institute of Dental Sciences & Research Center, Belgavi, Karnataka 590010, India.

Received: 1 May 2020 Accepted: 23 July 2020

Published online: 05 August 2020

References

- Nagai H, Kim YH (2017) Cancer prevention from the perspective of global cancer burden patterns. *Journal of Thoracic Disease* 9(1):448–451
- Nadaf SJ, Killedar SG (2018) Curcumin nanocholeates use of design of experiments, solid state characterization, in vitro apoptosis and cytotoxicity against breast cancer MCF-7 cells. *Journal of Drug Delivery Science and Technology* 47(1):337–350
- Kristjan O, Matevz P, Uros M (2017) Skin cancer and its treatment: novel treatment approaches with emphasis on nanotechnology. *J Nanomater* 1(1):1–20
- Yeap SK, Abu N, Mohamad NE, Beh BK, Ho WY, Ebrahimi S, Yusof HM, Alitheen NB (2015) Chemopreventive and immunomodulatory effects of *Murraya koenigii* aqueous extract on 4T1 breast cancer cell-challenged mice. *BMC Complement Altern Med* 15:306–316
- Javed I, Banzeer AA, Tariq M, Sobia K, Barkat A, Shah SA, Khalil AT (2017) Plant derived anticancer agents: a green anticancer approach. *Asian Pac J Trop Biomed* 7(1):1129–1150
- Kan XW, Justin JH (2017) Antiviral screen identifies EV71 inhibitors and reveals camptothecin-target, DNA topoisomerase 1 as a novel EV71 host factor. *Antivir Res* 143:122–133
- Li Y-Y, Chen S-W, Yang L-M (2010) The anti-HIV actions of 7- and 10-substituted camptothecins. *Molecules* 15:138–148
- Dong Q, Luo J, Qiu W (2016) Inhibitory effect of camptothecin against rice bacterial brown stripe pathogen *Acidovorax avenae* subsp. *avenae* RS-2. *Molecules* 21:978

9. Pommier YH (2012) DNA topoisomerases and cancer. *Cancer Drug Discovery and Development*:1–12
10. Pommier Y (2006) Topoisomerase I inhibitors: camptothecins and beyond. *Nat Rev Cancer* 6(19):789–802
11. Pommier Y (2013) Drugging topoisomerases: lessons and challenges. *ACS Chem Biol* 8(1):82–95
12. Tesaro C, Simonsen AK, Andersen MB (2019) Topoisomerase I activity and sensitivity to camptothecin in breast cancer-derived cells: a comparative study. *BMC Cancer* 19:1158
13. Liu L-F, Desai SD, Li TK, Mao Y, Sun M, Sim SP (2000) Mechanism of action of camptothecin. *Ann N Y Acad Sci* 922:1–10
14. Li F, Jiang T, Li Q, Ling X (2017) Camptothecin (CPT) and its derivatives are known to target topoisomerase I (Top1) as their mechanism of action: did we miss something in CPT analogue molecular targets for treating human disease such as cancer. *Am J Cancer Res* 7(12):2350–2394
15. Legha SS, Ring S, Bedikian A (1996) Treatment of metastatic melanoma with combined chemotherapy containing cisplatin, vinblastine and dacarbazine (CVD) and biotherapy using interleukin-2 and interferon. *Ann Oncol* 7(8): 827–835
16. Rosenberg SA, Yang JC, Schwartzentruber DJ (1999) Prospective randomized trial of the treatment of patients with metastatic melanoma using chemotherapy with cisplatin, dacarbazine, and tamoxifen alone or in combination with interleukin-2 and interferon alfa-2b. *J Clin Oncol* 17(3): 968–975
17. Margetts L, Sawyer R (2007) Transdermal drug delivery: principles and opioid therapy. The Board of Management and Trustees of the British Journal of Anaesthesia 7(5):171–176
18. Mishra U, Murthy PN, Pasa G, Kumar S (2011) Formulation development and standardization of herbal gel containing methanolic extract of *Butea frontosa*. *International Research Journal of Pharmacy* 2(11):126–129
19. Uma G, Balasubramaniam V (2012) GC-MS analysis of *Nothapodytes nimmoniana* [J. Graham] mabberly leaves. *J Chem Pharm Res* 4(9):4417–4419
20. Bhaisaikh IM, Galatage ST (2019) Floating microsponges as gastro retentive drug delivery system containing lafutidine to treat gastric ulcer. *Acta scientific pharmaceutical sciences* 32:3–12
21. Galatage ST, Killedar SG (2019) Development and characterization of micro sponge of amphotericin B for topical drug delivery. *RJPBCS* 10:1288–1300
22. Negi A, Sharma N, Singh MF (2012) Formulation and evaluation of an herbal anti-inflammatory gel containing Eupatorium leaves extract. *Journal of Pharmacognosy and Phytochemistry* 1(4):112–117
23. Fulzele DP, Satdive RK (2005) Comparison of techniques for the extraction of the anti-cancer drug camptothecin from *Nothapodytes foetida*. *J Chromatogr* 1063(21):9–13
24. Mulla RD, Kondawar MS (2012) Development and validation of uv-visible spectrophotometric method for quantification of camptothecin from *Nothapodytes nimmoniana*. *Inventi Rapid: Pharmaceutical Analysis & Quality Assurance Publication* 331:1–7
25. Sumeet G, Shailesh G (2012) Formulation and evaluation of herbal gel containing *Sesbania grandiflora* Poir. leaf extracts. *Acta Chimica Pharmaceutica Indica* 2(1):54–59
26. Das MK, Maurya D (2008) Evaluation of diltiazem hydrochloride loaded mucoadhesive microspheres prepared by emulsification-internal gelation technique. *Acta Pol Pharm Drug Res* 65(2):249–259
27. Mohammed HKP, Abraham A, Saraswathi R, Mohanta GP, Naya C (2011) Formulation and evaluation of herbal gel of *Basella alba* for wound healing activity. *International Research Journal of Pharmaceuticals* 1(1):15–20
28. Vats A, Sharma P (2012) Formulation and evaluation of topical anti acne formulation of coriander oil. *International Journal of Pharmacy and Pharmaceutical Science Research* 2(3):61–66
29. Missal G, Dixit G, Gulkari V (2012) Formulation and evaluation of herbal gel. *Indian J Nat Prod Resour* 3(4):501–505
30. Kumber VM, Peram MR, Kugaji MS, Shah T, Patil SP, Muddapur UM, Bhat KG (2020) Effect of curcumin on growth, biofilm formation and virulence factor gene expression of *Porphyromonas gingivalis*. *Odontology* 1:148–156
31. Peram MR, Jalalpure S, Kumber V, Patil S, Joshi S, Bhat K, Diwan P (2019) Factorial design based curcumin ethosomal nanocarriers for the skin cancer delivery: in vitro evaluation. *Journal of liposome research* 29(3):291–311
32. Patil A, Patil S (2014) UV, FTIR, HPLC confirmation of camptothecin an anticancer metabolite from bark extract of *Nothapodytes nimmoniana* (J. Graham). *American Journal of Ethnomedicine* 1(3):174–185
33. Thakral NK, Ray AR, Bar-Shalom D (2012) Soluplus-solubilized citrated camptothecin: a potential drug delivery strategy in colon cancer. *AAPS PharmSciTech* 13(1):59–66
34. Puri SC, Bhat BA (2005) Separation of 9-methoxycamptothecin and camptothecin from *Nothapodytes foetida* by semipreparative HPLC. *J Chromatogr Sci* 43(1):348–350
35. Ramesha BT, Amna T, Ravikanth G (2008) Prospecting for camptothecins from *Nothapodytes nimmoniana* in the Western Ghats, South India: identification of high-yielding sources of camptothecin and new families of camptothecins. *J Chromatogr Sci* 46(1):362–368
36. Nalluri BN, Devineni PK (2015) Studies on development of controlled release matrix tablets of camptothecin—an anticancer drug. *Indian Journal of Pharmaceutical Education and Research* 49(4):292–300

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Submit your manuscript to a SpringerOpen® journal and benefit from:

- Convenient online submission
- Rigorous peer review
- Open access: articles freely available online
- High visibility within the field
- Retaining the copyright to your article

Submit your next manuscript at ► [springeropen.com](https://www.springeropen.com)
