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ORIGINAL ARTICLE

Camptothecin: An Anticancer Drug from *Pestalotiopsis microspora* Mh458929 – An Endophytic Fungus Isolated from an Ethnopharmacologically Important Medicinal Plant *Cordia dichotoma* G. Forst

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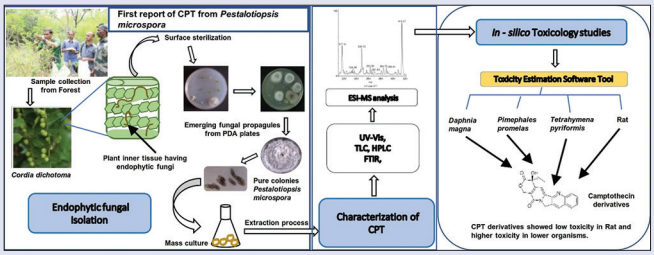
ABSTRACT

Background: Endophytic fungi that live asymptomatically inside the plant tissues have novel bioactive metabolites exhibiting a variety of biological activities, especially against cancer. *Cordia dichotoma* G. Forst. play a significant role in traditional medicines and therapeutics. Leaves and bark have been used as anti-inflammatory and anticancer agents. **Objectives:** Isolation, screening, and *in silico* toxicity evaluation of camptothecin (CPT) from the endophytic fungus *Pestalotiopsis microspora* MH458929. **Materials and Methods:** Endophytic fungus was isolated from leaves of *C. dichotoma* collected from Sathyamangalam Tiger Reserve forest (STRF), Tamil Nadu. The wild strain was identified by 18S rDNA sequencing. Modified potato dextrose broth was used as a screening medium for the presence of CPT. CPT was analyzed by high-performance liquid chromatography and electrospray ionization–mass spectrometry (ESI-MS). Compounds identified by ESI-MS from fungal extract were further studied for their *in silico* toxicity study against *Daphnia magna, Tetrahymena pyriformis, Pimephales promelas,* and *Rattus* sp. Bioaccumulation factors, developmental toxicity, and mutagenicity were studied by the quantitative structure–activity relationship model – Toxicity Estimation Software Tool. **Results:** Endophytic fungus *P. microspora* produced a maximum yield of 0.691 mg/L of CPT. CPT derivatives were identified at *m/z* of 349.10, 363.08, and 389.41 through ESI-MS analysis. *In silico* toxicity study revealed that compounds were of Category D and hence considered nontoxic to higher organisms. However, compounds showed high toxicity for lower organisms, with toxicity order *D. magna* > *T. pyriformis* > *P. promelas* > rat. **Conclusion:** The present study is the first report to screen, isolate, and analyze the CPT's *in silico* toxicity and its derivatives from endophytic fungus *P. microspora* from STRF. Further *in vitro* and *in vivo* studies are recommended to utilize CPT and its derivatives in pharmaceuticals.

Key words: Anticancer drug, camptothecin, *Cordia dichotoma* G. Forst, endophytic fungi, *Pestalotiopsis microspora*, quantitative structure–activity relationship toxicity

SUMMARY

- First report to ethnomedicinal studies from Sathyamangalam Tiger Reserve
- First report of an endophytic fungal Camptothecin *Pestalotiopsis microspora* from *Cordia dichotoma*.
- Maximum yield of CPT (0.691 mg/L) from wild strain of *P. microspora*
- *In silico* studies prove the nontoxicity of CPT and its derivatives to higher organisms.



Abbreviations used: STRF: Sathyamangalam Tiger Reserve forest; CPT: Camptothecin; ENVIS: Environmental Information System; NCBI: National Center for Biotechnology Information; BLAST: Basic Local Alignment Searching Tool; MEGA: Molecular Evolutionary Genetics Analysis; MPDB: Modified potato dextrose broth; TLC: Thin-layer chromatography; HPLC: High-performance liquid chromatography; FTIR: Fourier transform infrared spectroscopy; IR: Infrared spectroscopy; ESI-MS: Electrospray ionization–mass spectrometry; PDA: Potato dextrose agar; UV-Vis: Ultraviolet-visible; TEST: Toxicity Estimation Software Tool; QSAR: Quantitative structure–activity relationship; EPA: European Protection Agency; ATSDR: Agency for Toxic Substances and Disease Registry; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; R_f: Retention factor; U. S.: United States.

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INTRODUCTION

Medicinal plants are being used as curatives against various diseases.[1,2] Plant-based medicines are easily accessible, affordable, and acceptable with fewer side effects than allopathic medicine.[3] These plants are the primary source of active pharmaceutical compounds and recipients for drug delivery system.[4] The genus *Cordia* (Boraginaceae) has been widely explored for medicinal and ethnopharmacological purposes.[5-7] The inhibitory activity to prevent carcinoma by *Cordia verbenacea* and *Cordia dichotoma* leaves against tumor cells was investigated.[8,9] However, many medicinally important plants are underutilized. This is

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due to the non-availability of knowledge toward their ethnomedicinal importance. Thus, studying the ethnopharmacological benefits of *C. dichotoma* was attempted in this study.

Endophytes live inside the plant tissue for their lifetime without affecting the host plant.[10] Isolation of metabolites from endophytes is of increasing interest among taxonomists, mycologists, and chemists.[11,12] Endophytes provide beneficial effects to the host plants and are a significant source of bioactive metabolites.[13,14] The promising strain of endophyte can produce secondary metabolites similar to those in the host plant.[15] The study focuses on screening one such endophytic fungus *Pestalotiopsis microspora* for the synthesis of camptothecin (CPT), an anticancer drug.

According to the World Health Organization facts (2013) and GLOBOCAN (2018), the cancer is second leading and more prevalent disease throughout the world.[16] Thus, finding an anticancer compound with low-cost, easier extraction methodology with high production yield is of utmost importance. Taxol, CPT, vinblastine, podophyllotoxin, and vincristine are few anticancer compounds that are reported from endophytes.[17] CPT is a plant-based quinoline alkaloid used as an anticancer compound used against lung and refractory ovarian cancer.[18] Distribution of CPT in plants suggested the isolation of the metabolite for anticancer activity.[19] CPT from endophytes is an alternate method to produce the drug under *in vitro* conditions at a cheaper cost and aids in the prevention of loss of plant source.[20] Production of CPT from endophytic fungus was first reported from *Nothapodytes foetida*.[21]

However, studies on the effects of such natural compounds in different model systems are required in drug discovery, mainly because of unfavorable pharmacokinetic properties.[4] *In silico* toxicity studies provide an advantage over *in vitro* and *in vivo* methods, as they do not involve hazardous chemicals or the use of animal models. Quantitative structure–activity relationship (QSAR) models are *in silico* mathematical tools used to measure the toxicity of compounds based on their structure. Toxicity Estimation Software Tool (TEST) is a QSAR model recommended by the Environmental Protection Agency (EPA) to study the toxicity of compounds.

In the present study, ethnopharmacological importance of *C. dichotoma* was studied. Endophytes were isolated from the leaves of *C. dichotoma* and further screened for CPT production. The presence of CPT was confirmed from the fungus *P. microspora* and characterized by ultraviolet-visible (UV-Vis) spectrophotometry, thin-layer chromatography (TLC), Fourier Transform Infrared Spectroscopy (FTIR), high-performance liquid chromatography (HPLC), and electrospray ionization–mass spectrometry (ESI-MS). Further, the compounds detected by ESI-MS were studied for their toxicity using QSAR-TEST software.

MATERIALS AND METHODS

Reagents and chemicals

The general laboratory techniques followed in the present investigation were as those outlined by reagents and chemicals are Agar Agar type I (Himedia), Acetonitrile HPLC grade (Merck), CPT (Standard) >98% purity (Sigma-Aldrich), chloroform (Merck), dextrose (Himedia), ethanol (Merck), Ferrous sulfate heptahydrate (QualiTech), lactophenol cotton blue (Himedia), carbinol (Qualigens), magnesium sulfate heptahydrate (QualiTech), peptone (Himedia), potassium bromide (pellet) (Sigma-Aldrich), sodium hypochlorite (Rankem), and Water HPLC grade (Merck).

Geographical description of the study area

For the present study, Kottada–Mavallam beat of Hasanur range, Sathyamangalam Tiger Reserve (STR) forest, Tamil Nadu, was chosen as the study area. STR is a wildlife corridor between the Western and Eastern Ghats of the Nilgiri Biosphere Reserve, bordering the states of Tamil Nadu and Karnataka, India. Mountain ranges from Kottada beat of Hasanur range to Bejalatti beat of Thalamalai range are entirely restricted for migrants. The study area is located between the latitude and longitude coordinates of 11.58440° N–11.59351° N and 77.07143° E–77.10693° E with an elevation range of 1219–1283 m. There is no buffer zone and the core area is 11476.92 Ha. This region's temperature is relatively cool during winter (December to February) with an average temperature of 16°C–25°C. During summer and autumn, the weather is relatively high, i.e., 25°C–34°C. These forests are native to indigenous tribal people of the Irula and Soliga tribes. The selected region has diverse flora and fauna. However, ethnopharmacological information about the medicinal plants in these protected areas is not available.

Ethnobotanical studies

The ethnobotanical study was carried out at different ranges of STR (Germalam, Hasanur, Thalamalai) through face-to-face discussion with ten people from the local tribe [Table 1]. Data about the common name and the parts of the plant used for different ailments were recorded. For the present study, leaves of *C. dichotoma* were collected from the Hasanur range for isolation of endophytic fungi to screen CPT.

Collection and identification of medicinal plant

The medicinal plant *C. dichotoma* is commonly known as "Siru-naruvulli" in Kannada and Tamil. The plant was collected from a latitude and longitude of 11.62600° N–077.13343° E at 1072 m above sea level. The plant sample was transferred to sterile bags and processed further studies within 24 h. The plant specimen was authenticated as *C. dichotoma* G. Forst belonging to the family Boraginaceae by Botanical Survey of India, Southern Regional Centre, Coimbatore, India (Ministry of Environment, Forest and Climate Change, Government of India) (Authentication Number: BSI/SRC/5/23/2017/Tech/262/101 dated February 05, 2017). The plant was also identified with the Plant List database (www.plantlist.org), International Plant Names Index database (www.ipni.org), and Environmental Information System Programme, Government of India (www.envis.frlht.org).

Isolation of endophytic fungi

The modified methodology of surface sterilization protocol was employed for the isolation of endophytic fungi from leaves of *C. dichotoma*.[22] Fifty segments of the surface-sterilized plant tissues (0.5 cm × 0.5 cm) were placed on potato dextrose agar plates amended with 1% streptomycin. The plates were incubated for 2–3 weeks at 25°C ± 2°C in a light chamber with 12 h of dark and light cycle. The sporulated fungi were maintained as pure cultures and identified.

Morphological and microscopical identification of endophytic fungi

The identification of endophytic fungi was performed based on morphological and microscopic characters using standard manuals.[23,24] Photomicrograph was taken under light microscopy (Olympus CX31, Canon EOS 700D series) at the magnification of ×100. The cultures were deposited at Endophytic Fungal Metabolite Research Laboratory, Department of Biotechnology, Bannari Amman Institute of Technology, Sathyamangalam, Erode District, Tamil Nadu, India.

Selection of endophytic fungi for camptothecin screening

The isolates obtained from plant leaves of *C. dichotoma* were screened for their potency to produce CPT in modified potato dextrose broth (MPDB consists of potato extract (peeled and diced) 250.0 g/L, dextrose

Table 1: Geographical distribution and ethnopharmacological data of *Cordia dichotoma*

Range	Location/beat	GPS location	Altitude (m)	Vernacular name	Plant part	Disease curative
Hasanur	Kottada to Araepalayam-Mavallam beat Engineer Road (Core zone)	11.62600° N 077.13343° E	1072	Siru-naruvulli	Fruits	Wound healing, tumor killing, and anti-inflammatory
Thalamalai	Thimbam to Thalamalai Bejalatti Beat (Core zone)–Kaalithimbam	11.58606° N 077.10767° E	1240	Mukku-challi	Fruits	Diabetes, anti-inflammatory
					Leaves	Anti-fertility, tumor suppression, and cancer treatments
Germalam	Gethasal to Shooting Bungalow-Ooduthurai pallam Beat (Core zone)	11.74474° N 077.17918° E	1392	Kaadu-sellai	Leaves	Anti-fungal
					Roots	Gastric ulcer
					Fruits and bark	Anti-inflammatory, stomach disorders

20.0 g/L, peptone 10.0 g/L, $MgSO_4.7H_2O$ 0.5 g/L, and $FeSO_4.7H_2O$ 0.5 g/L (pH 5.6). The endophytic fungal cultures were inoculated in 100 mL of sterile MPDB medium and incubated in a light chamber on a rotary shaker (Orbitek) at 120 rpm, 26°C ± 2°C for 12 days.

Identification of camptothecin producing endophytic fungus by 18S rDNA analysis

Among the fungal endophytes isolated, the endophytic fungal strain with the highest yield of CPT was identified at the molecular level by 18S rDNA analysis and the sequence was submitted to the National Center for Biotechnology Information (NCBI) gene BankIT. A similarity search was performed using the Basic Local Alignment Searching Tool (BLAST). 18S rDNA fungal sequences with homology score >97% were classified under the same phylotype (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Construction of phylogenetic tree using Molecular Evolutionary Genetics Analysis X

Based on the homology scores, the phylogenetic tree was constructed using Molecular Evolutionary Genetics Analysis (MEGA) version X (https://www.megasoftware.net/) and NCBI (www.ncbi.nlm.nih.gov). The endophytic fungal taxa's evolutionary relationship was inferred using neighbor-joining method, and the bootstrap consensus tree was deduced from 500 replicates.[25] Maximum composite likelihood method was used to compute the evolutionary distance.[26,27]

Separation of cell-free bioactive metabolites

The mass culture of the endophytic fungus was extracted by solvent selection method.[28] The mass culture was centrifuged at 10,000 rpm, 4°C for 15 min, and the pellet was washed thoroughly with sterile distilled water. An equal volume of chloroform: methanol (4:1 *v/v*) mixture was added repeatedly four times to the supernatant. Ultrasonication (sonication, cell disruption, and lysis – High model: Life Care Equipments, ENUP-500A) was then performed twice at 50% frequency, 33 KHz for 5 min. An equal volume of $CHCl_3$-MeOH (4:1 *v/v*) was added to the sonicated pellet solvent mixture. This process was repeated thrice to collect fungal metabolites in a separating funnel, and the upper layer was separated. Using a rotary evaporator, the crude was concentrated at the pressure of 10 psi and temperature of 40°C. The crude was filtered using a syringe filter (0.2 µm), and the filtrate was maintained at 4°C until further analysis. The crude was redissolved in $CHCl_3$-MeOH (4:1 v/v) for the detection of CPT.

Analysis of camptothecin

Ultraviolet-visible spectrophotometry and thin-layer chromatography

The λ_{max} for fungal CPT and standard CPT was recorded using UV-visible spectrophotometer in the 200–700 nm wavelength range. For TLC, the samples were spotted on the silica-coated TLC plates and visualized at a long UV range of 365 nm under a UV-TLC chamber. 1 mg/mL stock solution of standard CPT (>98% purity of Sigma-Aldrich) was prepared and compared with fungal CPT spot based on the R_f values recorded.

Estimation of camptothecin metabolite using high-performance liquid chromatography

HPLC analysis was performed in a zorbax SB-C_{18} column (Phenomenex, Torrance, CA, USA). The sample (10 µL) was injected at a flow rate of 200 µL min^{-1} at 30°C. The mobile phase was water: acetonitrile in the ratio of 1:3. The peaks were studied in the UV detector with λ_{max} of 365 nm.

Molecular vibrational analysis of Infrared Spectroscopy

FTIR spectrum was recorded in the wavenumber range of 4000-400 cm^{-1} in FTIR spectrophotometer (Shimadzu, IR Affinity, Japan) at a resolution of 4 cm^{-1}. The sample was pelletized and formed as a thin disc using KBr pellet technique at room temperature. The functional groups were identified in reference to previous literature.[29]

Electrospray ionization–Mass spectrometry

Further confirmation of CPT and its derivatives was done using micromass QuattroII triple-quadrupole mass spectrometer with the operating conditions: sample flow rate 5 µL/min, capillary cone voltage 40 V, source temperature 120°C, desolvation temperature 300°C, and positive ionization mode. The spectrum was collected in 5-s scans. The compounds were identified regarding previous literature in PubChem and ChemSpider databases.

In silico toxicity analysis of fungal camptothecin and its derivatives

The toxicity of the compounds detected in ESI-MS analysis was studied using QSAR-TEST, version 4.1 (EPA, U. S.). This software includes various methods such as Caesar random forest, FDA, group contribution, hierarchical clustering, mode of action, nearest neighbor, single model, and consensus against different organisms such as *Daphnia magna, Pimephales promelas, Tetrahymena pyriformis,* and rat (oral). The toxicity values were correlated with the aquatic and mammalian toxicity scales described by Agency for Toxic Substances and Disease Registry (ATSDR, 2017). Further, their bioaccumulation factor, developmental toxicity, and mutagenicity were studied. Consensus method, which is generated based on each endpoint's methods, is presented in the study.[30]

RESULTS AND DISCUSSION

Ethnomedicinal knowledge of plant

Ethnomedicinal knowledge of medicinally essential plants is very much restricted. Hence, documentation of ethnobotanical information from indigenous population can aid in the conservation of plant sources, in addition to providing information on their potential scientific use.[31] In the present study, the ethnobotanical information of *C. dichotoma* was studied in different ranges of STR forest, Tamil Nadu, India. This study revealed that leaves, fruits, roots, and bark of the plant are traditionally being used for the management of diabetes, inflammation, gastric ulcer, and stomach disorders. Further, the leaves are used to treat cancer and suppression of tumors. Previous literature revealed that the fruits of *C. dichotoma* have antioxidant and antitumor activities.[32] The roots of *C. dichotoma* have antimicrobial, antimycobacterial, and antioxidant activities.[33] Parts of *C. dichotoma* from Seshachalam Biosphere Reserve are used for bronchial disorders and fever.[34] Hence, the study was focused on screening of an anticancer drug CPT by entophytic fungi from *C. dichotoma*.

Isolation of endophytic fungi from *Cordia dichotoma*

The endophytic fungi were isolated from the leaves of *C. dichotoma* from Hasanur range, STR. A total of 50 isolates belonging to 7 genera and 6 sterile forms, in total 13 fungal taxa, were obtained from 50 segments of *C. dichotoma*.

Identification of camptothecin producing strain *Pestalotiopsis microspora*

Among the endophytes, the isolate exhibiting the maximum yield of CPT was identified up to the molecular level. The CPT producing endophytic fungus has 1–2 apical appendages of 5–6 µm, with 1 basal appendage (2.92–4.5 µm long). Conidia were straight, clavate-fusoid, broad, 5-celled, with 15.69–29 × 6.73–9.5 µm. The intermediate colored cells were 15–20 µm long, guttulate, and amber, equally colored, with the lowest colored cell sometimes slightly paler and slightly constricted at septa. Based on the conidial and spore morphology, the species was identified as *P. microspora* [Figure 1]. A total of 417 base pairs were recorded in the sequence. Evolutionary analyses by BLAST similarity and MEGA X revealed 99% similarity to *P. microspora* (Gen Bank ID: MH458929; https://www.ncbi.nlm.nih.gov/nuccore/MH458929.1?report = genbank). The neighbor-joining circle tree of *P. microspora* is presented in Figure 2. Phylogenetic analysis indicates that *P. microspora* shifts to different hosts due to various external factors.[35] *Pestalotiopsis* sp. from *C. dichotoma* was previously reported from the Western Ghats.[36] Bioactive compounds such as taxol, pestaloside, and heteropolysaccharides have been recorded in *C. dichotoma*.[37,38]

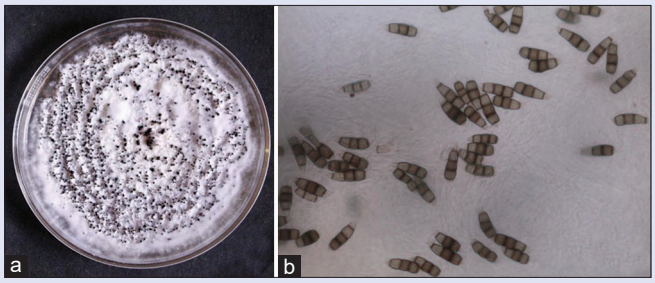

Figure 1: Camptothecin producing wild strain *Pestalotiopsis microspora* isolated from *Cordia dichotoma* G. Forst. (a) Petri plate containing axenic strain (b) spore morphology

Identification of camptothecin through ultraviolet-visible spectrophotometry and high-performance liquid chromatography analysis

Screening of CPT from the extracted fungal metabolite of *P. microspora* was carried out using UV-Vis spectrophotometry. The maximum absorbance was recorded at 349 nm, with a negligible difference in absorbance value of 0.10 between the standard and the fungal extracts [Supplementary Figure 3]. R_f values of the standard and fungal CPT were almost similar, with 0.40 and 0.37, respectively, as recorded by TLC. Two significant fractions were recovered, with that of CPT exhibiting a fluorescent green color. This was similar to the standard CPT. The presence of CPT was further analyzed by HPLC. Peaks were recorded at retention times of 6.078 and 6.029 min for the CPT produced by *P. microspora,* and the quantification of CPT was 0.691 mg/L. standard, respectively [Figure 3].

Fourier transform infrared spectroscopy analysis

In the present study, C–H = CH_2 bond vibrations were observed at 949.98 cm^{-1}. The peaks corresponding to C–H were recorded in the region 600–950 cm^{-1}, 1067.65 cm^{-1}, 1350-950 cm^{-1}, 2827.77 cm^{-1}, and 2966.65 cm^{-1}. The peaks in 1650-1200 cm^{-1} correspond to C-C stretch of the phenyl group. The C-H and C-C vibrations might attribute to one or more aromatic ring structure of the quinoline ring of CPT. The peak at 1529.62 cm^{-1} corresponds to C-N stretch. Similar C-N stretch was observed in aminomethyl quinolone.[39,40] The presence of two C = O stretches is evident at 1672.36 and 1689.72 cm^{-1}. In general, the narrow peak within 1800-1600 cm^{-1} belongs to carbonyl groups.[41] N-H and O-H groups were identified at 3445.98 and 3605.11 cm^{-1}, respectively. The FTIR spectrum recorded for *P. microspora* is presented in Figure 4 and the functional groups identified are presented in Table 2.

Structural elucidation and *in silico* toxicity analysis of camptothecin derivatives

Further confirmation of CPT and its derivatives was studied by ESI-MS analysis [Figure 5]. Seven out of eight isolates were CPT derivatives with *m/z* values of 334.66, 349.10, 363.08, 381.24, 384.75, 389.41, and 415.27. These compounds are known to act as cancer curative agents [Table 3]. The structures of the compounds identified are presented in Figure 6.

The compounds identified in *P. microspora* were toxic to lower organisms when compared to the higher organisms, as studied by QSAR-TEST. Experimental data were not recorded for any of the compounds against the endpoints studied. Of the 15 compounds studied, only one compound (C5) was extremely toxic (Category X) to *D. magna*. Very high toxicity (Category A) was exhibited by 3 compounds (C1, C6, and C9) while 5 compounds (C2, C3, C10, C12, and C15) were highly toxic (Category B). C1 and C9 were highly toxic against *T. pyriformis*. Moderate toxicity was exhibited by C6, C10, and C14. Most of the compounds studied (C1, C2, C3, C6, C10, C13, C14, and C15) showed high toxicity against *P. promelas*, whereas C5, C9, C11, and C12 were found to exhibit very high toxicity. Twelve compounds showed low toxicity against rat. C6 and C11 possessed high and moderate toxicities, respectively. The compound C8 had low toxicity (Category D). The toxicity order was observed to be *D. magna* > *T. pyriformis* > *P. promelas* > rat (oral). Table 4 represents toxicity values and the respective category recorded for the compounds. None of the compounds were bio-accumulative.

The compounds C3, C5, and C8 were considered as developmental nontoxicants. Five compounds, namely C1, C3, C5, C9, and C15, are mutagens [Table 5]. Endophytes produce bioactive and chemically novel compounds possessing therapeutic activities.[57] In particular, alkaloids

Table 2: Functional groups identified from Fourier transform infrared spectroscopy analysis of fungal camptothecin

Wavenumber (cm^{-1})	Type of vibrational assignment	Functional group	References
471.62	ϑ –γ	Out-of-plane ring bending, four hydrogen bonds	[42]
949.98	νs- γ- ω	$CH=CH_2$ out-of-plane wag	[43]
1067.65	M	C-H stretch	[43]
1529.62	S	NH_3^+deformation	[39]
1672.36	s-υ	C-N stretch	[44]
1689.72	s-υ	C=O stretch	[45]
2301.18	m-υ	N=N diazonium stretch	[45]
2827.77	M	CH_3 attached to O or N	[46,47]
2966.65	m-s-υ	C-H antisymmetric of CH_3 stretching	[40]
3445.98	m-s	N-Hin aromatic amines	[40]
3605.11	s-υ	-OH stretch in alcohols	[47]

ϑ: Variable; γ: Out-of-plane bending; m: Medium; νs: Very strong; υ: Stretching; ν_{sym}: Symmetric stretching; ω: Wag

Table 3: Compounds identified from electrospray ionization–mass spectrometry analysis

C ID	Molecular weight	Name of the compound	Molecular formula	Activity/assay	References
C1	221.10	1-Chloro-2-(3-chloro-2-butanyl)-4-fluorobenzene	$C_{10}H_{11}Cl_2F$	-	-
C2	243.25	N-[4-(Difluoromethoxy) phenyl]-2,2-dimethylpropanamide	$C_{12}H_{15}F_2NO_2$	-	-
C3	257.02	4-Chloro-6-(chloromethyl)-2-(difluoromethyl)-3-nitropyridine	$C_7H_4Cl_2F_2N_2O_2$	-	-
C4	272.21	N-(3-Oxo-2-{[(5-oxotetrahydro-2-furanyl) carbonyl] oxy}-1,2-oxazolidin-4-yl) acetamide	$C_{10}H_{12}N_2O_7$	Lactivicin derivative, Antibacterial activity	[48]
C5	279.16	(1E,2E)-Bis[(4-chlorophenyl)(^{2}H) methylene] hydrazine	$C_{14}H_8D_2Cl_2N_2$	-	-
C6	295.03	4-Bromo-3-fluoro-7-(trifluoromethyl)-1,5-naphthyridine	$C_9H_3BrF_4N_2$	-	-
C7	301.04	N-(6-Bromo-3-pyridinyl)-2,2,3,3-tetrafluoropropanamide	$C_8H_5BrF_4N_2O$	-	-
C8	309.27	(4S,5S,6R,7S,8R)-5-Acetamido-4,6,7,8,9-pentahydroxy-2-oxononanoic acid	$C_{11}H_{19}NO_9$	Pyruvate to aldehyde synthesis	[49]
C9	317.10	3-Beta-d-ribofuranosyl-3himidazo[4,5-g] quinazolin-8-amine	$C_{14}H_{15}N_5O_4$	Enzyme substrate derivatives	[50]
C10	334.66	7-[1-(Methoxycarbonyl) propyl] indolizino[1,2-b] quinoline-9 (11H)-one	$C_{20}H_{18}N_2O_3$	Anticancer activity	[51]
C11	349.10	(19S)-19-ethyl-19-hydroxy-17-oxa-3,6,13-triazapentacyclo [11.8.0.02,11.04,9.015,20] henicosa-1 (21),2 (11),3,5,7,9,15 (20)-heptaene-14,18-dione	$C_{19}H_{15}N_3O_4$	Anticancer activity	[52]
C12	363.4	10-Amino camptothecin	$C_{20}H_{17}N_3O_4$	Anticancer activity	[53]
C13	381.24	9 Amino camptothecin	$C_{20}H_{19}N_3O_5$	-	[54]
C14	384.3	(19S)-19-ethyl-6,7-difluoro-19-hydroxy-17-oxa-3,13diazapentacyclo [11.8.0.02,11.04,9.015,20] henicosa-1 (21),2 (11),3,5,7,9,15 (20)-heptaene-14,18-dione	$C_{20}H_{14}F_2N_2O_4$	Anti-tumor activity	[55]
C15	389.4	Camptothecin sodium salt	$C_{20}H_{18}N_2NaO_5^+$	Cytotoxic activity	[56]

Table 4: Toxicity values predicted for fungal compounds by consensus method

Compound ID	*Daphnia magna*		*Pimephales promelas*		*Tetrahymena*		Oral rat	
	LC_{50} (48 h) mg/L	Category	LC_{50} (96 h) mg/L	Category	IG_{50} (48 h) mg/L	Category	LD_{50} mg/kg	Category
C1	0.57	A	2.55	B	5.15	B	3225.14	D
C2	9.09	B	1.56	B	N/A	N/A	627.80	D
C3	7.2	B	1.82	B	N/A	N/A	1998.68	D
C4	N/A	N/A	N/A	N/A	N/A	N/A	654.4	D
C5	0.04	X	0.21	A	N/A	N/A	3098.96	D
C6	0.86	A	6.21	B	25.76	C	7.97	B
C7	96.38	C	86.85	C	N/A	N/A	204.54	D
C8	12,086.63	D	9232.38	D	34,462.56	D	16,944.21	D
C9	0.47	A	0.39	A	1.81	B	1056.66	D
C10	4.59	B	1.24	B	12.81	C	142.37	D
C11	23.58	C	0.91	A	N/A	N/A	71.1	C
C12	7.39	B	0.98	A	N/A	N/A	N/A	N/A
C13	12.20	C	1.22	B	N/A	N/A	675.39	D
C14	10.41	C	1.67	B	14.09	C	433.17	D
C15	6.53	B	2.91	B	N/A	N/A	208.97	D

X: Extreme toxicity; A: Very high toxicity; B: High toxicity; C: Moderate toxicity; D: Low toxicity; N/A: Not applicable

can act as medicines, poison, and potion, depending on microbes' chemical transformation. CPT, an alkaloid, is widely studied for its anticancer property. The compound is known to induce toxicity to cancer cells through inhibition of DNA and RNA synthesis in mammalian

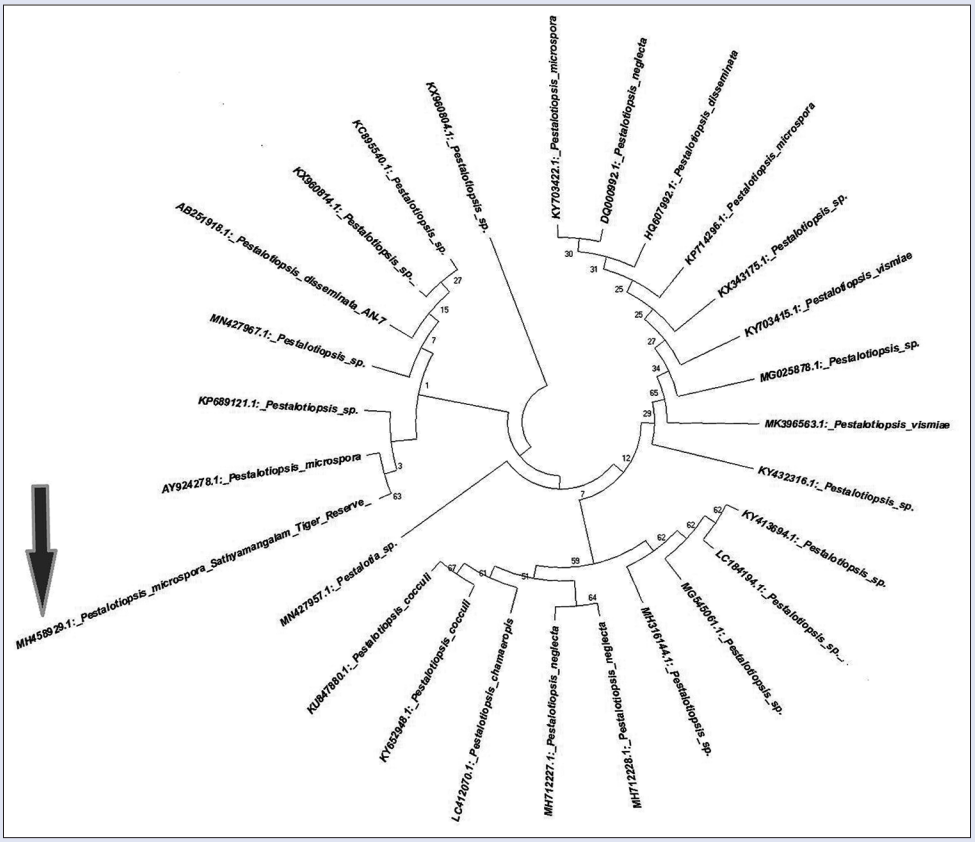

Figure 2: Neighbor-joining circle tree of *Pestalotiopsis microspora* MH458929. Ultraviolet-visible spectra of standard camptothecin compared with fungal camptothecin

Table 5: Values predicted for bioaccumulation factor, developmental toxicity, and mutagenicity of the compounds

Compound ID	Bioaccumulation factor	Developmental toxicity		Mutagenicity	
		Value	Result	Value	Result
C1	176.53	0.82	Toxicant	0.66	Positive
C2	24.50	1.04	Toxicant	0.15	Negative
C3	22.49	0.47	Nontoxicant	0.51	Positive
C4	N/A	0.54	Toxicant	0.60	Positive
C5	N/A	0.24	Nontoxicant	0.67	Positive
C6	108.64	0.78	Toxicant	0.27	Negative
C7	8.46	0.62	Toxicant	0.35	Negative
C8	0.02	0.31	Nontoxicant	0.14	Negative
C9	604.97	0.76	Toxicant	0.67	Positive
C10	N/A	0.97	Toxicant	0.31	Negative
C11	N/A	0.92	Toxicant	0.45	Negative
C12	N/A	1.00	Toxicant	0.42	Negative
C13	0.90	0.86	Toxicant	0.36	Negative
C14	N/A	0.77	Toxicant	0.36	Negative
C15	N/A	1.08	Toxicant	0.64	Positive

N/A: Not applicable

cells[58] by binding to topoisomerase I.[59] On the other hand, it is essential to study the toxicity of the compound against normal cells, so as to ensure its safety for use. Different delivery systems have been developed to increase the water solubility and reduce the toxicity of CPT.[60] The present study implies the use of *in silico* tool to study the toxicity of CPT and its derivatives prior to *in vitro* studies. It is evident that the derivatives of CPT and CPT itself are not toxic to higher organisms, such as rat.

CONCLUSION

Endophytic fungus and its secondary metabolites of CPT derivatives were isolated from forest sources and were used in this study. Endophytic fungus of *P. microspora* from *C. dichotoma* isolated from forest ecosystem can produce CPT and its derivatives. The compounds revealed low or no toxicity against rat (oral). Based on the results of the present study, cytotoxic analysis will be carried out

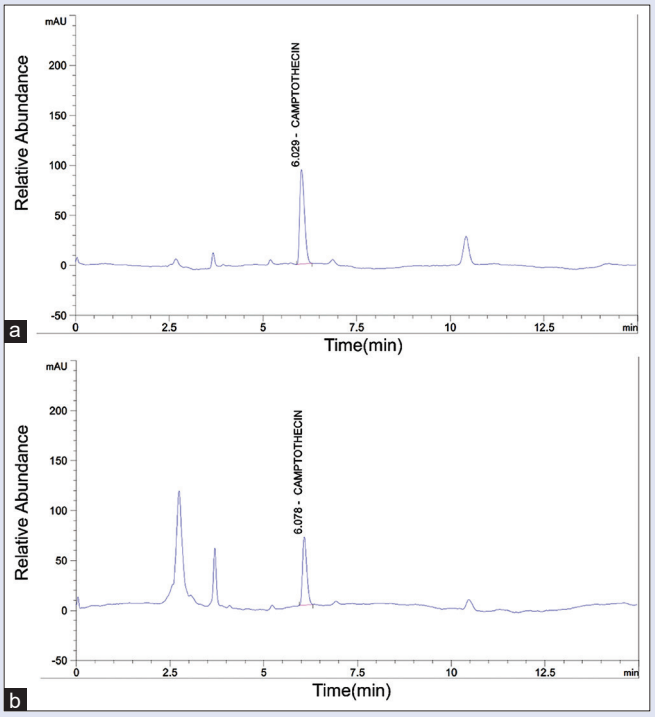

Figure 3: High-performance liquid chromatography chromatogram of (a) Standard camptothecin and (b) Fungal camptothecin

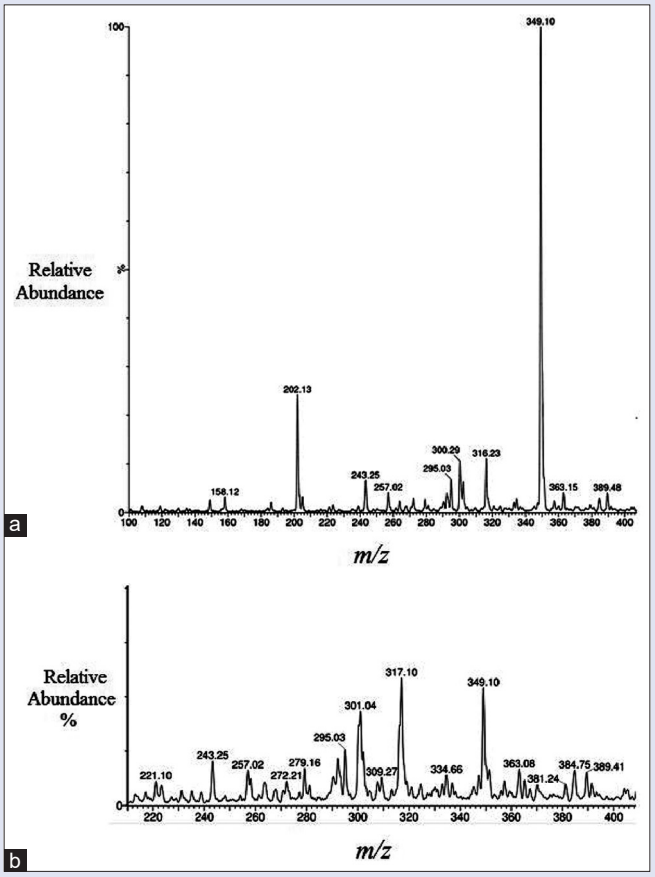

Figure 5: Electrospray ionization–mass spectrometry spectrum of (a) Standard camptothecin (b) Fungal camptothecin

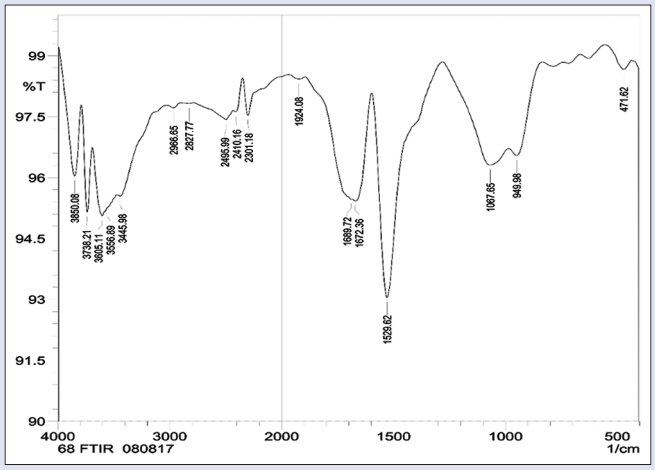

Figure 4: Fourier transform infrared spectroscopy spectrum of fungal camptothecin

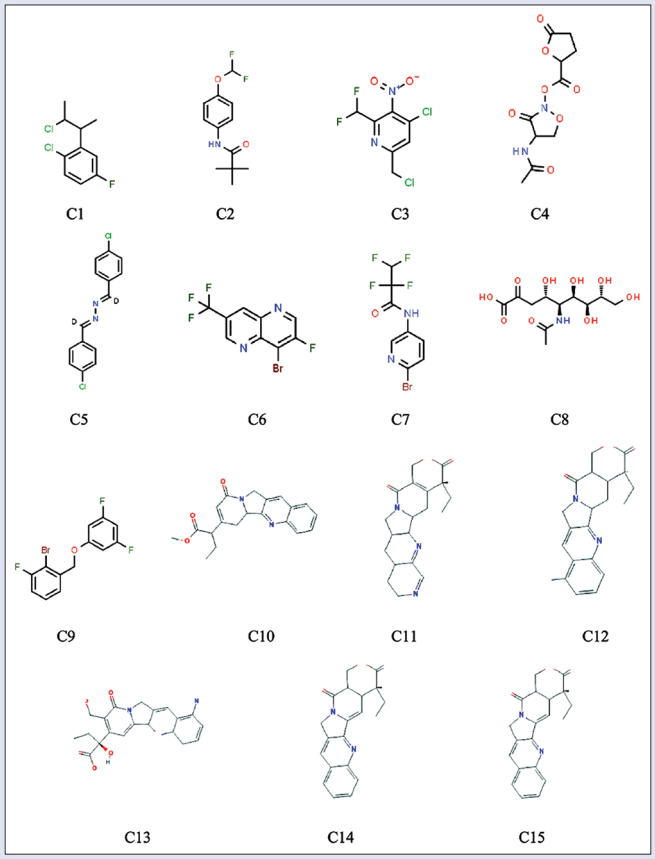

Figure 6: Compounds in fungal extract of *Pestalotiopsis microspora* identified by electrospray ionization–mass spectrometry analysis

and analyze the bio-efficacy against various cancer cells using MTT assays. The compounds will be used as anticancer studies and also these compounds will be utilized for pharmaceutical applications as its derivatives.

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Conflicts of interest

There are no conflicts of interest.

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