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Research Paper

Optimization of cultural conditions for the growth and maximum production of camptothecin, an anticancer agent by an endophytic fungus, *Glomerrella cingulata* from *Ophiorrhiza mungus* L.

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Abstract: Camptothecin (CPT) derived compounds are widely used clinically for chemotherapy of human cancer. Several plants such as *Camptotheca acuminata*, *Nothapodytes foetida* and *Ophiorrhiza* spp. are natural sources of CPT, but these plants produce very law amounts of CPT and its derivatives. Therefore, investigation into alternative sources of CPT has become important in recent years. Several reports are available on "*in vitro*" system of CPT production using tissue cultures of CPT-producing plants. Alternatively, endophytic fungi isolated from CPT producing plants were reported as promising sources of CPT in the recent years. *Enterospora enfrequens* isolated from *Nothapodytes nimmoniana* and *Fusarium solani* isolated from *Camptotheca acuminate* have been identified as CPT producers under "*in vitro*" conditions. However, no reports on the stability and optimization of cultural conditions for the growth and maximum production of CPT production by these fungi are available. *Ophiorrhiza mungus* L (Sinhala vernicular name; Dath katiya, family Rubiaceae) grown in Sri Lanka is also known to produce this alkaloid. In the present study, an endophytic fungus was isolated from field grown *O. mungus*. Stem tissues transplanted in Potato Dextrose Agar (PDA) medium resulted in the isolation of total of 19 individuals that belonged to six distinct species based on their colony characters. All 19 fungi were sub cultured in 100 ml Potato Dextrose Broth (PDB) and grown at 27 ^{o} C. Both the broth and mycelia were analyzed by HPLC for presence of CPT. only one fungal species (strain W3I28) was found to produce CPT, which was detected in the mycelium but not in the broth and content was similar to that produce by the host. The CPT was detected one week, two weeks as well as three weeks old cultures. Optimization of cultural conditions for the growth and maximum production of camptothecin by *Glomerrella cingulate* was performed by changing the physical and chemical parameters like incubation period, shaking conditions, incubation temperature and changing the chemical composition of the basal media (percentage of carbon source of the media). The highest content of CPT (9.70 ± 0.02 x 10^{-4} %) (Fresh weight basis) was produced when the sucrose percentage of Potato Sucrose Broth (PSB) medium was adjusted to 3% when compared to that (2.45 ± 0.05 x 10^{-4} %) (Fresh weight basis) produced in the standard PSB at 27^{o}c incubation temperature after 18 days from incubation. Further, the production of CPT was also observed in seven successive cultures of the fungus with no attenuation, indicating the production of CPT by the fungus is stable. This is the first report of the isolation and optimization of cultural conditions of CPT- producing endophytic fungus from *Ophiorrhiza mungus*.

Keywords: Camptothecin, *Ophiorrhiza mungus* L, Potato Dextrose Agar, HPLC, *Glomerrella cingulate*, optimization of cultural conditions.

Introduction

Camptothecin (CPT) is a monoterpenoid indole alkaloid (MIA) with broad-spectrum anti-cancer

activity, is produced from plants and endophytes. This compound was firstly discovered in 1966 from the bark of *Camptotheca acuminata* tree during the screening project for anti-tumor agents by the national cancer institute in the USA (Wall *et al*., 1966). CPT analogs and it's derivatives are effective anticancer agents due to the cytotoxicity of CPT itself, two derivatives of it, namely topotecan and irenotecan are used worldwide to treat various cancers (Martino *et al*. 2017).

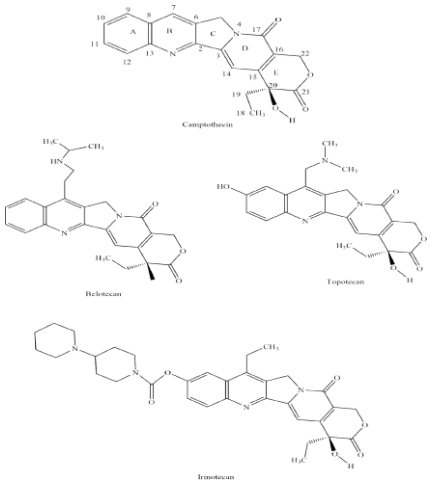


Figure 01. Structure of Camptothecin and its derivatives/ analogues (Akshatha Banadka *et al*., 2024)

CPT and its derivatives are known to inhibit topoisomerase 1 (Li *et al*., 2017).

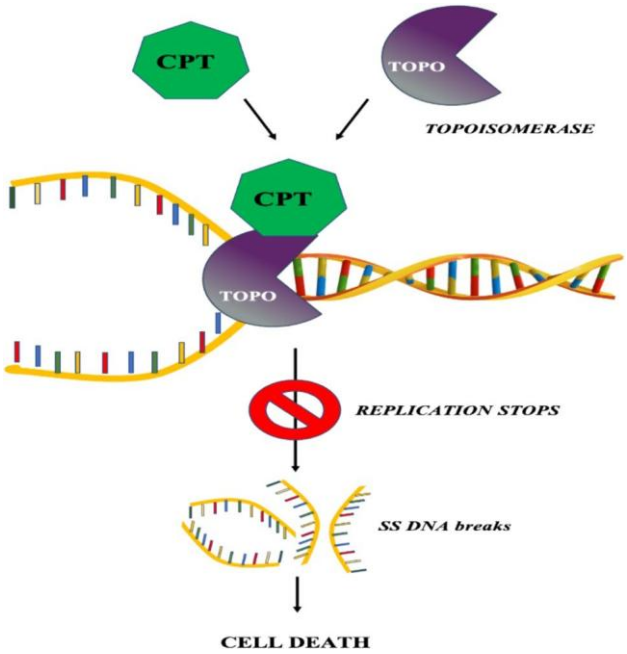


Figure 02. Mechanism of inhibition of Topoisomerase I activity by camptothecin (Akshatha Banadka *et al*., 2024)

In the pharmaceutical industry and academia, camptothecin and its derivatives have been drawing marvelous devotion and considered as the most promising anti-cancer treatment drugs due to their induction of DNA topoisomerase I dysfunction, especially Topotecan and Irinotecan have been approved by the Food and Drug Administration of the United States for the treatment of lung cancer, colorectal cancer and ovarian cancer (Qingyan Ruan *et al*., 2021). Recent studies have found that camptothecin also has a variety of other

pharmacological properties, including antiviral and antifungal activities (Qingyan Ruan *et al*., 2021). In addition to Nyssaceae family plant *C. acuminata*, various CPT-producing plant species have been discovered from different families such as *Apodytes dimidiata* (Icacinaceae), *Nothapodytes feotida* (Icacinaceae), *N. nimmoniana* (Icacinaceae), *N. pittosporoides* (Icacinaceae), *Pyrenacantha klaineana* (Icacinaceae), *P. volubilis* (Icacinaceae), *Merriliodendron megacarpum* (Icacinaceae), *Miquelia dentata* Bedd. (Icacinaceae), *Erratamia heyneana* (Apocynaceae), *Ophirrohiza pumila* (Rubiaceae), *O. mungos* (Rubiaceae), *O. hirsutula* (Rubiacea), *O. pectinate* (Rubiaceae), *O. trichocarpon* (Rubiaceae) (Arisawa *et al*., 1981; Aiyama *et al*., 1988; Mohinudeen *et al*., 2021; Devasia *et al*., 2021; Pu *et al*., 2019; Ramesha *et al*., 2013; Rajan *et al*., 2016; Shweta *et al*., 2010; Suma *et al*., 2014; Akshatha Banadka *et al*., 2024).

Table 01. Camptothecin production in different parts of the plant species

Plant Name	**Family**	**Site of production**	**Content CPT**	**of Reference**
Camptotheca acuminata	*Nyssaceae*	Shoot	0.042%	(Liu and Adams, 1996)
		Root	0.051%	
		Leaf	0.015%	
		Branch	0.062%	
		Seed	0.110%	
Camptotheca lowreyana S.Y.Li	*Nyssaceae*	Young leaves	0.5537%	(Li *et al*., 2002)
		Old leaves	0.118%	
Camptotheca yunnanensis Dode	*Nyssaceae*	Young leaves	0.4494%	
		Old leaves	0.059%	
Chonemorpha grandiflora G.Don	*Apocynaceae*	Stem bark	0.0013%	(Kulkarni *et al*., 2010)
		Leaves	0.0009%	
Nothapodytes nimmoniana	*Icacinaceae*	Shoots	0.075–0.81%	(Isah and Mujib, 2015a)
		Root	0.11–0.2%	
		Seeds	0.179%	
		Leaves	0.081-0.7%	
		Fruits	0.122%	
Mappia pittosporoides Oliv.	*Icacinaceae*	Leaves	0.238%	(Zeng *et al*., 2013)
		Fruits	0.102%	
		Roots	0.172%	
Ervatamia heyneana (Wall.) T.Cooke	*Apocynaceae*	Stem bark	0.00013%	(Gunasekera *et al*., 1979)
(Syn:*Tabernaemontana alternifolia* L.)			0.0003%	(Kulkarni, 2008)
		Leaves	0.0001%	
Merrilliodendron megacarpum (Hemsl.) Sleumer	*Icacinaceae*	Stem bark	0.053%	(Arisawa *et al*., 1981)
M. dentata	*Icacinaceae*	Leaf	0.024%	(Ramesha *et al*., 2013)
		Cotyledon	1.418%	
		Root	0.153%	

		Fruit	1.22%	
		Twig	0.003%	
Mostuea brunonis Didr.	*Loganiaceae*	Whole plant	0.01%	(Dai *et al*., 1999)
Pyrenacantha klaineana Pierre ex Exell & Men-	*Icacinaceae*	Stem	0.00048%	(Zhou *et al*., 2000)
donça		Fruit	0.488%	(Ramesha *et al*., 2013)
Ixora coccinea L.	*Rubiaceae*	Young leaves	0.4146 μg g^{-1}	(Saravanan and Boopalan, 2011)
		Mature leaves	5.0611 μg g^{-1}	
Ophiorrhiza. alata Craib	*Rubiaceae*	Leaves	83 μg g^{-1}	(Krishnakumar *et al*., 2020)
		Root	388 μg g^{-1}	
Ophiorrhiza rugosa var. *decumbens* (Gardner ex	*Rubiaceae*	Whole plant	4.20 μg g^{-1}	(Krishnakumar *et al*., 2020)
Thwaites) Deb & Mondal		Shoot	2 μg g^{-1}	
		Root	24 μg g^{-1}	
Ophiorrhiza rugosa var. *prostrata* (D.Don) Deb &	*Rubiaceae*	Stem	0.08%	(Gharpure *et al*., 2010)

In order to supply the immense demand of camptothecin in clinical practice, it is mainly extracted from the roots, stems, young leaves and fruits of these CPT-producing plants, which has increased the logging of these plants particularly *C. acuminata* in China and *N. nimmoniana* in India and is not conducive to the sustainable development of these plant resources (Mohinudeen *et al*., 2021; Pu *et al*., 2019). In addition, the growth of woody plants is very slow, and the traditional method of camptothecin extraction from plants is inefficient and expensive. Therefore, only plant-derived camptothecin is difficult to meet the growing global market demand.

In view of the limitations of plants as sources of camptothecin in productivity and efficiency, endophytes serve as the fast growth, high cost-effectiveness, good reproducibility, and feasible genetic manipulation, so they have the potential to meet the huge market demand of the pharmaceutical industry. Endophytes are the microorganism including fungi or bacteria living inside the healthy plant tissues in a symbiotic relationship (endosymbiont), and enhance host plants growth and nutrient gain but do not affect the plant growth and development (Aswani *et al*., 2020; Newman and Cragg, 2020; Uzma *et al*., 2018). In the past two decades, extensive research have found that plant endophytes are the most advantageous substitute for camptothecin sources, which not only improve the production efficiency, but also reduce the production cost of camptothecin (Akshatha Banadka *et al*., 2024; Newman, 2018; Rehman *et al*., 2008; Shweta *et al*., 2010, 2013). However, the amount of camptothecin producing by these endophytes are also very law. Therefore, additional researches are required to optimize the cultural conditions to enhance the production of CPT, which is producing by these endophytes. In order to increase the production of camptothecin, in the present study, summarized the isolation, identification and fermentation of CPT-producing endophyte, as well as the biosynthesis, extraction and detection of camptothecin from endophyte. Although, the optimization of cultural conditions for the growth and maximum production of camptothecin. The insights gained from these studies will be useful and provide an economical, eco-friendly and reliable alternative approaches to the discovery of natural product drugs.

Materials and Methods

Collection of the plant samples and isolation of endophytic fungi

Fresh leaves, stems and roots of *Ophiorrhiza mungus* were collected from botanical garden of University of Sri Jayawardhanapura, Sri Lanka. Plant materials were authenticated by comparison with herbarium type specimen in National Herbarium, Peradeniya, Sri Lanka.

Storage Conditions and Sample Collection

Figure 03. Mature plant of *Ophiorrhiza mungus*

These plant materials washed thoroughly with running tap water and sterile distilled water. The plant materials were segmented into small parts, surface sterilized with 70% ethyl alcohol for 1 min, and 2.5% sodium hypochlorite for 2 min, placed on the surface of Potato Dextrose Agar (PDA) supplemented with ampicillin (1 µg/mL), incubated for 7 days (Ashraf S. A. E., *et al.,* 2003) at 30 °C. The evolved fungal hyphal tips were collected and purified on PDA, and kept as slope cultures at 4 °C for further uses.

Fungus Identification

The recovered fungal isolates were morphologically identified according to their macroscopical and microscopical features. After growth in culture, fungi are identified based on visual characteristics such as colony morphology and color (based frequently on spore morphology such as color, septation, and different methods of spore production. In addition, the nature and morphology of sexual spores and the different spore types involved in the sexual and asexual life cycles are also used for fungal identification). Light microscopic observations although used to determine the presence of septate or nonseptate hyphae and fruiting structures for molds. Molecular identification was also carried out to identify the fungus.

Screening for the most putative recovered isolated fungi

The fungi were isolated from the tissues of *Ophiorrhiza mungus* transplanted in standard Potato Dextrose Agar (PDA) medium. The recovered fungal isolates were screened for camptothecin production by growing pure culture on Potato Dextrose Broth (PDB) medium (Extract of 200 g potato and 20 g dextrose per liter). Each fungal isolate (2 agar plugs of 5 mm) of 6 days old PDA culture were inoculated into 100 ml of Potato Dextrose Broth (PDB) medium/250 mL Conical flask, incubated at 27 °C for 18 days. Three biological replicates of each fungal isolate were conducted. After incubation, resulted in approximately 5g of fresh mycelia. Mycelia extracted with 61% of ethanol (Gowdiperu S. *et al*., 2019) at 60 ^{o}C in shaking water bath for 90 minutes and analyzed after filtration (Iso-DISC Filter 13mm x 0.45 µm) on an Agilent 12000 series HPLC. The purity and concentration of camptothecin were determined by Agilent 12000 series HPLC with reverse-phase C18 column (Eclipse Plus C18 4.6 mm × 150 mm, Cat. #959963-902), The mobile phase consisting of water (A) and acetonitrile (B) was run in the gradient as follows 90%A, 10% B; (v/v) at a flow rate 1.0 mL/min for 35 min, scanned by photodiode array detector (DAD)., the UV signal was recorded at λ_{max} 256 nm. Retention time of CPT was at 22 min. The identity and concentration of the putative sample of camptothecin were confirmed from retention time and peak area, normalizing to the authentic (standard) one at λ360 nm.

Optimization of cultural conditions for camptothecin production from *Glomerrella cingulate*.

Optimization of cultural conditions for the growth and maximum production of camptothecin by *Glomerrella cingulate* was performed by changing the chemical and physical parameters like growth medium, shaking conditions, incubation period, incubation temperature and changing the chemical composition of the basal media (percentage of carbon source of the medium). All experiments were carried out with ten replicates. Standard deviation was calculated according to the following formula,

$$s = \sqrt{\frac{\sum_{i=1}^{n}(x_i - \bar{x})^2}{n-1}}$$

Where:

- n - The total number of data points in the sample.
- x_i - Each individual data point.
- $\bar{x}$ - Mean (average) of the sample.

Effect of media on camptothecin production

The *Glomerrella cingulate* was grown for 18 days in different culture Medias like Potato Dextrose Broth (PDB), Potato Sucrose Broth (PSB), Nutrient Broth (NB), Malt Extract Broth (MEB). Twenty-five millilitres of the each medium was prepared and sterilized at 121 °C at 15 lbs pressure for 20 minutes, under aseptic conditions, the fungal culture was inoculated and incubated for 18 days by keeping the other parameters constant, adjusted to the PH of each media to 6.0. After incubation, the mycelial fresh weight and the camptothecin production were recorded. The growth media-exhibited maximum camptothecin production (mycelia fresh weight basis) was used as the optimized medium for further studies.

Shake flask experiment (Designed to find out the optimal kinetic condition for the production of CPT by *Glomerrella cingulate*)

The shake flask experiment with the fungal culture was conducted to standardize culture conditions to evaluate the optimal kinetic conditions for capacity to produce CPT. Data represented the average of 10 replicates. In order to study the production kinetics of CPT. Conical flasks (500 mL) were selected for the study. The Potato Sucrose Broth (PSB, 100 mL) medium (optimized in previous experiment) was prepared and sterilized at 121 °C at 15 lbs pressure for 20 minutes. One mL each of glycerol stock was inoculated into a 500 mL conical flask containing 100 mL of PSB medium, respectively, and cultured at 27 °C on a rotary shaker with 70 mm shaking diameter at 0 to 250 rpm, by keeping the other parameters constant. The fungal growth period was 18 days. All the conditions were decided according to the preliminary experiments observations and media selection was based on previous experiment results. After incubation, the mycelial fresh weight and the camptothecin production were recorded. The shaking speed-exhibited maximum camptothecin production (mycelia fresh weight basis) was used as the optimized shaking speed for further studies.

Effect of incubation period on camptothecin production (Designed to find out the optimal incubation period for the production of CPT by *Glomerrella cingulate*)

The *Glomerrella cingulate* was subjected to different periods of incubation (72hours (3 days) – 600 hours (25 days)), by keeping the other parameters constant to study the optimum period required for camptothecin (mycelia fresh weight basis) production. Twenty-five millilitres of the Potato Sucrose Broth (PSB) medium was prepared and sterilized at 121 °C at 15 lbs pressure for 20 min. Under aseptic conditions, the fungal culture was inoculated and incubated by keeping the other parameters constant. After incubation, the mycelial fresh weight and the camptothecin production were recorded every 24 hours from 72 hours (3 days) to 600 hours (25 days). All the conditions were decided according to the preliminary experiments observations and media selection; shaking conditions were based on previous experiment results. The period of incubation-exhibited maximum camptothecin production (mycelia fresh weight basis) was used as the optimized period of incubation for further studies.

Effect of temperature on camptothecin production (Designed to find out the optimal growth for the production of CPT by *Glomerrella cingulate*)

The *Glomerrella cingulate* was subjected to different incubation temperatures ranging from 20 °C to 45 °C, by keeping the other parameters constant to study the optimum incubation temperature required for camptothecin (mycelia fresh weight basis) production. Twenty-five millilitres of the basal medium was prepared and sterilized at 121 °C

at 15 lbs pressure for 20 min. Under aseptic conditions, the fungal culture was inoculated and incubated in unshaken conditions for 18 days (optimized in previous experiments), by keeping the other parameters constant. All the conditions were decided according to the preliminary experiments observations and media selection; shaking conditions; incubation period were based on previous experiment results. After incubation, the mycelial fresh weight and the camptothecin production were recorded. The incubation temperature-exhibited maximum camptothecin production (mycelia fresh weight basis) was used as the optimized incubation temperature for further study.

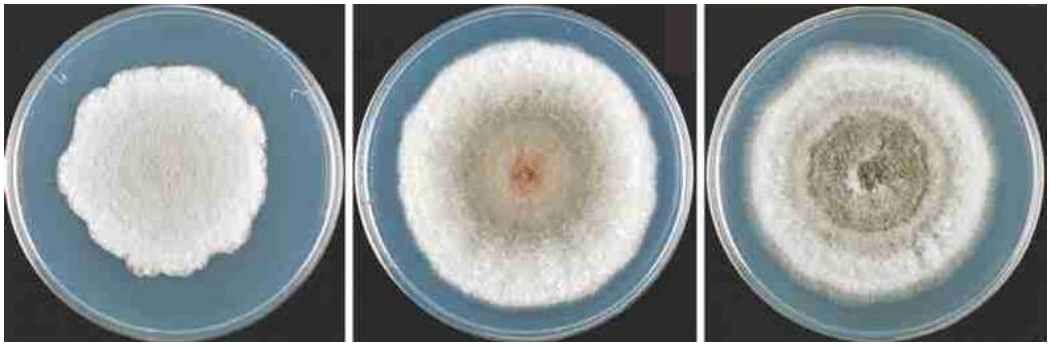
Effect of Sucrose percentage of the growth medium on camptothecin production (Designed to find out the optimal carban source/ sucrose percentage for the production of CPT by *Glomerrella cingulate*)

Standard Potato Sucrose Broth (PSB) is composed of Potato and Sucrose that encourage luxuriant fungal growth. In standard PSB 1000ml of distilled water containing 200g of potato and 20g of sucrose (2% of sucrose). In this experiment, The *Glomerrella cingulate* was subjected to different carbon source (sucrose) percentages containing PSB medias ranging from 1% to 5% of carbon source (sucrose) percentage, by keeping the other parameters constant to study the optimum carbon source (sucrose) percentages required for camptothecin (mycelia fresh weight basis) production. Twenty-five millilitres of the basal medium was prepared and sterilized at 121 °C at 15 lbs pressure for 20 min. Under aseptic conditions, the fungal culture was inoculated and incubated for 18 days in unshaken conditions at 35^{o}C incubation temperature. Except the sucrose percentage, all other conditions were optimized in previous experiments. Temperature range was decided according to the preliminary experiments observations. After incubation, the mycelial fresh weight and the camptothecin production were recorded.

Results and Discussion

Identification of the fungus

The isolated fungus from the stem tissues of *Ophiorrhiza mungus* was identified on the basis of morphological characteristics and microscopic observations as *Glomerrella cingulate* strain W3128 (Gene Bank Accession number GQ899146) deposited in the Biotechnology laboratory at the university of Sri Jayawardenepura. When *Glomerrella cingulate* cultured on potato dextrose media, this species can appear gray, orange, or pink in color, and will often exhibit concentric rings of growth radiating from the center. When asexual stage (anamorph) observed under dissecting microscope acervuli can often be spotted if the fungus has recently been under sporulating conditions. These acervuli will appear orange to pinkish in color, due to the masses of conidia being produced on the surface, and will have black, hair-like, setae spiking out in several directions. Under a compound microscope, conidia appear ovoid in shape. The fungus has thin hyphae (Figure 04). It produces abundant, verticillately branched (in a whorl) condiophores and phialospores are colorless, elongate-elliptical to cylindrical.



(a) (b) (c)

Figure 04. (a) Cultures of *Glomerrella cingulata* after 05 days from the inoculation on PDA, (b) Cultures of

Glomerrella cingulata after 10 days from the inoculation on PDA, (c) Cultures of *Glomerrella cingulata* after 15 days from the inoculation on PDA

Isolation, growth and CPT extraction of an endophytic fungus

Glomerrella cingulata is reported here as an endophytic fungus isolated from the stem tissues of *Ophiorrhiza mungus*. After the fungus grew on potato dextrose broth (PDB) medium for 7 days. CPT was detected in the mycelium but not in the broth and the content was similar to that produced by the host. Complete identification and characterization of CPTwas done through spectral and comparative studies. The HPLC profile and the spectrum of fungal camptothecin was compared with HPLC profiles and the UV spectrums of standard and host camptothecin (Figure 05, 06, 07 and 08).

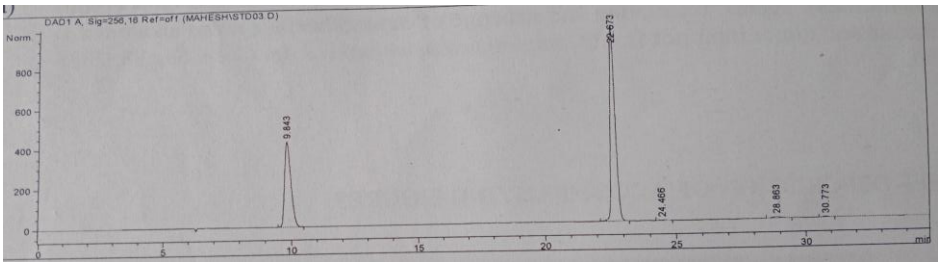


Figure 05. HPLC profile of authentic camptothecin

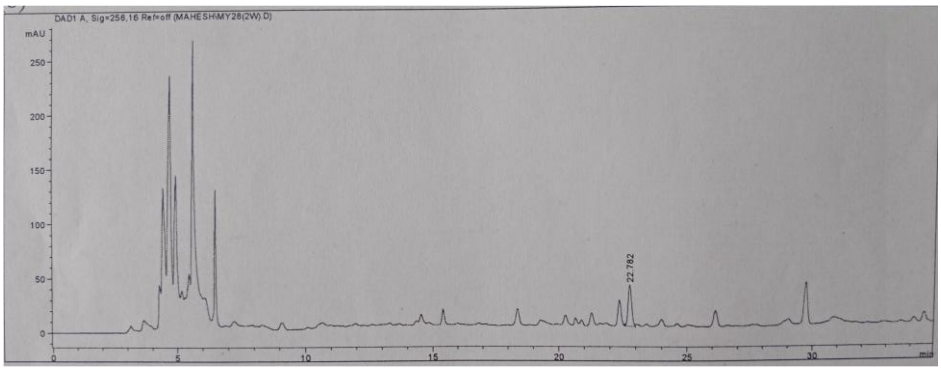


Figure 06. HPLC profile of fungal (*Glomerrella cingulate*) camptothecin

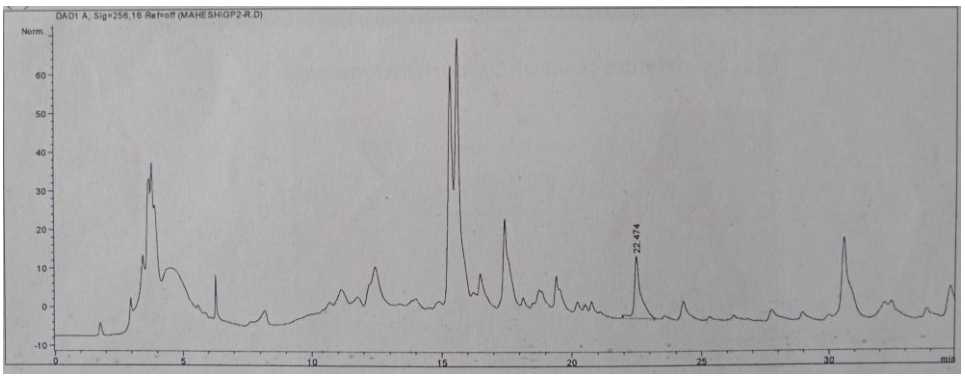


Figure 07. HPLC profile of host (*Ophiorrhiza mungus*) camptothecin

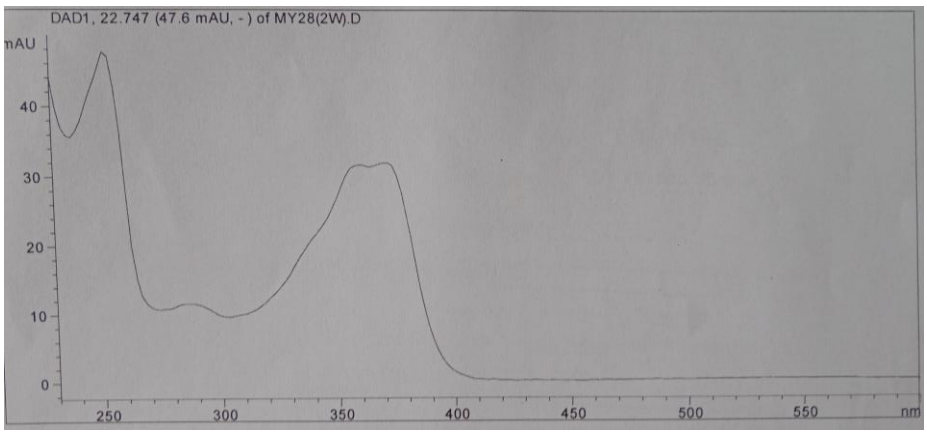


Figure 08. UV spectrum of camptothecin

The CPT was detected one week, two weeks as well as three weeks old cultures. Further, the production of CPT was also observed in seven successive cultures of the fungus with no attenuation, indicating the production of CPT by the fungus is stable. The production of CPT was confirmed by the HPLC profile and the spectrum of fungal camptothecin, which were compared with HPLC profiles and the UV spectrums of standard and host camptothecin.

It was observed that the fungus grew on many common Medias in 5–7 days. Potato Sucrose Broth (PSB) media was found to be the best-suited media for endophyte in terms of CPT production. Average production of CPT was observed on 18th day in terms of (2.45 ± 0.05 x 10^{-4} %) fresh weight basis of mycelia.

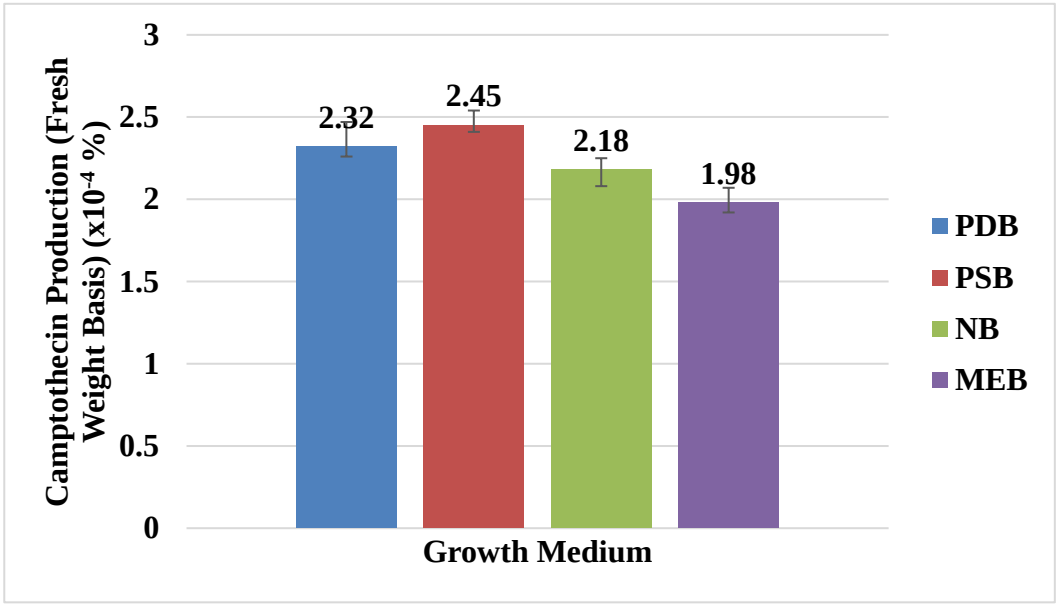


Figure 09. Production of camptothecin in different growth Medias.

In the present study, the endophytic fungus isolated from *Ophiorrhiza mungus* maintained significantly higher concentration of CPT at unshaken conical flasks compared with shaken flasks. After The fungal growth period of 18 days, Maximum production of CPT was observed on unshaken flasks. Average production of CPT was observed on 18th day in terms of (2.43 ± 0.05 x 10^{-4} %) fresh weight basis of mycelia (Figure10).

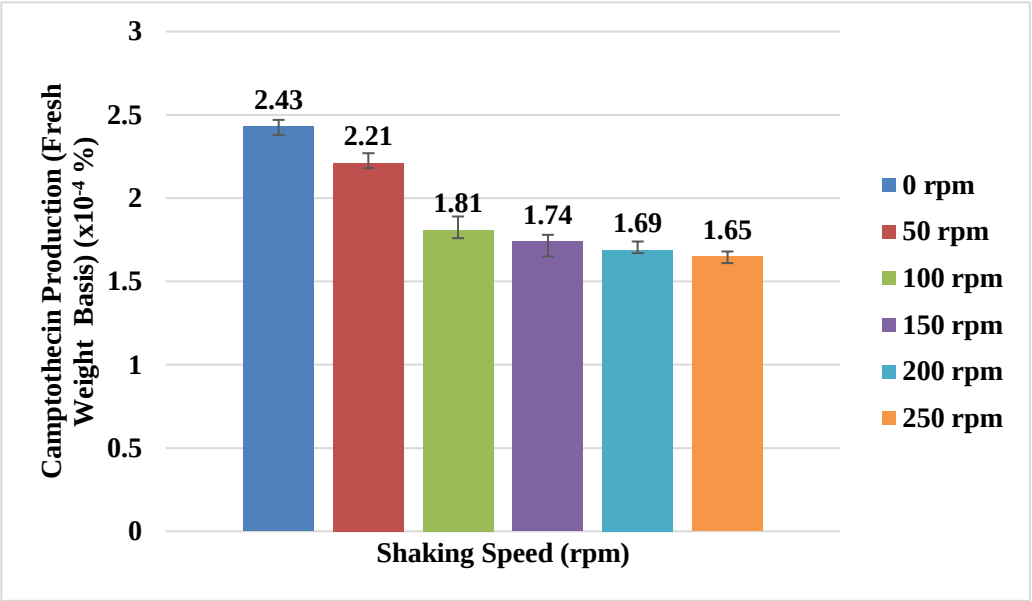


Figure 10. Production of camptothecin in different shaking conditions.

Formation of CPT started as early as 72 hours (3 days) (Figure 11). There was a steady increase in the production of CPT from day 3 to 18 in the conical flasks, but a maximum CPT was recorded in terms of (2.46 ± 0.02 x 10^{-4} %) fresh weight of mycelia at 432 hours (18 days) of fermentation. Conditions for the maximum production of CPT was observed to begin 384 hours (16 days) to 432 hours (18 days) after inoculation. However, Maximum production of CPT was observed on day 18 in terms of (2.46 ± 0.02 x 10^{-4} %) fresh weight of mycelia. Although, the CPT content rapidly declined after 432 hours (18 days) of incubation. the highest growth was observed to peak at day 18 in the conical flask, after which there was a rapidly fall in CPT production which may be due to partial lysis of cells after attaining stationary phase. The production of CPT started declining after day 18 and there was almost a stationary phase of CPT production after day 16 of fermentation (Figure 11).

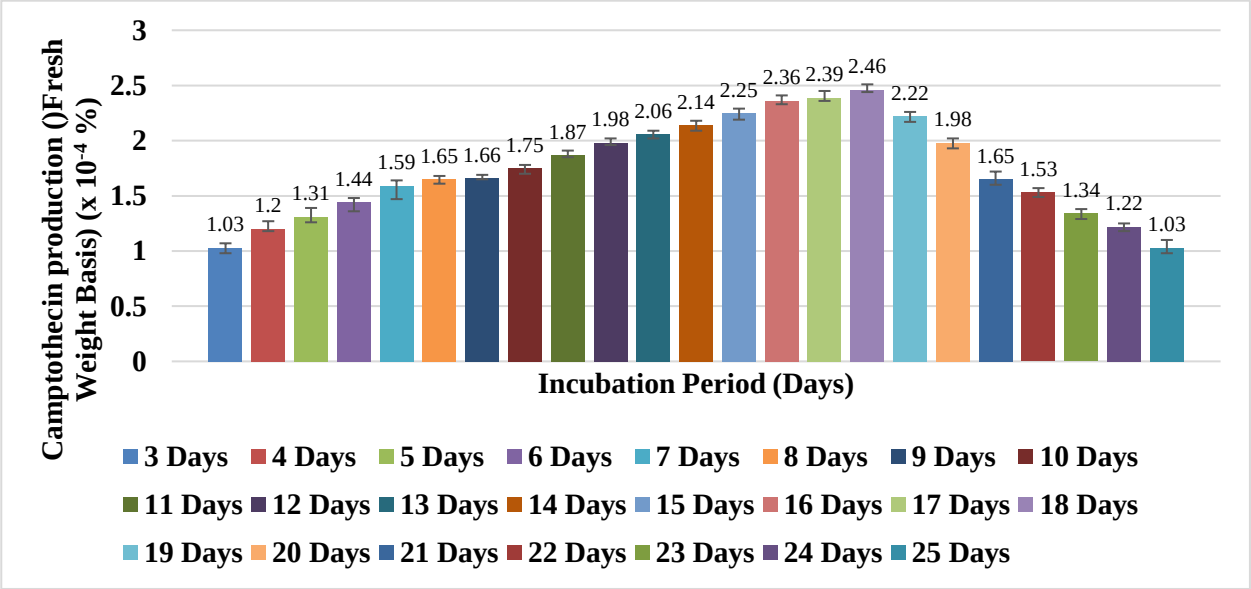


Figure 11. Production of camptothecin in different incubation periods.

There was a steady increase in the production of CPT from 25° C to 35° C incubation temperature, but a maximum CPT was recorded in terms of (5.31 ± 0.06 x 10^{-4} %) fresh weight of mycelia at 35° C of incubation temperature. Conditions for the high production of CPT was observed from 30° C and 35° C incubation temperatures, compared with other incubation temperatures. However, Maximum production of CPT was observed on 35° C in terms of (2.46 ± 0.06 x 10^{-4} %) fresh weight of mycelia. Although, the CPT content rapidly declined after 35° C of incubation. The highest growth was also observed to peak at 35° C in the conical flask, after which there was a rapidly fall in CPT production which may be due to high temperature stress (Figure 12).

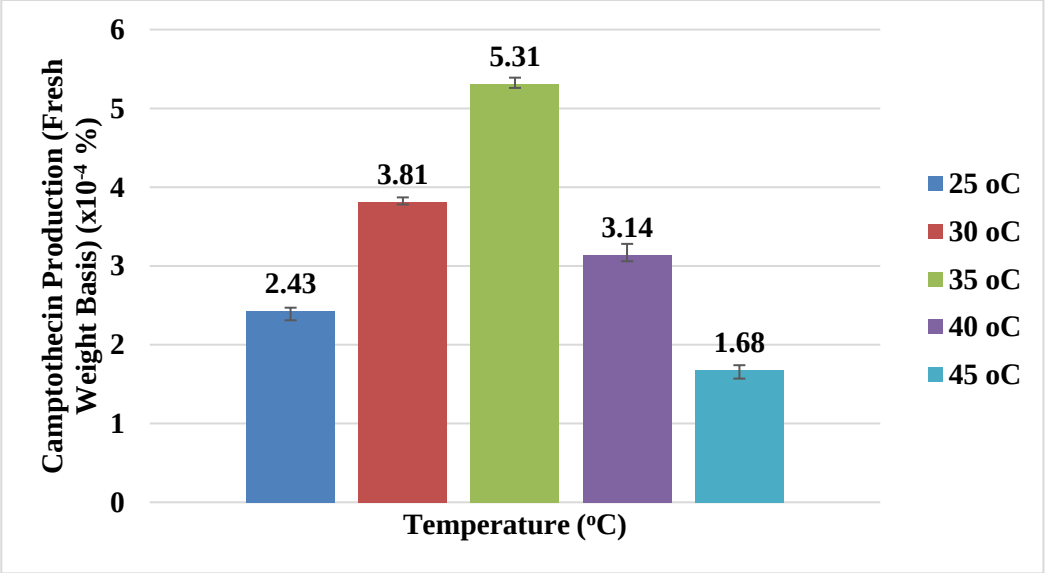


Figure 12.Production of camptothecin in different incubation temperatures

There was a steady increase in the production of CPT from 1% to 3% sucrose percentages of PSB, but a maximum CPT was recorded in terms of (9.70 ± 0.02 x 10^{-4} %) fresh weight of mycelia in 3% sucrose containing PSB medium. Conditions for the high production of CPT was observed in 3% and 4% of sucrose in PSB. However, Maximum production of CPT was observed on 3% of sucrose in terms of (9.70 ± 0.02 x 10^{-4} %) fresh weight of mycelia. Although, the CPT content rapidly declined after 4% of sucrose content in PSB, which may be due to loss of nutrient stress (Figure 13).

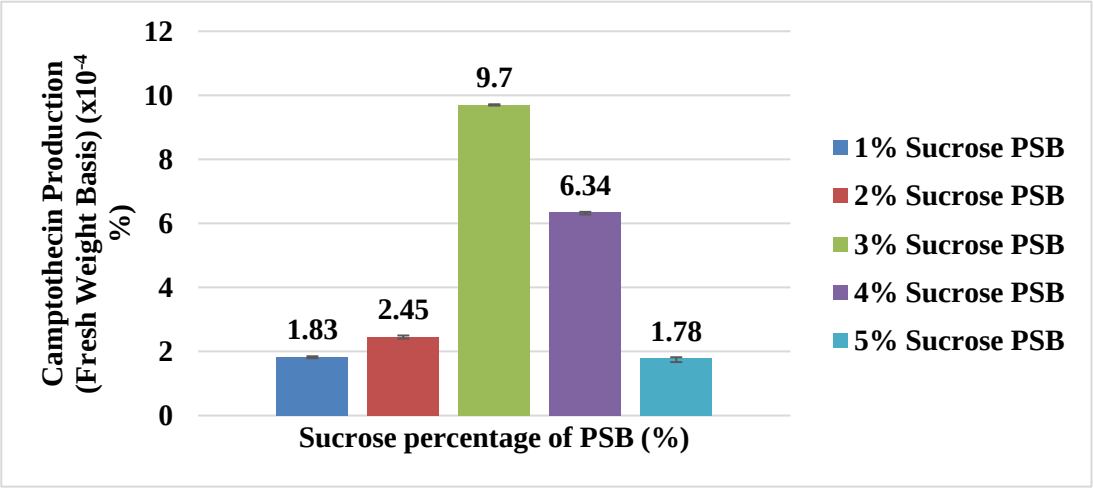


Figure 13. Production of Camptothecin in different carbon source (sucrose) percentages containing Pota to Sucrose Broth (PSB) Media

Conclusion and Discussion

This reported fungus was found to produce a well-known metabolite, CPT, an anti-cancer drug molecule. CPT is a promising anti-tumour agent. The major sources of camptothecin are the plants, for which a wide variety of valuable plants have been overexploited to meet the increased market demand, and that has resulted in their depletion. Although it is unfortunately only available in relatively insufficient concentration in the tree roots, which demands the uprooting of rare, 50–75-year-old trees from the forests. In this study, the cultural conditions, such as growth medium, shaking conditions, incubation period, incubation temperature and changing the chemical composition of the basal media (percentage of carbon source of the medium) have been optimize to obtain ($9.70 \pm 0.02 \times 10^{-4}$ %) (Fresh weight basis) of CPT from the endophytic fungi *Glomerrella cingulata* isolated from *Ophiorrhiza mungus*, which was much more than the content of that produced by the host *Ophiorrhiza mungus*.

According to the results of this study, Potato Sucrose Broth (PSB) media was found to be the best-suited media among the other medias (Potato dextrose broth (PDB), Nutrient broth (NB), Malt extract broth (MEB)), tested in this experiment for endophyte *Glomerrella cingulata* in terms of CPT production. PDB was resulted average production of CPT in terms of ($2.45 \pm 0.05 \times 10^{-4}$ %) fresh weight basis of mycelia under non optimized conditions of other factors.

Under the unshaking condition, maximum average production of CPT was observed on 18th day in terms of ($2.43 \pm 0.05 \times 10^{-4}$ %) fresh weight basis of mycelia, when growth media has been optimized. That is proving the unshaken incubation is the best condition compared with all other shaking conditions tested in this experiment in terms of CPT production of this endophyte. Although I found a negative correlation in between shaking speed and CPT production of endophytic fungus *Glomerrella cingulata*.

Optimum incubation temperature for maximum production of CPT was recorded as 432 hours (18 days) of fermentation with obtaining ($2.46 \pm 0.02 \times 10^{-4}$ %) of CPT (fresh weight of mycelia basis), when growth medium, shaking condition and incubation period have been optimized. The maximum CPT was recorded compared with other incubation temperatures tested, in terms of ($5.31 \pm 0.06 \times 10^{-4}$ %) fresh weight of mycelia at 35 ^{o} C of incubation temperature. Therefore, 35 ^{o} C of incubation temperature can be consider as the optimum incubation temperature of endophyte *Glomerrella cingulata* in terms of CPT production.

Maximum production of CPT was observed on 3% of sucrose, compared with other carbon source (sucrose) percentages tested, in terms of ($9.70 \pm 0.02 \times 10^{-4}$ %) fresh weight of mycelia, when growth medium, shaking condition, incubation period and incubation temperature have been optimized. That is proving there is a considerable effect of carbon source (sucrose) percentage on the production of CPT by endophyte *Glomerrella cingulata*.

Although, the results are suggesting the considerable effect of incubation temperature and carbon source (sucrose) percentage of growth medium on the CPT production by the endophyte. As well as shaking conditions of the growth medium is having negative effect on the production of CPT by the endophyte *Glomerrella cingulate*.

Finally, according to the results it can conclude, the maximum amount of CPT can obtain from endophyte *Glomerrella cingulata* by growing the fungus in 3% Potato Sucrose Broth (PSB) media under the unshaking condition, incubating for 432 hours (18 days) under 35 ^{o} C of incubation temperature. The microbial source that could produce an appreciable amount of the drug under the optimized cultural conditions, it could eliminate the need to harvest and extract the slow growing and relatively rare medicinal plants, and the price of CPT would also be reduced.

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