INTRODUCTION

Various plants having medicinal values have been in use for the treatment of multiple diseases either to completely cure or for the betterment of humans in general. Organic substances have recently become the centre of interest because of their versatility. The literature reveals that medicinal plants are the primary sources of drugs, nutraceuticals, and chemical entities for synthetic drugs. Bioactive compounds such as alkaloids, tannins, flavonoids define the potential of a plant to produce an explicit physiological activity in humans. Tinospora cordifolia (Willd.) Miers commonly known as heart-leaved moonseed belongs to the family Menispermaceae. It is an officinal flora that is autochthonic to the tropical regions of India, Myanmar and Sri Lanka. It has been reported that due to the plethora of medicinal properties, such as anti-diabetic, anti-spasmodic, anti-periodic, anti-inflammatory, anti-arthritic, anti-allergic, anti-stress, anti-malarial, anti-leprotic, hepatoprotective, anti-neoplastic and immunomodulatory activities, it is of great interest to the researchers across the globe. Traditionally, it has been in use to regulate the level of blood glucose for the patients with diabetes.

Anti-diabetic potential

T. cordifolia has innumerable potentials, but its role in anti-diabetic potential through extenuating oxidative stress, by advancing the secretion of insulin and also by inhibiting gluconeogenesis and glycolysis resulting in regulating the level of blood glucose has secured a prominent place in traditional medicine due to the existence of major phytoconstituents such as alkaloids, tannins, cardiac glycosides, flavonoids, saponins, and steroids.

The root extract of T. cordifolia decreases the levels of plasma thiobarbituric acid reactive substances, glycosylated hemoglobin, hydroperoxides, ceruloplasmin and vitamin E in diabetic mice. Oral administration of T. cordifolia extract in the “Bogen-Excel” formulation (Ayurvedic herbal formulation), consisting of eight medicinal plants, including Strychnosotatum, Curcumulonga, T. cordifolia, Salaciaoblonga,
Coscinium fenestratum, Vetiveria zizanioides, Andrographis paniculata, and Mimosa pudica, reduces glutathione (GSH) and vitamin C in blood\(^6\), and urine glucose and levels of lipids in the serum and in the tissues of alloxan diabetic rats with a subsequent decrease in body weight\(^7\). It also decreases the GSH, glutathione peroxidase (GPx), and superoxide dismutase (SOD), and the catalase activity is also reported in the heart and brain of diabetic mice\(^8\). T. cardifolia root extract (TCE) has been reported to cause an increase in body weight, total haemoglobin and hepatic hexokinase\(^9\). However, it decreases hepatic glucose-6-phosphatase, serum acid phosphatase (ACP), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) in diabetic mouseresulting in hypo- glycaemic and hypolipidaemic effect\(^9\).

**DPPH scavenging activity**

T. cordifolia extracts scavenge free radicals produced during aflatoxicosis\(^{10}\). Cyclophosphamide (CP), an anti-cancer drug, decreases the GSH content in both the liver and bladder and reduces the levels of cytokines Interferon-\(\gamma\) and IL-2, an increased levels of pro-inflammatory cytokine TNF-\(\alpha\)\(^{11}\). This effect could be reverted on T. cordifolia treatment indicating the role of T. cordifolia in overcoming CP-induced toxicities in cancer treatment\(^{11}\). Pre-treatment of HeLa cells by TCE has been shown to diminish the cell viability, enhance LDH and reduce GSH S-transferase activity\(^{12,13}\). Dihydrotestosterone (DHT), a sex steroid and androgen hormone, in TCE accelerate the growth and proliferation of human LNCaP cells—androgen-sensitive human prostate adenocarcinoma cells. Androgenic compounds found in TCE act generally via androgen receptor\(^{14}\).

Numerous studies reveal that dichloromethane extract of T. cordifolia has the radiosensitizing activity in Ehrlich ascites carcinoma (EAC) mice that enables tumor-free survival via the depletion of GSH and glutathione-S-transferase by exalted levels of lipid peroxidation and the modification of DNA of tumour cells\(^{10,15,16}\).**

**MATERIALS AND METHODS**

**Collection of sample**

*T. Cordifolia* (L.) plant material were collected from medicinal plants conservation area, Thandarai, Chengalpattu, Tamil Nadu, South India (12°41’04.6”N79°58’59.6”E) in the month of September 2014 (Fig. 1).

**Isolation of endophytic fungi**

The root and transition zone of *T. Cordifolia* were surface sterilized\(^{17}\). The samples were thoroughly washed with running tap water and all the visibly damaged materials were excluded. Plant parts were rinsed in 0.1% Tween 20 for 30 s and followed by bevistine (1%) for 2 to 3 min to inhibit the fungal growth, sequentially immersed in 0.1% sodium hypochlorite for 30 s and in 70% w/v ethanol for 3 to 5 min. After each treatment, the samples were rinsed three times in sterile distilled water. They were aseptically dissected to expose cortex region and placed onto Water Agar (WA) medium, supplemented with Streptomycin 250 mg/L to inhibit the growth of other organisms, incubated for 12 to 15 days at 28°C in dark. The emerging fungal propagules were isolated, purified and maintained by subsequent sub culturing.

**CULTURE MEDIA AND EXTRACTION**

The isolates were grown in 500 ml conical flasks containing 200 ml of potato dextrose broth (PDB). Three mycelial agar plugs (0.5 cm) were used as inoculum and the organism was grown at 25 ± 2°C statically for 21 days. The culture was extracted by using chloroform: methanol (3:1). The solvent layer was collected and then evaporated in a rotary evaporator under vacuum.

**PHYTOCHEMICAL ANALYSIS**

Endophytes produce a broad variety of secondary metabolites with unique structure, including alkaloids, benzopyranones, chinones, flavonoids, phenolic acids, quinines, steroids, terpenoids, tetralones and xanthones\(^{18}\). Thus, the presence of various phytochemicals in the extract was estimated (Table 1)\(^{19}\).

**ANTIBACTERIAL ACTIVITY**

A total of eight fungal isolates (TC-1 to TC-8) were screened for antibacterial activity against human Gram
positive pathogens such as *Bacillus subtilis* (ATCC 441), *Staphylococcus aureus* (ATCC 25923), and Gram negative pathogens *Escherichia coli* (ATCC 25922) and *Klebsiella pneumoniae* (ATCC 15380) using disc diffusion method\(^\text{20}\). Petriplates were prepared with 20 ml of sterile Mueller Hinton Agar (MHA) (Hi-media, Mumbai). The test culture (100 µl of suspension containing 108 CFU/ml bacteria) were swabbed and allowed to dry for 10 min. The discs were impregnated with 20 µl of extract (10 mg/ml) at different concentrations of (5, 2.5 and 1.25 mg/disc) and placed on the medium and incubated at 37°C for 24 h. Reference antibiotic Streptomycin (10 µg/disc) was used. Out of eight samples, one sample (TC-2) showed relatively good result compared to the others (Table 2), which was further analysed.

### Extraction of metabolite from endophytic fungi from the root of *T. cordifolia*

Chloroform–methanol crude extract of endophytic strain of TC-2 belongs to *T. cordifolia* showed significant antibacterial activity. Three-fold volume of the solvent was used for continuous extraction. The extraction repeated up to three times with the solvent. The solvent extracts were dried using rotary evaporator\(^\text{21}\).

### In vitro assay for anti-cancer activity (MTT assay)

Cells (1 × 10\(^5\)/well) were plated in 24-well plates and incubated in 37°C with 5% CO\(_2\) condition. After the cell reaches the confluence, the sample with various concentrations was added and incubated for 24 h. After incubation, the sample was removed from the well and washed with phosphate-buffered saline (pH 7.4) or MEM without serum. 100 µl/well (5 mg/ml) of 0.5% 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl–tetrazolium bromide (MTT) was added and incubated for 4 h. After incubation, 1 ml of DMSO was added in all the wells. The absorbance at 570 nm was measured with UV- Spectrophotometer using DMSO as the blank\(^\text{22}\). The % cell viability was calculated using the following formula:

\[
\text{% cell viability} = \frac{A_{570 \text{ of treated cells}}}{A_{570 \text{ of control cells}}} \times 100
\]

The results are tabulated in Table 3. A systematic study of root endophytic fungi of *Tinospora cordifolia* (Willd.) Miers against MCF-7 cancer cell lines and human pathogens in shown in Fig. 2.

### RESULTS AND DISCUSSION

Totally eight endophytic fungal strains were isolated from the root transition zone of *T. cordifolia*. Among them, the chloroform methanol (3:1) extracts of TC-1, TC-3, TC-4, TC-5, TC-6, TC-7, TC-8 (*T. cordifolia* endophytic...
fungi) did not show any significant antimicrobial activity in which the strain TC-2 exhibited significant activity.

Morphological study of the strain was typically possesses small hyphae, as a white colony mycelium, when young. The mycelia were filamentous, branched, septate and slow-growing; spores were cylindrical to oval, resembling with the genus Fusarium. However, to confirm the species, molecular identification of the endophytic strain will also be performed.

The Ehrlich ascites carcinoma mice tested under different concentration of TCE showed a dose-dependent elevation in tumor-free survival and a relatively higher number of survivors were also observed. In our study, the chloroform:methanol extract of root-endophytic fungi from T.Cordifolia (TC-2) shows an increased antimicrobial activity, compared with other isolates. Similar studies revealed that crude extracts from culture broth of endophytic microorganisms displays antibacterial, antifungal, antiviral, anti-inflammatory and anti-tumor activity. Since there are no earlier reports on the root endophytes from this plant, hence an attempt was made to isolate and test the isolated fungi for anti-microbial and anti-cancer property. They may have potent application in biotechnological or pharmaceutical processes. The future study can be conducted in other cell lines to determine the anticancer potential.

CONCLUSION
The root-endophytic fungi of T. cordifolia also produce similar compounds having significant effect against human pathogens, Bacillus subtilis and Staphylococcus aureus, and Klebsiella Pneumonia but not in MC7 cell lines as the aerial parts of it produce. They may have potent application in biotechnological or pharmaceutical processes. The future study can be conducted in other cell lines to determine the anticancer potential.
Action of root-endophytic fungi of Tinospora cordifolia (Willd.) Miers against cancer cell lines

Fig. 4  Cytotoxicity of live human breast adenocarcinoma (MCF-7) cells under different concentration.

AUTHOR CONTRIBUTIONS

A.T. and M.F. conceived and designed the experiments; A.T. performed the experiments and analyzed the data; M.M. contributed reagents/materials/analysis tools; A.T. wrote the paper M.F. reviewed.

CONFLICTS OF INTEREST

None declared.

ACKNOWLEDGEMENT

We thank Dr. Florida Tilton, Biozone Research Technologies, Chennai for helpful discussion and providing technical assistance.

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