**Dual Role of Endophytic Fungi from the Roots of Terminalia arjuna (Roxb.) Wight & Arn**

**ABSTRACT**

Plant endophytes constitute different crucial parts of microbial differences. Plant endophytic fungi are an imperative and novel asset of natural bioactive metabolites with their different potential applications in different industries including medicine. This article focuses on the root of Terminalia arjuna, the plant with rich ethnobotanical uses, for the nearness of endophytic organisms and to decide their antibacterial and anticancer potential from its common natural surroundings.

Considering the significance of this plant based on the various research, antibacterial activity was tested against four human pathogenic microorganisms in bioactive metabolites and were subjected to different tests to decide phytochemical constituents. After the screening, MTT was performed in the crude extract. The outcomes demonstrate that the crude extract of fungal endophytes of T. arjuna have both antibacterial effects and anticancer effect when tried in MCF-7 cell line.

**KEYWORDS** Terminalia arjuna (Roxb.) wight & Arn., root endophytes, phytochemicals, MTT, anticancer, antimicrobial

**INTRODUCTION**

Terminalia arjuna Roxburgh, a tropical woody tree growing all through India and traditionally known as Kumbuk; Arjuna in English, belongs to the Combretaceae family, which incorporates 250 types of Terminalia. The bark of the plant is known to have a crystalline compound, arjunic, a lactone, arjunetin, fundamental oil and lessening sugar. Other than these, it contains 34% calcium carbonate, 9% of different salts of calcium, 13% tannin and aluminum, magnesium, natural acids, shading matter and other substances. The plant shows an assortment of restorative properties, for example, fungicidal, antibacterial, antifertility and antihuman immunodeficiency infection incited sickness, hostile to dysenteric, antipyretic, astringent, cardiotonic, lithotriptic and tonic. Based on the nearness of different components, the plant is thought to be valuable in the treatment of cancer. It has as of now been accounted for that the powdered bark is customarily utilized as diuretic and general tonic in instances of cirrhosis of the liver. In this context, compounds of T. arjuna have cancer cell development inhibitory action against different cell lines, for example, P388, OVCAR-3, SF-295, A498, NCI-4460, KM20L2 and SK-MEL-5. The polar concentrate of the leaves of T. arjuna contains a pentacyclic compound, ursolic acid, and a glycoside, β-D-glycoside. The previous research indicated both hostile to leishmanial and anti-cancer activities.

**Antimicrobial potential**

Organic extract of T. arjuna bark and leaves might be utilized to treat the bacterial ear pathogens particularly Staphylococcus aureus, which has demonstrated more noteworthy inhibition zones than the herbal drops. The polar fraction of the bark could be utilized for the advancement of novel antimicrobial agents, especially against urinary tract infections, and candidiasis/candidaemia. Mandal et al. explored antioxidative and antimicrobial properties of methanolic concentrate of T. arjuna bark. The antimicrobial activity demonstrated that higher inhibition against gram negative microbes than gram positive microscopic organisms and demonstrated a
promising antioxidant activity, as ingestion of DPPH radicals diminished in DPPH free radical scavenging assay11. Therapeutic capability of T. arjuna bark concentrate enhanced myocardial capacity in streptozotocin (STZ)-instigated diabetic rats. In rats, it demonstrated a decrease in left ventricular weight (LVP), maximal rate of rise and fall in LVP (LV [dP/dt] max and LV [dP/dt] min), cardiac contractility index (LV [dP/dt] max/ LVP), and an ascent in LV end-diastolic pressure12. The impact of topical use of phytoconstituents fractionated from a hydroalcohol concentrate of the bark of the plant, T. arjuna, was surveyed on the recuperating of rodent dermal injuries utilizing as a part of in vivo models. The outcomes demonstrated a factually critical increment in the tensile strength the cut injuries and the percent epithelialization of excision wounds contrasted and control13.

Anticancer potential
A portion confined from T. arjuna was contemplated for its antimutagenic impact against 4-nitro-o-phenylenediamine (NPD), sodium azide and 2-aminofluorene (2AF), a promutagen utilizing analyzer strains of Salmonella typhimurium by applying the Ames assay in two distinct strains. The part repressed the mutagenicity of 2AF altogether in both strains while the revertant colonies instigated by NPD and sodium azide were decreased moderately14.

The fungal taxol obtained from a natural concentrate of the fungal culture had solid cytotoxic activity towards BT220, H116, Int 407, HL 251 and HLK 210 human tumor cells in vitro when tried utilizing an apoptosis assay15. Casuarinin, a hydrolyzable tannin separated from the bark of T. arjuna L. (Combretaceae), was researched for its antiproliferative movement in human breast adenocarcinoma MCF-7 cells. The outcomes demonstrated that casuarinin hindered the multiplication of MCF-7 by blocking cell cycle movement in the G0/G1 stage and instigating apoptosis16. T. arjuna prompted cytotoxicity in HepG2 cells in vitro. Apoptosis of HepG2 cells might be because of the DNA harm and articulation of apoptotic proteins. Consumption of GSH might be included in the enlistment of apoptosis of HepG2 cells17.

MATERIALS AND METHODS

Collection of sample
T. arjuna (L.) plant was collected from therapeutic plants preservation range, Thandarai, Chengalpattu, Tamil Nadu, South India (12°41’04.6”N79°58’59.6”E) in the month of September 2014 (Figure 1).

Isolation of endophytic fungi
The root and transition zone of T. arjuna were surface sterilized18. The specimens were altogether washed with running faucet water and all the unmistakably harmed materials were barred. Plant parts were flushed in 0.1% Tween 20 for 30 s and took after by bevistine (1%) for 2 to 3 min to hinder the fungal growth, successively inducted in 0.1% sodium hypochlorite for 30 s and in 70% w/v ethanol for 3 to 5 min. After every treatment, the specimens were flushed three times in sterile distilled water. They were aseptically dissected to uncover cortex area and set onto water agar (WA) medium, supplemented with streptomycin 250 mg/L to restrain the development of different living beings, hatched for 12 to 15 days at 28°C in dark. The developing fungal propogules were segregated, cleansed and kept up by consequent sub culturing.

Culture media and extraction
The isolates were grown in 500 ml flasks containing 200 ml of potato dextrose broth (PDB). Three mycelial agar plugs (0.5 cm) were utilized as inoculum and the organisms were developed at 25 ± 2°C statically for 21 days. The culture was extracted by using butanol. The dissolvable layer was gathered. Three-fold volumes of the solvent was utilized for persistent extraction. The extraction rehashed up to three times with the solvent. The solvent extract was dried utilizing rotational evaporator19.

Phytochemical analysis
Endophytes produce a broad variety of secondary metabolites with unique structure, including alkaloids, materials were barred. Plant parts were flushed in 0.1% Tween 20 for 30 s and took after by bevistine (1%) for 2 to 3 min to hinder the fungal growth, successively inducted in 0.1% sodium hypochlorite for 30 s and in 70% w/v ethanol for 3 to 5 min. After every treatment, the specimens were flushed three times in sterile distilled water. They were aseptically dissected to uncover cortex area and set onto water agar (WA) medium, supplemented with streptomycin 250 mg/L to restrain the development of different living beings, hatched for 12 to 15 days at 28°C in dark. The developing fungal propogules were segregated, cleansed and kept up by consequent sub culturing.

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Phytochemical analysis
Endophytes produce a broad variety of secondary metabolites with unique structure, including alkaloids,
benzopyranones, chinones, flavanoids, phenolic acids, quinines, steroids, terpenoids, tetrалones and xanthones. Thus, the presence of various phytochemicals in the extract was assessed (Table 1).

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

Cells (1 × 10⁵/well) were plated in 24-well plates and hatched in 37°C with 5% CO₂ condition. After the cell achieves the confluence, the specimen with different concentrations was added and hatched for 24 h. After incubation, the specimen was expelled from the well and washed with phosphate-supported saline (pH 7.4) or MEM without serum. 100 µl/well (5mg/ml) of 0.5% 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-tetrazolium bromide (MTT) was included and incubated for 4 h. After incubation, 1 ml of DMSO was added every one of the wells. The absorbance at 570 nm was measured with UV-Spectrophotometer utilizing DMSO as the blank. The % cell viability was ascertained utilizing the following equation:

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

### Table 1

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Name of test</th>
<th>Carbohydrates</th>
<th>Tannins</th>
<th>Saponins</th>
<th>Flavonoids</th>
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<th>Quinones</th>
<th>Flavonoids</th>
<th>Glycosides</th>
<th>Terpenoids</th>
<th>Triterpenoids</th>
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### Table 2

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Plant: <em>Terminalia Arjuna</em></th>
<th>Zone of Inhibition (mm)</th>
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<tr>
<td>B1</td>
<td>B4</td>
<td>B7</td>
</tr>
<tr>
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<td>−</td>
</tr>
<tr>
<td>TA-2</td>
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</tr>
<tr>
<td>TA-3</td>
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</table>

B1: Bacillus subtilis (+); B4: Staphylococcus aureus (+); B7: *E. Coli* (-); B9: Klebsiella pneumonia (-).

### Table 3

<table>
<thead>
<tr>
<th>Sample concentration (mg/mL)</th>
<th>% Cell viability</th>
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<tbody>
<tr>
<td>0</td>
<td>100</td>
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<tr>
<td>50</td>
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<td>28.99</td>
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Dual role of endophytic fungi from the roots of *Terminalia arjuna* (Roxb.) Wight & Arn

Fig. 2. A systematic study of root endophytic fungi of *Terminalia arjuna* (Roxb.) Wight & Arn. against MCF-7 cancer cell lines and human pathogens.

Fig. 3. Cell viability measured by MTT assay showing cytotoxicity of on MCF-7 cell lines treated with different concentration described in Table.

The outcomes are classified in Table 3. A deliberate investigation of root endophytic organisms of *T. arjuna* (Roxb.) Wight & Arn against MCF-7 growth cell lines and human pathogens is shown in Fig. 2.

**RESULTS AND DISCUSSION**

Absolutely, three endophytic contagious strains were confined from the root move zone of *T. arjuna*. Among them, the butanol concentrate of TA-1 (*T. arjuna* endophytic fungi) did not demonstrate any critical antimicrobial action in which the strain TA-2 showed noteworthy action against *E. coli* alone yet not with different pathogens. Among all the three confines, TA-3 demonstrated fabulous movement against all the four positive and negative pathogens (Table 2). TA-1 indicated more grounded movement than the other two specimens (TA-2 and TA-3) in scavenging activity. In this manner, in view of the DPPH, cell cytotoxicity test was performed.

Morphological study and molecular identification of the endophytic strain will likewise be performed to affirm the species. At the point when the methanolic concentrate of *T. arjuna* and piperine segregated from black pepper is dealt with separately, they are not dynamic against A549 cell line. Be that as it may, when the concentrates given in mix demonstrate strong antitumor property than the standard Cisplatin. It is clear that the methanolic concentrate of *T. arjuna* is found to have both cell antioxidant and antidiabetic properties.

It has been seen from the past report that the bark, leaves, and stem extract have greatest anticancer and antibacterial potentials. Since there are no prior reports on the root endophytic fungi from this plant, henceforth an endeavor was made to disengage and test the detached growths for hostile to microbial and against tumor property. The breast cancer cells (MCF-7) treated with the fungal extracts sufficiently demonstrated cytotoxic impact under lower fixation going between 0 μg/ml and 500 μg/ml (Figures 3 and 4).

Subsequently of this first review, as far as anyone is concerned, led in root-endophytic organisms concentrate of *T. arjuna* demonstrated noteworthy anticancer action against MCF-7 cells yet insufficient antibacterial activity with the majority of the pathogens.
CONCLUSION

All the isolated root-endophytic fungi of *T. arjuna* may not produce the same or nearly the same characteristics compounds having critical impact against human pathogens, *Bacillus subtilis* (+), *Staphylococcus aureus* (+), and *Klebsiella pneumoniae* (−) and *E. coli* (−), however in MCF-7 cell lines as the aerial parts of it produce. They may have powerful application in biotechnological or pharmaceutical procedures. As of now, little work has been done on the antimicrobial movement and conceivable restorative uses of the phytochemical mixes, and henceforth extensive examinations are required, for example, in vivo studies on endophytic growth of this plant is important to determine toxicity of the active constituents, their symptoms, pharmacokinetic properties to exploit the bioactive standards, for remedial utility in treating different illnesses.

AUTHOR CONTRIBUTIONS

AT and MF conceived and designed the experiment; AT performed the experiment and analyzed the data; AT wrote the paper MF reviewed.

CONFLICTS OF INTEREST

None declared.

ACKNOWLEDGEMENTS

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REFERENCES

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