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Research Article

Evaluation of Antibacterial Activities of Endophytic Fungus *Chaetomium jodhapurense* Isolated from Medicinal Plant *Tridax procumbens* (L.)

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ABSTRACT

Tridax procumbens (L.) is a promising species that produces secondary metabolites reported to have a variety of medicinal uses including among others, anti-anemic, anti-inflammatory, anti-diabetic, and anesthetic properties. Endophytes are plant-associated microorganisms that live within the living tissues of their host plants without causing any harm to them. They stimulate the production of secondary metabolites with a diverse range of biological activities. The objective of the present research was to investigate fungal endophytes harboring in the tissues of the medicinal plant *Tridax procumbens (L.)* and assess its antimicrobial activity. In the present research article one of the isolated endophytic fungus *Chaetomium jodhapurense* was evaluated for its antibacterial potential. To isolate the endophytic fungus, the surface sterilization method was used. The isolate was identified on the basis of morphological characters and molecular analysis. The isolate was then cultivated on potato dextrose broth for 3 weeks and aqueous extract was analyzed for antibacterial activity against five bacterial strains i.e., *Staphylococcus aureus* (ATCC-6538), *Klebsiella pneumonia* (ATCC-4352), *Escherichia coli* (ATCC-8739), *Streptococcus pneumoniae* (ATCC-6303) and *Salmonella typhimurium* (ATCC-14028). The aqueous extract (25 ul) of the endophytic fungus *Chaetomium jodhapurense* showed broad-spectrum activity against five human pathogenic bacteria.

Keywords: *Chaetomium jodhapurense*; *Tridaxprocumbens (L.)* medicinal plant; Antibacterial activity

INTRODUCTION

Plants may serve as a reservoir of large numbers of microorganisms known as [1]. Endophytes are microorganisms (mostly fungi and bacteria) that inhabit plant hosts for all or part of their life cycle. de Bary in 1866 first defined all organisms that colonize internal plant tissues as endophytes. The study of endophyte distribution, biodiversity, and their biochemical characteristics are of immense importance in plant biology to understand and also to improve plant fitness. They colonize the internal plant tissues beneath the epidermal cell layers without causing any apparent harm or symptomatic infection to their host, living within the intercellular spaces of the tissues and it seems that they may penetrate the living cells [2].

Endophytic fungi are an ecological, polyphyletic group of highly diverse fungi, mostly belonging to ascomycetes and anamorphic fungi [3]. Individual plants may be host to one or more endophytes, and many endophytes may colonize certain hosts, suggesting that there may be many undiscovered endophyte species [4]. Most of endophytes produce a plethora of bioactive metabolites that may be involved in the host-endophyte relationship. These metabolites may serve as sources of novel natural products for exploitation in medicine, agriculture, and industry. The described populations of endophytic strains are few, which means there are good opportunities to find new endophytes that colonize plants in different niches and ecosystems. Endophytic fungi represent an important and quantified component of fungal biodiversity and are known to affect plant community diversity and structure [5,6].

Tridax procumbens (L.), also known as "coat buttons" is a perennial plant from the Asteraceae family, native to Central and South America [7]. Since ancient times, this species has been used in Ayurveda in India [8]. Different substances such as oils, teas, and skin poultices, among others, have been manufactured using this species. *Tridax procumbens* (L.) has diverse pharmacological properties including but not limited to

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immunomodulatory, anti-oxidant, anti-hepatotoxic, analgesic, antidiabetic, anti-inflammatory, antifungal, and antimicrobial activities [9,10]. The fungal endophyte *Chaetomium jodhapurense* was isolated from this ethnomedicinal plant and same was assessed for its antibacterial potency against five bacterial isolates: *Staphylococcus aureus* (ATCC-6538), *Klebsiella pneumonia* (ATCC-4352), *Escherichia coli* (ATCC-8739), *Streptococcus pneumoniae* (ATCC-6303) and *Salmonella typhimurium* (ATCC-14028).

MATERIALS AND METHODS

Sampling area and collection of plant materials

Mature healthy plant material such as leaves, stems, roots, and flowers of *Tridax procumbens (L.)* was collected from different localities of the Amravati district of Maharashtra state (India). The samples were collected during pre-monsoon (Feb-May), monsoon (June-Sept), and post-monsoon (Oct-Jan) seasons and subjected to surface sterilization. Samples were collected from symptomless plants, placed in zip-lock plastic bags carefully brought to the laboratory, and processed immediately thereafter or stored at 40°C so as to reduce the chances of contamination.

Processing of sample for isolation of endophytes

Collected plant samples were washed in running tap water to remove soil particles and adhered debris, and finally washed with distilled water. The stem, leaves, root, and flowers were cut into segments (0.5-sterilized by agitating in 70% ethanol (5 s), followed by treatment with 4% NaOCl (90 s), and then rinsed in sterile distilled water (10 s). 50 segments (leaf, stem, and root samples) from the *Tridax procumbens (L.)* plant was processed for the isolation of endophytic fungi. Leaf, stem, and root segments were then placed in petri dishes containing Potato Dextrose Agar (PDA) media, amended with Streptomycin 150 mg/l. The Petri dishes were sealed using para film and incubated at 28°C. The efficiency of surface sterilization was ascertained for every segment of tissue following the imprint method.

Isolation and identification of endophytic fungi

Endophytic fungi usually begin to produce hyphal filaments after 5-6 days of incubation at 20-280°C. The hyphal tips appeared and were carefully transferred to potato dextrose agar plates for further growth. The endophytic fungal isolates were stained with lactophenol cotton blue and were morphologically identified based on spore morphology with the help of a standard manual. Identification was authenticated from Agharkar Research Institute Pune.

Mass production of antibacterial metabolites

150 ml of Potato Dextrose Broth was prepared in 250 ml flasks and autoclaved at 15 lbs psi for 20 min. The medium was inoculated with isolated fungus culture and incubated at 28 ± 1°C in the incubator. After 7, 14, and 21 days of incubation, the crude culture broth was filtered and tested for antibacterial activity against the six pathogenic bacteria by using agar well diffusion methods.

Antibacterial activity

The agar well diffusion assay method was used to assess the antibacterial activity of the fungi. In this method, wells were aseptically made in the seeded media using a sterile cork borer, and the appropriate amount of the bioactive metabolite was dropped in the prepared wells and incubated at 37°C in a bacteriological incubator for 24 hours. Finally, plates were observed for zones of inhibition, and their diameter was measured with the help of the Hi-Antibiotic zone scale, Hi-Media Laboratories Mumbai.

There were five strains of pathogenic bacteria such as *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, *Escherichia coli*, *Salmonella typhimurium*, and *Staphylococcus aureus* were used to evaluate the antibacterial activity of fungi. These bacterial strains were taken from the American Type Culture Collection (ATCC).

RESULTS AND DISCUSSION

Since ancient times *Tridax procumbense (L.)* has been used as a medicinal plant in Ayurveda. *Tridax procumbens* has a long history of traditional use but isolation and evaluation of each phytochemical has not been properly related to its pharmacological properties and could show difficulty in reproducibility after isolation and evaluation [11]. Different substances such as oils, teas and skin poultices, among others, have been manufactured using this species. An array of natural products have been characterized from endophytes, including anti-cancer, anti-fungal, anti-bacterial, anti-viral, anti-oxidant, anti-insecticidal, and immunosuppressant. In the present investigation, an endophytic fungus *Chaetomium jodhapurense* was isolated from plant parts of *Tridax procumbens (L.)*. Five endophytic fungi from *Tridax procumbens (L.)-Aspergillus japonicus*, *Fusarium sp*., *Aspergillus niger*, *Alternaria sp*., and *Penicillium sp*. and assessed *Aspergillus japonicus* for antibacterial activities were also reported [12]. Two species of *Chaetomium* on *Tridax procumbens* but the endophytic fungus *Chaetomium jodhapurense* is reported for the first time by authors [13] (Table 1).

The aqueous extract (25 µl) of endophytic fungus *Chaetomium jodhapurense* was assessed for antibacterial activity against five isolates of pathogenic bacteria-*Staphylococcus aureus* (ATCC-6538), *Klebsiella pneumonia* (ATCC-4352), *Escherichia coli* (ATCC-8739), *Streptococcuspneumoniae* (ATCC-6303) and *Salmonella typhimurium* (ATCC-14028) showed the broad-spectrum activity against all five pathogenic bacteria. The aqueous extract of *Chaetomium jodhapurense* showed a zone of inhibition of 33 mm against *Staphylococcus aureus*, 32 mm against *Klebsiella pneumoniae*, 30 mm against *Escherichia coli*, 25 mm against *Streptococcus pneumoniae*, 22 mm *Salmonella typhimurium*. This activity may be due to the presence of different metabolites in the genus *Chaetomium*. The *Chaetomium* genus of kingdom fungi is considered to be a rich source of unique bioactive metabolites. These metabolites belong to chemically diverse classes, i.e., chaetoglobosins, xanthones, anthraquinones,

chromones, depsidones, terpenoids, and steroids. *Chaetomium* through the production of diverse metabolites can be considered as a potential source of antitumor, cytotoxic, antimalarial, antibiotic, and enzyme-inhibitory lead molecules for drug discovery [14].

It is known that the endophytic fungi existing in the plant are potential sources of antimicrobial substances and this has also been demonstrated in earlier studies but the antibacterial activities of the endophyte isolate *Chaetomium jodhapurense* is reported for the first time [15,16].

Pathogenic bacterium	Zone of inhibition(mm)
Staphylococcus aureus	33
Klebsiella pneumoniae	32
Escherichia coli	30
Streptococcus pneumoniae	25
Salmonella typhimurium.	22

Table 1: Screening of *Chaetomium jodhapurens* against five pathogenic bacteria.

CONCLUSION

The study showed that the *Chaetomium jodhapurens* show antibacterial activities against *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, and *Salmonella typhimurium*. Further investigation may provide evidence of biochemicals in the form of secondary metabolites responsible for this antibacterial activity.

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