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STUDIES ON THE ANTIMICROBIAL ACTIVITY OF THE SOLVENT EXTRACT OF GARLIC'S ENDOPHYTIC FUNGAL ISOLATES

KALIDOSS RAJENDRAN[1*], MURUGAN THIRUMALAI[2], HAANIA ISHAQ[3], SAHANA R K[4], KALPANA RAMAMURTHY[5], MUTHUKRISHNAN P[6] and MARIRAJ MURUGAN[7]
[1]Department of Microbiology, PSG College of Arts & Science, Coimbatore, TN, India
[2]Department of Sciences, Mathematics and Education, St. Joseph University in Tanzania,
[3]Department of Biology, The University of Haripur, Haripur, Pakistan
[4]Department of Biotechnology, SRM Institute of Science and Technology, TN, India
[5] Department of Biology, Arsha Vidhya Mandir, Guindy, Chennai, India
[6]Department of Microbiology, PSG College of Arts & Science, Coimbatore, TN, India
[7]Department of Botany, Sri Kaliswari College, Sivakasi, T.N, India
[*]**Corresponding Author's Email:** doss_rk@yahoo.com

This study was aimed at exploring and observing the antimicrobial activities of secondary metabolite compounds (bioactive) of endophytic fungi from *Allium sativum* as common medicinal plants in Tanzania against *Staphylococcus aureus* and *Escherichia coli* together with the fungus *Candida albicans*. Healthy, undamaged leaves and stems of the *Allium sativum* plants were collected from the botanic garden and processed separately. Ethyl acetate extracts of garlic leaves and garlic stems of endophytic fungi were extracted from potato dextrose broth and tested against *Candida albicans*, *Escherichia coli*, and *Staphylococcus aureus* species by using the Kirby-Bauer disc diffusion method. The diameter of the zone of inhibition was measured in millimeters (mm). The study reveals that the crude extract of endophytic fungal isolate from both leaves and stems of *Allium sativum* shows moderate antimicrobial activity against *Candida albicans, Staphylococcus aureus,* and *Escherichia coli.*

Keywords: Secondary metabolite, Ethyl acetate extract, potato dextrose broth, Endophyte, and Kirby-Bauer disc diffusion.

Introduction

The Greek terms "endon" (inside or within) and "phyton" (plant) are the source of the word "endophyte. They are the microorganisms showing a kind of relationships in plant hosts throughout the entire part of lives or during a particular phase. They populate the internal plant tissue underneath epidermal cell layers without producing any symptomatic infection or obvious harm to their host, surviving within the tissue's intercellular gaps and appearing to penetrate living cells[1]. Endophytes create inconspicuous infections within stem, root and leaf tissues without showing any external infections on the host plants. They don't show any symptoms until the hosts are disturbed. The word "endophyte" refers to bacteria and fungi found in the tissues of seemingly healthy plant hosts at a given time[2]. Endophytes create a variety of secondary metabolites, some of which are antibiotics, poisons, pigments, and waste products, Secondary metabolites produced by these endophytes serve a variety of functions in various fields, including agriculture (crop protection by inducing disease resistance, plant growth stimulants by increasing phosphorus uptake, siderophores production, plant hormones and nitrogen fixation. Endophytes offer bioactive chemicals that are employed in the production of medicines for anticancer and antibacterial properties[3-5]. There is more

than one endophyte colonizing every plant within the hosts inter or intracellular tissues, which secretes bioactive compounds[6-7]. Therefore, the aim of this study is to isolate the endophytic fungi from *Allium sativum*, a common medicinal plant in Tanzania, as well as its antimicrobial activities.

Some of the conventional herbal remedies and endophyte derivatives that are still in use today for medicinal purposes include quinine, aspirin, and opium. According to the World Health Organization (WHO), herbal medicine is used by 80% of the population in some Asian and African countries as part of their basic health care[8]. The search for novel compounds with a wide variety of pharmacological and therapeutic properties that are produced from wild resources has been ongoing for a while now.

This sparked extensive research into organic molecules produced by a wide range of plants and bacteria that flourish in different environments and exhibit a variety of behaviors. From the endophytic fungal isolates of medicinal plants, numerous potentially innovative therapeutic substances are produced. Plants grow slowly, whereas plant-derived fungal isolates can be cultured quickly. Because the extraction and purification of chemicals from endophytic fungus is simpler than with slow-growing medicinal plants, it is therefore a possible substitute. Moreover, by cultivating endophytic fungi, one can preserve natural biodiversity rather than exploiting medicinal plants for human use. Furthermore, using a microbiological source to manufacture a high-value phytochemical is simpler and less expensive, resulting in greater product availability and lower market prices[1]. Tanzania is also rich in natural medicinal plants, such as garlic (*Allium sativum*), which is used for a range of purposes, including economics and the treatment of ailments including cough, abdomen discomfort, chest relief, lung infection, cold, and flu. So, the goal of this research is to analyze and assess the microbial susceptibility towards the secondary metabolic chemicals or compounds (bioactive) of endophytic fungi from garlic.

So, the goal of this study is to look into and analyze the antibacterial activity of secondary metabolite chemicals (bioactive) found in endophytic fungi from garlic against clinical bacteria, as well as fungal pathogens. The study's goal is to isolate and test for antibacterial properties of the solvent extracts of endophytic fungal isolate from *Allium sativum*, as well as to morphologically identify the endophytic fungal isolate.

Material and Methods

Endophytic fungal isolate was cultured from individual plant parts, such as leaves and stems of the garlic plant, using procedures outlined by Maheswari and Kokila[9]. The separated pieces were thoroughly cleaned with tape water to remove any debris or soil contamination before being air-dried. The samples were chopped into small 5cm pieces and washed for a few seconds with tween 20 (0.1%). The surface sterilization of the sample was carried out by soaking the leaves and stems for 3 minutes in 70% ethanol, for 60 seconds in 0.4% sodium hypochlorite and for 2 minutes in 70% ethanol, followed by rinsing the samples three times with sterile distilled water. A lamina air flow chamber was used for drying the samples. The outer tissue was removed, and the interior tissue, measuring about 0.5cm, was dissected. Potato Dextrose Agar (PDA) was used for culturing the endophytic fungal isolates and the plates were stored in an incubator at 27 ± 2 ºC for 4-6 weeks in the dark. The petri dish was checked frequently to monitor the growth of fungal isolate. Each fungal isolate was purified using the hyphae tip method, which involves cutting the hyphal tip of a

developing endophytic fungus with a sterile blade and transferring it to a half strength PDA plate, followed by 7-14 days of room temperature incubation. The pure culture was subsequently obtained by serial subculture.

In each 250 mL conical flask with 200 mL of Potato Dextrose Broth, colonies of endophytic fungal isolates were inoculated. The flask was held at 25°C for two weeks in a mechanical shaker. After the incubation period, metabolites from the fungus fermentation broth were extracted with ethyl acetate and ethanol as organic solvents. Approximately 200ml of ethyl acetate was added to the broth, vigorously shaken for 15 minutes, and held in a separating funnel for 15 minutes until two distinct immiscible layers formed. The initial aqueous layer was drained from the separating funnel and placed in a clean beaker, followed by the organic layer containing the extracted chemicals. The beaker containing the secondary metabolite was dried for 6 days until all of the ethyl acetate was gone evaporated, leaving only the bioactive molecules. The bioactive chemicals obtained were diluted in 1 mg/ml dimethyl sulphoxide and used as a stock solution for an antibacterial experiment[10].

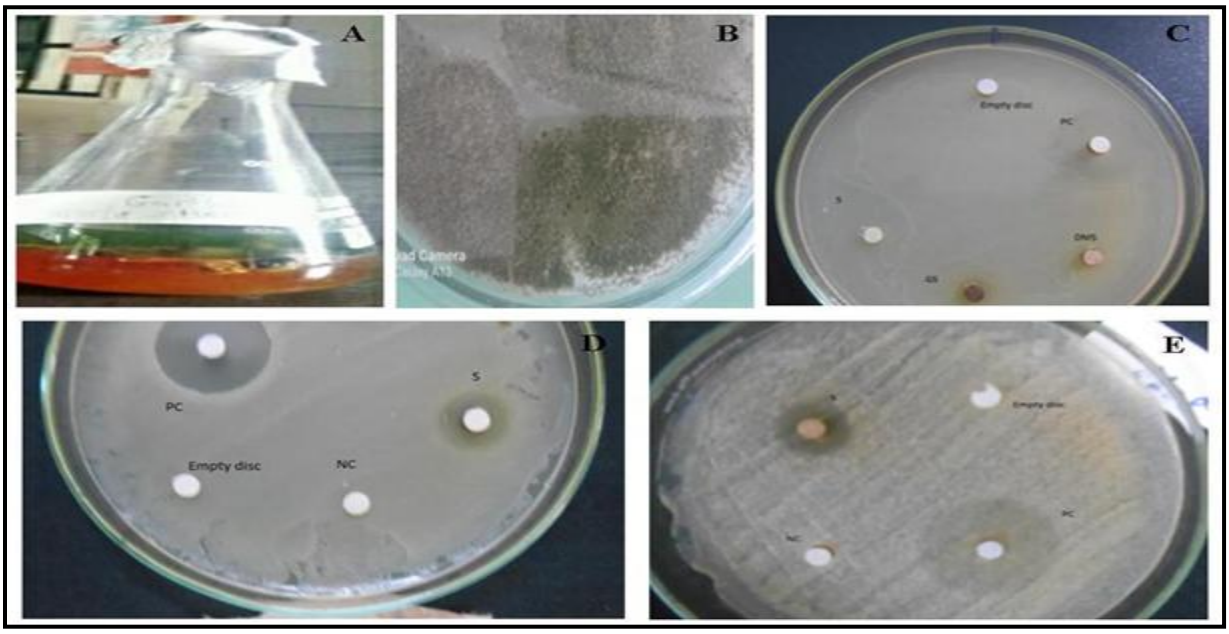

Figure 1: Shows Endophytic fungal isolates and antimicrobial activity of garlic leaf extract
A) *Aspergillus* spp. of a stem; B) *Aspergillus* spp. of leaves; C) Petri dishes showing zone of inhibition (mm) of garlic plant extract: Endophytic fungal isolate of garlic leaf ethyl acetate extract against *Staphylococcus* bacteria; D) Petri dishes showing zone of inhibition (mm) of garlic plant extract: Endophytic fungal isolate of garlic leaf ethyl acetate extract against *Escherichia coli;* E) Petri dishes showing zone of inhibition (mm) of garlic plant extract: Endophytic fungal isolate of garlic leaf ethyl acetate extract against *Candida albicans* (S – sample, NC – Negative control, PC – Positive control, DMS- Dimethyl sulfoxide)

Antimicrobial test

The antimicrobial test was done using the Kirby Bauer disc diffusion method (the reference method used in laboratories to determine the sensitivity of isolates) with certain modifications. About 100µl of prepared overnight fungal and bacterial test strains were swabbed on potato dextrose

agar and nutrient agar plates, respectively. Sterile Whatman discs 6 mm were added with ethyl acetate crude extract, left to dry, and then placed on prepared petri dishes containing the test strain. Discs containing dried water served as a negative control and reference antimicrobial drug (ampicillin for bacteria 30 µg/ml and flucanazole for *Candida*, 50 µg/ml) served as positive control (Table.1).

Table 1: Shows the antimicrobial activity of ethyl acetate extract.

S. No.	Plant Samples	Inhibitory Zone		
		S. aureus	***E. coli***	***C. albicans***
1	*Aspergillus* spp. isolate from garlic leaf	23±1.00	7.00±1.00	8.00±2.00
2	*Aspergillus* spp. isolate from garlic stem	12±1.53	10±2.51	4±3.78

Result and Discussion

Pure endophytic fungus *Aspergillus* spp. was characterized using both macromorphological observations of colony color and micromorphological observations of hyphae morphology and spore morphologies under a binocular microscope. Based on the shape and culture features, an endophytic fungal isolate was identified. The vegetative structure was showing septate hyphae. The conidia were subspherical to spherical and carried long chains called conidial heads. The conidiophores had shown a long, thin structure that was unbranched. The conidiophores had shown a distinctive bottlebrush-like structure. The vegetative structure was showing septate hyphae. The colony morphology of garlic leaf and stem fungal isolates were light green and dark green, respectively as shown in Figure 1 A and B. The Department of Microbiology at the St. Joseph University in Tanzania, Boko campus provided the *Escherichia coli*, *Staphylococcus aureus* and the fungi *Candida albicans*. To obtain bacterial inoculums of the test strains, cultures were cultured overnight in a nutrient broth and 0.5 McFarland units (about 10^6 CFU/ml for fungi and 10^2 CFU/ml for bacteria) was set as a standard to compare the microbial turbidity.

In this work, solvent extracts of endophytic fungal isolates cultured from *Allium sativum* (leaves and stem) were tested against two human bacterial pathogens (*Staphylococcus aureus* and *Escherichia coli*) and one fungal pathogen (*Candida albicans*). The study discovered that the ethyl acetate crude extract had shown an excellent antibiotic action against *S.aureus*, *E. coli*, and *C. albicans*, as shown in Figures 1 C, D and E. Furthermore, the crude extracts result reveals strong antibacterial activity against Staph as opposed to *E. coli*, as revealed by other prior studies, which also reported that Gram-negative bacteria are resistant to most antibacterial activity due to the presence of lipopolysaccharide protein in the cell and the presence of a thinner layer of peptidoglycan, which makes its cell more complex than Gram-positive bacteria[11]. This discovery is consistent with prior study, which discovered that garlic has high antibacterial and antifungal activities due to the presence of allicin (made by the phosphopyridoxal enzyme allinase) and ajoene bioactive chemicals. Thus, the suppression of fungus seen in this study could

be attributed to allicin or ajoene, which block the activity of several enzymes crucial to fungi[12-13]. A similar study conducted by Sathiyaseelan *et al.* showed that the ethyl acetate extract of *Paraconiothyrium brasiliense* had contained flavonoid and phenolic compounds[14].

Conclusion

It is apparent from observation that garlic stems and leaves contain endophytic fungi that produce a variety of secondary metabolites that fight against human bacterial and fungal infections, with variations in response that could be attributable to structural composition variances.

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