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Assessment of Antibacterial and Antifungal Efficacy of Endophytic Fungi Isolated from *Psoralea Corylifolia* Linn.

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Abstract

Endophytic fungi that inhabit host plants symbiotically and cause no harm to the host plants. In past few decades variants of endophytic fungi have been isolated and identified with medicinal properties finding wider application as bio-control agents, bio-fertilizer, bio-stimulant and natural products. In lieu of this, the present study aims to isolate, identify, and evaluate the antibacterial and antifungal activities of endophytic fungi obtained from *Psoralea corylifolia* Linn. Total 4 endophytic fungi were isolated from the root, stem, leaves and fruits of host plant that is *Piriformospora indica, Alternaria alternata, Aspergillus niger, Penicillium citrinum.* Antibacterial activity of three crude extracts including butanol, ethyl acetate, and methanol of different fungal endophytes was appraised against *Escherichia coli, Proteus vulgaris, and Salmonella typhi*, *Bacillus subtilis*, and *Staphylococcus aureus* and Antifungal efficacy of different crude extracts of fungal endophytes was also appraised against three pathogenic fungal strains including *Aspergillus niger, A. fumigatus* and *Candida albicans.* The result revealed that the maximum antibacterial efficacy was found in methanol extract of *Piriformospora indica* in the test organism *bacillus subtilis* that was 20.3±0.57 followed by ethyl acetate that was 18.66±0.57. The maximum antifungal activity was found in methanol extract of *Alternaria alternata* in the test organism *Aspergillus niger* that was 43.1 followed by *Penicillium citrinum* in the test organism *Aspergillus fumigatus* that was 42.8. More study will be done in future on exploration and exploitation of these microorganisms for pharmaceuticals applications and also used as a biocontrol agent. The present study is the first report on the investigation of antifungal and antibacterial properties of endophytic fungus that have been isolated from *Psoralea corylifolia.*

Keywords: *Psoralea corylifolia*, endophytic fungi, bio-control agent, microbial ecology, antimicrobial efficacy

Introduction

De Bary first coined the word "endophyte" to describe all organisms that reside inside plant tissues in 1866 (Hyde and Soytong, 2008; Aswani *et al*., 2020). The term was coined during the study of endophytic tree leaf fungi and was used to describe fungi that can colonise the internal tissues of plants without appearing to harm them, including latent pathogens that can live asymptomatically in their host for a while (Petrini, 1991). From the arctic to the tropics, from agricultural fields to the most biotically diverse tropical forests, endophytic fungi are found in the above-ground tissues of liverworts, hornworts, mosses, lycophytes, equisetums, ferns, and seed plants. Endophytes are found in all of the plant species that have been studied to date and are rich in biodiversity. Numerous of them have the ability to incorporate bioactive substances that plants can utilise to defend themselves against infections, and some of these substances have been shown to be helpful in the search for new drugs. According to their diverse functional components, the majority of natural endophyte products have so far been discovered to be antibacterial agents, anticancer agents, and other bioactive substances (Guo *et al*., 2008). Endophytic fungi contain a wide range of structurally varied chemical substances, including peptides, alkaloids, polyketides, terpenoids, and phenolics, among their many antibacterial leads (Deshmukh *et al*., 2022; Rai *et al*., 2023).
Numerous plant species have had their potential medical uses thoroughly examined in recent years. Because traditional medicine systems are thought to be safe for use by both humans and the environment, and because they are a rich source of essential substances, they have gained international attention. Plants have medicinal significance because of their phytochemical components, which may be used to cure illnesses in humans. A member of the Fabaceae family, *Psoralea corylifolia* is also referred to as "Babchi". This is a 60–100 *cm* tall annual plant that is upright. Psoralen-containing sticky, oily pericarp envelops the seeds. It is used locally for psoriasis, eczema, laparoscopy, alopecia, albinism, cardiac, vasodilator, pigment, antibacterial cytotoxic, and anti-helminthic properties. The Fabaceae family plant *Psoralea corylifolia* L. is important to Chinese and Ayurvedic medicine. Indigenous medical traditions use its seeds to treat a variety of illnesses (Chopra *et al*., 2006). The presence of bakuchiol in the *P. corylifolia* seed extract was found to be responsible for its antibacterial activity against both Gram-positive and Gram-negative bacteria. The seeds are used to treat fevers and have anti-helminthic, aphrodisiac, diuretic, and laxative qualities. The whole part of plant *Psoralea corylifolia* have medicinal value as many bioactive compounds are found in this plant. According to Kuo *et al*., 2021, total 113 fungal strains were isolated from six fungal genera. These six fungal genera are *Penicillium, Colletotrichum, Diaporthe, Daldinia, Alternaria* and *Didymella*. The present study was the first work on this plant to isolate and identified the endophytic fungi present inside the plant and the antibacterial and antifungal activities of that isolated fungus.

Material and Methods

Plant collection

Fresh stem leaves, roots and fruits of *Psoralea corylifolia* Linn. (RUBL10309) were collected from Nahargarh Biological Park Kukus Jaipur Rajasthan. The collected plant's authenticity was validated by assigning a voucher specimen number, which was recorded in the herbarium of the Department of Botany at the University of Rajasthan in Jaipur. Samples were delivered to the laboratory of Nims University Rajasthan Jaipur.

Sample sterilization, media preparation and isolation

The plant materials were surface sterilised in order to eliminate external pollutants. Plant materials were washed with distilled water. Surface sterilization was carried out by dipping in 75% ethanol for 1 minute. The sample further sterilize by sodium hypochloride (NaOCl) for 3 minutes then washed with distilled water. Again, surface sterilize in 75% ethanol for 30 second and washed with distilled water. The sterilised plant materials were chopped into tiny pieces under sterile circumstances (such as in a laminar flow hood and the size of pieces were 2 and 5 mm long). Placed the chopped plant pieces in Petri plates on top of an appropriate culture medium. Potato Dextrose Agar was a frequently used medium for this fungus growth (PDA). To prevent bacterial growth, antibiotics such as penicillin was added to the medium. The sealed Petri plates kept in incubator for four days for growth of fungi (Abdelwahab *et al*. 2021, Abdelkader *et al*. 2022). The media was subcultured to discern the ultimate outcome. Once pure cultures have been obtained, used their morphological features to identify the fungi.

Identification of endophytic fungi

Endophytes are diverse and often grow readily in culture. For characterisation of the morphology of fungal isolates, slides were prepared from cultures and they were stained with lactophenol blue reagent and examined under a brightfield and phase-contrast microscope, identified by referring standard manuals (Barnett and Hunter, 1972). Identification of isolated culture of endophytic fungi was based on morphological characteristics such as growth pattern, characteristics of the spores, presence of mycelium and production and characteristics of spores. Microscopic observation of endophytic fungi was isolated in the present study was performed by preparing of temporary mount of staining of lactophenol cotton blue. Prepared slides were observed under light microscope at 40x and some 100x magnification and photographed.

Test organisms

Disc diffusion method was used for antibacterial assay. Both Gram positive and Gram-negative bacteria were used for this assay: *Bacillus subtilis, Bacillus subtilis, Escherichia coli, Proteus vulgaris, Salmonella typhi* and *Staphylococcus aureus* pathogen. Tube dilution method was used for screening of anti-fungal activity. For antifungal assay, *Aspergillus niger, A. fumigatus* and *Candida albicans* were used.

Antibacterial Assay

The disc diffusion method was used to measure the antibacterial activity (Bauer *et al*., 1966; Mishra *et al*., 2016a, b). Using this technique, microbial culture plates were infected with sterile discs containing antimicrobial agents. The strength of the endophytic fungal extracts was determined by measuring the width of the inhibitory zone around the discs. The inoculating wire was used to select the test bacterial colony, which was then placed into a regular saline tube. After comparing the tube's turbidity to that of the 0.5 McFarland opacity tube, the tube's concentration or dilution was adjusted. After allowing the inoculation plates to dry for a minimum of five to ten minutes, Whatman filter paper discs with a diameter of six mm were put on the plates using sterile forceps. The discs had been previously saturated with 10µl of each endophytic fungal extract (200 *mg/ml*). A variety of antibiotics, including ampicillin, streptomycin, and chloramphenicol, were used as positive controls for the bacterial strains. Utilising just DMSO as a negative control was done. The plates were incubated at 37°C for 24 hours after being put in the incubator. Using a ruler, the IZD (inhibition zone diameter) around each disc was measured to the closest “mm”

Antifungal Assay

Tube dilution method was used for screening of anti-fungal activity (Blank and Rebell., 1965). Stock solutions were prepared by dissolving each extract, of each endophytic fungus in Dimethyl sulfoxide (DMSO). For the purpose of promoting the development of fungi, 4 milli-litres of SDA (Sabouraud Dextrose Agar) were added to each screw-cap test tube, and the tubes were autoclaved for 15 minutes at 121°C. After allowing the tubes to cool to 50°C, 66.66μl of endophytic fungal extracts from the stock solution were added to the melted SDA. The extract's final concentration in the medium was 200 *mg/ml*. The tubes were left at room temperature to harden in the tilted orientation. To obtain a 200$\mu g/ml$ concentration, 83μl of Nystatin (12 *mg* dissolved in 1 *ml* of DMSO) was added to a tube as a positive control. A 100μl test tube containing pure DMSO served as the negative control. Four milli-meter-diameter inoculums extracted from a test fungal culture that had been growing for seven days were introduced into each tube. Every fungal strain growth was treated with the streaking technique. For seven days, all of the tubes were incubated at 27–29°C. 3–7 days later, results were recorded. After incubation, the fungus on slant was assessed for linear growth (measured in millimetres), and the negative control was used as a reference to calculate growth inhibition.

Results

The result revealed that total 4 endophytic fungi were isolated from root, stem, leaf samples of *Psoralea corylifolia* viz *Piriformospora indica, Alternaria alternata, Aspergillus niger* and *Penicillium citrinum.* The result showed that the data on the frequency of colonization by fungal endophytes and the number of isolation cases observed in various regions of *Psoralea corylifolia*. The high incidence of isolation cases was reported in *Piriformospora indica*. Among the endophytes, *Piriformospora indica* was found to be the only dominating species, with a colonizing frequency of 37.03% in root and among the leaf portions, it was observed that *Alternaria alternata* exhibited a comparatively greater frequency of occurrence that is 22.22% (Fig 1). The colonization frequency of the stem sections was found to be greatest for *Aspergillus niger* that is 18.51%. The plant species exhibited the lowest rate of isolation for the endophytic fungus *Penicillium citrinum* that was 0.03, whereas the greatest rate was reported in *Piriformospora indica* that was 0.13 (Fig 2).

From the results it was observed that the butanol, methanol and the ethyl acetate extract showed a better inhibitory activity against almost all the tested bacterial strains. Antibacterial activity of three crude extracts including butanol, ethyl acetate, and methanol of different fungal endophytes was appraised against both Gram-negative bacterial strains. Among the endophytic fungal isolates (*Piriformospora indica*, *Alternaria alternata*, *Aspergillus niger* and *Penicillium citrinum*) of *Psoralea corylifolia,* it was observed that the methanolic extract of *Piriformospora indica* showed a highest IZD against *B. subtilis* although the activity of the extracts of other stains also was higher against the same strain. The result showed that the maximum antibacterial efficacy was found in methanol extract of *Piriformospora indica* in the test organism *bacillus subtilis* that was 20.3±0.57 followed by ethyl acetate that was 18.66±0.57 (Table 1).
Antifungal efficacy of different crude extracts of fungal endophytes was also appraised against three (3) pathogenic fungal strains including *Aspergillus niger, A. fumigatus* and *Candida albicans*. From the results it was observed that *A. niger* was the most sensitive fungal pathogen for almost all the extracts of the fungal endophytes as its growth was inhibited the most. The maximum antifungal activity was found in methanol extract of *Alternaria alternata* in the test organism *Aspergillus niger* that was 43.1 followed by *Penicillium citrinum* in the test organism *Aspergillus fumigatus* that was 42.8 (Table 2).

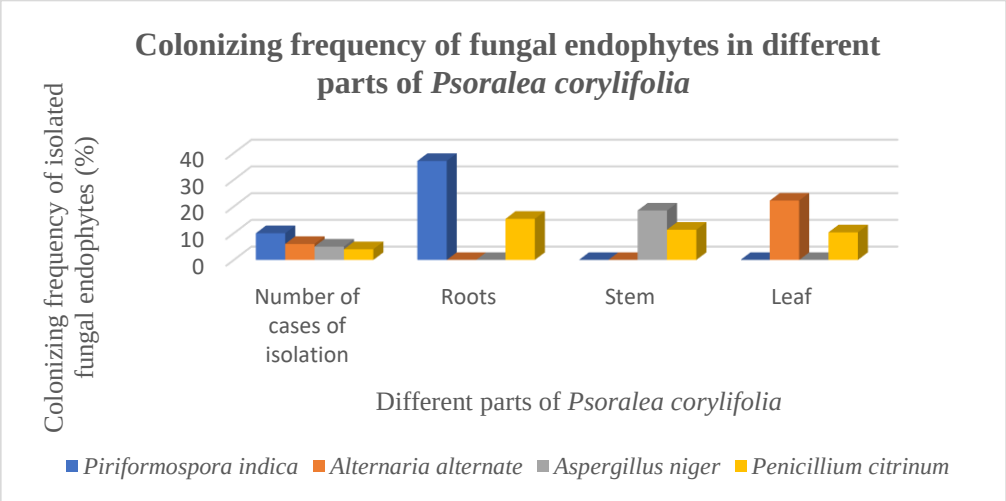

Figure 1. Colonizing frequency of fungal endophytes in different parts of *Psoralea corylifolia*

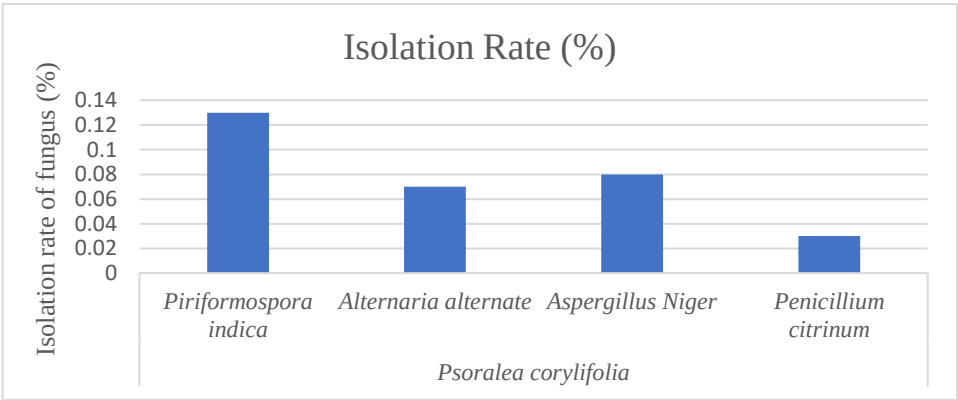

Figure 2. Isolation rate of *Psoralea corylifolia*

Table 1. Antibacterial activity of various extracts (200*mg/ml*) of Fungal Endophytes isolated from *Psoralea corylifolia*.

Fungal Endophytes	**Test Organisms**	**Extracts**		
		Butanol	**Ethyl acetate**	**Methanol**
Piriformospora indica	*Bacillus subtilis*	16.66±0.57	18.66±0.57	20.3±0.57
	Escherichia coli	13.66±1.52	12.66±1.15	20±1
	Proteus vulgaris	12±1.73	7±1	16.66±0.57
	Staphylococcus aureus	7.66±0.57	8.6±0.57	7±1
	Salmonella typhi	13±1	16.33±1.15	17.66±0.57
Alternaria alternata	*Bacillus subtilis*	14±1.73	17.33±1.52	17±1
	Escherichia coli	10.33±1.15	11.33±0.57	9.66±0.57

	Proteus vulgaris	7.33±1.52	7±1	7.66±1.15
	Staphylococcus aureus	7.66±0.57	14.66±0.57	12.66±0.57
	Salmonella typhi	12.66±0.57	9.66±0.57	19.33±0.57
Aspergillus niger	*Bacillus subtilis*	11.33±1.52	8.66±0.57	17±1
	Escherichia coli	11.33±1.15	10.33±0.57	13±1
	Proteus vulgaris	7.66±0.57	7.33±0.57	7.66±0.57
	Staphylococcus aureus	16.33±0.57	18.33±1.15	18.33±1.52
	Salmonella typhi	13.33±0.57	18.66±0.57	15±1
Penicillium citrinum	*Bacillus subtilis*	9.33±0.57	9.33±0.57	8.66±0.57
	Escherichia coli	7.66±1.15	9±1	8.66±0.57
	Proteus vulgaris	11.6±1.15	11.33±0.57	14.33±0.57
	Staphylococcus aureus	8.66±0.57	9.66±0.57	9.33±0.57
	Salmonella typhi	12.33±0.57	10.33±0.57	7.33±0.57

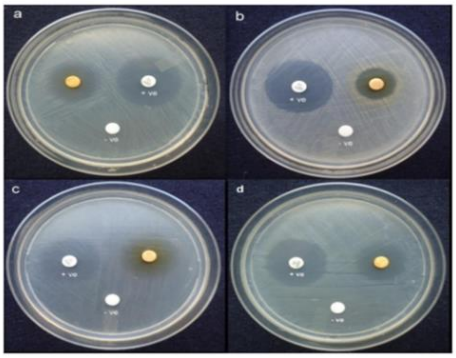

Figure 3. Antibacterial Activity of crude extracts of Fungal Endophytes isolated from *Psoralea corylifolia*

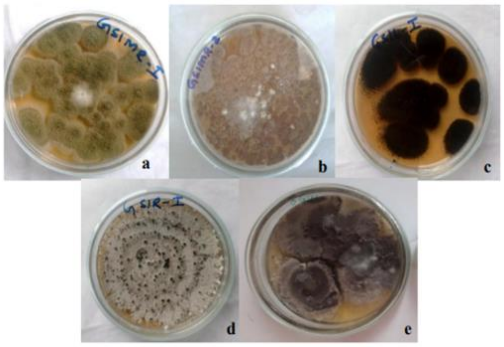

Figure 4. Plant associated fungal isolates obtained from leaf (d), root (e), stem (c) and Fruit (a, b) of *Psoralea corylifolia*

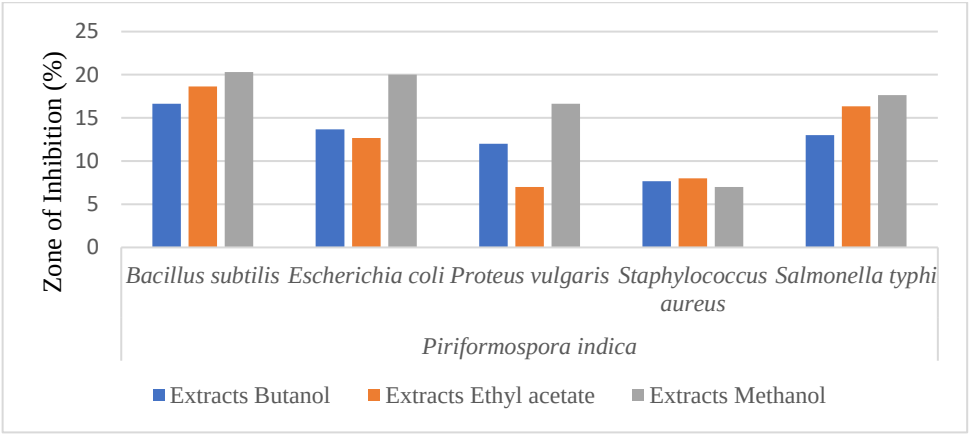

Figure 5. Antibacterial efficacy of several extracts derived from *Piriformospora indica*

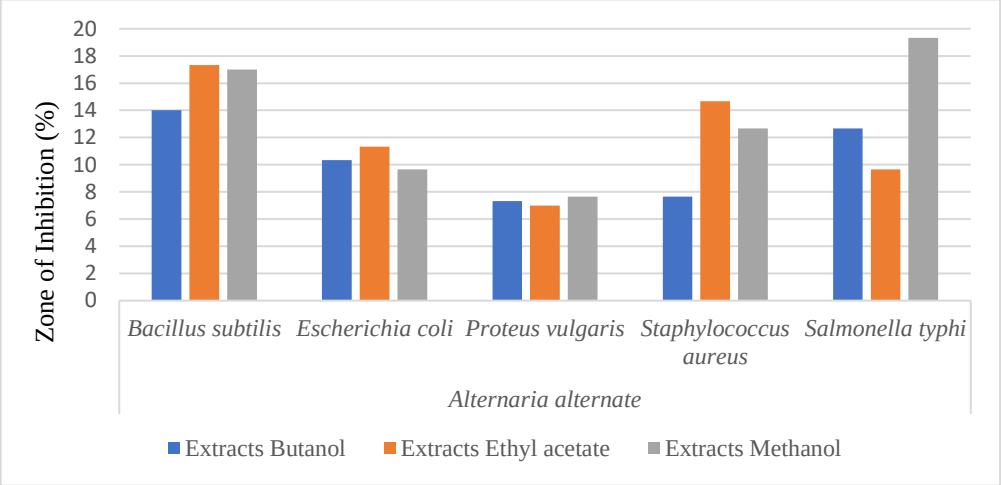

Figure 6. Antibacterial efficacy of several extracts derived from *Alternaria alternata*

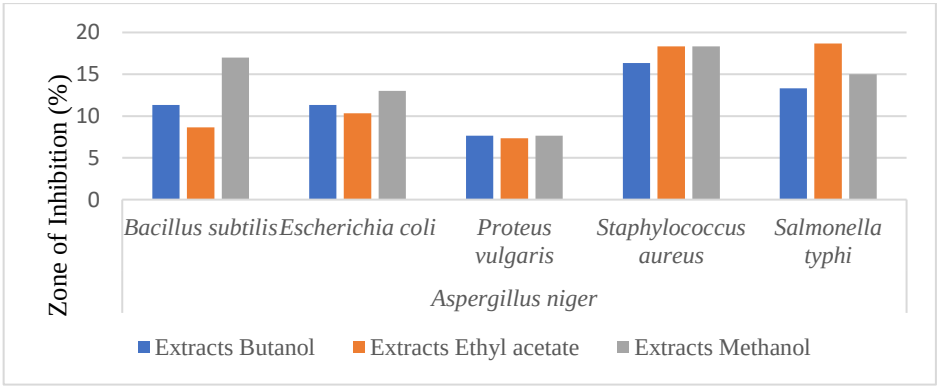

Figure 7. Antibacterial efficacy of several extracts derived from *Aspergillus niger*

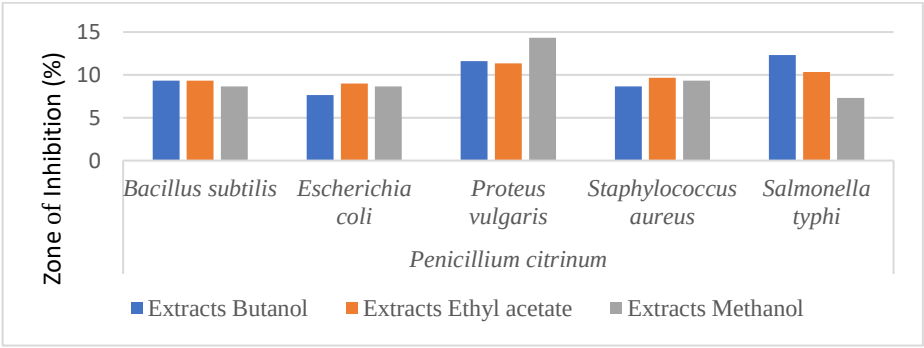

Figure 8. Antibacterial efficacy of several extracts derived from *Penicillium citrinum*

Table 2. Antifungal activity of various extracts (200*mg/ml*) of Fungal Endophytes isolated from *Psoralea corylifolia*

Fungal Endophytes	**Test Organism**	**Extracts**		
		Butanol	**Ethyl acetate**	**Methanol**
Piriformospora indica	*Aspergillus niger*	30.5	41.0	35.0
	Candida albicans	17.5	29.3	24.8
	Aspergillus fumigatus	27.4	33.1	31.5
Alternaria alternata	*Aspergillus niger*	24.3	15.66	43.1
	Candida albicans	25.4	38.1	30.4
	Aspergillus fumigatus	23.8	20.5	15.3
Aspergillus niger	*Aspergillus niger*	25.5	41.1	17.6
	Candida albicans	34.6	39.2	25.2
	Aspergillus fumigatus	27.8	41.2	33.2
Penicillium citrinum	*Aspergillus niger*	25.5	31.3	21.8
	Candida albicans	37.8	43.0	32.9
	Aspergillus fumigatus	42.8	22.9	36.8

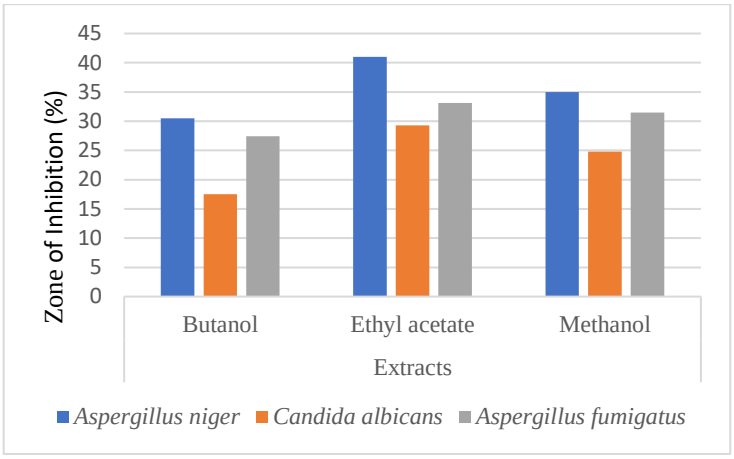

Figure 9. Antifungal efficacy of three extracts derived from *Piriformospora indica*

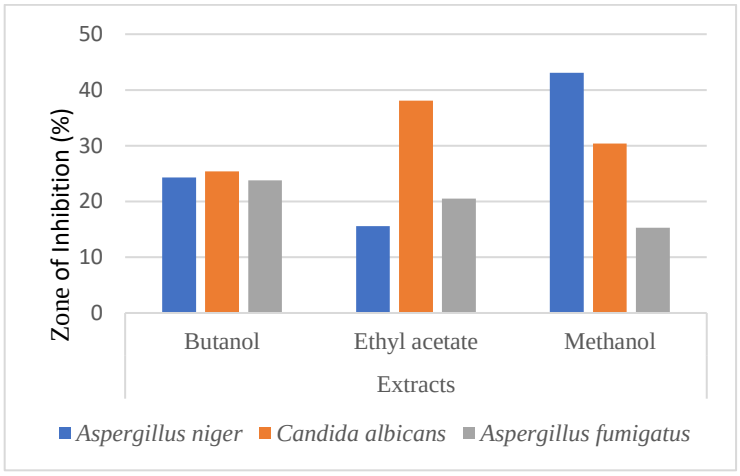

Figure 10. Antifungal efficacy of three extracts derived from *Alternaria alternata*

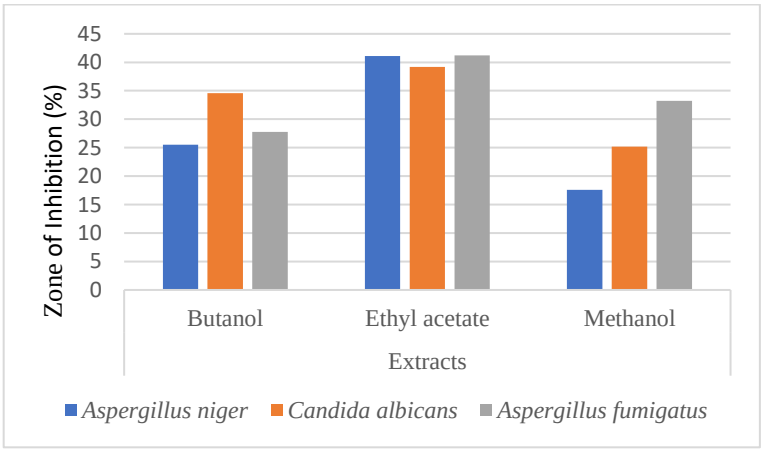

Figure 11. Antifungal efficacy of three extracts derived from *Aspergillus niger*

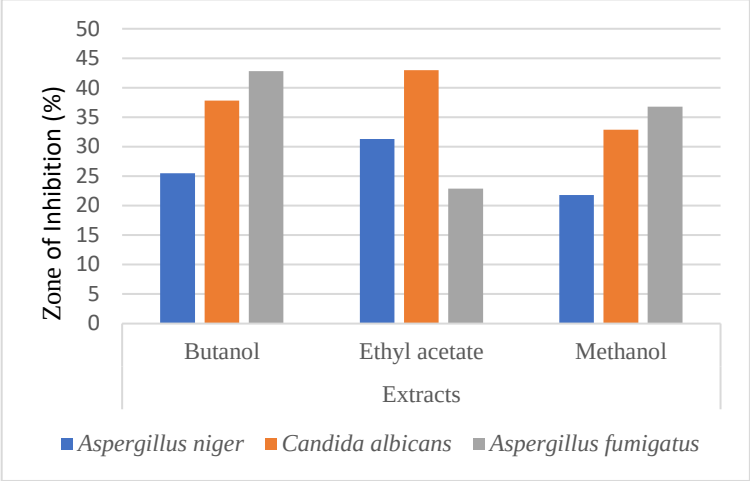

Figure 12. Antifungal efficacy of three extracts derived from *Penicillium citrinum*

Discussion

The objective of this work was to extract endophytic fungus from these medicinal plants and evaluate the antibacterial and antifungal activities. The samples of root, stem and leaf from *P. Corylifolia* collected from Nahargarh Biological Park Kukus Jaipur, Rajasthan. According to similar results that support the current investigation, extracts of *Cladosporium* sp. from several medicinal plants showed strong antibacterial action against *Proteus* sp., *Bacillus subtilis*, and *Bacillus cereus*. The fungal endophytes *Acremonium curvulum, Aspergillus chraceus, Gibberella fujikuroi, Myrothecium verrucaria,* and *Trichoderma piluliferum* were linked to *Bauhinia forficate*, and their extracts showed antimicrobial activity against *Streptococcus pyrogenes* and *Staphylococcus aureus* (Raut *et al*., 2021). Using 20 different plant species, Sudirga *et al*., 2023 found that six plant species—*Piper nigrum, Piper ornatum, Piper retrofractum, Ficus septica, Samanea saman,* and *Tithonia diversifolia*—were able to stop the growth of *C. acutatum* fungus. In their study, Cui *et al*., (2011) recorded the presence of fungal endophytes in *Aquilaria sinensis*, with the majority of these endophytes belonging to the genus Fusarium. Ola *et al*. 2020 carried out the antibacterial test, fungal extract extraction, fungal culturing, endophytic fungal isolation, and metabolite identification. Using a 1 L Erlenmeyer flask, a pure colony of endophytic fungi was cultivated on solid rice media. Ethyl acetate was used to extract the cultivated fungus. The antibacterial qualities of the crude ethyl acetate extract were next examined chemically. *Aspergillus niger* was determined to be the endophytic fungal species through both macroscopic and microscopic examination.
Similarly, Elghaffar *et al*. (2022), reported that *Alternaria alternata* was isolated from *Ziziphus spina-christi*. Antimicrobial results illustrated that EA crude extract exhibited promising antimicrobial activity against Gram-negative bacteria (*Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa,* and *Klebsiella pneumonia*; Gram-positive bacteria (*Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis)* and unicellular fungi *Candida albicans.* Here in the present study, endophytic fungal isolates from *Psoralea corylifolia* on investigating antibacterial and antifungal activity showed highest efficacy against bacterial strains and lesser efficacy against the fungal strains respectively.

Conclusion

In order to isolate four distinct fungal species, this study set out to explore the fungal diversity from the plant *Psoralea corylifolia*. Against a panel of harmful bacteria and fungi, all of these fungal extracts showed notable antibacterial and antifungal activity. The variety of antimicrobial chemicals found in this study illustrates the complex interactions among microbial populations and their adaption mechanisms in natural environments, in addition to providing a viable path for the development of new antibiotics. These endophytic fungi will be helpful in future as a biocontrol agent and these are useful in pharmaceutical industries. Additionally, it is possible that the medicinal properties of this plant are a result of the capability of its endophytic microorganisms to generate biologically active secondary metabolites. Additional research is required to determine the specific chemical molecules that are responsible for producing antibacterial effects, with the ultimate goal of innovating uncovering novel medications.

Acknowledgement

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Conflicts of Interest

The authors declare that there are no conflicts of interest.

References

1. Abdelkader DH, Negm WA, Elekhnawy E, Eliwa D, Aldosari BN, Almurshedi AS. Zinc oxide nanoparticles as potential delivery carrier: Green synthesis by *Aspergillus niger* endophytic fungus, characterization, and in vitro/in vivo antibacterial activity. Pharmaceuticals. 2022 Aug 26;15(9):1057.
2. Abdelwahab GM, Mira A, Cheng YB, Abdelaziz TA, Lahloub MF, Khalil AT. Acetylcholine esterase inhibitory activity of green synthesized nanosilver by naphthopyrones isolated from marine-derived *Aspergillus niger*. Plos one. 2021 Sep 10;16(9):e0257071.
3. Aswani, R.; Vipina Vinod, T.; Ashitha, J. Benefits of plant–endophyte interaction for sustainable agriculture. In Microbial Endophytes: Functional Biology and Applications; Kumar, A., Radhakrishnan, E., Eds.; Elsevier INC.: Amsterdam, The Netherlands, 2020; pp. 35–55.
4. Barnett, H. L. Illustrated genera of imperfect fungi (3rd ed). Burgess publishing company, California.1972. 1-700.
5. BLANK H, REBELL G. Griseofulvin-containing medium for simplified diagnosis of dermatophytosis. Archives of dermatology. 1965 Sep 1;92(3):319-22.
6. Chopra RN, Chopra IC. Indigenous drugs of India. Academic publishers; 1994.
7. Cui J, Wang C, Guo S, Yang L, Xiao P, Wang M. Evaluation of fungus-induced agilawood from *Aquilaria sinensis* in China. Symbiosis. 2013 May;60:37-44.
8. Deshmukh SK, Dufossé L, Chhipa H, Saxena S, Mahajan GB, Gupta MK. Fungal endophytes: A potential source of antibacterial compounds. Journal of Fungi. 2022 Feb 8;8(2):164.
9. Elghaffar RY, Amin BH, Hashem AH, Sehim AE. Promising endophytic *Alternaria alternata* from leaves of *Ziziphus spina-christi*: phytochemical analyses, antimicrobial and antioxidant activities. Applied Biochemistry and Biotechnology. 2022 Sep;194(9):3984-4001.
10. Guo B, Wang Y, Sun X, Tang K. Bioactive natural products from endophytes: a review. Applied Biochemistry and Microbiology. 2008 Mar;44:136-42.
11. Hyde KD, Soytong K. The fungal endophyte dilemma. Fungal divers. 2008 Nov 30;33(163):e173.
12. Kuo J, Chang CF, Chi WC. Isolation of endophytic fungi with antimicrobial activity from medicinal plant *Zanthoxylum simulans* Hance. Folia Microbiologica. 2021 Jun;66:385-97.
13. Mishra S, Anuradha J, Tripathi S, Kumar S. In vitro antioxidant and antimicrobial efficacy of Triphala constituents: *Emblica officinalis*, *Terminalia belerica* and *Terminalia chebula*. Journal of Pharmacognosy and Phytochemistry. 2016a;5(6):273-7.
14. Mishra S, Anuradha J, Tripathi S, Nathiya D, Kumar S. In vitro effect of triphala constituent in sodium selenite-induced cataractogenesis. International Journal of Herbal Medicine 2016b;4(6):193-197.
15. Ola AR, Sugi Y, Lay CS. Isolation, identification and antimicrobial activity of secondary metabolites of endophytic fungi from Annona leaves (*Annona squamosa* L.) growing in dry land. InIOP Conference Series: Materials Science and Engineering 2020 Apr 1 (Vol. 823, No. 1, p. 012039). IOP Publishing.
16. Petrini O. Fungal endophytes of tree leaves. InMicrobial ecology of leaves 1991 (pp. 179-197). New York, NY: Springer New York.
17. Rai, S, Anuradha, J, Sanjeevi, R. Investigation on the Larvicidal Potential of Endophytic Fungi Isolates from *Psoralea corylifolia* and *Leptadenia reticulata* Against *Aedes Aegypti*. Journal of Advanced Zoology, 2023, Vol 44, 255.
18. Răut I, Călin M, Capră L, Gurban AM, Doni M, Radu N, Jecu L. *Cladosporium* sp. isolate as fungal plant growth promoting agent. Agronomy. 2021 Feb 23;11(2):392.
19. Sudirga SK, Wijaya IM, Darmadi AA, Astiti NP. Assessment of plants' leaf extracts for antifungal activity against the fungus *Colletotrichum acutatum* in chili (*Capsicum annuum* L.). SABRAO Journal of Breeding & Genetics. 2023 Dec 1;55(6).