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Antifungal Activity of *Etlingera fenzlii*against its Rhizospheric Fungal Isolates.

Aravind S[1*] and Nasreen K[2]
[1]*Department of Botany, Annamalai University, Tamil Nadu, India.*
[2]*Department of Botany, JNRM, Andaman and Nicobar Islands, India.*

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ABSTRACT: Microbial colonies in the rhizospheric soil play a significant role in bringing unforeseen advantages or detriment to the plant. The presence of *Aspergillus clavatus*, *Penicillium chrysogenum*, *Rhizopus oligosporus*, and *Rhizopus arrhizus*was clearly demonstrated in the current investigation of soil samples collected from the rhizosphere of the indigenous medicinal plant *Etlingera fenzlii* for screening and identifying fungal diversity. The antifungal activity of *E. fenzlii* methanolic leaf extract was tested against four rhizospheric isolates using the well diffusion method and found to be most effective against *P. chrysogenum* and *R. arrhizus*. The revelation of pharmacological actions in *E. fenzlii* will eventually boost the value of this potent Shompen's traditional medicinal plant.

KEYWORDS: Antifungal activity, Endemic plant, Medicinal plant, Shompen, Rhizospheric fungi.

I. INTRODUCTION

The physiological activities for plant development are correlated by the rhizospheric niche, which also harbours a mesotrophic environment that represents microbiome colonisation. The Rhizosphere effect is triggered when the dynamic microbiota gain access to the plant's root exudates. The fungal microbiome plays a vital role in biomass and can provide unintended benefits or harm to the plants.

The prolific soil tenant Fungi microbiota espouses distinct sorts of adaptation to harsh and adverse scenarios due of its versatility and potential to maintain carbon and nutrient balance by producing a wide range of exoenzyme to deteriorate organic soil components [1]. Beneficial soil fungus has the unusual capacity to function as an efficient biosorbent of harmful metals whilst still contributing to the growth of their host. Fungi is ubiquitous and exist in a wide variety of pH and temperature conditions and may marked as cosmopolitan. Beside benign fungus, the most concerning scenario is pathogenic fungi infesting and harming root exudates, impeding plant development.

Etlingera fenzlii (Kurz) K. Schum., prior known as *Amomum fenzlii*formerly delineate by Kurz on the Kamorta collection of Nicobar in 1876. The provincial name of this plant in Nicobar tribal language is Hami, which is a perennial erect medicinal herb endemic to Andaman Nicobar Islands belongs to the family Zingiberaceae. The plant is leafless at the base with green swollen leafy shoot. The leaves are in the axial part with mucronate sheaths, striate, glabrous with pubescent margin. The leaf was dark green oblong to ovate with wavy margin which is slightly incurved with tiny dense golden hairs. The rhizome is thick covered with reddish brown triangular scales and dense with white villous. [2] It also exhibits a vivid array of therapeutic properties whilst being an effective insect repellent.

The plant is traditionally utilised by Shompen (the native inhabitants of Great Nicobar Island's interior, a part of Andaman and Nicobar Islands.) as a bee repellent. The practical application by Shompen was adopted in order to obtain honey while avoiding being stung related to potential insect repellent. The plant leaves are chewed and sprayed over the bee hives, which calmed the bees and protects them [3] while cough, fever, respiratory disorders, and skin problems are all treated with rhizome juice by the Nicobari tribes [4]. Furthermore, numerous studies have shown that the volatile components of the leaves are effective eco-friendly insect and pest control agents. The essential oil of *E. fenzlii*exhibited larvicidal activity [5] and hepatoprotective effect [6]. The genus *Etlingera*with around 150–200 species in the global pool and diverse species have been reported in Indonesia, including 48 species in Sulawesi and 6 species in Java [7]. The subsequent species of *Etlingera*demonstrated antimicrobial in *E. elatior*[8] and anticancer properties in *E.corneri*[9] which eluted the scrutiny on antifungal properties in *E. fenzlii.*

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The potentialities of traditional and medicinal plants, though both are cost-effective and non-toxic, might be considered as an alternative source for producing natural fungicides instead of industrial fungicides. In contrary, the plant fungal disease represses agriculture productivity, a concerning tragic event for farmers but use of industrial fungicide results in mass amalgamation in agriculture products. As a corollary, the development of antifungal medicines is a luxury that can aid in the control of infectious diseases.

Since, *E. fenzlii*is utilised by tribes and has numerous therapeutic properties, it is assumed that the microbial diversity of the plant's rhizospheric soil expected to be significant as well. However, there is no report on antifungal effects of the leaf extract of *E. fenzlii*. In this context, the present study investigated the antifungal activity of *E. fenzlii* methanolic leaf extracts against four rhizospheric fungal strains.

II. MATERIALS AND METHOD

Collection of Plant Material and Rhizospheric soil Sample.

The fresh leaves and the rhizospheric soil sample of *Etlingera fenzlii*were collected from the Botanical Garden of the Botanical Survey of India (BSI), Nayashahar, South Andaman. The plant materials were sterilized using mild detergent and rinsed thoroughly in running tap water. The sterilized leaves were then shade dried for about 7 days and grounded to fine powder. The powder was stored in the dark before extraction.

Identification and Purification of fungal isolates

For the preparation of media, inoculation, a nd culture maintenance, general laboratory procedur eswere used [10,11]. The rhizospheric soil sample (1 gm) was suspended in Sterile distilled water (10 ml). The suspension was serially diluted till 10^{-6} factor.200 µl of suspension was taken from the dilution factor of 10^{-1} 10^{-2}, 10^{-3} and $10^{-4}$and spread plated aseptically on PotatoDextrose Agar (PDA) plates using sterile L- rod. The isolation plates were incubated at room temperature (30±2°C) for 48 hours. On PDA plates, distinct single fungal colonies were sub-cultured. To inhibit bacterial growth, 1% Ampicillin solution was added to the medium before pouring into petri plates. Riddle's standard slide culture technique was used to identify fungi isolates [12]. Color and texture of hyphae, stolon, Sporangiophore sporangia, and Columellae were examined. The observed attributes were assessed using the Mycobank database.

Preparation of leaf extracts

The fine coarse powder was followed by methanolic extraction method. In a 1:10 ratio, powdered plant leaf material (10 gm) was dissolved in 100 ml of methanol. The suspension was transferred to a conical flask and encased in aluminium foil for containment. The suspension was agitated in a shaker for 48 hours. Thereafter, the suspension was filtered with filter paper (Whatman No. 1). By mixing the leftover crude with methanol in a 1:10 ratio and repeating the operation, the filtrate was produced. The filtrate was then kept in an aseptic condition for one week while the solvent evaporated in an open bowl. After complete drying, the dried raw layer was scraped from the bowl and dissolved in 5% Dimethyl sulfoxide (DMSO).

Antifungal test: Well diffusion method

The antifungal activity of the methanolic crude extract of *E. fenzlii*against an isolated rhizospheric fungal strains were assessed using Well diffusion method. In each PDA plates, fungal disc (5 mm) from pure culture was implanted in the four wells using aseptic cork borer [13]. With DMSO, 1 ml of crude at three distinct concentrations viz. 5%, 10% and 50% was prepared. 200 µl of crude sample was taken from each concentration and loaded into three wells while 200 µl was loaded in one of the four as control. The activity was evaluated after three days of incubation.

III. RESULT AND DISCUSSIONS

Methanolic extracts of Leaves of *E. fenzlii* displayed substantial antifungal action against four isolated rhizospheric fungal strains: *Aspergillus clavatus*, *Penicillium chrysogenum*, *Rhizopus oligosporus*, and *Rhizopus arrhizus*(Figure 1). These extraction techniques are straightforward and low-cost to produce, and they offer the potential for technical advancement in Agro-industries. The morphological features of the rhizospheric fungal isolates are represented in Table 1 with Mycobank ID.

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Table 1: Morphlogical characters of Rhizospheric fungi strains.

FUNGAL STRAIN	MORPHOLOGICAL CHARACTERS		MYCOBANK ID
Aspergillus clavatus	Colony	Bluish-green	211530
	Hyphae	Non-septate	
	Conidiophore	Smooth walled, elongated club shaped, uncoloured.	
	Sterigmata	Uniseriate, dense.	
	Conidia	Elliptical, smooth walled.	
	Vesicles	Clavate.	
	Phialides	Entire.	
Penicillium chrysogenum	Colony	Dull green	165757
	Conidiophore	Thin smooth- walled, Terverticillate	
	Conidia	Elliptical	
	Phialides	Flask Shaped	
Rhizopus oligosporus	Colony	White	155475
	Hyphae	Colourless, black	
	Sporangiophore	Straight or curved,	
	Sporangia	Opaque, spherical, white to black	
	Columella	Round or flatten with basal collar	
	Spores	Colourless, oval	
Rhizopus arrhizus	Colony	Brownish White	167790
	Hyphae	Non-septate	
	Sporangiophore	Curved, branched, smooth walled	
	Sporangia	Globose, yellowish brown	
	Columella	Globose, smooth walled	
	Spores	Elliptical	
	Stolons	Smooth	
	Rhizoid	Short, Finger-shaped	

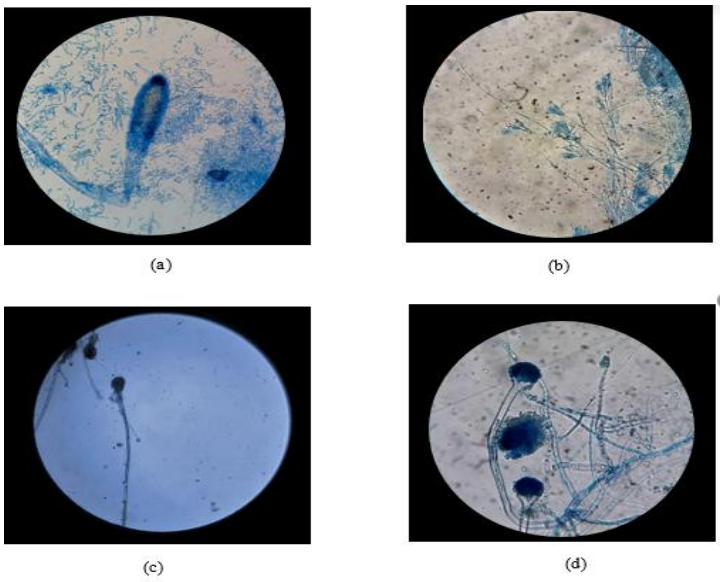

Figure 1: showing the microscopic photograph of the rhizospheric fungal isolates - (a) *Aspergillus clavatus* (b) *Penicillium chrysogenum* (c) *Rhizopus oligosporus* (d) *Rhizopus arrhizus*

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Aspergillus and *Mucor* species are abundant in rhizosphere niche colonies, and they are regarded dominant members of the Mucoraceae and Trichocomaceae families [14] similar to the current study, where the dominating species were *A. clavatus, P. chrysogenum, R. oligosporus,* and *R. arrhizus.* In tropical areas, the diversity of most fungus groups tends to grow, but extensive studies are still in their adolescence.

The results showed that the methanolic fractions of the leaf extract of *E. fenzlii*effectively varied inhibiting fungi growth. The bio composition of chemical components of plant extracts, i.e., secondary metabolites of plants, even when collected from the same species, might result in varied responses, particularly in terms of microbe inhibition capability [15] and many research indicates that medicinal plants' antibacterial property may be related to the presence and synergistic activity of a variety of bioactive metabolites [16].

The antifungal effects of *E. fenzlii* methanolic leaf extracts at concentrations of 5, 10, and 50 µg/ml on four rhizospheric fungal strains were dosage dependent. *R. arrhizus* growth suppression was more pragmatic than other strains at 5g/ml, however at 40 µg/ml; there was a greater significant difference than the positive control where *P. chrysogenum* and *R. arrhizus* growth was inhibited to the maximum, revealing antifungal effect of *E. fenzlii*. In all the concentrations, the remaining two strains, A. clavatus and R. oligosporus, showed the least zone of inhibition. However, the crude concentration of 10 and 50% showed antagonistic activity against the isolated fungal strains but *R. arrhizus*exhibited antagonistic action at 5%.

Table 2: Showing the antifungal activity of *E. fenzlii*leaf extract.

Antifungal Activity (Zone of Inhibition)				
Sl. No	**Rhizospheric Fungal Isolates**	**Methanolic Extracts of *Etlingera fenzli* (µg/ml)**		
		5	**10**	**50**
1	*Aspergillus clavatus*	-	+	+
2	*Penicillium chrysogenum*	-	+	++
3	*Rhizopus arrhizus*	+	+	++
4	*Rhizopus oligosporus*	-	+	+

Given the current need for alternative bio rational fungicides, it was thought that evaluating the antifungal properties of endemic plant extracts would be worthwhile, where *E. fenzlii* demonstrated moderate inhibition, which was encouraging and may possess an unambiguous latent for new effective fungicides.

It is conceivable that the low diversity is related to particular root exudates produced by this plant species, which do not favour the survival of a varied fungus community. Furthermore, the single kind of microbial media utilised in the current study might have resulted in decreased fungal diversity.

To the best of my knowledge, no investigations on *E. fenzlii* rhizospheric soil fungus have been reported. As a consequence, it is difficult for me to compare the current study's findings to some mainstream published literature.

IV. CONCLUSION

The outcomes of this study suggests that the methanolic leaf extract of *E. fenzlii*may successfully showed antifungal action, and this research leads the way to show the diversity of these microflora in the rhizosphere of this endemic plant. The fast growing and concurrent appearance of these species reveals that these fungal strains are much adapted to the soil sites. The phytochemicals studies will open up new avenues for better utilization of this endemic plant and envision various pharmacological activities in near future.

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