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Screening of secondary metabolites and evaluation of its biological activity of isolated endophytic fungi *Aspergillus niger* from *Alangium salviifolium* (L.f.) Wangerin

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Abstract
Endophytic fungi are the storehouse of bioactive compounds that are of significantly important. *Aspergillus niger* is one the important microbe that have wide range of industrial applications and of pharmaceutical importance. The present study was carried out to screen the secondary metabolites and to evaluate the antimicrobial and antioxidant activity of endophytic fungi *Aspergillus Niger* isolated from *Alangium salviifolium* (L.f.) Wangerin. The ethyl acetate extract of *Aspergillus niger*showed the presence of alkaloids, tannins, flavonoids, phenols, anthraquinones and terpenoids. The DPPH assay revealed that the fungal extract has 78% of inhibition at 300 µl concentration. The crude extract was effective and showed good inhibition zone of around 9 to 15mm against both gram negative and grampositive bacteria. It also showed considerable antifungal activity.

Keywords: endophytic fungi, *Aspergillus niger*, secondary metabolites, dpph, antimicrobial activity

Introduction

Naturally derived metabolites from microbes, plants and other natural sources are exploited by human for thousands of years for treating various diseases. Bacteria and fungi live inside the tissues of plants as an endophyte and are benefitted mutually (Hyde and Soytong, 2008). About 300,000 plant species are present in the earth. Each and every plant is a home for one or many endophytes. Endophytes synthesize variety of secondary metabolites which confers drought resistance, disease resistance, growth promoting factors to the host plant (Rodriguez *et al.* 2012) and are capable of producing the host metabolites (Kusari *et al*., 2012; Heinig *et al*., 2013) [7, 3]. Fungi are important part of our ecosystem and play a vital role for the sustainable development. Fungi are known to harbor thousands of pharmaceutically important metabolites. *Aspergillus niger* is one of the most important fungi and harbors pharmaceutically important metabolites and economically important enzymes (Nielsen *et al*., 2009) [9]. Antimicrobial metabolites such as helvolic acid, ergosterol, monomethyl sulochrin, and 3 β hydroxy-5 ά ergosterol, ochratoxin, brefeldin A and cytotoxin are housed in *Aspergillus niger* (Gao *et al*., 2007; Wang *et al*., 2002) [2, 16]. Even though considerable research has been done on *Aspergillus niger* of various origin including the endophytes, but the bio-prospecting studies on the *Aspergillus niger* isolated endophytes *Alangium salviifolium* (L.f.) Wangerin leaves was not reported so far. Moreover, the host plant *A. salviifolium* is an ethnomedicinally important plant and belongs to Alangiaceae family. The anti-arthritic, anti-cancer, anti-diabetic, antiepileptic, antihelminthic, anti-inflammatory, antimicrobial, antioxidant, antiulcer and hepatoprotective activity of *A. salviifolium* was reported (Pandian *et al*., 2006; Parida *et al*., 2010; Vineet *et al*., 2010; Jubie *et al*., 2008; Dinakar *et al*., 2011; Rajesh *et al*., 2011; Ronok *et al*., 2011; Sreekanth *et al*., 2011; Venkateshwarlu *et al*., 2011; Ahad *et al*., 2012; Pandey, 2012; Prusty *et al*., 2012; Meenakshi and Rajesh, 2015; Shravya *et al*., 2017) [11]. Bavya *et al*., (2021) [1] isolated the endophytes *Aspergillus niger*, *Diaporthe longicolla* and *Schizophyllum commune* from *Alangium salviifolium*. With this background, the present study was aimed to reveal the phytoprofile and evaluate the antimicrobial and antioxidant potential of endophytic fungi *Aspergillus niger* isolated from *Alangium salviifolium* (L.f.) Wangerin.

Materials and Methods

Isolation of endophytic fungi

The endophytes *Aspergillus niger* was isolated from the leaves of *Alangium salviifolium* (L.f.) Wangerin as described by Bavya *et al*., (2021) [1].

Fig 1: Isolated endophytic fungi *Aspergillus niger* from the leaves of *Alangium salviifolium*

Preparation of extracts

The fungal hyphae grown in Potato dextrose broth was centrifuged for 10 min at 8000 rpm. The supernatant was mixed with equal volume of ethyl acetate. The crude extract was stored after concentrating on rotary vacuum evaporator.

Phytochemical analysis

Harborne (1998) method was employed to confirm the presence or absence of alkaloids, flavonoids, phenols, tannins, saponins, quinones, glycosides, terpenoids, coumarins, steroids and anthraquinones in the ethyl acetate extracts of *Aspergillus niger*. The results were tabulated.

Quantitative Phytochemical Analysis

Total flavonoid, phenolics and tannins content of ethyl acetate extracts of *Aspergillus niger* was evaluated by using the standard method described by Kaur & Singh (2015) [5]. The quercetin, gallic acid and tannin acid was employed as standard for the quantitative determination of flavonoids, phenolics and tannins respectively.

Antioxidant Activity

DPPH assay was used to evaluate the antioxidant capacity of ethyl acetate extracts of *Aspergillus niger* and ascorbic acid was utilized as standard (Tiwari *et al*., 2006) [12]. Various concentrations (100, 200, 300 µg/ml) of extract were added to 0.1 mM DPPH solution and the solution was incubated at room temperature for 30 mins. Absorbance (Abs) was measured at 517 nm. Percentage of inhibition was calculated using the formula

$$\% \text{ of Inhibition} = \frac{\text{Abs of control} - \text{Abs of ethyl acetate extract}}{\text{Abs of control}} \times 100$$

Antimicrobial assay

Agar well diffusion method was adopted to assess the antimicrobial activity of ethyl acetate extracts of *Aspergillus niger*. The human pathogens (bacteria and fungi) were obtained from the department of microbiology, VIT University, Vellore. Mueller Hinton Agar medium was poured onto the Petridish and 0.1ml of bacterial pathogens was swabbed. 30 µL / well (1mg/ml concentration) of ethyl acetate extracts of *Aspergillus niger* was poured onto the wells and Streptomycin was employed as positive control and DMSO as negative control. It was incubated for 24 hours at 37ºC and zone of inhibition was measured. Similar method was followed for antifungal activity with PDA as culture medium and Fluconazole as positive control. For antifungal activity, PDA plates with incubated for 48 hours before measuring the zone of inhibition.

Results and Discussion

The endophytic fungi isolated from the leaves of Alangium salviifolium was identified as Aspergillus Niger (Genbank accession number MN821058) using the BLAST analysis (Bavya *et al*., 2021) [1]. The preliminary phytochemical analysis confirmed the existence of alkaloids, tannins, flavonoids, phenols, anthraquinones and terpenoids in the ethyl acetate extracts of Aspergillus niger. The quantitative analysis determined the phenolics (17.3 ± 0.38 µg of GAE /mg), tannin (1.87 ± 0.31 µg of TE /mg) and flavanoids (13.77 ± 0.68 µg of QE /mg) contents of ethyl acetate extracts of *Aspergillus niger*. Phenols are important antioxidants because of their redox property that helps in neutralizing free radicals (Zheng and Wang, 2001) [18]. Flavonoids are responsible for various biological activities like free radical scavenging, hepatoprotective, anti-inflammatory, antiviral, anticancer activities. Flavonoids act as an antioxidant by chelating metal ions and/or by scavenging the free radicals because of the presence of functional hydroxyl group (Kumar and Pandey, 2013) [6]. Tannins are astringent, water soluble polyphenols which act as secondary antioxidant that retards the chain reaction. The existence of the metabolites suggests various biopotencial applications of ethyl acetate extracts of *Aspergillus niger*. Similar to the present study Satari *et al*., (2018) [10] also found phenolics and falvonoids in the *Aspergillus niger* isolated from *Achilea millefolium*. Our results coincide with Shakthi devi *et al*., (2014) observations in the endophytes of *Alangium salviifolium*. The researchers observed the variation in quantity of metabolites contents of endophytes *Aspergillus niger* with reference to host plant (Shakthi devi *et al*., 2014 and Satari *et al*., 2018) [10].

An antioxidant is a molecule that donates electron to the free radical thereby preventing the damages caused free radicals (Lobo *et al*., 2010) [8]. Secondary metabolites like phenols, flavonoids act as an antioxidant and help us to prevent cellular damage (Kasote *et al*., 2015) [4]. The DPPH scavenging activity result of *Aspergillus niger* ethyl acetate extracts exhibited a dose dependent scavenging activity. The DPPH scavenging activity of *Aspergillus niger* ethyl acetate extracts were as follows 300 µg/mL (78.33%) > 200 µg/mL (77.7%) > 100 µg/mL (75.88%). Similar scavenging activity (72%) was observed in the *Aspergillus niger* endophyte isolated from *Eugenia jambolana* (Yadav *et al*., 2014) [17]. The *Aspergillus niger* ethyl acetate extracts was effective against almost all tested pathogens (Table 1 & 2). *Aspergillus niger* ethyl acetate extracts showed the maximum zone of inhibition against *Proteus vulgaris* with 17 mm (Table 1). Next to that, 15 mm zone of inhibition was observed against *Micrococcus luteus* and *Salmonella typhimurium* (Table 1). *Aspergillus niger* ethyl acetate extracts displayed a moderate zone of inhibition (11 – 14 mm) against *Vibrio parahaemolyticus*, *E. coli*, *Yersinia enterocolitica*, *Bacillus subtilis*, *Enterobacter aerogenes* and *Klebsiella pneumoniae* respectively (Table 1). *Aspergillus Niger* ethyl acetate extracts illustrated the least zone of inhibition (9 mm) against *Staphylococcus epidermidis*. But the *Aspergillus niger* ethyl acetate extracts showed minimal activity against the tested pathogenic fungi *viz*., *Aspergillus flavus*, *Candida albicans* and *Curvularia lunata* with 1- 3 mm of zone of inhibition (Table 2). A dose dependent activity was demonstrated in the antibacterial and antifungal activity of *A. niger* ethyl acetate extracts.

Table 1: Antibacterial activity of Endophytic Fungi *Aspergillus niger*

S. No.	Test organism	Concentration of extract (µg/ml)	Zone of inhibition (mm)
1	*Staphylococcus epidermidis*	Control*	12 ± 0.43
		25	7 ± 0.53
		50	8 ± 0.8
		75	8 ± 0.53

		100	9 ± 0.56
2.	*Escherichia coli*	Control*	15 ± 0.53
		25	11 ± 0.8
		50	11 ± 0.7
		75	12 ± 0.6
		100	13 ± 0.73
3.	*Klebsiella pneumoniae*	Control*	15 ± 0.53
		25	10 ± 0.2
		50	11 ± 0.48
		75	11 ± 0.36
		100	11 ± 0.61
4.	*Bacillus subtilis*	Control*	11 ± 0.61
		25	10 ± 0.32
		50	10 ± 0.62
		75	11 ± 0.53
		100	12 ± 0.41
5.	*Enterobacter aerogenes*	Control*	14 ± 0.83
		25	11 ± 0.62
		50	11 ± 0.35
		75	11 ± 0.12
		100	12 ± 0.48
6.	*Vibrio parahaemolyticus*	Control*	10 ± 0.57
		25	10 ± 0.7
		50	10 ± 0.3
		75	12 ± 0.31
		100	14 ± 0.35
7.	*Micrococcus luteus*	Control*	15 ± 0.6
		25	11 ± 0.36
		50	12 ± 0.32
		75	13 ± 0.63
		100	15 ± 0.31
8.	*Salmonella typhimurium*	Control*	14 ± 0.34
		25	10 ± 0.54
		50	12 ± 0.52
		75	13 ± 0.45
		100	15 ± 0.22
9.	*Proteus vulgaris*	Control*	18 ± 0.25
		25	10 ± 0.5
		50	13 ± 0.32
		75	14 ± 0.36
		100	17 ± 0.24
10.	*Yersinia enterocolitica*	Control*	10 ± 0.5
		25	10 ± 0.35
		50	10 ± 0.37
		75	11 ± 0.4
		100	13 ± 0.42

Control*-Streptomycin

Table 2: Antifungal activity of endophytic fungi *Aspergillus niger*

Sample Concentration (mg/ml)	Zone of Inhibition (mm)		
	Candida albicans	***Curvularia lunata***	***Aspergillus flavus***
Control (Flucanozole)	02 ± 0.7	03 ± 0.5	03 ± 0.43
25	01 ± 0.54	01 ± 0.9	01 ± 0.55
50	01 ± 0.6	02 ± 0.75	03 ± 0.64
75	01 ± 0.64	02 ± 0.68	01 ± 0.75
100	02 ± 0.8	03 ± 0.75	01 ± 0.55

Conclusion

The results of present study explored the secondary metabolites and bioactivity of *Aspergillus niger* isolated from the leaves of *Alangium salviifolium*. From the results we conclude that due to the presence of various secondary metabolites phenolics, tannins and flavonoids, the endophyte exhibited potent antioxidant and antimicrobial activity. These results could be the basis for further exploration of their bioactivity which could lead to potent drug molecule.

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