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Antagonistic Activity Of Endophytic Fungi Isolated From *Syzygium Cumini* (L.) Skeels

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Abstract: **In the present study, endophytes have been analyzed as a source of antimicrobial agents for the treatment and prevention of plant and human diseases. The endophytic fungi were isolated and then fungal culture broth, fungal mycelia and the crude extract was evaluated for antimicrobial activity against twelve test pathogens using agar well diffusion bioassay. An endophytic fungal isolate obtained from the leaf of *Syzygium cumini* showed antimicrobial activity against seven bacterial and five fungal test pathogens (involving two plant fungal pathogens). The antimicrobial compound was broad spectrum in action and also showed activity against the fungal test pathogens which could not be inhibited by the standard antibiotic fluconazol (in 1mg/ml concentration). The minimal inhibitory values of the crude extract ranged from 0.234 to 32000µg/ml.**

Keywords: **Endophytes, human diseases, crude extract, antimicrobial compound, broad spectrum.**

I. INTRODUCTION

Endophytes are the microorganisms that colonize the internal tissues of the plant but they do not show any symptom of their existence in the plant. They are harmless and are generally in symbiotic association with the host plant. The endophytes are ubiquitous, i.e, they have been isolated from almost every plant studied so far. The close association of the endophytes with the host plant led to the spectaculations of their importance in the therapeutic role of the host plant. [1]

Syzygium cumini also known as jambul, jambolan, jamblang, or jamun is an indigenous evergreen tropical tree of India. The plant is an intergral part of various alternative healing systems like Ayurveda, Unani and Chinese medicine. The leaves are antibacterial, prevent gingivitis and strengthen the teeth and gums. The fruits and seeds are sweet, acrid, sour, tonic, and cooling, and are used in diabities, diarrhoea and ringworm. Wine and vinegar are also made from the fruit. It is a high source of vitamin A and vitamin C. The bark is astringent, controls blood pressure as it is diuretic. It is sweet sour, digestive and anthelmintic. Studies have shown that various extracts of jamun exhibits pharmacological effects such as anti-inflammatory, antifungal, antiviral, anti-inflammatory, antiulcerogenic, cardioprotective, anti-allergic, anticancer, radio protective, antioxidant and hepato-protective properties [2]

The hunt for the new and improved alternatives to deal with the health issues was prompted by the demand for natural, chemical free and less harmful treatment strategies. [3] Endophytes can be viewed as a source of novel antimicrobial compounds. Various studies on the endophytes and their bioactivities have been conducted. The present study deals with the evaluation of antimicrobial activity of endophytic fungi isolated from *Syzygium cumini (L.) Skeels* plant.

II. MATERIAL AND METHODOLOGY

A. Collecting plant Sample:

A random selection of plant samples (leaf/stem) was done from different locations of Kurukshetra University, Kurukshetra, Haryana. The plant sample was cut using a sterile surgical blade and was bought into the laboratory for further use in an air tight bag. [4]

B. Surface Sterilization:

The sample was washed under running tap water and then the epiphytes were removed by sterilizing the surface of the plant sample in laminar air flow. The plant sample was cut into pieces of 1×1cm using sterile surgical blades. Then the sample fragments were successively surface sterilized by immersion in 70% ethanol for 1-3 min, sodium hypochlorite for 5-10 minutes, followed by rinsing with 70% ethanol and double distilled water respectively. Finally the sample fragments were allowed to air dry under aseptic conditions. [4]

C. Isolation of endophytic fungi:

The sample pieces (3-4) were placed on potato dextrose agar plates amended with 100mg/l ciprofloxacin. The plates were kept in a biochemical oxygen demand incubator at 25-27˚C for 5-7 days. [5]

D. Purification of Endophytic Fungi:

Each fungal colony that appeared on the potato dextrose agar plate was subcultured onto a fresh media plates and incubated in a B.O.D incubator at 25-27˚ C for 5-7 days. The slants of the pure cultures were prepared on potato dextrose agar and were stored in the refrigerator for further use. [5]

E. Fungal cultivation:

Each pure culture obtained was inoculated in 250ml Erlenmeyer flasks containing 100ml potato dextrose broth. The broths were incubated at 25±2˚C for 7-10 days. The broth cultures were filtered to separate the mycelia and the filtrate. The filtrate was used for the preliminary screening for antimicrobial activity. [4],[5]

F. Procurement of Test Pathogens:

The various bacterial and fungal pathogens were procured from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, which included Gram positive bacteria, *Streptococcus mutans* (MTCC 497), *Streptococcus pyogenes* (MTCC 1924), *Bacillus megaterium* (MTCC 428) and *Bacillus subtilis* (MTCC 121); Gram negative bacteria *Escherichia coli* (MTCC 40) and *Pseudomonas fluorescens* (MTCC 1748); fungal pathogens *Candida albicans* (MTCC 227) and *Candida glabrata* (MTCC 3814).Two phytopathogenic fungi *Alternaria solani* (MTCC 10690) and *Fusarium graminearum* (MTCC 2089) were also obtained. The slants of brain heart infusion agar were made to preserve the cultures. All the slants were kept at 4ºC in the refrigerator for further studies.

G. Assessment of antimicrobial activity:

The fungal culture broth, crude extract broth and the fungal mycelia were screened for their antimicrobial activity. The inoculums of different test pathogens were adjusted according to 0.5 McFarland standard and the antimicrobial activity was determined by using agar well diffusion method.

The cell free filtrate was extracted with equal volumes of ethyl acetate in a separating funnel by vigorous shaking for 20-30 minutes. The ethyl acetate was evaporated in a rotaevaporator set at 45 ˚C and 100 rpm. The resulting metabolite was dried in a water bath followed by dehydration with $MgSO_4$. Twofold serial dilutions in DMSO were prepared and the minimum inhibitory concentration was calculated using agar well diffusion method. [6],[7]

III. RESULTS AND DISCUSSION

The antimicrobial compounds extracted from the endophytes isolated from the medicinal plants indicate the extent of possible association of some of the fungal metabolites with the host plant. Gomes-Figueiredo in 2007 observed that the metabolic extracts of an endophyte isolated from the *Maytenus ilicifolia* (which is a small, shrubby, evergreen, herbal tree with immense therapeutic value) exhibited antimicrobial activity against different human parthogens as well as against a plant pathogen *Guignardia citricarpa*. In 2005, Li and coworkers tested the antitumour and antifungal activity of 130 endophytic fungi isolated from the Chinese medicinal plants. 9.2% of the isolated endophytic fungi showed antitumour activity while 30% presented antifungal activity against test fungal pathogens. Similarly, Teles and his coworkers (2006) and Hoffman and colleages (2008) also isolated the compounds (from the endophytes) with inhibitory action against the pathogenic organisms. [8],[9],[10]

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In the present study, antimicrobial activity of an endophytic bacterium isolated from the medicinal plant *Syzygium cumini* (L.) Skeels was evaluated against twelve test pathogens; five gram–ve, two gram+ve bacteria, three opportunistic fungal human pathogens and two plant pathogens. The cell free filtrate of the endophytic fungal isolate inhibited the growth of ten out of twelve test pathogens. This fungal isolate was obtained from the leaf sample of the *Syzygium cumini* plant. While all the seven bacterial test pathogens (involving both gram positive and gram negative bacteria) were controlled by the fungal metabolites. Two of the opportunistic fungal pathogens; *Candida glabrata* (MTCC 3814) and *Candida tropicalis* (MTCC 3421) could not be inhibited by the cell free fungal broth filtrate. However, clear halo was observed in the plates inoculated with opportunistic fungal pathogen; *Candida albicans* (MTCC 227). The maximum inhibition zone obtained was 23 mm. in diameter and was observed against) *Staphylococcus aureus* (MTCC 7443) and the lowest zone of inhibition were 12 mm. in diameter detected against *Streptococcus mutans* (MTCC 497), *Bacillus megaterim* (MTCC 428), *Pseudomonas fluorescens* (MTCC 1748) and *Alternaria solani* (MTCC 10690) (Table1).

The fungal mycelium was also evaluated for the presence of antagonistic activity and clear halos were observed against all the twelve test pathogens used in the study. The fact that fungal biomass exhibited the antimicrobial activity states that the antimicrobial metabolite was partially or completely secreted as intracellular compound. The zones of inhibition ranged between 11mm. to 15 mm. (in diameter).

The standard antibiotic ciprofloxacin inhibited the growth of six of the twelve test pathogens, *viz*., *Streptococcus mutans* (MTCC 497), *Streptococcus pyogenes* (MTCC 1924), *Bacillus megaterim* (MTCC 428), *Bacillus subtilis* (MTCC 121), *Escherichia coli* (MTCC 40) and *Pseudomonas fluorescens* (MTCC 1748) except *Staphylococcus aureus* (MTCC 7443) which was found resistant to 1 mg/ml of ciprofloxacin. On the other hand, the standard fungal antibiotic fluconazole in 1 mg/ml concentration could not inhibit the growth of any of the test pathogens. The endophytic mycelia inhibited all whereas the broth filtrate inhibited the growth of three of the five fungal pathogens (Table1).

Table 1- Antimicrobial activity exhibited by endophytic fungal isolate against test pathogens

Code of the sample	Zone of inhibition (in mm.)											
	Test pathogens											
	Bacteria							Fungi				
	Gram-positive					Gram-negative		Opportunistic human pathogens			Plant pathogens	
	Bm	Bs	Sa	Sm	Sp	Ec	Pf	Ca	Cg	Ct	As	Fg
SYZ. C. 4L broth	12± 0.57	18± 0.33	23± 0.57	12± 0.89	17± 0.51	14± 0.16	12± 0.89	18	NZ	NZ	12± 0.33	15± 0.33
SYZ. C. 4L Mycelia	14±0.33	15± 0.51	15± 0.66	12± 0.33	12± 0.33	11± 0.57	14± 0.16	13± 0.79	15± 0.33	15± 0.66	11± 0.57	13± 0.88
Positive control	48	35	NZ	39	38	40	50	NZ	NZ	NZ	NZ	NZ

Values are mean inhibition zone (mm) ± S.D of three replicates

Legend: NZ: no zone of inhibition; negative control: Dimethyl sulphoxide; positive control: ciprofloxacin as antibacterial agent (1mg/ml); fluconazol as antifungal agent (1mg/ml); B: Bean; L: Leaf; S: Stem; Sm: *Streptococcus mutans* (MTCC 497); Sa: *Staphylococcus aureus* (MTCC 7443); Sp: *Streptococcus pyogenes* (MTCC 1924); Bm: *Bacillus megaterim* (MTCC 428); Bs: *Bacillus subtilis* (MTCC 121); Ec: *Escherichia coli* (MTCC 40); Pf: *Pseudomonas fluorescens* (MTCC 1748); Ca: *Candida albicans* (MTCC 227); Cg: *Candida glabrata* (MTCC 3814); Ct: *Candida tropicalis* (MTCC 3421); As: *Alternaria solani* (MTCC 10690); Fg: *Fusarium graminearum* (MTCC 2089).

Although the zones of growth inhibition showed by the standard antibiotic were much larger than the endophytic fungal isolate but the endophytic fungus could inhibit the growth of all the test pathogens; involving those which were resistant to the standard antibiotic. (Table 1)

The minimal inhibitory values of crude ethyl acetate extract of the endophytic fungal isolate ranged from 0.234 to 32000µg/ml. the antimicrobial compound produced by the endophytic fungal isolate is broad spectrum as it inhibited a wide range of microorganisms; including both Gram positive and Gram negative bacteria. (Table 2)

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Table 2- Minimal inhibitory concentration demonstrated by the organic extract of antimicrobial compound.

Organic extracts of antimicrobial metabolite	Minimal inhibitory concentration (in µg/ml.)											
	Test pathogens											
	Bacteria							Fungi				
	Gram-positive					Gram-negative		Opportunistic human pathogens			Plant pathogens	
	Bm	Bs	Sm	Sp	Sa	Ec	Pf	Ca	Cg	Ct	As	Fg
Ethyl acetate extract	0.9375	16000	0.234	4000	32000	0.234	32000	8000	16000	0.937	0.937	8000

Legends: Sm: *Streptococcus mutans* (MTCC 497); Sa: *Staphylococcus aureus* (MTCC 7443); Sp: *Streptococcus pyogenes* (MTCC 1924); Bm: *Bacillus megaterim* (MTCC 428); Bs: *Bacillus subtilis* (MTCC 121); Ec: *Escherichia coli* (MTCC 40); Pf: *Pseudomonas fluorescens* (MTCC 1748); Ca: *Candida albicans* (MTCC 227); Cg: *Candida glabrata* (MTCC 3814); Ct: *Candida tropicalis* (MTCC 3421); As: *Alternaria solani* (MTCC 10690); Fg: *Fusarium graminearum* (MTCC 2089); NA: No activity.

The presence of clear halos in the plates of test pathogens for which no zone of inhibition was observed in broth filtrate could be explained by the high degree of dilution, permeability troubles and the presence of impurities in the crude extracts.

Since, the crude extract obtained showed the inhibition of all the twelve test pathogens and minimal inhibitory value observed was as low as 0.234µg/ml, this antimicrobial metabolite obtained from endophytic fungal isolate may be considered as a potent antimicrobial agent.

IV. CONCLUSION

An endophytic fungal isolate obtained from the leaf of the *Syzygium cumini* plant showed the inhibition of all twelve test pathogens included in the study. It is broad spectrum antimicrobial compound. Therefore, it demonstrates the potential to become a commercial antimicrobial drug. The antimicrobial compound is also effective against two major plant pathogens; *Alternaria solani* and *Fusarium graminearum* and may provide aid in agriculture. Further studies needs to be performed to increase the production of the antimicrobial compound, improve its properties and lower the costs of production. The antimicrobial compound obtained from endophytic fungus needs to be evaluated for its safety and efficacy in humans and plants.

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REFERENCES

[1] Strobel G, Daisy B. Bioprospecting for Microbial Endophytes and their Natural Products.(2003) Microbiol Mol Biol R 67: 491–502.

[2] Stephan A. (2012) Syzygium cumini (L.) Skeels: A multipurpose tree, its phytotherapic and pharmacological uses. Journal of Phytotherapy and Pharmacology 1(4): 22-32.

[3] Jain P, Sharma P (2012) Probiotics and their efficacy in improving oral health: a review. Journal Applied Pharmaceutical Sciences (11): 151-163.

[4] Sharma P., Jain P. (2014) Evaluation Of Antimicrobial Activity Of An Endophytic Aspergillus Sp. Isolated From Azadirachta Indica Against Plant And Human Pathogens. World Jornal of Pharmaceutical Research 3 (9): 808-814.

[5] KR Aneja, "Experiments in microbiology plant pathology and biotechnology". 4th ed. New age international publishers: New Delhi, 2003.

[6] Bonev B, Hooper J , Parisot J (2008) Principles of assessing bacterial susceptibility to antibiotics using the agar diffusion method. Jounal of Antimicrobial Chemotherapy 61(6): 1295-1301.

[7] Sen A, Batra A (2012) Determination of antimicrobial potentialities of different solvent extracts of the medicinal plant: Phyllanthus amarus Schum. And Thonn. International Journal of Green Pharmacy 6 (1): 50-56.

[8] Gomes-Figueiredo J, Pimentel IC, Vicente VA et al. (2007) Bioprospecting highly diverse endophytic Pestalotiopsis spp. with antibacterial properties from Maytenus ilicifolia, a medicinal plant from Brazil. Can J Microbiol 53: 1123-1132.

[9] Li H, Qing C, Zhang Y, Zhao, Z (2005) Screening for endophytic fungi with antitumour and antifungal activities from Chinese medicinal plants. World J Microbiol Biotechnol 21: 1515-1519.

[10] Hoffman AM, Mayer SG, Strobel GA et al. (2008) Purification, identification and activity of phomodione, a furandione from an endophytic Phoma species. Phytochemistry 69: 1049-1056.