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ANTIMICROBIAL EFFICACY OF SECONDARY METABOLITES FROM *GLOMERELLA CINGULATA*

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ABSTRACT

Fungi are known to produce a vast array of secondary metabolites that are gaining importance for their biotechnological applications. Early reports suggest that *G. cingulata* has the capability to transform many compounds by various enzymatic actions. Therefore, the focus of this study was to determine the antibacterial and antifungal activity of crude ethyl acetate extract of *G. cingulata* using agar cup bioassay method. Crude extract of *G. cingulata* exhibited remarkable antifungal activity against *Rhizopus oryzae*, *Chrysoporium tropicum and Beauveria bassiana* but no antifungal activity was found against *Alternaria tenuissima* and *Aspergillus niger* at any concentrations. The crude extract presented no antibacterial activity against Gram positive and Gram negative bacteria at any concentration.

Key words: Antimicrobial activity, *Glomerella cingulata*, Agar cup bioassay

INTRODUCTION

Identification of microorganisms that produce bioactive compounds is of great interest in the development of new molecules to fight against many pathogens (2). Fungi produce a wide range of secondary metabolites with high therapeutic value as antibiotics, cytotoxic substances, insecticides, compounds that promote or inhibit growth, attractor, repellent etc., (1). Secondary metabolites produced from fungi vary in production, function and specifity to a particular fungus (4). These metabolites are being exploited in different fields of medicine and industries (3,10). Among fungi classes, ascomycetes are reported to be active producers of antimicrobial compounds, which have high therapeutic values (9). Among the ascomycetes, *Glomerella cingulata* has been reported for the potential of biotransformation and production of many bioactive molecules (6,7,8). Hence, in the present study we have tested the antibacterial and antifungal properties of *G. cingulata* crude extract against some selected bacterial and fungal strains.

MATERIALS AND METHODS

Fungi

Six fungal organisms *Glomerella cingulata* (MTCC No. 2033), *Rhizopus oryzae* (MTCC No. 262), *Chrysoporium tropicum* (MTCC No. 2821), *Beauveria bassiana* (MTCC No. 984) *Alternaria tenuissima* (MTCC No. 2802) and *Aspergillus niger* (MTCC No. 281) were obtained from the Institute of Microbial Technology, Chandigarh, India. Cultures were maintained on potato dextrose agar slants and were sub-cultured in Petri dishes prior to testing. The nutrient agar and the potato dextrose agar media were provided by M/S Himedia, Mumbai, India.

Bacteria

Six test organisms, *Staphylococcus aureus* (MTCC No. 96), *Bacillus subtilis* (MTCC No. 441), *Bacillus sphaericus* (MTCC No. 511), *Klebsiella aerogenes* (MTCC No. 39), *Psuedomonas aeruginosa* (MTCC No. 741) and *Chromobacterium violaceum* (MTCC No. 2656) were obtained from the Institute of Microbial

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Technology, Chandigarh, India. Cultures were maintained on nutrient agar slants and were sub-cultured in Petri dishes prior to testing.

Fungal growth and extraction of crude extract

A portion of mature agar slant was inoculated in 100ml potato dextrose broth in a 500 ml Erlenmeyer flask and incubated at 30ºC on a rotary shaker at 250 rpm for 8 days. The fermented broth (3 liters) was treated with ethyl acetate (2 liters) for 2 hours followed by cheesecloth filtration to remove the biomass. The organic extract was separated and dried over anhydrous sodium sulfate and concentrated *in vacuo* to yield a crude yellow solid (0.3g).

Antibacterial and antifungal studies

Penicillin G (100 µg/ml) and streptomycin (100 µg/ml) were used for Gram negative and for Gram positive bacteria respectively as controls. For fungi, clotrimazole (100 µg/ml) was used as control. The crude extract of *G. cingulata* was made to different concentrations (100 - 1500 µg/ml) and tested against the bacterial and fungal strains, following the procedure of Linday, 1962 (5). The diameter of the minimum zone of inhibition was measured in mm. For each test, three replicates were performed.

RESULTS AND DISCUSSION

Fungi are well known to show antibacterial, antifungal, larvicidal, molluscicidal, antioxidant and free-radical scavenging activities (4). A vast number of fungi have been utilized for biotransformation process and many more to be explored for isolation of some potential compounds. In the present study, different concentrations of crude ethyl acetate extract of *G. cingulata* were tested against 6 bacterial strains and 6 fungal strains. It is found that there was no antibacterial activity exhibited by any concentrations of *G. cingulata* crude extract (Table 1) whereas, there were some good antifungal activities found from 1300 to 1500 µg/ml against *Rhizopus oryzae*; 1100 to 1500 µg/ml against *Chrysosporium tropicum* and from 1200 to 1500 µg /ml against *Beauveria bassiana* (Table 2). However, these antifungal activities against these fungi were shown to be equal or less activity when compared to the control clotrimazole (100 µg/ml). This differential action of antifungal property of crude extract of

Table 1. Testing of antibacterial activity of *G. cingulata* against some selected gram (+ve and –ve) strains.

Test organisms	Concentration (µg/ml)											Penicillin-G
Gram positive	100-500	600	700	800	900	1000	1100	1200	1300	1400	1500	100 µg/ml (Control)
Staphylococcus aureus	-	-	-	-	-	-	-	-	-	-	-	18
Bacillus subtilis	-	-	-	-	-	-	-	-	-	-	-	20
Bacillus sphaericus	-	-	-	-	-	-	-	-	-	-	-	19
Gram negative												Streptomycin 100 µg/ml (Control)
Psuedomonas aeuginosa	-	-	-	-	-	-	-	-	-	-	-	34
Klebsiella aerogenes	-	-	-	-	-	-	-	-	-	-	-	30
Chromobacterium violaceum	-	-	-	-	-	-	-	-	-	-	-	28

*Negative control (DMSO) – No activity; Zone of Inhibition values are indicated in mm.

Table 2. Testing of antifungal activity of *G. cingulata* against some selected fungal strains.

Test organisms	Concentration (µg/ml)											Clotrimazole
	100-500	600	700	800	900	1000	1100	1200	1300	1400	1500	100 µg/ml
Rhizopus oryzae	-	7	9	11.5	12.5	15	16	18	20	20.5	21.5	22
Alternaria tenuissima	-	-	-	-	-	-	-	-	-	-	-	28
Aspergillus niger	-	-	-	-	-	-	-	-	-	-	-	23
Chrysosporium tropicum	-	-	7	8	11	13	14.5	16	18	19.5	20	20
Beauveria bassiana	-	-	-	-	7	9	11	12.5	13	16	18	29

*Negative control (DMSO) – No activity; Zone of Inhibition values are indicated in mm.

G. cingulata may be depending upon the active compounds on the specific fungus. Hence, the present study gives an idea to test against more number of organisms and to find out the actual antifungal compound from the *G. cingulata*.

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RESUMO

Eficiência antimicrobiana do extrato bruto de *Glomerella cingulata*

Fungos são conhecidos produtores de uma vasta coleção de metabólitos secundários que vem mostrando importância crescente na sua aplicação biotecnológica. Publicações anteriores sugerem que *G. cingulata* tem a capacidade de transformar vários componentes por diferentes ações enzimáticas. Logo, o foco deste estudo foi determinar a atividade antibacteriana e antifúngica do extrato bruto de *G. cingulata* obtido por acetato de etila utilizando-se um método envolvendo bloco de agar. O extrato bruto de *G. cingulata* demonstrou marcante atividade antifungica contra *Rhizopus oryzae*, *Chrysoporium tropicum* e *Beauveria bassiana* entretanto, não foi possível detectar, em nenhuma concentração, atividade antifungica contra *Alternaria tenuissima* e *Aspergillus niger*. O mesmo extrato não apresentou atividade antibacteriana, em nenhuma concentração, contra bactérias Gram negativa e positiva.

Palavras chave: atividade antimicrobiana, Glomerella cingulata, bloco de agar

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