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Research Article

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Isolation and detection of anti-bacterial activity of endophytic fungi from *Bombex cebia and Argemone mexicana*

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ABSTRACT

Bioactive compounds of endophytic fungi exhibit antimicrobial, antioxidant, anticancerous, antiviral and insecticidal properties. They harbor inter or intra-cellular in the plants epidermal layer. They are the most potent microorganism in establishing interrelationship with plant without harming them or asymptomatically. They have the mechanism which enables them to establish association with the plant and produce similar bioactive compound as plant produce. This research paper focused on the isolation, production, screening and separation of exploitable bioactive compounds from the plants Bombex cebia and Argemone mexicana. Total 15 fungi were isolated from segments of the plants in which fungal isolate SR2 isolated from leaf of Bombex cebia, AMS2 and AMS3 isolated from stem of Argemone mexicana showed best antibacterial result against six bacterial strains of Ethyl acetate crude extract. SR2 showed maximum zone of inhibition (27mm) against B. subtilis, AMS2 showed maximum zone of inhibition against K. pnumoniae (21mm), AMS3 showed maximum zone of inhibition against K. pneumoniae and S. typhimurium (19mm).

Keywords: Endophytic fungi, *Bombex cebia*, *Argemone mexicana*, Ethyl acetate crude extract, Anti-bacterial activity.

INTRODUCTION

There is need to search new ecological niches for potential of natural bioactive agents for different pharmaceutical, agriculture and industrial application; these should be renewable, eco-friendly and easily obtainable natural products discovery in the search for new drugs, and is the most potent source for the discovery of novel bioactive compounds [1]. Thus, a large number of bioactive compounds are isolated from the plants, bacteria, fungi and many other organisms. In which endophytic fungi being the most promising of this have been a source of various such bioactive compounds [2]. The endophytic fungi live inside the host without causing any symptoms and provide protection to their host against a number of pathogens by secreting bioactive secondary metabolites [3, 4]. They are present almost all parts of plant and contribute by producing plethora of known and novel biologically active metabolites. The secondary metabolites produced can be utilized in modern medicine, agriculture and industry [5]. The novel bioactive compounds produced by endophytic fungi consist of anti-bacterial, anti-fungal, anti-viral, anti-inflammatory and anti-tumor properties. These compounds belong to alkaloids, flavenoids, terpenoids derivatives and some other types of structural compounds [6, 5, 7, 8].

Bombex cebia belongs to Order: Malvales, Family: Malvaceae and *Argemone mexicana* belongs to Order: Ranunculales, Family: Papaveraceae. Both of these plants are known for their active ingredients of traditional

medicinal products and plant extracts. The plant products currently been used in screening programmes in pharmaceutical companies, universities and various research institutes. *Bombex cebia* (Shemul) has been known well for treatment of infections in human body. Mostly plant parts used in the treatment of diseases as diarrhea, dysentery and stomach troubles in folk medicines [9, 10, 11]. Along this the plant extracts have been used in the treatment of diseases like urinary troubles, leucorrhoea, gonorrhea, boils, body wash, chicken pox, dental caries, conjunctivitis, guinea worm, cough, leprosy have been reported to be treated. *Argemone mexicana* (prickly poppy) seeds are utilized as antidote to snake venom. Smokes of seed are helpful in treatment of toothache, freshly produced yellow milky seeds are used in curing diseases like warts, cold sores, cutaneous infections, skin diseases, itches, dropsy and jaundice [12].

EXPERIMENTAL SECTION

Sample Collection

Bombex cebia L. was collected from Dumna and *Argemone mexicana* from R.D. University, Jabalpur (M.P.). The tissue samples of the plants were then collected in sterile bags and processed in laboratory. Freshly collected plant materials were utilized for the isolation of the endophytic fungi to reduce the chance of contamination.

1solation of endophytic fungi

Isolation of endophytic fungi was done by some modifications according to the methods described by Peterni [13]. Each sample of roots, stems, leaves and buds were rinsed gently for 5-10 min. in running water to remove the dust and debris. After proper washing stem, root, and leaf samples of different medicinal plant part were cut into 3-4mm x 0.5-1mm separately. Simultaneously the fragments were disinfected with 70% ethanol for 2 minutes. Immerge it in sodium hypochlorite for 2 minutes. Then rinse it with distilled water followed by drying it on filter paper then the samples were to PDA plates amended with Streptomycin (40-50 mg/l). Inoculated plates were then provided temperature range of 28±1°C for 7 days. Then the isolated fungi were preserved in PDA slants at 4°C.

Test Organisms

Six test bacteria were selected to test the effectiveness of the metabolites. The selected bacterial species were *Escherichia coli*, *Klebsiella pneumoniae*, *Bacillus subtilis*, *Salmonella typhimurium*, *Enterrococcus* sp. and *Streptococcus pyogenes* were maintained on slants for 24 hrs. at 37°C in bacteriological incubator. The source of these cultures was (MTCC) Chandigarh, India.

Production of secondary metabolite and extraction

Static flask were used for the concerned growth of culture, spores from 7 days old culture were transferred into 500 ml Erlenmeyer flask containing 250 ml of Potato Dextrose Broth (PDB). The seeded flasks were incubated at 28±1°C for 7, 14, and21 days under stationary conditions. The cultures were harvested and filtered through Whatman no. 1 filter paper to give clear filtrate that was exposed to the extraction process. The culture filtrate was used for preliminary evaluation of antibacterial activity using an agar well diffusion method [14] against 6 pathogenic species of bacteria. The extract residue was dissolved in Ethyl acetate and stored at 4°C to be used as stock solution for anti-bacterial activity.

Anti-bacterial activity

For antibacterial evaluation, agar well diffusion method was performed by standard method [15]. Nutrient Agar plates were inoculated with overnight culture of each bacterial suspension, by evenly spreading out with sterile Borosil glass spreader. The Agar wells were prepared by scooping out the media with a sterile cork borer (10mm in diameter). The wells were then filled with 60 µl, of the crude fungal extract pre dissolved Ethyl acetate. The plates were then incubated at 37°C for 24 hrs and the zone of inhibition was recorded by Himedia antibiotic Zone Scale and compared with the control.

RESULTS AND DISCUSSION

The plant materials were collected from Priyadarshani colony, Dummna and R.D. University campus, Jabalpur. About 50 segments (10 pieces of buds, 20 pieces the branches and 20 pieces of root part) of *Bombex cebia and Argemone mexicana were* processed for the isolation of endophytic fungus. Total of 15 endophytes were isolated in the present experiment from *Bombex cebia and Argemone mexicana* plant (Table 1).

Table 1 Enophytic fungal isolated from *Bombex cebia and Argemone Mexicana*

Plant name	Plant part	Isolate fungi
Bombex cebia	Bud	SBU1
		SBU2
		SBU3
		SBU4
	Bark	SB1
		SB2
		SB3
		SB4
	Root	SR1
		SR2
Argemone Mexicana	Stem	AMS1
		AMS2
		AMS3
		AMS4
	Root	AMR1

Recently, Sandhu *et al*. [16] isolated 12 endophytic fungi from the *Clatropis procera* and evaluate the anti-bacterial activity against *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Bacillus subtilis and Staphylococcus epidermidis* of DMSO crude extracts of fungal endophytes from this plant. Sandhu *et al*. [17] till now also isolated endophytic fungi from *Ricinus communis* they investigated their potential antibacterial activity of 10 endophytic fungal species. Similarly, Bagyalakshmi *et al*. [18] isolated endophytic fungus *Pestalotiopsis* sp. from the leaves of *Pinus caneriensis*. The crude culture extract of hexane, ethyl acetate, dichloromethane and methanol were screened for antimicrobial activity.

Table 2 Anti-bacterial screening of Isolated Endophytic fungal

S. No.	**Name of Endophytic fungi**	**Diameter zone of inhibition (in mm)**					
		S. typhimurium	***K. pneumoniae***	***S. pyogenes***	***B. subtilis***	***E. coli***	***Enterrococcus* sp.**
1.	SB1	6	10	6	8	6	20
2.	SB2	9	11	9	10	15	14
3.	SB3	9	13	12	11	8	11
4.	SB4	10	7	8	5	17	8
5.	SBU1	11	8	10	18	13	9
6.	SBU2	7	6	9	12	8	7
7.	SBU3	12	8	10	9	8	7
8.	SBU4	13	14	10	11	10	8
9.	SR1	5	10	7	12	9	7
10.	SR2	26	23	26	27	21	21
11.	AMS1	7	9	6	10	-	12
12.	AMS2	20	21	19	20	18	18
13.	AMS3	19	19	18	18	18	15
14.	AMS4	-	10	12	-	-	9

Anti-bacterial screening of total isolated endophytic fungi

During screening of the endophytic fungi objective was to obtain the anti-bacterial activity produced by various endophytic fungi. These results might be attributed either to the anti-microbial potency of the Ethyl acetate extract or to the high concentration of unidentified active principle in the extracts. The technique utilized was agar well diffusion method against six bacterial strains. SR2 showed the maximum zone of inhibition against *S. typhimurium, K. pneumoniae, B. subtilis, S. pyogenes, E. coli and Enterrococcus* sp. AMS2 showed the maximum activity against *S. typhimurium, K. pneumoniae* and *B. subtilis*. AMS3 showed the maximum activity against *S. typhimurium* and *K. pneumoniae*. AMS4 showed the maximum activity against *S. typhimurium, K. pneumoniae* and *B. subtilis*. SB1 showed the maximum activity against *K. pneumonia*. SB2 showed the maximum activity against *E. coli* and *Enterrococcus* sp. SB3 showed maximum activity against *K. pneumoniae* and *S. pyogenes*. SB4 showed the maximum activity against *E. coli*. SBU1 and SBU2 showed maximum activity against *B. subtilis*. SBU3 showed maximum activity against *S. typhimurium*. SBU4 showed maximum zone against *K. pneumonia*. SR2 showed the

maximum anti-bacterial activity against *B. subtilis* (Table 2). All the above strain was found potent at some extent to inhibit the bacterial population but somehow some strains gave exceptionally best results the fungal isolate were SR2, AMS2, AMS3.

Broad spectrum anti-bacterial activity of endophytic fungi

As depicted in (Table-3 and Figure-1) antibacterial activity results of isolated endophytic fungi SR2 showed 27mm zone against *E. coli*, 26mm zone against *S. typhimurium* and *S. pyogenes* respectively, 23mm zone against *K. pneumoniae*, 21mm zone against *E. coli* and *Enterrococcus* sp. AMS2 showed 20mm zone against *S. typhimurium*, 21mm zone against *K. pneumoniae*, 19mm zone against *S. pyogenes*, 18mm zone against *E. coli* and *Enterrococcus* sp. respectively. AMS3 showed 19mm zone against *S. typhimurium* and *K. pneumoniae*, 18mm zone against *S. pyogenes*, *B. subtilis* and *E. coli* respectively, 15mm against *Enterrococcus* sp. In the present study, the Ethyl acetate extract of SR2, AMS2 and AMS3 isolated from the *Bombex cebia and Argemone mexicana* exhibited significant antibacterial activity against bacterial pathogens (Figure 2). The results revealed that the metabolites of all three isolated fungi are the potential source for the development of new antibacterial compounds.

Table 3 Broad spectrum of effective secondary metabolite producing endophytic fungi against six medically concerned bacterial strains

S. No.	Name of endophytic fungi	Diameter zone of inhibition (in mm)					
		S. typhimurim	*K. pneumoniae*	*S. pyogenes*	*B. subtilis*	*E. coli*	*Enterrococcus* sp.
1.	SR2	26	23	26	27	21	21
2.	AMS2	20	21	19	20	18	18
3.	AMS3	19	19	18	18	18	15

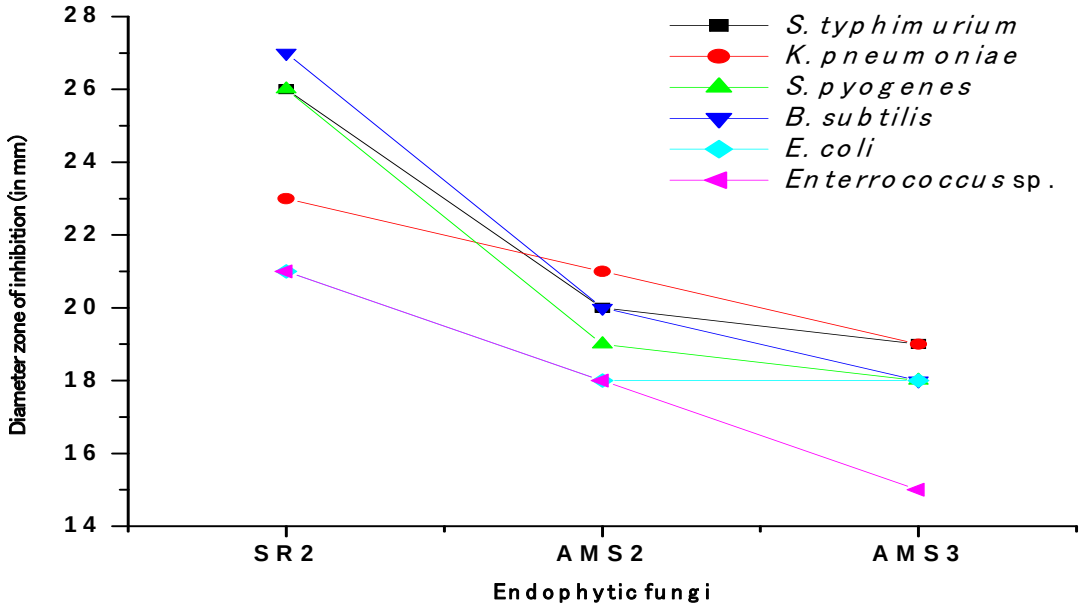
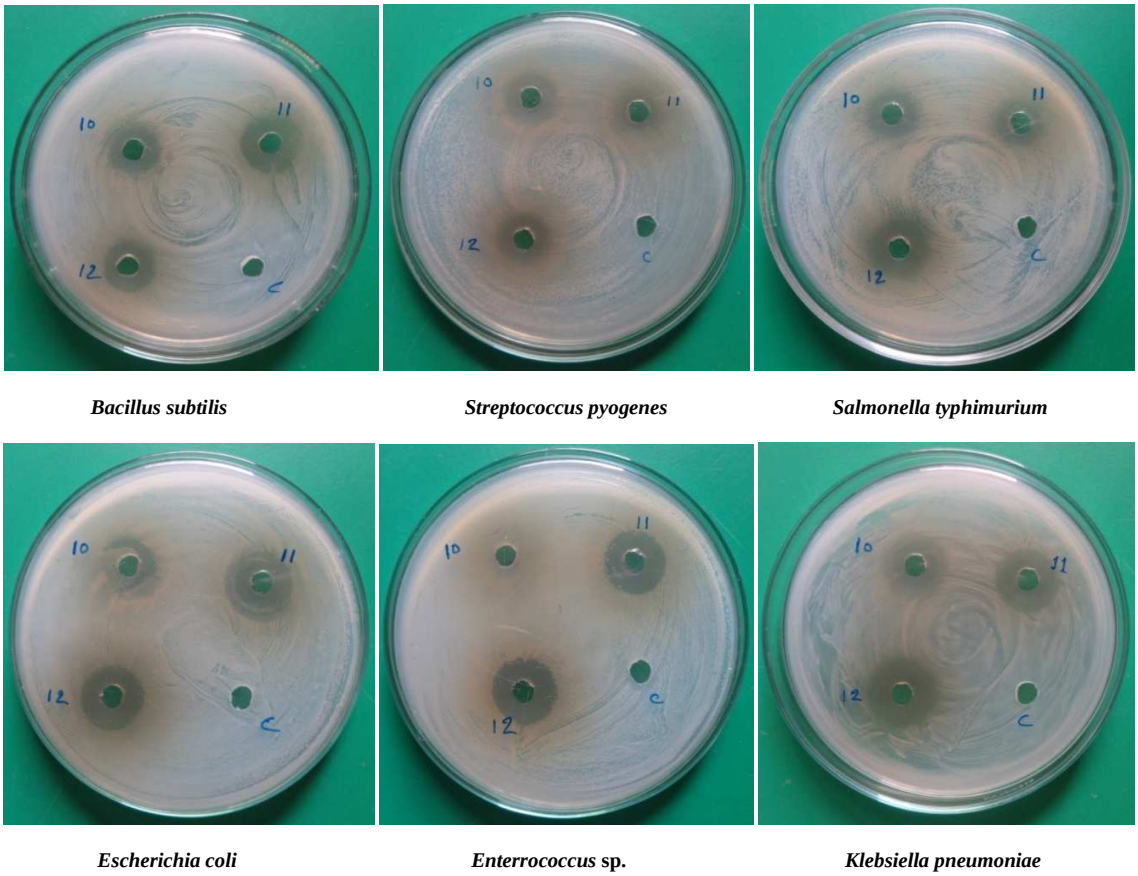


Fig. 1 Anti-bacterial activity of endophytic fungi against six pathogenic bacteria



Bacillus subtilis ***Streptococcus pyogenes*** ***Salmonella typhimurium***

Escherichia coli ***Enterrococcus* sp.** ***Klebsiella pneumoniae***

Fig. 2 Anti-bacterial activity crude extract of isolated fungi against 6 pathogenic bacteria

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