International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.10 No.4, pp 430-435, 2017

In Vitro Antimicrobial Potential of *Aspergillus niger* (MTCC-961)

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Abstract : In vitro antibacterial and antifungal activity of fungal extract at different concentrations were screened against four gram positive bacteria, *Bacillus coagulans, Staphylococcus aureus, Bacillus licheniformis,Corynibacterium glutamicum,* four gram negative bacteria, *Escherichia coli, Pseudomonas fluorescence, Klebsiell apneumoniae, Sphingomonas paucimobilis,* and three fungal species *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus* by cup plate method. The extract were found to inhibit the growth of all the bacteria and fungal organisms test. The antimicrobial activity of fungal extract were comparable with the standard antibacterial agent, gentamycine and chloramphenicol as standard antifungal agent and were found to be active against all the organisms tested.
Key words : *Aspergillus niger,* Fungal extract, Antibacterial activity, Antifungal activity.

Introduction:

Overuse of antibiotics and increased resistance to drugs in microorganisms as becoming a serious global concern[1]. It leads to urgent search for new sources of antibiotics that are cheap, effective, non-toxic, and have little environment impact. More over, the frequency of advent infectious diseases has increased world wide accompanied with an increasing extent of environmental degradation, spoilage of land, industry sewage and poisonous gases[2].

Aspergillus niger is a versalite filamentous fungus found in the environment all over the world in soil and decaying plant material, and it has been reported to grow on a large number of foods and feeds[3]. Phytochemical studies have shown that fungi with antimicrobial activity contain bioactive constituents such as tannins, flavonoids, alkaloids, saponins[4]. Since microorganisms grow in unique and extreme habitats, they may have the capability to produce unique and unusual metabolites. Generally, the reason why they produce such metabolites is not known, but it is believed that many of these metabolits may act as chemical defense as an adaptation of fungi competing for substrates[5]. The antimicrobial properties of secondary metabolites derived from various groups of fungi are widely reported[6-9]. Antimicrobial activities of an endophytic Aspergillus sp. Against some clinically significant human pathogens have been reported[10].

Microorganisms are highly diverse source of bioactive natural products. Endophytic microbes are fungi and bacteria that colonize internal tissues of living plants without causing any harm to its host[11]. Bioactive natural compounds isolated from fungal endophytes have been playing a promising role in the search for novel drugs and becoming an inspiring source for researchers due to their enormous structure diversity and complexity. The present study to investigate in vitro antimicrobial potential of Aspergillus niger extract against different gram positive, gram negative bacteria and fungi.

Materials and Methods:

Culture collection and maintenance : Pure cultures of *Aspergillus niger* (MTCC No-961) were purchased from MTCC, Chandigarh, India and were immediately transferred to sterile agar slants of potato dextrose agar media. The strains were grown in potato dextrose media.The *Asperigillus sps.*, culture from potato dextrose broth was streaked on a Potato dextrose agar slant and it was incubated at 27oC for 72 hours. It was then sub cultured and was stored in refrigerator for further use.

Preparation of fungal extracts : The fungi were mass cultured on Potato Dextrose Broth (PDB) media for 10 days at 27^{o}C on a shaker at 160 rpm. After incubation the mycelium were filtered and then the filterate were then extracted with organic solvent ethyl acetate (1:1) by using cold percolation for 48-72hr. The obtain extract was separated by using separating funnel and then concentrated under vacuum at 40^{o}C by using rotary evaporator.

Antibacterial activity : The Bacterial cultures of *Bacillus coagulans(MTCC-5856), Staphylococcus aureus(MTCC-3160), Bacillus licheniformis(MTCC-429),Corynibacterium glutamicum(MTCC-2745), Escherichia coli (MTCC-443), Pseudomonas fluorescence(MTCC-2453), Klebsiell apneumoniae(MTCC-452), Sphingomonas paucimobilis(MTCC-6363)* grown overnight at 370 temperature were used for testing the antibacterial activity. Nutrient agar medium (High media) was dissolved in water this was distributed in 100ml conical flask and were sterilized in an autoclave at 121^{o}C 15lbp for 15min after autoclaved the media poured sterilized petriplates. Gentamycin was taken as positive control and DMSO was taken as negative control for antibacterial activity. The antibacterial activity of fungal extract was evaluated by Agar well diffusion method[12]. Inoculums were spread over the surface of agar plates with sterile glass spreader. 5 wells were made at equal distance using sterile cork borer. To test the antibacterial activity of extracts were made a final concentrations of 100mg/ml. , 60µg/ml, 80µg/ml, 100µg/ml of extract was poured on each well and then plates were incubated for a period of 24h at 37^{o}C in incubater after incubation diameter (mm) of the clear inhibitory zone formed around the well was measured.

Antifungl activity : The fungal cultures of *Aspergillus niger(MTCC-961), Aspergillus flavus(MTCC-3396), Aspergillus fumigatus(MTCC-2584)*grown overnight at 370 temperature were used for testing the antibacterial activity. Potato dextrose broth (High media) was dissolved in water this was distributed in 100ml conical flask and were sterilized in an autoclave at 121^{o}C 15lbp for 15min after autoclaved the media poured sterilized petriplates. Chloramphenicol was taken as positive control and DMSO was taken as negative control for antibacterial activity. The antifungal activity of fungal extract was evaluated by Agar well diffusion method (Perez et al., 1990). Inoculums were spread over the surface of agar plates with sterile glass spreader. 5 wells were made at equal distance using sterile cork borer. To test the antibacterial activity of extracts were made a final concentrations of 100mg/ml. , 80µg/ml, 100µg/ml, 120µg/ml of extract was poured on each well and then plates were incubated for a period of 24h at 37^{o}C in incubater after incubation diameter (mm) of the clear inhibitory zone formed around the well was measured.

Results and Disscussion:

The fungal extract showed considerable antibacterial and antifungal activities. The fungal extract were tested for their antimicrobial activity against four gram positive bacteria and four gram negative bacteria and fungal test organisms, by Agar well method. The results of antimicrobial activity against tested pathogens were tabulated in the tables 1,2 for the crude extract of *Aspergillus niger* for antibacterial and antifungal activity respectively. Among the gram positive organisms *corynibacterium glutamicum* (100µg/ml) showed maximum zone of inhibition (17mm), *Bacillus licheniformis* (60µg/ml showed minimum zone of inhibition when compared to the standard antibiotic and among the gram negative organisms *Staphylococcus aureus* (100µg/ml) showed maximum zone of inhibition (18mm), *Escherichia coli*(60µg/ml) showed minimum zone of inhibition (10mm) compared to standard antibiotic.

Among the fungal organisms *Aspergillus fumigatus* (120µg/ml) showed maximum zone of inhibition (12mm), *Aspergillus niger* (80µg/ml) showed minimum zone of inhibition(6mm) when compared to standard antibiotic.

A previous study by Rao et al., 2000[13] showed antibacterial activity of *Aspergillus terreus* against *S.aureus, Enterococcus faecalis, B.Subtilis, Pseudomonas aeruginosa and E.coli. Aspergillus* genera are a major contributor of antimicrobial compound of fungal origin (Bungni *et al*., 2004).

From the above preliminary studies on the antibacterial and antifungal activities, the crude extracts of the fungi showed promising activity against all the test pathogens, promising a future scope for the use of these organism against a range of microbial populations. The work can further be extended to reveal its source of secondary metabolites that attributes to the antimicrobial activity.

Table-1Antibacterial activity of *Aspergillus niger* extract:

S.No	Test Organism	Zone of Inhibition (mm)				
		60µg/ml	80µg/ml	100µg/ml	Standard (Gentamycin)	Control (DMSO)
1	*Staphylococcus aureus (MTCC-3160)*	12	14	18	17	-
2	*Klebsiella pneumonia (MTCC-452)*	10	14	16	16	-
3	*Escherichia coli (MTCC-443)*	10	12	15	15	-
4	*Bacillus coagulans (MTCC-5856)*	11	13	15	17	-
5	*Spinghomonas paucimobilis (MTCC-6363)*	12	13	15	16	-
6	*Pseudomonas fluorescens (MTCC-2453)*	14	15	17	19	-
7	*Corynibacterium glutamicum (MTCC-2745)*	12	15	17	16	-
8	*Bacillus licheniformis (MTCC-429)*	8	10	11	19	-

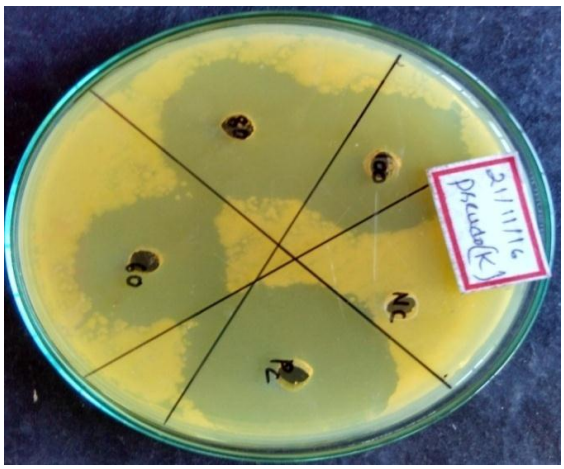

Fig-1-Antibacterial activity of *A.niger* extract against *Pseseudomonas flurescence*

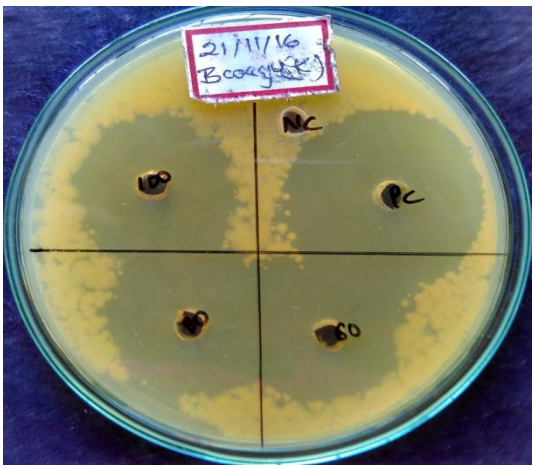

Fig-2-Antibacterial activity of *A.niger* extract against *Bacillus coagulans*

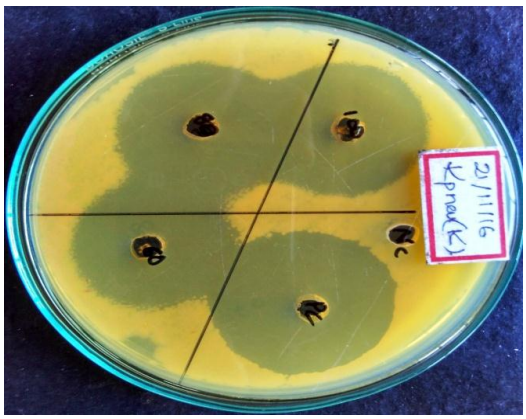

Fig-3-Antibacterial activity of *A.niger* extract against *Klebsiella pneumoniae*

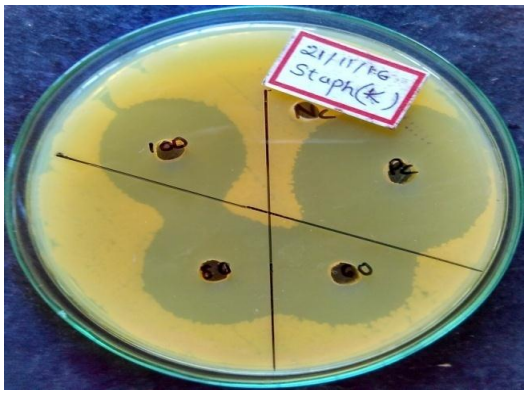

Fig-4-Antibacterial activity of *A.niger*extract against against *Staphylococcus aureus*

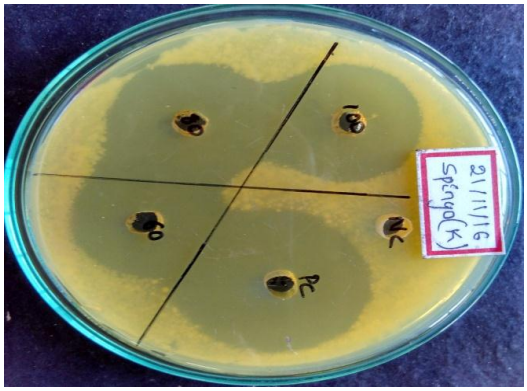

Fig-5-Antibacterial activity of *A.niger* extract *Spinghomonas paucimobilis*

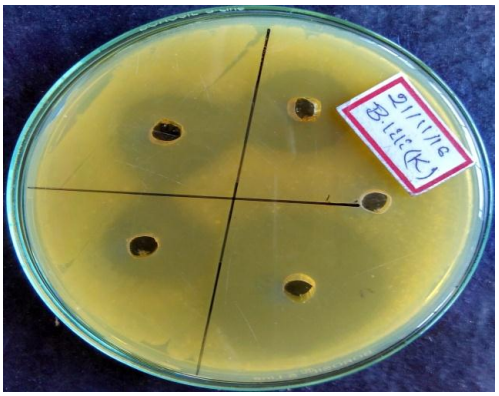

Fig-6-Antibacterial activity of *A.niger* extract against *Bacillus licheniformis*

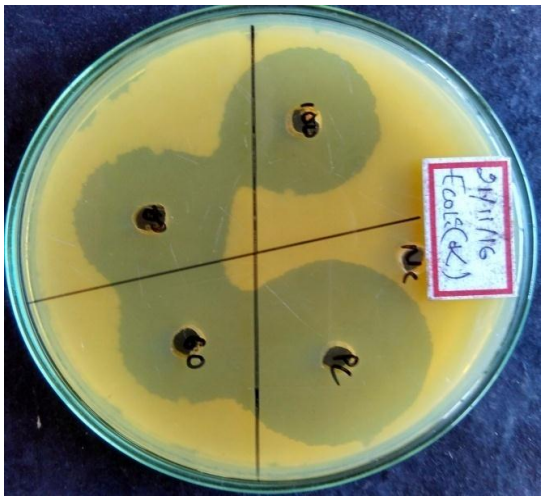

Fig-7-Antibacterial activity of *A.niger* extract against *Escherichia coli*

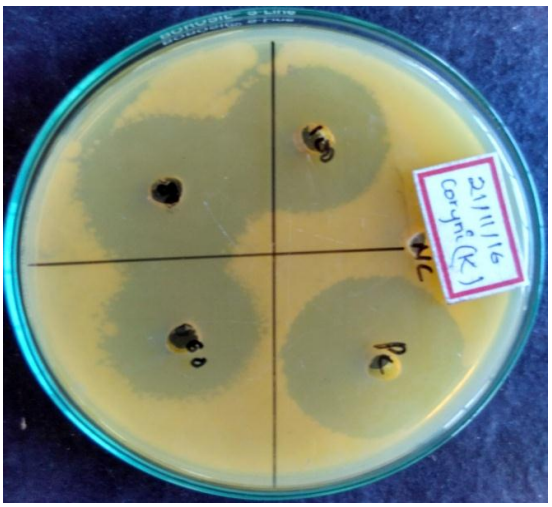

Fig-8-Antibacterial activity of *A.niger* extract against *Corynibacterium glutamicum*

Table-2-Antifungal activity of *Aspergillus niger* extract:

S.No	Test Organism	Zone of Inhibition (mm)				
		80µg/ml	100µg/ml	120µg/ml	Standard (Chloramphenicol)	Control (DMSO)
1	*A.niger(MTCC-961)*	6	9	10	13	-
2	*A.flavus(MTCC-3396)*	8	9	11	15	-
3	*A.fumigatus(MTCC-2584)*	9	10	12	17	-

Conclusion:

The present work was attempted to A.niger which capable of producing efficient antibacterial. Findings of our study indicates that fungi are a rich source of natural antimicrobial compounds. Antimicrobial compound isolation from fungi can meet the ever growing need of antimicrobial novel compounds.

References:

1. Aksoy DY, Unal.S. New antimicrobial agents for the treatment of Gram-positive bacterial infections. Clin Microbio Infect, 2008; 14:411-420.
2. Guo B, Wang Y, Sun X, Tang K, Bioactive natural products from endophytes: A review. App Biochem and Microbiol, 2008; 44(2):136-142.
3. Sorensen, K., Kim, K.-H. and Takemoto, J.Y.1996. In vitro antifungal and fungicidal activities and erythrocytes toxicities of Pseudomonas syringae pv. Syringae. Antimicrob Agents Chemother 40, 2710-2713
4. Chukwuka, K.S., J.O.Ikheloa, I.O.Okonko, J.O.Moody, T.A.2011. Mankinde, Advances in applied science research, 2(4):37-48
5. Gallo ML, Seldes AM, Cabrera GM. Antibiotic longchain α-unsaturated aldehydes from the culture ofthe marine fungus *Cladosporium* sp. *Biochem. Systemat. Ecol.* 32, 2004, 551-554.
6. Sekiguchi, J. and Gaucher, G. M. 1977. Conidiogenesis and secondary metabolism in *Penicilliumurticae*. *Appl Environ Microbiol* 33:147-158.
7. John, M., Krohn, K., Florke, U., Aust, H. J., Draeger, S. and Schulz, B. 1999. Biologically activesecondary metabolites from fungi. 12. (1) Oidiolactones A-F, labdane diterpene derivativesisolated from *Oidiodendron truncate*. *J Nat Prod* 62:1218-1221.

8. Schulz, B., Boyle, C., Draeger, S., Rommert, A. and Krohn, K. 2002. Endophytic fungi: a source ofnovel biologically active secondary metabolites. *Mycol Res* 106:996-1004.
9. Keller , N. P ., Turner, G. and Bennett, J. W. 2005. Fungal secondary metabolism - from biochemistryto genomics. *Nat Rev Microbiol* 3:937-947.
10. Tayung, K. and Jha, D. K. 2007. Antimicrobial activity of a compound produced by *Aspergillus* sp.DEF 505, an endophyte on *Taxus baccata*. *J Microbial World* 9: 287-292.
11. Kaneko T, Minamisawa K, Isawa T, Nakatsukasa H, Mitsui H, Kawaharada Y et al. Complete genomic structure of the cultivated rice endophyte Azospirillum sp. B510. DNA res, 2010;17:37-50
12. Perez C, Pauli M, Bezevque P.An antibiotic assay by agar well diffusion method. Act Biol Med Exp, 1990;!5;113-115.
13. Rao KV, Sadhukhan AK, Veerender M, Mohan EVS, Dhanvantri SD, Itaramkumar S et al Butyrolactones from Aspergillus terreus. Chem Pharm Bull, 2000;48(4):559-562.

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