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Production of antibacterial agent from fungi isolated from park soil sample by fermentation under optimized conditions

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ABSTRACT

The present study aimed to isolate fungi having antibacterial activity from park site soil sample and production of antibacterial agents by solid and submerged state fermentation under optimized conditions. Antibacterial activity of laboratory isolated and produced antibacterial agent was compared with other commercialized antibiotics to check the efficiency of laboratory produced antibacterial agent. For isolation and characterization of fungal isolates APHA standard was followed. Antibacterial activity was determi ned by using disc diffusion and agar disc diffusion method. On the basis of morphological and microscopic characteristics six fungal isolates belongs to five different genus species i.e. *Aspergillus sp.* (F3, F6), *Penicillium sp.* (F4), *Alternaia sp.* (F1), *Candida sp.* (F5) and *Fusarium sp.* (F2) and they were tested against six bacterial isolates i.e. *Streptococcus sp.* (B1), *Bacillus sp.* (B2), *Staphylococcus sp.* (B3), *Bacillus sp.* (B4), *Bacillus sp.* (B5) and *Enterococcus sp.* (B6). Except B4 all bacterial isolates growth were inhibited by fungal isolates. Under optimized conditions maximum zone of inhibition i.e. 78mm against B3 and B5 shown by F1 and F6 at 1% and 3% glucose concentration at respective pH 10 and 4. When comparison was made between commercialized antibiotics and lab produced antibacterial agents it was observed lab produced antibacterial agent was more efficient in terms of zone of inhibition. The present study demonstrated that lab isolated antibacterial agents isolated from six fungal isolates seems to be a stable and potent antibacterial and can be used as alternative to expensive commercialized antibiotics.

Keywords: Antibacterial; bacteria; disc diffusion; fungi; fermentation.

INTRODUCTION

Antibiotic is a medicinal substance used to treat infections caused by microbes that can cause disease to higher level of eukaryotic organisms. Antibiotic functions to break down the bacterial cells that cause certain disease because antigenic substances are present on the cell wall of bacteria. In fact, to maintain their niche and territory, bacteria produce antibiotic as secondary metabolite. There are few classes of microorganisms that can be used as sources for clinically useable antibiotics (Lihan et al., 2014).

Today at global level healthcare is fa cing the problem of emergence of multi-resistant bacteria thus threat of epidemics and pandemics (Svahn et al., 2012). Several factors have favored this scenario, such as extensive and often inappropriate use of antibiotics, poor hygienic conditions, emigration/immigration of travelers, increased numbers of immunocompromised patients, and delay in diagnosis of infections (dos Santos et al.,

* Corresponding Author Email: anupamabhardwaj25@yahoo.co.in Contact: +91- 9888007909 Received on: 13-01-2017 Revised on: 08-03-2017 Accepted on: 14-03-2017 2015). The tremendous increase in antibiotics resistant bacterial species lead to invention of new sources of antibiotics through microorganisms such as bacteria, fungi and actinomycetes (Sheikh, 2010). The antibiotics produced by microbes have been gaining importance by many researchers. Soil is considered one of the most suitable habitat or environments for microbial growth. The pace of development of genuinely new antimicrobial agent is disappointingly slow.

For the human health and nutrition fungi are well known to produce both beneficial and deleterious natural agents and continue to be explored as useful sources of natural antimicrobial agents. In comparison to plants, microorganisms are highly diverse but narrowly explored. Till date only about 1% of bacteria and 5% of fungi have been characterized while rest remain unexplored for their contribution to the human wel fare (Chioma et al., 2016). In Recent years, the isolation of fungi from soil and screening of antimicrobial activity has gained more attention.

Based on the above-mentioned literatures, this study is an attempt to isolate fungi from park site soil sample and investigate the antimicrobial activity of certain soil microorganisms i.e. fungi isolated from the soil sample. Crude extract of fungi were prepared and were tested for antibacterial activities against a set of test bacteria. To evaluate their antibacterial efficiency, antibacterial activity was compared with commercially available antibiotics. Further, antibacterial agent from isolated fungi was produced by solid state and submerged fermentation.

MATERIALS AND METHODS

Procurement of wastewater samples

Sampling of soil sample was done from park according to standard procedures from American Public Health Association (APHA, 1989) (Sonune and Garode, 2015).

Procurement of chemicals, media, reagents and instruments

All the chemicals and analytical reagent grade were commercially obtained from HIMEDIA[®], Loba Chemie[®], SRL[®](Sisco Research Laboratories) and Qualigens [®] and used as received without further modification. Doubledistilled water was filtered through a Millipore membrane filter before being used. The instruments were purchased from Remi equipments Ltd., Esico, M.S. Electronics India Pvt. Ltd. and Bio-age equipments and services.

Isolation of bacterial isolates

Soil sample bacteria were isolated by Koch method based on the serial dilution and spreading on Nutrient agar. From the individual colonies resulted on the media, bacterial pure cultures were obtained by inoculation in test tubes with sloping medium surface at $37 \pm 1.0^{\circ}$ C, 48hrs.

Isolation of fungal isolates

Dairy wastewater fungi were isolated by Koch method based on the serial dilution and spreading on potato dextrose agar. From the individual colonies resulted on the media, fungal pure cultures were obtained by inoculation in test tubes with sloping medium surface at $25 \pm 1.0^{\circ}$ C, 72hrs.

Inoculum preparation

Each bacterial isolate with 0.1 ml suspension $(3.1 \times 10^5 \text{ CFU/ml})$ was inoculated in 100 ml inoculum medium. The flasks were kept on rotary shaker at 150 rpm for 24 h at 37 °C. Similarly, 0.1 ml suspension of fungal isolate was inoculated in 100 ml inoculum medium. The flask was kept on rotary shaker at 150 rpm for 3days at 25 °C.

To study the biodegradation efficiency of the microbial isolates, biomass of actively growing cells was prepared. For the same, each bacterial isolate was grown on 50ml nutrient broth and 50ml potato dextrose broth for fungi at 150 rpm on rotary shaker at 32 °C/25 °C for 24 to 72hrs. Actively growing bacterial culture of each isolate was centrifuged at 10,000 rpm for 10 min to get wet pellet of each isolate and pellet was washed thrice with sterile distilled water. The pellet was resuspended in sterile deionized water till turbidity reaches at or above that of McFarland 0.5 standard (Porwal et al., 2015).

Cultivation for screening and isolation of secondary metabolites

Each fungal isolate was inoculated into a 1000ml Erlenmeyer flask containing 300 ml of PDB media. Depending on the fungal growth, cultures on liquid medium were incubated for three to four weeks. The fermentation was brought to an end by adding 250 ml ethyl acetate to the culture flask and standing closed for at least 24 h.

Extraction of fungal liquid cultures

250 ml EtOAc were added to each Erlenmeyer flask containing 300 ml culture medium and left overnight to stop cell growth. Crude extract of fungal isolates prepared according to Kalyanasundaram et al. (2015) and crude extracts were subjected to antimicrobial assays.

Screening of fungal isolates for antibacterial activity

Antagonistic assay for fungal extract against bacteria

Antagonistic assay was done by an agar disk diffusion and disk diffusion method. Isolated fungal extract were tested for the antibacterial activity. Bacteria were spread on MHA plates. Then disks (dipped in crude fungal extract) were placed on media and 2mm agar disk were also placed on media. Antagonistic activity was detected after an incubation of 24 to 48 h at 30°C. The presence of zone clearance on agar plates was used as an indicator for the antibacterial activity. The strains which showed the maximum zone of clearance was chosen for further study (Kalyanasundaram et al., 2015).

Identification of potential fungal isolates

For characterization of the morphology of fungal isolates, slides prepared from cultures were stained with lactophenol cotton blue reagent and examined under light microscope (Kalyanasundaram et al., 2015).

Identification of test bacterial isolates

Characterization of unknown bacterial cultures

The identification and characterization of unknown bacteria was done on the basis of microscopic observation (morphology and arrangement of cells, cultural growth characteristics on agar), gram's stain and various biochemical tests as given by Bergey's (1984) manual of determinative bacteriology (Bhattacharya et al., 2014) and online ABIS software.

Submerged fermentation

Fungal isolates inoculum was introduced aseptically into each sterile flask containing the following ingredients (g/l): glucose (20); KNO_3 (2.0); K_2HPO_4 (0.8); MgSO₄-7H₂O (0.7) and KCI (0.5). The pH was adjusted at 7.0 before sterilization. After 5 days of incubation at 30 °C. Filtration was carried out through cotton wool and followed by centrifugation at 5000 rpm for 15 min (Atta, 2015).

Extraction

The culture filtrates were extracted twice with n-Butanol and the pooled solvent extracts were evaporated to dryness under vacuum to yield a crude residue.

Optimization of media parameters for efficient biosynthesis of antibacterial agent

Incubation pH

Effect of different pH ranging from 4, 7 and 10 was examined on the biosynthesis of the selected fungal isolates.

Glucose concentration

Biosynthesis of antibacterial agent by the selected fungal isolates was studied at different glucose concentrations such as 1%, 2% and 3%.

Solid state fermentation

The static experiments were conducted in Erlenmeyer flasks containing 10 g wheat bran (particle size 500 μ m-1mm) as substrate. 1% ammonium sulphate was added to the substrate as additional nutrient. After sterilization inoculated the substrate with 1ml inoculum and moistened with appropriate amount of autoclaved seawater, so that the final moisture content was 60% (w/v). Fermentation was carried out for 7 days at 30°C. After incubation the extraction and the antibiotic assays were carried out.

Extraction of antibiotic (crude extract)

Extraction of antibiotic was done by the addition of 50 ml distilled water to the fermented substrate (10g). Filtration was done using cheese cloth and the filtrate was subjected to centrifugation at 10,000 rpm for 10 min. The supernatant was used for antibiotic assay (Smitha and Philip, 2014).

Compare of antibacterial activity of fungal isolates with commercialized antibacterial agents on the basis of zone of inhibition

Determination of MIC value was carried out using disk diffusion method. Antibacterial agent disk (fungal isolates) was placed onto the MHA media inoculated with test bacteria. Seven commercialized antibiotic discs i.e. erythromycin, fluconazole, voriconazole, cefotaxime, ceftriaxone, cefixime, nalidic acid were also placed onto the MHA media inoculated with test bacteria. The plate was incubated at 30°C for 24-48 hours. The diameter of the inhibition zone formed around the disc was measured (Lihan et al., 2014).

RESULTS AND DISCUSSION

Sample collection, isolation, and identification of fungi and bacteria at genus level A systematic study about the fungal biodiversity in arksoil was carried out to evaluate their capacity to produce the bioactive compound. A total of six fungi were isolated from soil sample by spreading and streaking method using potato dextrose agar media for culti vation. Six bacteria were also isolated from soil sample by spreading and streaking method using nutrient agar media and used as test organisms on which fungi were screened for their antifungal activity. The colony morphology and microscopic characterizati on of each bacterial isolate and fungal isolate is illustrated in Table 1 and Table 2. Dos santos et al. (2015) isolated 65 fungi from the leaves of *I. suffruticosa* and screened them for their antimicrobial activity. Svahn et al. (2012) isolated 61 fungal isolates from highly antibioticcontaminated river sediments and their screened fungal isolates for their antibacterial activity.

Screening of fungal isolates having antibacterial property

Antibacterial activity of crude extract by agar disc diffusion and paper disc method

The antibacterial activity at concentration of 25 mg/ml of ethylacetone extracts of fungi were tested against six bacteria i.e. B1, B2, B3, B4, B5, B6 had shown broad-spectrum activity which has been reported in Table 4. The crude extract of F1, F2, F3, F4, F5, F6 produced the highest zone of inhibition 76, 33, 60, 72, 69 and 77 mm respectively, against bacteria B2, B5 and B6 respectively. The crude extract F6 from ethylacetone solvent has shown the highest zone of inhibition of 77 mm against B2.

Solid-state fermentation

Optimization of antibacterial agent production

Optimization for maximum production of antibacterial agent was done at different incubation time i.e. 2, 4, 6 days. The results obtained from the six day, solid-state fermentation is the best. This contain maximum amount of supernatant, which is responsible for the antibacterial activity.

Table 5: Maximum antibacterial agent concentration was achieved after 6 days of incubation. Least antibacterial agent concentration was observed after 2 days of incubation. Maximum antibacterial agent was produced by F4 followed by F1, F5, F3, F6 and F2.

Test for sensitivity

Six bacteria were tested for their sensitivity of fungal extract. The antibacterial potency was determined by the agar disc diffusion method. Table 6 shows diameters of inhibition zones exerted by the various extracts towards challenged bacteria. Clear zones against B.I.1, B.I.2, B.I.3, B.I.5, B.I.6 but in B.I.4 showed the hazy zones i.e. not clear.

Table 6: As compared to values of zone of inhibition (mm) of bacteria shown by six fungal isolates illustrat-

ed in table 4 with the values given in table 6, it was observed that under optimized treatment condition increment in zone of inhibition was noticed. From table 4 it was observed that F1 maximally inhibit bacteria growth around 76mm while under optimized condition 77mm. F2 maximally inhibit bacteria growth around 33mm while under optimized condition 34mm. F3 maximally inhibit bacteria growth around 60mm while under optimized condition 64mm. F4 maximally inhibit bacteria growth around 72mm while under optimized condition 72mm. F5 maximally inhibit bacteria growth around 69mm while under optimized condition 70mm. F6 maximally inhibit bacteria growth around 77mm while under optimized condition 79mm.

Submerged state fermentation

Optimization for maximum production of antibacterial agent was done at different pH values i.e. 4, 7 and 10 and at different glucose concentrations i.e. 1%, 2% and 3%. The results obtained from the pH 4, 7, 10 and glucose concentration 1%, 2%, 3% submerged fermentation is the best. This contain maximum amount of supernatant, which is responsible for the antibacterial activity.

Table 7: Six fungal isolates produced maximum antibacterial agent at variable pH range. Maximum antibacterial agent produced by F1 at pH 10 i.e. 0.69 while F2 at pH 4 i.e. 0.27. Maximum antibacterial agent produced by F3 at pH 7 i.e. 0.70 while F4 at pH 7 i.e. 0.60. Maximum antibacterial agent produced by F5 at pH 7 i.e. 0.57 while F6 at pH 4 i.e. 0.35. Least antibacterial agent concentration produced by F1 at pH 4 i.e. 0.24 while F2 at pH 10 i.e. 0.01. Least antibacterial agent concentration produced by F3 at pH 10 i.e. 0.08 while F4 at pH 10 i.e. 0.14. Least antibacterial agent concentration produced by F5 at pH 10 i.e. 0.06 while F6 at pH 7 i.e. 0.07. Maximum antibacterial agent was produced by F3 followed by F1, F4, F5, F6 and F2.

Table 8: Six fungal isolates produced maximum antibacterial agent at variable glucose concentration. Maximum antibacterial agent produced by F1 at 1% glucose concentration i.e. 1.08 while F2 at 3% glucose concentration i.e. 0.17. Maximum antibacterial agent produced by F3 at 1% glucose concentration i.e. 0.63 while F4 at 2% glucose concentration i.e. 0.15. Maximum antibacterial agent produced by F5 at 2% glucose concentration i.e. 0.27 while F6 at 3% glucose concentration i.e. 0.56. Least antibacterial agent concentration produced by F1 at 3% glucose concentration i.e. 0.22 while F2 at 2% glucose concentration i.e. 0.02. Least antibacterial agent concentration produced by F3 at 2% glucose concentration i.e. 0.13 while F4 at 1% glucose concentration i.e. 0.03. Least antibacterial agent concentration produced by F5 at 3% glucose concentration i.e. 0.01 while F6 at 1% glucose concentration i.e. 0.05. Maximum antibacterial agent was produced by F1 followed by F3, F6, F5, F2 and F4.

Test for sensitivity

Six bacteria were tested for their sensitivity of fungal extract. The antibacterial potency was determined by the agar disc diffusion method. Table 9 shows diameters of inhibition zones exerted by the various extracts towards challenged bacteria. Clear zones against B.I.1, B.I.2, B.I.3, B.I.5, B.I.6 but B.I.4 in showed the hazy zones i.e. not clear.

As compared to values of zone of inhibition (mm) of bacteria shown by six fungal isolates illustrated in table 4 with the values given in table 9, it was observed that under optimized treatment condition increment in zone of inhibition was noticed. From table 4 it was observed that F1 maximally inhibit bacteria growth around 76mm while under optimized condition 79mm. F2 maximally inhibit bacteria growth around 33mm while under optimized condition 38mm. F3 maximally inhibit bacteria growth around 60mm while under optimized condition 68mm. F4 maximally inhibit bacteria growth around 72mm while under optimized condition 76mm. F5 maximally inhibit bacteria growth around 69mm while under optimized condition 73mm. F6 maximally inhibit bacteria growth around 77mm while under optimized condition 80mm.

Desale and Bodhankar (2013) isolated seventeen fungi i.e. Colletotrichum gloeosporioides Penz, Phomopsis archeri B. Sutton, Aspergillus flavus gr., Nigrospora sphaerica (Sacc.) Mason, Nonsporulating dematiaceous form, Colletotrichum gloeosporioides Penz., Alternaria raphani JW Groves, Penicillum sp., Mucor hiemalis Wehmer, Monodictys paradoxa (Corda) Hughes, Nigrospora state of Khuskia oryzae H.J. Hudson from Different Parts of Vitex negundo L. Antibacterial activity of fungal isolates were screened against six bacteria viz. E. coli (NCIM No.2345), S. typhimurium (NCIM No.2501), B. cereus (NCIM No.2155), S. aureus (NCIM No.2079), K. pneumoniae (NCIM No.2706) and B. subtilis (NCIM No.2063). The crude extract of seventeen fungal isolates with Hexane, Ethyl acetate and Methanol were screened for their antimicrobial potential. The extract of Phomopsis archeri B. Sutton in ethyl acetate showed significant antimicrobial activity against E.coli, S. typhimurium, B. cereus, B. subtilis, K. pneumoniae and S. aureus. The antimicrobial activity was highest against E. coli (24mm), followed by S. typhimurium (22mm), and B. cereus (16mm). The hexane and methanol extracts showed moderate activity against all the pathogenic organisms.

Bisht et al. (2016) isolated five fungal endophytes from living symptomless needle of *Cupressus torulosa* D. Don and characterized as such as *Aspergillus* sp., *Fuzaium* sp., *Cladosporium* sp., *Curvularia* sp., *and Diaporthe* sp. In which, only *Cladosporium* sp. (PCTS23) and *Curvularia* sp. two (WCTS21) endophytic fungal isolates were able to show strong antagonism activity against fungal pathogen. The WCTS21 crude extract produced the highest zone of inhibition 12 mm

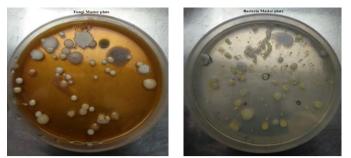


Figure 1: Fungal and Bacterial growth on PDA and NA plates after spreading



Figure 2: Growth of six fungal isolates on PDA plates

Table 1: Morphological	and microscon	ic analysis	of fungal isolates	s
Table 1. Morphological	and microscop	ne analysis	or rungar isolate.	

Isolates	Colony characteristics	Zonation	Sporulation	Tentative fungi
F1	Grayish- green with gray edges	Concentric	Moderate	Alternaria sp.
F2	Floccose, Magenta pink	With concentric zones of dark and light reddish colouration	Poor	Fusarium sp.
F3	Velvety, Light Yellow	With venation like radial furrows	Nil	Aspergillus sp.
F4	Powdery, Olivaceous green with sterile white margin	Radially furrowed	Heavy	Penicillium sp.
F5	Creamy white	Radially furrowed	Moderate	Candida sp.
F6	Velvety, White with typical black spores	Heavily furrowed on the reverse	Heavy	Aspergillus sp.

Table 2: Morphological and microscopic analysis of bacterial isolates

Isolates	Colony characteristics	Gram Reaction				
B1	1 Branching growth pattern with yellowish white appearance					
B2	B2 Circular, convex, smooth, golden yellow					
B3	B3 Small, round, regular, red color pigmented					
B4	B4 Small, circular, regular, pale yellow color pigmentation					
B5	B5 Large, irregular, greenish yellow fluorescent colonies					
B6	Rod shaped, moist colony with irregular edges	Negative				

for *S. aureus,* whereas crude extract of PCTS23 has shown the highest zone of inhibition of 10 mm against *S. aureus.*

Similarly Sandhu et al. (2014) isolated and screened ten fungal isolates viz. *Aspergillus fumigates, Aspergillus japonicas, Aspergillus niger, Fusarium semitectum, Cur-* vularia pallescens, Phoma hedericola, Alternaria tenuissima, Fusarium solani, Drechslera australien and Aspergillus repens from medicinal plant Ricinus communis for their antibacterial activity against six human pathogenic bacterial strains Bacillus subtilis, Enterococcous sp., Klebsiella pneumoniae, Escherichia coli, Salmonella typhimurium and Staphylococcus aureus.

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	Catalase	Citrate	MR	VP	Indole	Nitrate	H₂S	Tentative bacteria				
B1	+	+	-	-	-	+	-	Streptococcus sp.				
B2	+	+	+	1	-	+	-	Bacillus sp.				
B3	+	-	-	+	-	+	-	Staphylococcus sp.				
B4	+	+	-	1	-	+	-	Bacillus sp.				
B5	+	-	-	-	-	+	-	Bacillus sp.				
B6	-	-	-	+	-	+	-	Enterococcus sp.				

Table 3: Biochemical characterization of bacterial isolates

Table 4: Antibacterial activity shown by fungal isolates against test bacteria

	Zone of inhibition (mm) shown by fungal isolates against test bacteria									
	F1	F2	F3	F4	F5	F6				
B.I.1	0	0	0	0	0	0				
B.I.2	0	33	46	72	69	77				
B.I.3	10	13	0	10	62	45				
B.I.4	24	0	40	7	36	17				
B.I.5	60	0	60	0	3	26				
B.I.6	76	23	20	51	0	0				

Table 5: Antibacterial agent concentration at variable incubation period 2, 4 and 6 days

Incubation period (days)	F1	F2	F3	F4	F5	F6		
incubation period (days)	Optical density at 600nm							
2	0.11	0	0	0	0	0		
4	0.28	0.09	0.01	0.12	0.05	0.09		
6	0.65	0.22	0.06	0.26	0.16	0.28		

Table 6: Antibacterial activity shown by fungal isolates against test bacteria under optimized condition

	Zone of inhibition (mm) shown by fungal isolates against test bacteria									
	F1	F2	F3	F4	F5	F6				
B.I.1	0	0	0	0	0	0				
B.I.2	0.8	34	48	72	70	79				
B.I.3	14	15	0.8	14	64	48				
B.I.4	25	0	42	10	37	20				
B.I.5	62	0.7	64	0.9	8	28				
B.I.6	77	25	24	53	0	0				

Table 7; Antibacterial agent concentration at variable pH range 4, 7 and 10

рH	F1	F2	F3	F4	F5	F6			
рп	Optical density at 600nm								
4	0.24	0.27	0.04	0.28	0.55	0.35			
7	0.36	0.07	0.70	0.60	0.57	0.07			
10	0.69	0.01	0.08	0.14	0.06	0.20			

Table 8: Antibacterial agent concentration at variable glucose concentrations 1%, 2% and 3%

Cluster concentration (9/)	F1	F2	F3	F4	F5	F6			
Glucose concentration (%)	Optical density at 600nm								
1	1.08	0.04	0.63	0.03	0.08	0.05			
2	0.30	0.02	0.13	0.15	0.27	0.12			
3	0.22	0.17	0.16	0.06	0.01	0.56			

Table 9: Antibacterial activity shown by fungal isolates against test bacteria under optimized condition

	Zone of inhibition (mm) shown by fungal isolates against test bacteria									
	F1	F2	F3	F4	F5	F6				
B.I.1	27	0	45	39	42	20				
B.I.2	65	0	64	10	9	30				
B.I.3	78	27	12	0	0	0				
B.I.4	0	0	0	0	0	0				
B.I.5	0	37	51	75	73	78				

	Zone of inhibition (mm) shown by fungal isolates against test bacteria								
	ER	FZ	VZ	СТ	СХ	CF	NA		
B.I.1	24	0	0	40	31	0	27		
B.I.2	55	52	0	25	28	0	25		
B.I.3	48	0	0	46	46	22	49		
B.I.4	0	0	0	17	27	10	15		
B.I.5	11	0	0	19	27	0	40		
B.I.6	17	0	0	15	28	0	0		

Table 10: Antibacterial activity shown by commercialized antibiotics against test bacteria

Comparative analysis of antibacterial activity of fungal isolates and commercialized antibiotics

In the present study seven commercialized antibiotics i.e. erythromycin (ER), fluconazole (FZ), voriconazole (VZ), cefotaxime (CT), ceftriaxone (CX), cefixime (CF), nalidic acid (NA) were used against six test bacteria and zone of inhibition was observed after 24, 48 and 72h of incubation period. Zone of inhibition shown in table 10.

When comparison was made between antibacterial agent produced from lab isolated fungal strains and commercialized antibiotics, it was observed except bacterial isolate B.I.1 all bacterial strains were inhibited by all fungal isolate's antibacterial agent more efficiently as compared to commercialized antibiotics. B.I.1 showed maximum zone of inhibition against antibiotic cefotaxime i.e. 40mm. B.I.2 showed maximum zone of inhibition against antibiotic erythromycin i.e. 55mm while against fungal isolate antibacterial agent i.e. 80mm. B.I.3 showed maximum zone of inhibition against antibiotic erythromycin i.e. 48mm while against fungal isolate antibacterial agent i.e. 68mm. B.I.4 showed maximum zone of inhibition against antibiotic ceftriaxone i.e. 27mm while against fungal isolate antibacterial agent i.e. 47mm. B.I.5 showed maximum zone of inhibition against antibiotic nalidic acid i.e. 40mm while against fungal isolate antibacterial agent i.e. 68mm. B.I.6 showed maximum zone of inhibition against antibiotic ceftriaxone i.e. 28mm while against fungal isolate antibacterial agent i.e. 79mm.

CONCLUSION

This study raises the possibility of exploring fungal species having antibacterial activity and used them as alternative to commercialized antibiotics. Furthermore, our observations indicate that fungi from pharmaceutical soil sample have pharmaceutical potential as they produce antibacterial compounds, and that the medicinal properties of these fungi may be a consequence of the capacity to produce biologically active secondary metabolites. Further studies are now needed to identify the active compounds produced in order to discover new drugs with antibacterial activity.

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