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CHARACTERIZATION AND ANTAGONISTIC POTENTIAL OF ACTINOMYCETES AGAINST PATHOGENS OF HUMAN MYCOSIS

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ABSTRACT

This study was conducted to isolate and identify the biologically potential actinomycetes and also this study confirm that the isolate AP-27 have enormous potential for the production of antifungal agent. Total of 70 cultures were isolated from different regions of Gwalior (M.P.). For isolation SCA media was proved to be the best medium. Primary and secondary screening revealed that out of 70 isolates 16 isolates showed antifungal activity against dermatophytes. Finally one promising isolate designated as AP-27 was selected as a most effective and highly active isolate on the basis of broad spectrum of antifungal activity and maximum zone of inhibition against M. canis, M. gypseum, and T. rubrum. Different media were used to understand culture characteristics of isolate AP-27 where it was concluded that SCA media supported the maximum growth of isolate. Morphological, microscopic and biochemical analysis was done to understand the characteristics of isolate AP-27 which revealed that isolate had the similar characteristics to Streptomyces genus and supposed to belong this group and this confirmed by 16S rRNA sequence analysis which concluded that isolate AP-27 belongs to streptomyces genus. This study concluded that Gwalior region has good source of potent actinomycetes with antidermatophytic activity.

Keywords: *Actinomycetes, Gram-Positive, Metabolite etc.*

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INTRODUCTION

Actinomycetes are Gram-positive bacteria with high G+C content showing filamentous growth like fungi. They are aerobic and have diversity in ecological habitats such as soil, fresh water, sewage and marine water. Actinomycetes are producer of theruptically used compound and widely used antibiotics. Production of secondary metabolite is an important characteristic feature of actinomycetes. Many antibiotics like streptomycin, gentamycin, rifamycin, erythromycin and many other well-known anticancer drugs are the product of secondary metabolite by actinomycetes (Valan *et al*., 2012). Many other commercially used compounds like antibiotics, antiparasitic, antifungal agents, anticancer and immunosuppressive agents are produced by actinomycetes. (Bundale *et al.,* 2015 and Safey *et al.,* 2013).

About 20% to 25% world's population have the problem of Dermatomycosis commonly known as ringworm infection. It is an infection of skin, hair and nails. Keratinized tissues are the main target of dermatophytic fungi. Three genera of dermatophytes known as *Microsporum, Tricophyton*, and *Epidermatophyton* are responsible of all dermatophytic infection in human and animals. Opportunistic infection becomes a big problem in the immunocompromised host, so new, safe and more effective compounds are focus of research. Antibiotic resistance to pathogenic fungi is also becomes a big problem. Because of human mycoses dermatophytes get attention of medical epidemiologists. Many antifungal agents like azole, allylamine etc. are used to manage these infection but many drawbacks becomes a serious problem like drug resistance and severe side effects (Ndako *et al.*, 2012 and Sherien *et al.,* 2016).

MATERIALS AND METHODS

Sample collection:

Soil samples were collected from the different region of District, Gwalior M.P., India. These sites were Playground soil, Medicinal plant soil, Agricultural soil, Poultry farm soil, Industrial waste soil and Sewage soil. Soil samples were collected at the depth of 5-10 cm, top surface layer of soil was removed and central portion of soil was collected in sterile plastic bags (Mohanashrinivasan *et al.,* 2013). All samples were labelled and kept in the BOD incubator at 4^{o}C for further use.

Isolation of Actinomycetes:

Isolation of actinomycetes was done by the serial dilution and pour plate technique (Safey et *al.*, 2013). For isolation of actinomycetes different media were used like starch casein agar (SCA), Glycerol Asparagine Agar (GAA), Actinomycetes Isolation Agar (AIA), and soil extract agar (SEA). All plates were incubated at 30°c for 7 to 21 days. After incubation isolated colonies were purified on respective fresh media and stored at 4°C until further use (Alimuddin *et al*., 2011 and Baskaran *et al*., 2011).

Isolation and identification of test dermatophytes:

Dermatophytes used as test organisms like *Microsporum canis*, *Microsporum gypseum*, *Microsporum fulvum*, *Tricophyton rubrum* and *Tricophyton mentagrophyte* were isolated originally from hospital waste land and drainage soil, collected from 4 hospital of Gwalior regions. Isolation of dermatophytes was done by hair-bait technique by placing 50g of each soil sample into a sterile Petri dish and baited with sterilized hair piece and moistened with 5-10 ml of sterilized distilled water and Plates were incubated at room temperature (Zarei *et al.*, 2008). Isolated fungal culture were grown on Sabouraud dextrose agar plates and incubated at 28°C for 6-7 days. Isolated cultures were identified by morphology, culture characteristics and microscopic examination (Frey *et al.*, 1886., Rippon *et al.*, 1988 and Laron, 1995). Identified fungal culture were then transferred into slant of SDA and kept at 4°C for further use (Kannan *et al.*, 2013 and Shahita *et al*., 2013)

Screening of soil actinomycetes:

All isolated actinomycetes were screened for their antifungal activity against dermatophytes. Screening was done by primary and secondary method. Primary screening was done by double layer method. In this method starch casein agar medium was prepared and thin layer was poured into the plate then actinomycetes were inoculated by spot inoculation method in the centre of medium. plates were incubated for 3 to 4 days at 30°C then second layer of SDA was poured on same plate and culture of dermatophytes were spread by spread plate method, plates were

incubated for 6-8 days at 28° C and after incubation zone of inhibition was measured (Adel *et al.,* 2012).

Secondary screening was performed by agar well diffusion method. Two media were prepared i.e. Sabourauod dextrose agar and starch casein broth. Most promising isolate was inoculated into starch casein broth and incubated in a rotary shaker under agitation at 30°C ±1 for 5-7 days at 200 rpm. The fermented broth was centrifuged at 10,000 rpm for 10 min, filtered through a Whitman No.1filter paper. The mycelium free culture filtrate was tested for antifungal activity (Kannan *et al.*, 2013). Two wells were made on sabouraud dextrose agar plate by well cutter and broth culture of tested fungal pathogen was spread by spread plate technique, then wells were loaded with starch casein broth (150 µl) and one with cyclohexamide as a positive control. All plates were incubated at 28°C and zone of inhibition was measured after 7 days of incubation (Bharti *et al.,* 2010).

Cultural, Morphological characteristics of Isolate AP-27

Cultural characteristics of AP-27 such as colour of aerial mycelium, colour of substrate mycelium, and pigmentation were studied on different media like Starch casein agar, Tryptone-yeast agar (ISP-1), Maltose Yeast extract agar, Potato Dextrose Agar, Nutrient Agar, Czapex dox agar, Starch agar medium and Sabouraud dextrose agar (Safey et *al*., 2013). To study the morphological characteristics Gram staining was done and spore structure was examined by electron microscopy (Scanning electron microscope) (Khanna *et al.,* 2011 and Sowndharanjan *et al.,* 2012).

Biochemical characteristic

After screening, promising isolate was selected for Biochemical testing. Biochemical test used were hydrolysis of gelatin, Casein hydrolysis, Methyl red and Voges Proskauer Test, Nitrate reduction, Hydrogen sulfide production and Fermentation of carbohydrates like - Glucose Fructose, Sucrose, Ribose, Galactose, Maltose, Xylose, Rahaminose, Raffinose and their acid gas production was also observed (Safey *et al.,* 2013).

Identification of isolate by I6S rRNA Sequencing

The selected isolate AP-27 was subjected to molecular characterization and phylogenetic analysis. Firstly genomic DNA was isolated, extracted and amplified by using PCR. PCR product was sequenced by using universal primers (forward primer -5'-GCCTAACACATGCTGG-5' and reverse primer -5'-GTATTACCGCGGCTGCTGG-5'). Molecular Evolution Genetics Analysis (MEGA) software version 7 was used to carry out phylogenetic analysis of the alignment **(**Nanjwade *et al.,* 2010 and Safey *et al.,* 2013).

RESULTS AND DISCUSSION

A Total of 70 soil actinomycetes cultures were isolated from soil samples. Starch casein agar medium was proved to be the best medium for isolation. All isolates were grouped based on different color groups of aerial mycelium, substrate mycelium and soluble pigment.

Total of 5 fungal cultures were identified as keratinophilic or dermatophilic fungi they were *Microsporum canis, Microsporum gypseum, Microsporum fulvum, Tricophyton ruburum* and *Tricophyton mentagrophyte*. Identification was done on the basis of morphological, cultural and microscopic examination and it was found that all dermatophytic fungi shown different morphological features like colony appearance and colony colour. *M.canis* and *M.gypseum* shown long incubation period from 7-10 days as compare to other 3 fungi. Results are shown in Table 1.

Among the 70 isolates, 16 actinomycetes showed antifungal activity against *M.canis, M.gypseum* and *T.ruburum* in primary screening. In secondary screening 6 actinomycetes were active against *M.canis, M.gypseum* and *T.rubrum.* Among these 6 isolates one isolate was selected as a most promising isolate designated as AP-27 on the basis of maximum zone of inhibition (6- 22 mm) against maximum no. of dermatophytes. All results are shown in Table 2.

Morphological and cultural analysis of isolate AP-27 on starch casein agar medium suggested that isolate produced white colored spore mass and aerial mycelium color with light yellow colored substrate mycelium. Isolate AP-27 produced pink colored pigment soluble in media. Light microscopy of isolate was observed and concluded as gram positive and dichotomously branched spore chain (Figure 1). Observation under scanning electron microscope (SEM) shown spiral spore chain, terminal of spores was open and surface was smooth (Figure 2). Isolate shown maximum and fast growth on starch casein agar medium as

compare to other medium, pigment production was also varied with change in media like on SCA medium isolate produced light pink colored pigment while colour of pigment was dark pink with starch agar medium. Biochemical study of isolate AP-27 suggested that isolate had the ability to degrade casein and starch and isolate also shown positive results for Simmon citrate and MRVP test while H_2S and nitrate reduction was not observed. Many carbon sources was utilized by isolate AP-27 like Glucose, Fructose, Sucrose, Ribose, Maltose, Xylose while Rhamnose and Raffinose were not used. Results of morphological, cultural and biochemical characteristics are shown in Table 3, 4 and 5 respectively. From cultural, morphological and biochemical characterization it was concluded that isolate belongs to *Streptomyces* genus.

Molecular characterization suggested that 16S rRNA sequence of isolate AP-27 had 98% sequence identity with 16S rRNA gene sequence from several *Streptomyces sp*. This result clearly suggests that the isolate AP-27 belongs to the genus *Streptomyces* sp. Phylogenetic analysis shown that isolate AP-27 closely related to *Streptomyces* sp. and showed high similarity towards *Streptomyces griseu.* (Figure 3).

From the previous research it was concluded that many effective antifungal compounds were produced by group of actinomycetes (Valan *et al*., 2012).) Some researchers isolated actinomycetes like *Streptomyces hygroscopicus* from soil of Saudi Arabia active against many pathogens like Gram positive bacteria and fungi (Safey *et al* 2013). Therefore in the present study 70 different actinomycetes were isolated from soil of Gwalior region (M.P.). Dermatophytes were isolated from hospital soil and 5 culture of dermatophytes were obtained and many other workers used similar method for isolation of keratinophilic fungi from soil and suggested as a best method, they isolated and identified *M.canis* and *T.rubrum* from public parks in Ahvaz and identified fungal pathogens on the basis of morphology, culture characteristics, and microscopic examination by lacto phenol cotton blue stain. The presence of these keratinophilic fungi in hospital soil may be result of human keratin residues present in soil (Zarei *et al.*, 2008). Ekwealor *et al.*, (2015) also isolated dermatophytes from fungal infected wounds and lesions and identified as *M. audoinii, M. Ferrugineum, T. megninii, T. tonsurans* and *T. rubrum* on the basis of morphology and microscopic examination and concluded that micro and macroconidia formation is an important characteristic feature of fungal pathogen. In present study it was found

that 16 actinomycetes isolate shown maximum zone of inhibition against *M. canis*, *M. gypseum* and *T. ruburum*. Among the antagonists, the isolate designated as AP-27 reported as higher antagonistic activity against *M. canis, M. gypseum* and *T. rubrum,* causative agent of many skin diseases (Zarei *et al.,* 2008). Many species of actinomycetes have the ability to inhibit the growth of various fungus (Adel *et al.,* 2012). Bharti *et al.,* (2010) screened 94 actinomycetes out of 316 isolated from Gharwal region uttarakhand and found that isolates shown antifungal activity against *C. albicans, T. ruburum, A. fumigatus M. canis, M. gypseum,* and *A. flavus,* they also suggested that agar well diffusion method was very effective. Further all studies like morphological and biochemical characterization shown that isolate AP-27 was gram positive with spiral spore chain which suggested that isolate belongs to *Streptomyces* group. Same identification was suggested by Alimuddin *et al.,* (2011) they reported in their studies that morphological, cultural and biochemical characteristics play important key role in identification of actinomycetes, they characterize the actinomycetes on the basis of aerial mycelium colour, substrate mycelium colour and soluble pigment. They find grey colored aerial mycelium and concluded that *Streptomyces* group was dominant. Same findings were also confirmed by other workers (Bharti *et al.,* 2010) they used both microscopic and macroscopic parameters to identified the isolates from Gharwal region and suggested that each isolate had specific characteristics, colour of aerial mycelium were cream, brown, pink and white while reverse mycelium colour were white, brown, reddish and yellow, they also observed pigment production, some isolates produced brown pigment. In their studies microscopic analysis revealed that spore chain with 7-12 spores rod, spiral, they identified three genera as *Saccarothrix* sp., *Streptomyces* sp. and *Kitasatospora* sp. Valan *et al.,* (2012) identified *Streptomyces* sp. active against pathogenic bacteria and dermatophytes from coast of Bay of Bengal on the basis of biochemical and physiological characteristics, they find that isolates utilized different carbon sources and reduce nitrate while H_2S production was not observed, all isolates shown different growth on different media and SCA media was shown good growth of isolates, in their studies all parameters indicated that isolates belongs to *Streptomyces* genus. Finding of new bioactive compound is never ending process. Present study was focused on screening and identification of actinomycetes isolated from different soil samples of Gwalior region (M.P.). From the present study, it could be demonstrated that actinomycetes isolated from Gwalior soil sample have good

potential to produce antifungal compound against dermatophytes. The morphological and 16S rRNA sequencing study clearly suggested that the isolate AP-27 belongs to the genus *Streptomyces*. The *Streptomyces* sp. AP-27 had strongest inhibitory activity effect on growth of dermatophytes. Therefore isolation and screening of actinomycetes from such area may contribute the discovery of novel antifungal compound. Potent antifungal against dermatophytes from AP-27 Isolate could contribute a lot to fight against antibiotic resistant dermatophytes. Selected isolate have been shown strong and promising antidermatophytic activity, so further purification, optimization, structural elucidation and characterization is under progress to know the chemical structure, quality, novelty and commercial value of this compound.

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Table 1 Morphological and Microscopic characteristics of isolated Dermatophytes

Isolate	Colony appearance	Reverse colony or pigment production	Microscopic study	Dermatophytes Identified as
Pd1	White to dark cream,	Light yellow to brown	Macroconidia produced with spherical shaped thick wall.	*M. canis*
Pd2	Cream to brown colored.	Yellow brown pigment	Macroconidia with spiny thin wall.	*M.gypseum*
Pd3	Light brown surface with white border.	Dark red surface	Longer bullet shaped Macroconidia. Some clavate Microconidia produced.	*M.fulvum*
Pd4	White colored fluffy and sticky.	Deep red wine colored.	Small Pyriform Microconidia were present. Macroconidia not seen.	*T.ruburum*
Pd5	White powdery surface.	Yellow to light brown	Thin smooth walled Microconidia were present.	*T.mentagrophyte*

Table 2 Antifungal activity of isolates against dermatophytes

Zone of inhibition (mm)			
Isolates	*M.canis*	*T.ruburum*	*M.gypseum*
AP-7	22	--	20
AP-10	–	14	12
AP-19	12	--	12
AP-27	26	20	22
AP-35	16	16	--
AP-49	8	12	--

Table 3 Morphological and culture characteristics of isolate AP-27

Properties	characteristics of AP-27
Gram's staining	Gram positive
Hyphae	Present
Mycelium	Present
Color of aerial mycelium	White
Color of substrate mycelium	Light yellow
Spore mass colour	White
Pigment production	Light pink (soluble in media)
Spore morphology	Spiral(open)
Spore surface	Smooth

Table 4 Culture characteristics of isolate AP-27 on different media

S. No. Medium	Growth	AMC	Substrate mycelium colour	Pigment production
Starch casein agar (SCA)	Excellent	White	yellow	light pink
Tryptone-yeast agar(ISP-1)	--	--	--	--
Maltose Yeast extract agar	--	--	--	--
Potato Dextrose Agar(PDA)	Average	White	yellow	Light pink
Nutrient Agar(NA)	--	--	--	--
Czapex dox agar	--	--	--	--
Starch agar medium	Good	White	light yellow	Dark pink
Sabouraud dextrose agar	Good	white	light yellow	Light pink

*AMC - Aerial mycelium colour and -- denotes negative

Table 5 Biochemical characteristics of isolate AP-27

Biochemical Characteristics	Isolate AP-27
Casein hydrolysis	+ve
Simmon citrate	+ve
Methyl red	+ve
Voges Proskauer	+ve
Nitrate reduction	-ve
H_2Sproduction	-ve
Starch hydrolysis	+ve
Fermentation of carbohydrates	**AG formation**
Glucose	AG
Fructose	AG
Sucrose	G
Ribose	G
Galactose	AG
Maltose	AG
Xylose	G
Rhamnose	G
Raffinose	G

*AG- Acid Gas and G- Gas

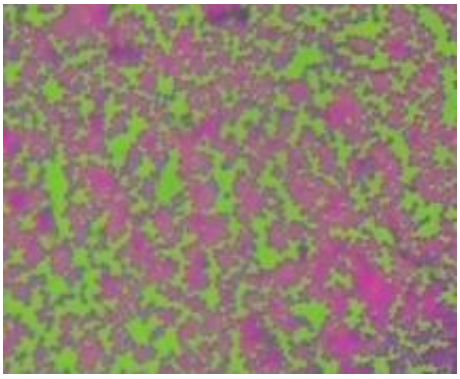

Fig 1 Gram's stain of AP-27

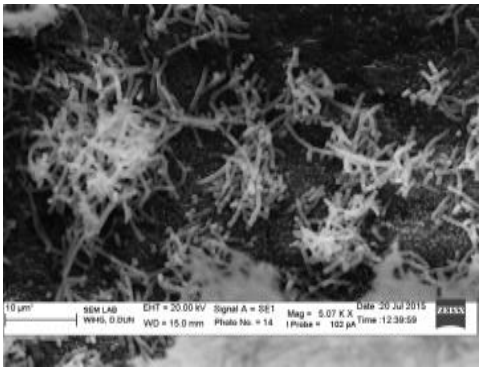

Fig 2 Scanning Electron micrograph of AP-27

(Microscopic view of AP-27 under 100 x resolution)

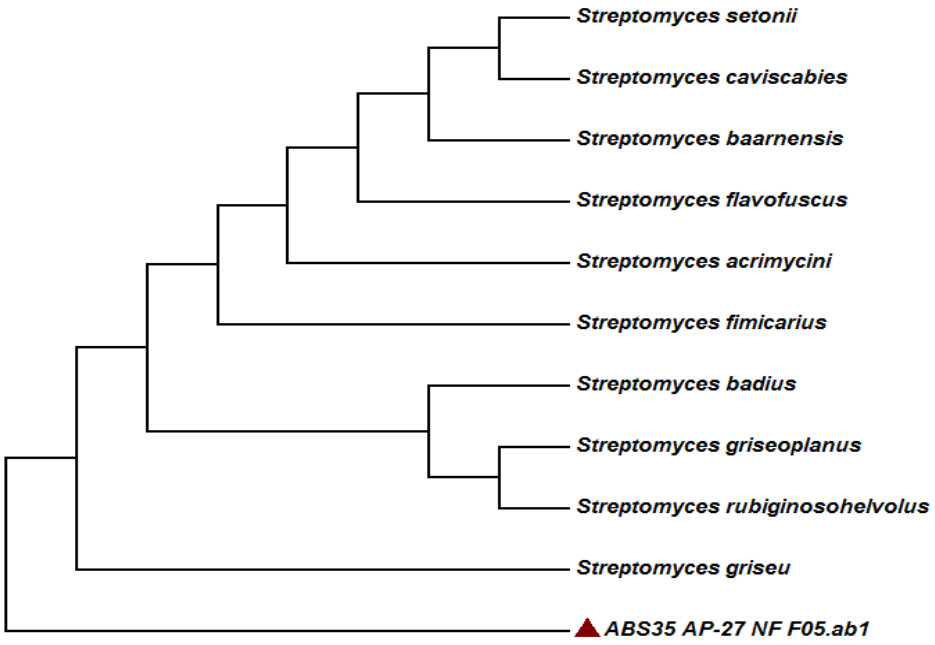

Fig 3 Phylogenetic tree based on 16S rRNA gene sequences, showing the phylogenetic relationship of isolate AP-27 with recognized member of the genus Actinomycetes.

REFERENCES

Adel, A., Houda, M. and Kirane, G.D. 2012. Identification and antifungal activity of *Streptomyces sp*. S72 isolated from Lake Qubeira sediments in North-East of Algeria. *Afr. J. Biotechnol*. 11(2), 305-311.

Alimuddin, Widada, J., Widya, A., Mustofa. 2011. Antifungal Production of a Strain of Actinomycetes *spp* Isolated from the Rhizosphere of Cajuput Plant: Selection and detection of exhibiting activity against tested fungi. *Indonesian J. of Biotechnol*. 16 (1), 1-10.

Baskaran, R, Vijaykumar R, Mohan, P.M. 2011. Enrichment method for the isolation of bioactive actinomycetes from mangrove sediments of Andaman Islands, India. *Mal. J. of Microbol*. 7(1), 26-32.

Bharti, A., Kumar, V., Gusain, O. and Bisht, G.S. 2010. Antifungal Activity of Actinomycetes isolated from Garhwal Region. *J of Sci. Engg. & Tech. Mgt*. 3(1), 1-9.

Bundale, S., Begde, D., Nashikka, N., Kadam, T. and Upadhyay, A. 2015. Optimization of Culture conditions for production of bioactive metabolites by *Streptomyces spp*. isolated from soil. *Adv Microbiol.* 5, 441-451

Ekwealor, C.C. and Oyeka, C.A. 2014. *In vitro* Anti Dermatophyte activities of crude methanol and aqueous extracts of Lawsonia inermis. *Int J Pharm Sci & Drug Res*. 7(1), 59-62.

Frey, D., Oldfield, R.J., and Bridger, R.C. 1986. A color atlas of pathogenic fungi. Wolfe Medical Publication. 66-73.

Kannan, P., Janaki, C. and Selv, G.S. 2006. Prevalence of dermatophytes and other fungal agents isolated from clinical samples, *Indian J Med Microbiol*. 24 (3), 212-5.

Khanna, M., Solanki, R. and Lal, R. 2011. Selective isolation of rare actinomycetes producing novel antimicrobial compounds. *Int J Advanced Biotechnol Res*. 2(3), 357-375.

Laron, D.H. 1995. Medically important fungi: a guide to identification. Society of Microbiology Washington.

Mohanashrinivasan, V., Sriramkalyan, P., Subhadradevi, C., Selvaranjan, E.., Suganthi, V and Naine, J. 2013. Fermentative production of extracellular pigment from *Streptomyces coelicolor*. *Res. J Biotechnol*. 8(4), 1-4.

Nanjwade, B. S., Chandrasekhar, Ali M Shamarez., Goudanawar S P. and Manvi,V. F. 2010. Isolation and morphological characterization of antibiotic producing actinomycetes. *Tropical J Pharm Res*. 9(3), 231-236.

Ndako, J.A., Osemwegie, O., Spencer, T.H., Olopade, B. and K., and Yunusa, G.A. 2012. Prevalence of Dermatophytes and other associated Fungi among school children. *Global Adv Res of Medi and Med Sci*. 1(3), 049-056.

Rippon, J.W. and Saunders, W.B. 1988. The pathogenic fungi and pathogenic actinomycetes Saunders Company, Philadelphia, USA.

Safey, M.El., Safey, El., Atta, M.H. and AlJaralah, M.K. 2013. Antibiotic production by *Streptomyces hygroscopicus* M 121 isolated from Kingdom of Saudi Arabia. *Life Science J*. 10 (2), 1157-1163.

Shahitha, S., Saranya, M. and Poornima, K. 2013. Isolation and identification of Dermatophyte from clinical samples and antidermatophytic activity of *Lawsoniainermis (Henna plant). Int .J. Pharm. chem . sci*. 2, 23-31.

Sherein, G.G., Nermien, E.H., Seddek, and Shereen, M.M. 2016. Activity of Some Natural Oils on Dermatophytes Isolated from Assuit University Hospitals Egyptian. *J Med Microbiol.* 2(25), 85-91.

Sowndharanjan, K, Kang, S. C. 2012. *In vivo* antagonistic potential of *Streptomyces* sp. against plant and human pathogens. *J of Agr. Chem. and Environ.* 1, 41-47.

Valan, A.M., Asha, K.R., Duraipandiyan, V., Ignacimuthu, S., and Agastian, P. 2012. Characterization and phylogenetic analysis of novel polyene type antimicrobial metabolite producing actinomycetes from marine sediments: Bay of Bengal India. *Asian Pac J Trop Biomed*. 2(10), 803-810.

Zarei, A.M., and Zarrin, M. 2008. Isolation of dermatophytes and related keratinophilic Fungi from the two public parks in Ahvaz Jundishapur. *J Microbiol*. 1(1), 20-23.