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ISOLATION OF ACTINOMYCETES FROM MARINE ECOSYSTEM AND STUDY OF THEIR BIOACTIVE COMPONENT PRODUCTION POTENTIAL

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ABSTRACT

Antibiotics are generally isolated from microbes, used as antimicrobial and antitumor agents against pathogenic bacteria, fungi and tumors. In present study, nine isolates of Actinomycetes were isolated using spread plate method on Starch Casein Agar (SCA) plates from seven marine water samples collected from Tithal Beach, Valsad. They were used to perform screening for the production of bioactive compounds i. e., antibiotics. These bioactive compounds present in the supernatant were tested against four test organisms. Antibacterial activities were performed using Nutrient Agar (NA) plates by Agar Diffusion Assay with the test organisms. Bioactive compounds were extracted from the supernatant by using two solvents, ethyl acetate and methanol. From the antimicrobial assay, A2 was selected for further study because; it shows good antibacterial activity on Nutrient Agar plate. Mutations were carried out with both physical and chemical mutagens for A2 isolate. A2 was found potential producer of bioactive compound/s.

KEYWORDS: *Actinomycetes, antibacterial assay bioactive compound, extraction, mutation*

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INTRODUCTION

Oceans are highly complex environment with extreme variations in pressure, salinity and temperature which are habitats of diversified of microbes.[1] Marine Actinomycetes act as good source of bioactive compounds. The adaptation of marine Actinomycetes towards the harsh sea environment resulted such that most of these microbes show the requirement of seawater for growth, in compared to the terrestrial Actinomycetes.[2] Based on this unique adaptation to the high salinity in the sea, it is assumed that marine Actinomycetes might be able to produce unique secondary metabolites for their successful survivability. In the ocean, marine Actinomycetes are widely distributed where it can be found in seawater, sediments and also in association with many marine organisms.[3] The marine water is a good source for the isolation of microorganisms to producing antibiotics as a secondary metabolites and used for the production of novel bioactive compounds.[4] Various organism produce various types of antibiotics with different anti-potential activity with unique properties and applications.[5]Actinomycetes are aerobic, gram positive, filamentous and high G+C content containing bacteria which are earlier termed as ray fungi.[6] This secondary metabolites contains bioactive compounds, which are used as an antibiotics. Actinomycetes have been isolated from marine water sample by screening methods, separated, purified and used as antifungal, antibacterial and antitumor activities to inhibit the growth of pathogenic fungi, bacteria and parasites.[7]About 80% of the naturally occurring antibiotics are produced by Actinomycetes and among them two most potential producers are *Streptomyces* and *Micromonospora*.[8] Nowadays multi drug resistant strains of pathogens are emerging more quickly than the rate of discovery of antibiotics. Methicillin and Vancomycin resistant strain of *Staphylococcus aureus*[9] and penicillin resistant *Streptococcus pneumonia* has been reported since long time.[10] To overcome this problem, scientists and many pharmaceutical industries trying to perform new screening programmes for the discovery of novel Actinomycetes from untouched environment like marine water.[11] This study was focused on the isolation of different species of marine Actinomycetes on marine agar (Starch Casein Agar) from different water sources, subjected to the production of secondary metabolites i.e., antibioticsthrough broth inoculation and antibacterial potential of fermented broth was tested using agar well diffusion method.[12]

MATERIALS AND METHODS

Collection

A total number of seven marine water samples were collected from Tithal beach, Valsad. The marine water samples were collected in sterile glass container to avoid chances of contaminations.

Selective Isolation of Actinomycetes

Isolation and enumeration of Actinomycetes were performed by serial dilution and spread plate technique by using starch casein agar plates and streptomycin was added to inhibit the unwanted fungal growth. The marine water sample was serially diluted up to 10^{-}□ dilution.0.1 ml of each dilution was spreaded on Starch Casein Agar plates and the plates were incubated at 28□C for 15 days.[6] After incubation, the colonies were identified and purified on Starch Casein Agar plates and preserved in refrigerator for further use.

Fermentation Process

Submerged fermentation was performed with inoculating each the isolate of Actinomycetes using Starch Casein broth. Flasks were incubated on the shaker at 150 rpm at 28°C for 7 days. The broths were harvested and centrifuged at 10,000 rpm for 15 min to remove cell debris and supernatants were transferred aseptically into screw capped bottles and kept at 4°C for antimicrobial activities.[13]

Antibacterial activity study

Antibacterial screening of Actinomycete isolates were performed by agar well diffusion method on Nutrient Agar (NA) plate using *Escherichia coli, Salmonella typhi, Staphylococcus aureus* and *Bacillus subtilis* as test organisms. Twenty four hours old grown test organisms were mixed with melted nutrient agar and poured over sterile empty petri dishes and allowed to solidified. The wells (8 mm diameter) were made using sterile cup borer, 0.5ml crude extracts (supernatant) were added in each well and incubated at 37°C for 24 hours. After incubation, plates were observed for inhibition zone and results were recorded.[14]

Extraction

The bioactive compounds were recovered from the fermentation broth by solvent extraction method. Antibiotic extraction was done using ethyl acetate and methanol. The filtrate from each isolate was mixed with equal volume of ethyl acetate and shaken vigorously for 1 hour and separated by Separatory funnel. The solvent phase that may contain antibiotic was separated from the aqueous phase after stabilization of mixture. Extracts were concentrated by evaporation of ethyl acetate.[15]In another method, 1g charcoal powder was added in 10ml of each supernatant, mixed well and filtered with filter paper. Then 5ml methanol was added to filtered charcoal powder. The mixture was shaken vigorously, centrifuged and supernatant was used to perform antibacterial activity.

Mutational study in order to increase antibiotics production

Mutations were carried out by physical mutation and chemical methods. In physical mutation Ultraviolet rays were used as mutagen and organism was exposed to Ultraviolet rays for 40 and 60 seconds. In chemical mutation, isolate was treated with different mutagenic dyes like, acridine orange (50µg/ml) and ethidium bromide (25µg/ml). 4 flasks containing Starch Casein broth were inoculated with mutated organisms and were incubated for 7 days at 28°C on shaker at 150 rpm. Antibacterial activity from supernatant was performed as above.

RESULTS

Actinomycetes are good source of bioactive compounds and secondary metabolites. A total of nine isolates of Actinomycete A1 to A9 were collected from seven marine water samples collected from Tithal Beach, Valsad. The isolates were identified by microscopic and macroscopic examination. The Actinomycete isolates developed large, round, rough, irregular, convex, opaque, leathery colonies in general with various types of pigments like, chalky white, pink dotted white, brown color etc.(figure 1). Isolates were Gram-positive, rod shape, branched, filamentous, spore former, aerobic bacteria. From microscopic observation the isolated Actinomycetes were look like *Nocardia, Micromonospora* or *Streptomyces spp.* (figure 2)

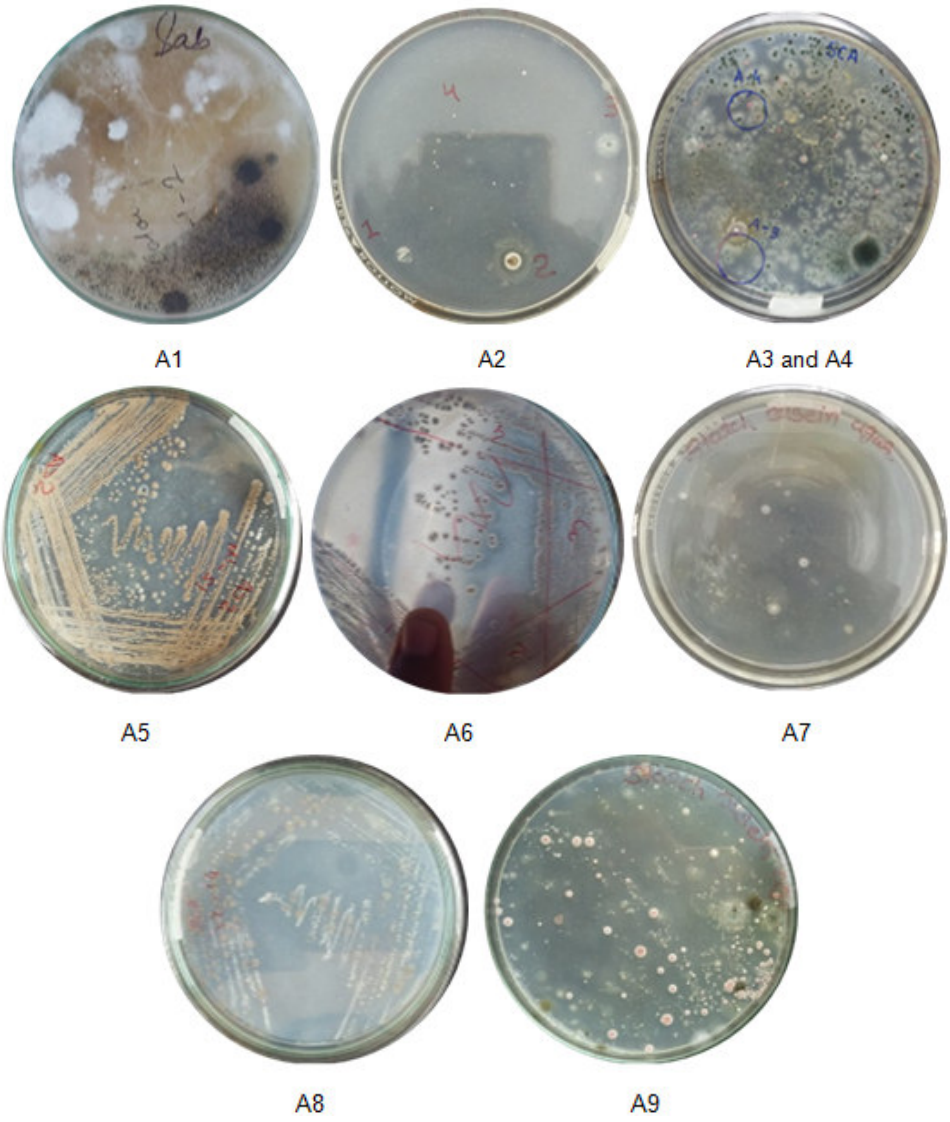

Figure 1
Isolates of Actinomycete on Starch Casein Agar (SCA) Plate

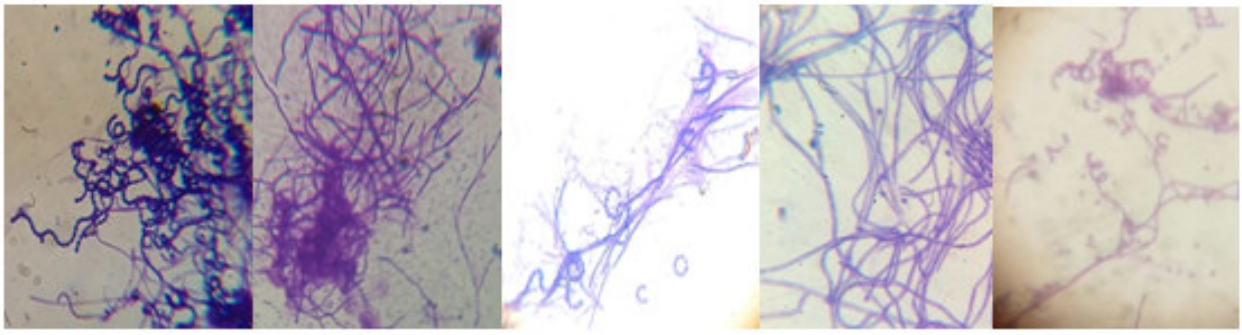

Figure 2
Gram-staining of some Actinomycete Isolates

During 7 days submerged fermentation, Actinomycete isolates developed growth in a form of beads like structures as well as growth was also mainly found on the surface too as shown in figure 3.

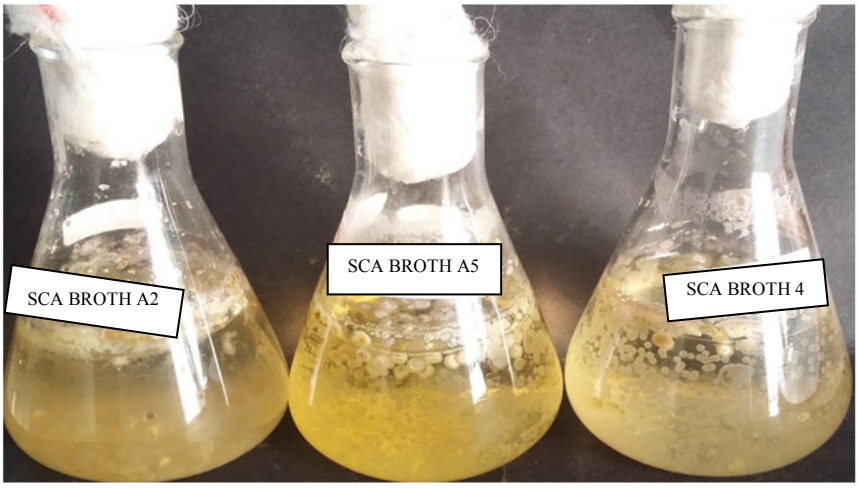

Figure 3
Fermentation Broths (Starch Casein broth medium)

Antimicrobial assay were performed by agar diffusion method using all nine isolates. The antimicrobial activity was tested against various test organisms (*Escherichia coli, Salmonella typhi, Staphylococcus aureus* and *Bacillus subtilis*) using supernatant obtained from fermented broth, as it may possibly contain bioactive compound. Highest antibacterial activity was found against test organism *Escherichia coli.* Out of a nine crude extracts, six extracts has given zones of inhibition and highest inhibition zones 15mm and 14mm were given by isolates A2 and A5 respectively. The inhibition zones measured, ranging from 10mm to 15mm. But another Gram-negative test organism *Salmonella typhi* did not give zone of inhibition against any of the nine crude extracts. *Staphylococcus aureus* also gave zone of inhibition around 10mm with three crude extracts of A2, A4and A5, whereas *Bacillus subtilis* gave inhibition zone only with crude extract of A2 (Table 1 and Figure 4). These may occur due to number of influencing factors such as incubation time, temperature, pH, oxygen availability etc.

Table 1
Antibacterial activity given by crude supernatants of Actinomycetes

Test organisms/Isolates	A1	A2	A3	A4	A5	A6	A7	A8	A9
Escherichia coli	+ (10mm) ±0.8165	+ (15mm) ±0.471405	-	+ (11mm) ±0.471405	+ (14mm) ±0.163299	+ (10mm) ±0.0	-	+ (10mm) ±0.163299	-
Salmonella typhi	-	-	-	-	-	-	-	-	-
Staphylococcus aureus	-	+ (12mm) ±0.188562	-	+ (10mm) ±0.0	+ (10mm) ±0.94281	-	-	-	-
Bacillus subtilis	-	+ (12mm) ±0.057735	-	-	-	-	-	-	-

Values are mean ± SD of three replications.

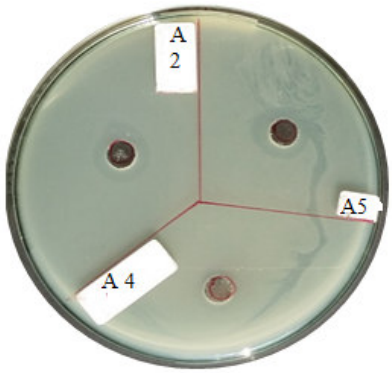

Figure 4
Zones of Inhibition on Nutrient Agar (NA) Plate with the test organism Escherichia coli

Treatment with both solvents, ethyl acetate and methanol, which were used for the extraction of bioactive compound were used for antibacterial activity. Ethyl acetate did not give any result on Nutrient agar (NA) plates (Table 2). Ethyl acetate of any of the nine supernatants did not exhibit inhibitory effect on any test organism *Escherichia coli* and gave entirely negative results. Extracted broth with methanol shows antibacterial activity only against test organism *Escherichia coli*. Only A2 shows 12mm zone of inhibition (Figure 5).

Table 2
Antibacterial activity given by solvent treated supernatants of Actinomycetes

Solvents/Isolates	A1	A2	A3	A4	A5	A6	A7	A8	A9	Control
Ethyl acetate	-	-	-	-	-	-	-	-	-	-
Methanol	-	+ (12mm) ±0.94281 -1.38≤x>4.04	-	-	-	-	-	-	-	-

Values are mean ± SD of three replications.

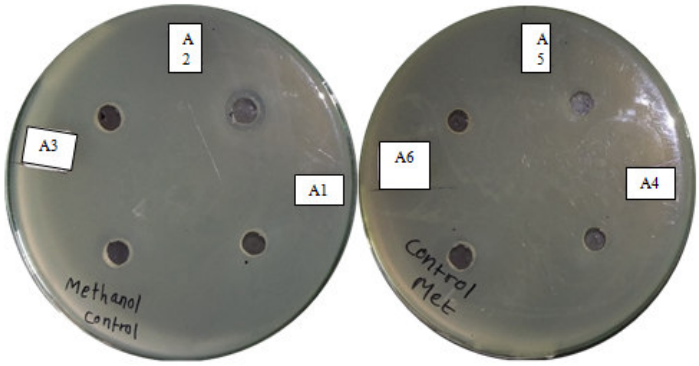

Figure 5
Antibacterial activity given by methanol treated supernatant of A2 and other isolates against Escherichia coli

Mutations of A2 were carried out by both methods. With UV rays as physical mutagen isolate A2 gave 11mm and 14mm inhibition zone at 40 and 60 seconds exposure respectively against *Escherichia coli*.(Table 6)

Table 6
Results of Physical mutation study of the selective isolate A2

Physical mutation (Ultraviolet light)	At 40 seconds	At 60 seconds
Zone diameter	+(11mm) ±0.8165	+(14mm) ±0.235702

Values are mean ± SD of three replications.

Table 7
Results of Chemical mutational study of the selective isolate A2

chemical mutation(mutagenic dyes)	Acridine orange	Ethidium bromide
Zone diameter	_	_

Whereas there was negative impact of both mutagenic dyes on chemical mutation of the test organisms.(Table 7)

DISCUSSIONS

Natural product drug discovery using marine microbes prevailed for many years in pharmaceutical industry. Among the diverse marine microbial communities, Actinobacteria have occupied a prominent and significant position as potential producers of structurally complex and unique metabolites.[16]After more than 50 years of widespread use, however, many antimicrobials are not as effective as they used to be. Over time, some bacteria have developed ways to circumvent the effects of antibiotics. Widespread use of antibiotics is thought to have spurred evolutionarily adaptations that enable bacteria to survive these powerful drugs. Antimicrobial resistances provide a survival benefit to microbes and make it harder to eliminate infections from the body. Pathogens are gaining resistance to existing antibiotics, hence; there is a desperate need of screening Actinomycetes for antimicrobial compound.[17]Nine isolates were collected from seven marine water

samples of Tithal Beach, Valsad. Low numbers of isolates were isolated and it may happen due to rainy season, because marine water diluted and may be the concentration of organisms also decreased. Some Actinomycetes may have failed to grow on SCA plates. The SCA plates are good source of nutrition for marine Actinomycetes. Fermentations were carried out using nine isolates inoculated in Starch Casein broth. A2, A4 and A5 shows good antibacterial activity specifically against test organism *Escherichia coli*, which shows higher inhibition zone son Nutrient Agar (NA) plates. Treatment with ethyl acetate did not show any significant results with test organism. It suggests that ethyl acetate was not proved a good solvent for antibiotic/s produced by the isolate. Supernatant treated with charcoal powder and methanol did not give significant results. So, antibiotic may be either not produced. Methanol is not a suitable solvent for the antibiotics produced by Actinomycetes. So, A2 isolate was selected for the further study because, it shows good results.A2 gave 11mm and 14mm inhibition zone against *Escherichia coli*, when exposed to 40 and 60 seconds to UV light. But supernatant without any mutagenic treatment also gave 14mm and 15mm zone. So there was no positive impact of UV mutagenesis over bioactive compound production. In case of chemical mutagenesis no inhibition zones were obtained against Escherichia coli. This may be due to destruction of genes/factors responsible for antibiotic production. Further purification process is significant to get pure antibacterial substance for the application of treatment of different pathogenic microorganisms and also effective against some multidrug resistant pathogens.

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CONCLUSION

Marine microbial community is vast and remains to be richest resource for bioactive compounds which are untrapped by the marine biologist. The critical marine environments are the reason for the production of secondary metabolites by these microbial groups adding more significance than terrestrial environments. The hunts for potent compound from the marine ecosystem for treatment of various drug resistant pathogenic diseases are still in progress even through several compounds successfully marketed. Considering the outcome of the present findings, it was concluded that marine water is a rich source for Actinomycetes that are able to produce different bioactive compounds and drug produce by them are used for the treatment against pathogens. Identification of potential Actinomycetes producing bioactive compounds to genus level and further studies along with these findings are underway. The isolate A2 shows good potency of antibacterial activity against *Escherichia coli.*

CONFLICT OF INTEREST

Conflict of interest declared none.

REFERENCES

1. Olano C, Méndez C, Salas JA. Antitumor compounds from marine actinomycetes. Mar. Drugs. 2009 Jun 11;7(2):210-48.
2. Fenical W, Jensen PR, Palladino MA, Lam KS, Lloyd GK, Potts BC. Discovery and development of the anticancer agent salinosporamide A (NPI-0052). Bioorg. Med. Chem. 2009 Mar 15;17(6):2175-80.
3. Abdelmohsen UR, Pimentel-Elardo SM, Hanora A, Radwan M, Abou-El-Ela SH, Ahmed S, Hentschel U. Isolation, phylogenetic analysis and anti-infective activity screening of marine sponge-associated actinomycetes. Mar. Drugs. 2010 Feb 26;8(3):399-412.
4. Ravi RK, Vasantba J, Bhoomi M, Bonisha T, Bhumika C. Antibacterial potentials of Actinomycetes isolated from Gujarat. Int J Pharm Sci Rev Res. 2015;30:78-83.
5. Mohan YS, Sirisha B, Haritha R, Ramana T. Selective screening, isolation and characterization of antimicrobial agents from marine actinomycetes. Int. J. Pharm. Pharm. Sci. 2013;5:443-9.
6. Duddu MK, Guntuku G. Isolation and partial characterization of actinomycetes from mangrove sediment sample. Journal of Global Biosciences. 2015;4(7):2921-9.
7. Ravikumar S, Inbaneson SJ, Uthiraselvam M, Priya SR, Ramu A, Banerjee MB. Diversity of Endophytic Actinomycetes from Karangkadu mangrove ecosystem and its antibacterial potential against bacterial pathogens.J. Pharm. Res. 2011; 4(1):294-96.
8. Stackebrandt E, Rainey FA, Ward-Rainey NL. Proposal for a new hierarchic classification system, Actinobacteria classis nov. Int J Syst Evol Microbiol 1997 Apr 1;47(2):479-91.
9. Gordon RJ, Lowy FD. Pathogenesis of methicillin-resistant Staphylococcus aureus infection. Clin. Infect. Dis.. 2008 Jun 1;46(Supplement 5):S350-9.
10. Bizuye A, Moges F, Andualem B. Isolation and screening of antibiotic producing actinomycetes from soils in Gondar town, North West Ethiopia. Asian Pacific Journal of Tropical Disease. 2013 Oct 31;3(5):375-81.
11. Moncheva P, Tishkov S, Dimitrova N, Chipeva V, Antonova-Nikolova S, Bogatzevska N. Characteristics of soil actinomycetes from antarctica. Journal of Culture Collections.;3(1):3-14..
12. Devi NK, Rajendran R, Sundaram SK. Isolation and characterization of bioactive compounds from marine bacteria. Indian J Nat Prod Resour. 2011; 2(1):59-64.
13. Baskaran R, Vijayakumar R, Mohan PM. Enrichment method for the isolation of bioactive actinomycetes from mangrove

sediments of Andaman Islands, India. Malaysian J Microbiol. 2011;7(1):1-7.
14. Poosarla A, Krishna RM. Isolation of potent antibiotic producing Actinomycetes from marine sediments of Andaman and Nicobar Marine Islands. J Microbiol Antimicrob. 2013 Jan 31;5(1):6-12.
15. Atta HM, Ahmad MS. Antimycin-A antibiotic biosynthesis produced by Streptomyces sp. AZ-AR-262: taxonomy, fermentation, purification and biological activities. Aust J Basic Appl Sci 3: 126–135; 2009.
16. Abirami M, Gopiesh Khanna V, Kannabiran K. Antibacterial activity of marine streptomyces SP. Isolated from andaman & nicobar Islands, India. Int J Pharma Bio Sci. 2013;4(3):B280-6.
17. PANWAR D, SAINI VK. Antagonistic activity of soil actinomycetes against common human pathogens. Int J Pharm Bio Sci. 2012;3(4):500-6.

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