CHINESE JOURNAL OF MEDICAL GENETICS
ISSN: 1003-9406 Vol. 33 Iss. 1 2024

ANTIMICROBIAL AND ANTIOXIDANT POTENTIAL OF ACTINOMYCETES FROM MEDICINAL PLANT RHIZOSPHERE SOILS

Sunita C Mesta[1], Maria Nashrah[1] and Satish Kumar J
1 Department of Microbiology, JSS Academy of Higher Education and Research, Mysuru-570015
2 Department of Environmental Sciences, JSS Academy of Higher Education and Research, Mysuru-570015

Abstract
The actinomycetes are a group of prokaryotic organisms that share both the traits of fungus and bacteria. They may be isolated from the rhizosphere of plants and are found in vast areas including terrestrial soil, marine water and sediments. They produce various bioactive compounds with antimicrobial properties. The present study focus on antimicrobial and antioxidant activity of actinomycetes from medicinal plant rhizosphere soils collected from local area of Mysuru, Karnataka. The rhizosphere soils of medicinal plants were used for screening of actinomycetes. Serial dilution technique was employed for the isolation of actinomycetes using Actinomycetes Isolation Agar (AIA). A total of thirty two isolates were recovered. These isolates were subjected for morphological and biochemical analysis. Majority of the isolates belonged to Streptomyces. Using the agar well diffusion method and the cross streak method, the antibacterial activity was investigated against gram positive and gram negative bacterial organisms. Ethyl acetate solvent was used to extract the extracellular metabolites from the potent isolates after they underwent fermentation.The DPPH radical scavenging assay was used to measure the antioxidant activity. The outcomes revealed antioxidant activity that was dose dependant.

Key Words: Actinomycetes, Medicinal plants, Streptomyces, Rhizosphere soil, Antioxidant activity.

Introduction

The most common causes of health issues are infectious infections with high morbidity and death rate in developing countries. One significant issue in the treatment of infectious diseases is antibiotic resistance caused by morbific microbes. Multidrug resistance poses a challenge, necessitating an urgent focus on research and the development of novel bioactive chemicals to tackle these infections (Mesta et al., 2017)[1]

The area of soil around a plant root where the roots have an impact on the chemistry and biology of the soil is known as the rhizosphere. Plants harbour a vast range of microorganisms, including bacteria, fungus, actinomycetes, and protozoans. This functions as the microbial seed bank where microorganisms convert organic and inorganic compounds in the rhizosphere into available plant nutrients. The actinomycetes from rhizosphere soils are qualitatively and quantitatively predominant (Raut and Kulkarni 2018)[2] and acquire particular significance as the most effective source of natural compounds with medicinal value, bioactive secondary metabolites, and antibiotics (Jayaprakashvel 2012[3] ; Suzuki et al., 2000)[4].

Actinomycetes are saprophytic bacteria that are gram positive, free-living, and ubiquitous in nature. The majority of them are located in soil, rhizospheres of plant roots and marine sediments (Elshafie and Camele, 2022)[5] fresh waters and the water bodies' surface and also in sea water (Sharma et al., 2014)[6]. The majority are referred to as saprophytic soil dwellers (Takisawa et al., 1993)[7]. At a mature stage in their life cycle, they develop branching mycelium of two types: substrate mycelium and aerial mycelium with a characteristic long chain of arthrospores. The DNA of these bacteria has a high G + C concentration (>55%) (Patel et al., 2022[8]; Wang et al., 1999[9]; Tistechok et al., 2023[10]). The actinomycetes from rhizosphere soils are rich source of siderophore, antifungal compounds and Indole acetic acid (Khamna et al., 2009)[11]. In addition to safeguarding people from a wide range of illnesses and degenerative disorders, antioxidants are crucial in scavenging and inhibiting free radicals. Oxidative stress conditions result from a diet low in antioxidants. The DPPH scavenging method has been extensively used to study the samples' ability to scavenge free radicals (Priya et al., 2010)[12]. Considering the present scenario, the present work is centred on antioxidant and antibacterial properties from medicinal plant rhizosphere soils.

Material and Methods

Collection of soil samples

The rhizosperic soil samples were collected at a depth of 10-15cm from 7 different medicinal plants available at the local area of Mysuru. Samples of soils were gathered and stored in sterile ziplock bags and brought to the laboratory for further analysis. The soil samples were air dried at room temperature to remove moisture (Krishnakumari et al., 2006)[13].

Isolation of Actinomycetes

The collected soil samples were further subjected for serial dilution plate technique. The diluted aliquotes were plated on Actinomycetes Isolation Agar (AIA) (Himedia) for isolation of actinomycetes. The inoculated plates were kept in an incubator at at 30°C for 7 to 14 days. The isolates were purified by streak plate method. The isolates were sub- cultured and stored at 4°C for further examination. The media was supplemented with Fluconazole and Griseofulvin antifungal agents to avoid fungal contamination (Aneja, 1996)[14].

Identification of actinomycetes

The tough and powdery Colonies were chosen and recognized by cultural, morphological (Cover Slip Method) upto genus level (Holt et al., 2000)[15].

Staining and Biochemical Tests

All the isolates were subjected through Gram's staining techniques. Different biochemical tests namely starch hydrolysis, catalase test, gelatin hydrolysis, carbohydrate utilisation test (glucose, lactose, and starch) were performed (Aneja 1996[14]; Cappuccino and Sherman 1996[16]; Holt et al., 2000[15]).

Antimicrobial activity of actinomycetes

The preliminary screening for antimicrobial activity was done by cross streak method towards five pathogenic bacteria. Peptone and beef extract were added to the AIA medium to promote bacterial growth.. The actinomycetes isolates were inoculated in the middle of the AIA agar plates, then an

incubation period at 30°C for 3-5 days. The test bacteria were streaked perpendicular to the confluent growth of actinomycetes and followed by incubation at 37°C for 24 hours. Results were recorded on the basis of suppression of test bacteria as a result of therapeutic compound secreted by actinomycetes, considered to be positive for secreting anti- microbial constituents, whereas those showing strong growth near the confluent growth of actinomycetes without area of clearance were regarded to have a shielding effect towards anti- biotic producers (Sahin and Ugur 2003[17]; Williams et al.,1923[18]).

Fermentation and Solvent Extraction

The potential strains were added to a 250 ml Erlenmeyer flask containing starch casein nitrate broth and incubated at 30° C for 7 -10 days. The flasks were continuously checked for any signs of contamination. Following incubation, Whatmann No. 1 filter paper was used to aseptically filter the broth culture. The crude extracts were subjected for solvent extraction in separating funnel. The culture filtrate was combined with the ethyl acetate solvent in a 1:1 (v/v) ratio and mixed thoroughly. The solvent and filtrate layer were separated by allowing the separating funnel to stand for roughly thirty minutes. Further, the solvent layer was separated, dried and its biological activity was measured. (Sahin and Ugur 2003[17]).

Secondary screening

The agar well diffusion method was used to do the secondary screening for antibacterial activity. The wells of 6mm diameter were punched in Nutrient agar plates, pre-inoculated with test microbes, using sterile cork borer. The solvent extract of the potent isolates were carefully added in each well using micropipette. Plates were allowed to diffuse for about 15min before incubating. The plates were incubated for 24 hours at 37°C for bacteria. The zones of inhibition developed around the well on the plates were measured in millimetres. (Mesta et al., 2017[19]).

Antioxidant Activity of solvent extracts of Actinomycetes

The solvent extracts showing promising antibacterial activity were subjected to antioxidant activity by DPPH (1, 1- biphenyl – 2- picrylhydrazyl) assay was done to evaluate free radical scavenging activity of the metabolite. Different concentrations of solvent extract was added to 3ml of 0.004% DPPH. After roughly 30 minutes of dark incubation at room temperature, the tubes' absorbance at 517 nm was measured spectrophotometrically. Ascorbic acid was used as standard. The absorbance of DPPH without extract/ standard was noted. The scavenging activity (%) of each concentration was calculated using the formula, Scavenging activity (%) = [(A-B)/A] x 100 Where A refers to absorbance of DPPH control and B refers to absorbance of extract/ standard respectively (Braca et al., 2001[20]).

Results

Medicinal plants: The rhizospheric soil samples from 7 different medicinal plants were collected viz Salvia rosmarinus, Aloe barbadensis, Bacopa monnieri, Coleus amboinicus, Costus igneus, Bryophyllum pinnatum and Azadirachta indica.

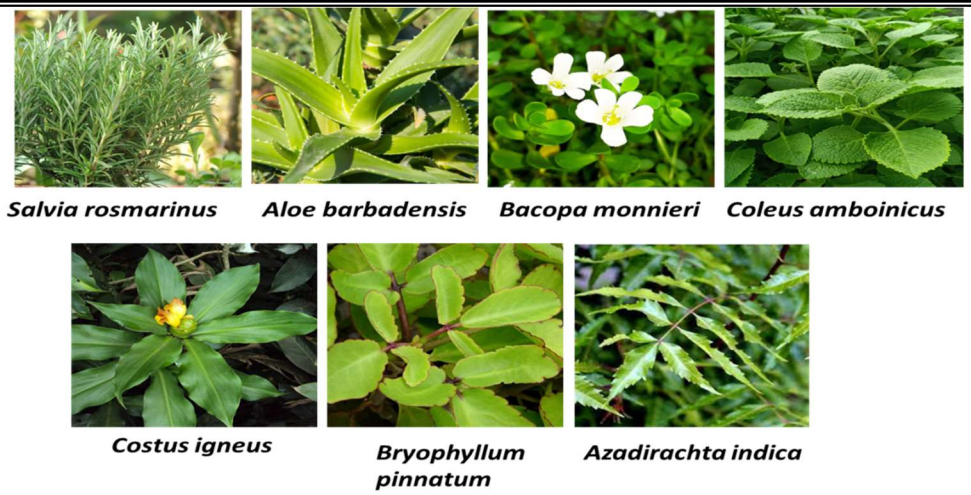


Fig 1: Medicinal plants selected for the study.

Isolation of Actinomycetes: A total of 34 actinomycetes species were isolated. The initial dilutions yielded highest number of colonies followed by the higher dilutions. Costus igneus, Bryophyllum pinnatum yielded higher number of isolates compared to others.

Table 1: Distribution of actinomycetes isolates in various rhizosphere soil samples of medicinal plants.

Sl No	Medicinal Plants	Number of Isolates
1	Salvia rosmarinus (Rose mary)	02
2	Aloe barbadensis (Aloe vera)	02
3	Bacopa monnieri	03
4	Coleus amboinicus	03
5	Costus igneus (Insulin plant)	12
6	Bryophyllum pinnatum	11
7	Azadirachta indica (Neem)	01

Identification of actinomycetes : Out of 34 actinomycetes isolated, 15 isolates were subjected for further studies. The results revealed a diverse morphological characteristics with varied spore colours, colony morphology, aerial and substrate mycelium colourations. The spore morphology showed different spore arrangement varying from rectus, flexibilis, retinaculum aperatum – open loops, hooks and spira– simple spirals, short and compact spirals (Table 02; Fig 2 and 3). The isolates belonged to genus Streptomyces based on the spore chain arrangement.

Table 2: Morphological Characteristics of isolated Actinomycetes species

Isolates	Aerial mycelium	Substrate mycelium	Colony morphology	Spore Arrangement
SM-1	Grey	White	Raised, Powdery	Rectus
SM-2	Yellow	White	Discrete	Flexibilis
SM-3	White	White	Powdery	RA-hooks

SM-4	Grey	Grey	Powdery	Flexibilis
SM-5	Brown	Brown	Radiating	Rectus
SM-6	Dark grey	White	Discrete	Rectus
SM-7	Pink	White	Velvety	Rectus
SM-8	Grey	White	Discrete	Flexibilis
SM-9	White	White	Discrete	Flexibilis
SM-10	Blue	White	Powdery	Simple spira
SM-11	Grey	Grey	Raised, Powdery	Spiral
SM-12	White	White	Granular	Simple spira
SM-13	Yellow	Grey	Velvety	Rectus
SM-14	Grey	White	Granular	Rectus
SM-15	White	Grey	Powdery	Rectus

Figure 2: Representative Actinomycetes isolates from rhizhosphere soils of Medicinal plants.

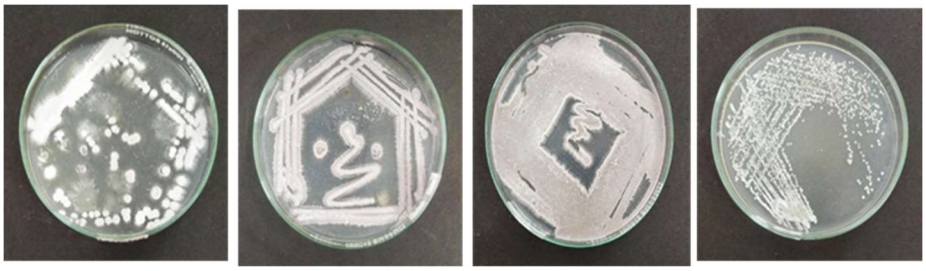
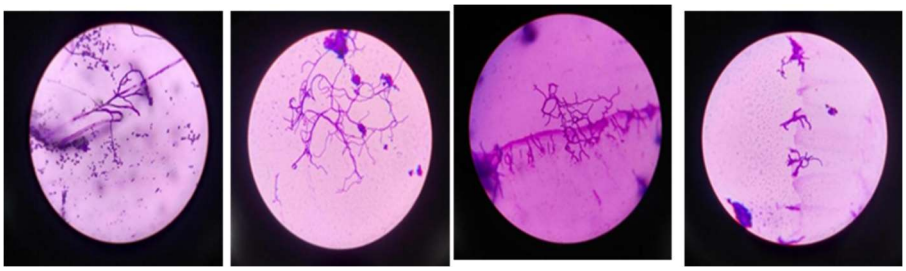


Figure 3: Spore arrangement (1- Rectus, 2- Retinaculum aperatum -Hooks, 3-Spira, 4-Flexibilis)



Staining and Biochemical Tests: All the isolates were found to be gram positive. The biochemical tests showed varied activities of the actinomycetes isolates. All the isolates were positive for catalase and hydrolyzed the starch by amylase production. Twelve isolates were positive for gelatin hydrolysis (Table 3).

Table 3: Biochemical characteristics

Isolates	Catalase Test	Starch Hydrolysis	Gelatin hydrolysis	Carbohydrate utilization test								
				Glucose			Lactose			Starch		
				A	G	Al	A	G	Al	A	G	Al
SM-1	+	+++	+	-	-	+++	-	-	+++	-	-	+++

SM-2	+	++	-	-	-	+++	-	-	+++	-	-	+++
SM-3	+	+++	+	+	-	-	+	-	-	+	-	-
SM-4	+	+++	+	-	-	+++	-	-	+++	-	-	+++
SM-5	+	+++	+	++	-	-	-	-	+++	-	-	-
SM-6	+	++	+	-	-	+++	-	-	+++	-	-	+++
SM-7	+	+++	+	-	-	++	-	-	+++	-	-	++
SM-8	+	+++	+	-	-	+++	-	-	+++	-	-	+++
SM-9	+	+++	+	-	-	+++	-	-	++	-	-	+++
SM-10	+	+++	+	-	-	+++	-	-	+++	-	-	+++
SM-11	+	++	-	-	-	+++	-	-	+++	-	-	+++
SM-12	+	+++	+	+	-	-	+ +	-	-	+	+	-
SM-13	+	++	-	-	-	+++	-	-	+++	-	-	+++
SM-14	+	+++	+	-	-	+++	-	-	++	-	-	+++
SM-15	+	+++	+	-	-	+++	-	-	+++	-	-	+++

('-' Negative, '+' Positive, '++' Good, '+++' Very good, A-Acid, G- Gas, Al- Alkali)

The carbohydrate utilization test showed varied results. The results showed acid, gas and alkali production. The acid and gas production was noted by the colour change from red to yellow and accumulation of gas in the durham's tube. The alkali production was observed by the change of colour from red to deep pink. Three isolates were positive for acid production in glucose. Most of the isolates showed positive results for alkali production in all the sugars tested, alkali production was considerably high in monosaccharide, disaccharides and polysaccharides (Table 3).

Antimicrobial activity: The primary screening of the isolates revealed antagonistic activity against the test organisms to varied level. The antagonistic activity was measured as the interaction between the actinomycetes isolates and the test bacteria. The antibiosis was seen as a clear zone around the actinomycetes isolates inhibiting the growth of test bacteria. Majority of the isolates showed the inhibition of one or more. Isolate numbers SM-8, SM-10 and SM-14 showed marked inhibition against all the test organisms. Isolate SM-1, SM-7, SM-11 and SM-13 were ineffective against all the tested organisms (Table 4).

Table 4: Primary screening of actinomycetes isolates

Isolate No	Extent of Inhibition				
	B1	B2	B3	B4	B5
SM-1	-	-	-	-	-
SM-2	-	+	+	-	+
SM-3	+	+	+	-	+
SM-4	+++	-	+++	-	-
SM-5	-	-	++	-	-
SM-6	+	++	-	++	+
SM-7	-	-	-	-	-

SM-8	+++	+++	+++	+++	+++
SM-9	-	-	-	-	+
SM-10	+++	+++	+++	+++	+++
SM-11	-	-	-	-	-
SM-12	-	+++	-	+++	-
SM-13	-	-	-	-	-
SM-14	+++	+++	+++	+++	+++
SM-15	+++	+	+++	-	+

Figure 4: Primary screening of actinomycetes isolates against microbial pathogens

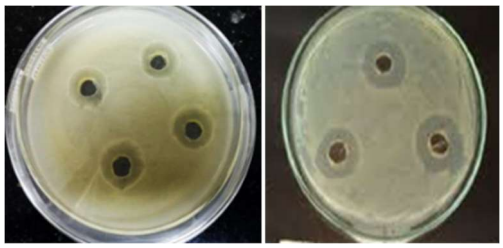
Secondary screening: The secondary screening results showed the diversity of antibiotic metabolites produced by Actinomycetes species. The antibacterial metabolites with both broad spectrum and narrow spectrum activities were obtained. The inhibitory zone was observed around the well and was taken as positive and absence of zone was considered negative. The zone of inhibition for antimicrobial activity was measured in mm using antibiotic zone measuring scale (Himedia), the zone of inhibition varied from 0 to 22 mm (Table 5).

Table 5: Secondary screening of solvent extracts of potent Actinomycetes isolates

Isolate number	Zone of inhibition (mm)				
	B1	B2	B3	B4	B5
SM-8	22	00	18	00	10
SM-10	11	12	00	00	13
SM-14	05	00	15	04	08

B-1 V. cholera, B-2 S. aureus, B-3 E. coli, B-4 S. typhi, B-5 B.cereus

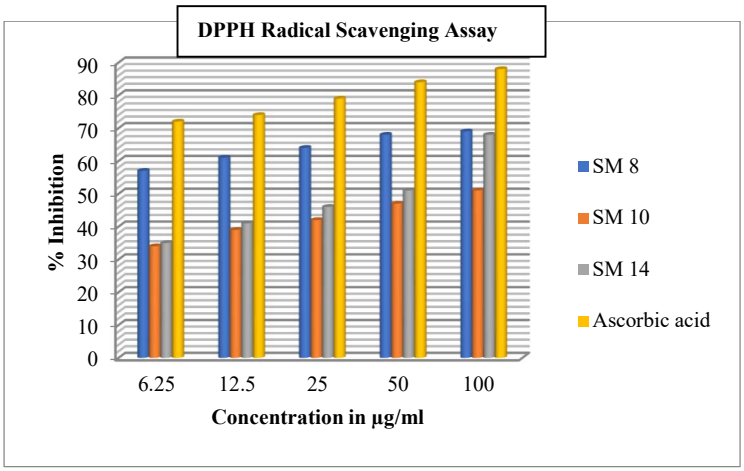
Figure 5: Secondary screening of solvent extracts of potent Actinomycetes isolates



Antioxidant activity (DPPH assay): The DPPH assay was carried out using the extracts of actinomycetes that showed bacterial suppression activity. DPPH is light sensitive; handling was

carried out in dark cool place. The reduction capability of DPPH radicals was determined by decrease in absorbance at 517nm induced by antioxidants. The dose dependent scavenging effect was observed. The percent inhibition of DPPH radicals by extracts from SM 8, SM 10, SM 14 and standard ascorbic acid at 100µg/ml was 69.26%, 51.61%, 68.02% and 88.28% respectively (Fig 06).

Figure 6: DPPH radical scavenging activity of SM 08, SM 10, SM 14 and Ascorbic acid



DISCUSSION

The rhizosphere is rich in organic materials and nutrients from root exudates; it provides an optimal environment for the growth of microorganisms. Rhizome soils are often home to actinobacteria, which are capable of producing a wide range of beneficial secondary metabolites and compounds with various characteristics (Sriragavi et al., 2023) [21]. Antimicrobial resistance increases morbidity, death, and health care costs in addition to making therapy ineffective. The rise of antibiotic resistance in pathogens, their high cost, and their adverse effects have prompted study into the discovery of natural sources of antimicrobials. The natural products can be effectively used as promising alternates to fight drug resistance (Mesta et al., 2017) [1].

In the present study a total of 32 actinomycetes isolates were obtained from rhizosphere soil of medicinal plants, among which Streptomyces were the dominant species. Similar studies have shown that diverse group of actinomycetes are present in rhizosphere soils of crop plants (Dicko et al., 2013[22]); medicinal plants (Khamna et al., 2009[11]). The preliminary screening for antibacterial activity revealed that the isolates SM 8, SM 10 and SM 14 were effective against the test organisms including both gram positive and gram negative bacteria. The secondary screening showed broad spectrum activity against the organisms tested. Ibnouf et al. (2022)[23] reported that actinomycetes isolated from dried sandy soils were effective against Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus vulgaris, Salmonella typhimurium. Rammali et al. (2022)[24] revealed that actinomycetes isolates from extremely cold and microbiologically unexplored terrestrial environment were more active against Gram-positive bacteria than Gram-negative bacteria. Similar antimicrobial activity results

were reported by Chaudhary et al. (2013)[25] against multidrug resistant bacteria. Similarly Tistechok et al. (2023)[10] reported antibiotic properties of actinomycete isolates from the rhizosphere soil of Juniperus excelsa.

Oxidative stress is a condition caused by the body's accumulation of free radicals. These free radicals can be scavenged and inhibited by antioxidants. Natural antioxidants derived from microorganisms and plants are safe medicines for human life (Rao and Rao, 2013)[26]. It has been discovered that actinomycetes are potent antioxidant producers (Jayaprakashvel, 2012[27]; Praptiwi et al., 2023[28]). The actinomycetes species isolated from various habitats such as coastal, mangrove soil, and humus soil are known to possess antioxidant activity. The DPPH radical scavenging assay is a simple, rapid and sensitive method to screen and evaluate the antioxidant nature of molecule (Kekuda et al., 2015)[29]. The antioxidant activity of extracts from rhizosphere actinomycetes isolates SM 08, SM 10, and SM 14 were evaluated by DPPH radical scavenging activity. In the present study, the scavenging activity of ethyl acetate extracts were found to be dose dependent i.e., higher the concentration, the scavenging activity was more. The results obtained are in agreement with the results reported by Praptiwi et al. (2023)[28] with actinomycetes isolated from Dacrycarpus imbricatus plant. Nagaseshu et al. (2016)[30] reported antioxidant capacity of Actinobacteria methanol extracts obtained from marine sediments collected along the coast of Kakinada. Additionally, they correlated the extract's antioxidant activity with its cytotoxic and antiproliferative properties.

CONCLUSION

The microbial natural products are the primary sources of chemicals for drug development. Among the microorganisms, the actinomycetes are the main producers of antibiotics. The rhizosphere soils of medicinal plants provide a rich source of diversity of actinomycetes. Screening of microorganisms for the production of novel antibiotics has been intensively pursued for many years by scientists. The generation of secondary metabolites by actinomycetes is affected by the temperature at which they are incubated, the culture conditions used, and the type of the target pathogen. The results of the present study indicated the potential of rhizosphere actinomycetes containing the bioactive principles to exhibit antimicrobial activity against pathogenic microorganisms and free radicals that cause oxidative damage. In-depth research on the diversity of actinomycetes is still needed, as they may provide valuable insights for the pharmaceutical sector.

BIBLIOGRAPHY

1. Mesta, S., and Onkarappa, R. Characterization and Antimicrobial Potential of Marine Actinomycetes from Estuaries of Uttara Kannada District, Karnataka. Journal of Drug Delivery and Therapeutics. 2017. 7(6): 27-37. DOI https://doi.org/10.22270/jddt.v7i6.1527

2. Raut, R.A., and Kulkarni, S.W. 2018. Isolation, Characterization and biodiversity of actinomycetes from rhizosphere soil of some medicinal plants, International Journal of Recent Trends in Science and Technology. 13-18.
3. Jayaprakashvel, M. 2012. Review Article – Therapeutically Active Biomolecules from Marine Actinomycetes. **Journal of Modern Biotechnology**. 1(1): 1-7.
4. Suzuki, S., Yamamoto, K., Okuda, T., Nishio, M., Nakanishi, N., and Komatsubara, S. Selective isolation and distribution of Actinomadura rugatobispora strains in soil. Actinomycetology. 2000. 14:27–33.
5. Elshafie, H.S., and Camele, I. Rhizospheric Actinomycetes Revealed Antifungal and Plant-Growth-Promoting Activities under Controlled Environment. Plants (Basel). 2022 Jul 18;11(14):1872. doi: 10.3390/plants11141872. PMID: 35890505; PMCID: PMC9317816
6. Sharma, M., Dangi, P., and Choudhary, M. Actinomycetes: Source, Identification, and Their Applications. International Journal of Current Microbiology and Applied Sciences, 2014, 3(2): 801-832
7. Takisawa, M., Colwell, R.R., and Hill, R.T. Isolation and diversity of actinomycetes in the Chesapeake Bay. Appl Environ Microbiol. 1993. 59: 997–1002
8. Patel, K., Upadhyay, D., Bhattacharya, I., Thakur, A., and Andhare, P. Isolated Actinomycetes from Soil. Bull. Env.Pharmacol. Life Sci., Vol Spl Issue [3] 2022: 114-118.
9. Wang, Y., Zhang, Z. S., Ruan, J. S., Wang, Y. M., & Ali, S. M. (1999). Investigation of actinomycete diversity in the tropical rainforests of Singapore. Journal of Industrial Microbiology and Biotechnology, 23(3), 178-187.
10. Tistechok, S., Roman, I., Fedorenko, V., Luzhetskyy, A., Gromyko, O., Diversity and bioactive potential of Actinomycetia from the rhizosphere soil of Juniperus excelsa, Folia Microbiologica (2023) 68:645–653. https://doi.org/10.1007/s12223-023-01047-x
11. Khamna, S., Yokota, A., and Lumyong, S. 2009.Actinomycetes isolated from medicinal plant rhizosphere soils: diversity and screening of antifungal compounds, indole-3-aceticacid and siderophore production. World J Microbiol Biotechnol, 25:649–655DOI 10.1007/s11274-008-9933-x
12. Priya, A.J., Sagadevan, E., Dhanalakshmi, P., Kumar, S.S., Karthikeyan, V., and Arumugam, P. Detection of Antioxdant and Antimicrobial activities in marine actinomycetes isolated from Puducherry Coastal Region. Journal of Modern Biotechnology 2012; 1(2): 63-69
13. Krishnakumari, K., Ponmurugan, P., and Kannan, N. Isolation and characterisation of Streptomyces sp. from soil samples for secondary metabolite production. Biotechnology. 5(4); 2006: 478-480.
14. Aneja, K.R. (1996). Experiments in Microbiology, Plantpathology, Tissue culture and Mushroom cultivation. 2nd Edition. Vishwa Prakashan, New Delhi.

15. Holt, J.G., Krieg, R.N., Sneath, P.H.A., Staley, J.T., and Williams, S.T. Bergeys manual of determinative Bacteriology, Lippincot Williams and Wilkins, Baltimore, Maryland. 9 th edn; 2000.
16. Cappuccino and Sherman. Microbiology a Laboratory Manual. P. Fourth edition. The Benjamín/Cummings Publishing Company Inc. 1996.
17. Sahin N, and Ugur A. Investigation of the Antimicrobial activity of some Streptomyces isolates. Turkish Journal of Biology 2003; 27: 79-84.
18. Williams, T.S., Sharpe, E.M., and Holt, G.J. (eds). Bergey's Manual of Systemic Bacteriology. Williams and Wilkins. Baltimore, Maryland. 1923; 2451- 2508.
19. Mesta,S.C., Onkarappa, R., Chittara, S., Dinesh, J., and Chaitra, K. 2017. Screening of antimicrobial and antioxidant activities of endophytic actinomycetes isolated from Rhizophora mucronata and Sonneratia caseolaris. International Journal of Pharmaceutical & Biological Archives. 8(4): 22-28.
20. Braca, A., Tommasi, Nunziatina De, Bari, Lorenzo Di, Pizza, Cosimo, Politi, Mateo, and Morelli I. 2001. Antioxidant principles from Bauhinia terapotensis. **Journal of Natural Products.** 64: 892-895.
21. Sriragavi, G., Sangeetha, M., Santhakumar, M., Lokesh, E., Nithyalakshmi, M., Saleel, C.A., Balagurunathan, R. 2023. Exploring Antibacterial Properties of Bioactive Compounds Isolated from Streptomyces sp. In Bamboo Rhizosphere Soil, ACS Omega, 36333-36343. https://doi.org/10.1021/acsomega.3c04954
22. Dicko ,A.H., Babana, A.H., Maïga, K., Kassogué, A., Traoré ,D., and Faradji, F.A. 2013. Isolation and characterization of crop rhizospheric actinomycetes with antimicrobial activity in Mali. African Journal of Applied Microbiology Research, 2(1): 1-8.
23. Ibnouf, E.O.,Aldawsari, M.F., and Waggiallah H.A. Isolation and extraction of some compounds that act as antimicrobials from actinomycetes Saudi J Biol Sci. 2022 Aug; 29(8): 103352 doi: 10.1016/j.sjbs.2022.103352
24. Rammali, S., Hilali, L., Dari, K. et al. Antimicrobial and antioxidant activities of Streptomyces species from soils of three different cold sites in the Fez-Meknes region Morocco. Sci Rep **12**, 17233 (2022). https://doi.org/10.1038/s41598-022-21644-z
25. Chaudhary HS, Yadav J, Shrivastava AR, Singh S, Singh AK, Gopalan N. Antibacterial activity of actinomycetes isolated from different soil samples of Sheopur (A city of central India). J Adv Pharm Technol Res. 2013 Apr;4(2):118-23. doi: 10.4103/2231-4040.111528.
26. Rao, K.V.R., and Rao, T.R. 2013. Molecular characterization and its antioxidant activity of a newly isolated Streptomyces coelicoflavus BC 01 from mangrove soil. **Journal of Young Pharmacists.** 5: 121-126.
27. Jayaprakashvel, M. "Therapeutically Active Biomolecules from Marine Actinomycetes" Journal of Modern Biotechnology, 2012, 1(1), 1-7.
28. Praptiwi, P., Fathoni, A., Putri, A.L., Wulansari, D., and Agusta, A. Biological potency of actinomycetes extracts from rhizosphere soil of Dacrycarpus imbricatus from Toba

Samosir, North Sumatra. J Appl Pharm Sci, 2023; 13(06):146–153. https://doi.org/10.7324/JAPS.2023.106853

29. Kekuda, P.T.R., Onkarappa, R., Gautham, S.A., Mesta, S.C., and Raghavendra, H.L. 2015. Antimicrobial, Antioxidant and Cytotoxic activity of Streptomyces species from Western Ghat Soils of Karnataka, India. Science, Technology and Arts Research Journal. 4(2): 164-180.
30. Nagaseshu, P., Gayatridevi, V., Kumar, A. B., Kumari, S., Mohan, M. G., and Malla, R. (2016). Antioxidant and antiproliferative potentials of marine actinomycetes. Int. J. Pharm. Pharm. Sci. 8, 277–284.