Antioxidant Potential of Extra Polymeric Substances From Marine Actinobacteria *Streptomyces* Species

Lasya Ganta a, Lakshminarayanan Arivarasu b* and P. Sivaperumal b

a Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai, India.
b Department of Pharmacology, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai, India.

Authors’ contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

**Introduction:** The Actinobacteria are a phylum of Gram-positive bacteria. They can be terrestrial or aquatic. They are of great economic importance to humans because agriculture and forests depend on their contributions to soil systems. They are more abundant in soils than other media, especially in alkaline soils and soils rich in organic matter, where they constitute an important part of the microbial population.

**Material and Methods:** The sediment sample was collected from the Gulf of Mannar, Tamilnadu. The collected sample was sun dried for 48 hrs and turned into fine powder by mortar and pestle. The colour of the mature sporulating aerial mycelium was recorded in naked eye.

**Results:** The antioxidant potential of actinobacterial EPS was determined on the basis of their scavenging activity of the stable 1,1- diphenyl-2-picryl hydrazyl (DPPH) free radical. Different concentrations (0.5-3mg/ml) of samples were mixed with 2.9ml diphenylpicrylhydrazyl (DPPH) solution (120µM) in methanol and incubated in darkness at 37°C for 30 minutes. The absorbance was recorded at 517 nm. Data were implied as mean ± SEM.

**Conclusion:** The produced melanin pigment from the Actinobacteria of *streptomyces* species was found to have potent antioxidant potential activity. Further characterisation and bio active properties should be done in the further studies, and more articles in future are yet to come in various properties of melanin pigment.

*Corresponding author: E-mail: lakshmi@saveetha.com;
1. INTRODUCTION

The Actinobacteria are a phylum of Gram-positive bacteria. They can be terrestrial or aquatic. They are of great economic importance to humans because agriculture and forests depend on their contributions to soil systems. They are more abundant in soils than other media, especially in alkaline soils and soils rich in organic matter, where they constitute an important part of the microbial population. Actinobacteria can be found on both the soil surface and at depths of more than 2 m below ground. They produce a number of enzymes that help degrade organic plant material, lignin, and chitin. Antioxidant activity is defined “as an limitation of the oxidation of proteins, lipids, DNA or other molecules that occurs by blocking the propagation stage in oxidative chain reactions” and primary antioxidants directly scavenge free radicals, while secondary antioxidants indirectly prevent the formation of free [1-3].

*Streptomyces* is the largest genus of actinobacteria and the type genus of the family streptomycaceae. Over 500 species of *Streptomyces* bacteria have been described. As with the other Actinobacteria, streptomycetes are gram-positive, and have genomes with high GC content. Found predominantly in soil and decaying vegetation, most streptomycetes produce spores. They produce over two-thirds of the clinically useful antibiotics of natural origin. *Streptomyces* is the largest antibiotic-producing genus, producing antibacterial, antifungal, and antiparasitic drugs, and also a wide range of other bioactive compounds, such as immunosuppressants [4-6]. Almost all of the bioactive compounds produced by Streptomycetes are initiated during the time coinciding with the aerial hyphal formation from the substrate mycelium. Microbial EPS are biosynthetic polymers (biopolymers). EPS were defined (GEESEY 1982) as “extracellular polymeric substances of biological origin that participate in the formation of microbial aggregates”. Another definition was given in a glossary to the report of the Dahlem Workshop on Structure and Function of Biofilms in Berlin 1988 (CHARACKLIS AND WILDERER 1989). Here, EPS were defined as “organic polymers of microbial origin which in biofilm systems are frequently responsible for binding cells and other particulate materials together (cohesion) and to the substratum (adhesion)” [6-9].

The vast majority of microorganisms live and grow in aggregated forms such as biofilms and flocs (“planktonic biofilms”). This mode of existence is lumped in with the somewhat inexact but generally accepted expression “biofilm”. The common feature of all these phenomena is that the microorganisms are embedded in a matrix of extracellular polymeric substances (EPS). The production of EPS is a general property of microorganisms in natural environments and has been shown to occur both in prokaryotic (Bacteria, Archaea) and in eukaryotic (algae, fungi) microorganisms [10,11]. Biofilms containing mixed populations of these organisms are ubiquitously distributed in natural soil and aquatic environments, on tissues of plants, animals and man as well as in technical systems such as filters and other porous materials, reservoirs, plumbing systems, pipelines, ship hulls, heat exchangers, separation membranes, etc. EPS are mainly responsible for the structural and functional integrity of biofilms and are considered as the key components that determine the physicochemical and biological properties of biofilms [12,13]. Our team has extensive knowledge and research experience that has translated into high quality publications [14-16].

The present study focuses on the antioxidant property of extracellular polymeric which is derived from the marine Actinobacteria *Streptomyces* species. Previous studies on antioxidants and the other properties had shown that extracellular polymeric has a potent antioxidant and cytotoxic effects. These properties of extracellular polymeric had increased the use of extracellular polymeric substances over industries and all over the country.

2. MATERIALS AND METHODS

2.1 Antioxidant Potential of EPS from Marine Actinobacteria *Streptomyces* sp.

2.1.1 Sample collection and preparation

The sediment sample was collected from the Gulf of Mannar, Tamil Nadu. The collected sample was sun dried for 48 hrs and turned into fine powder by mortar and pestle.
2.1.2 Isolation of actinobacteria

Isolation and enumeration of actinobacteria were carried out in Kuster's agar medium (KUA) supplemented with 0.5% (W/v) NaCl. To minimize the fungal and bacterial contamination, KUA medium was supplemented with cycloheximide (10 µg/ml) and nalidixic acid (10 µg/ml) respectively (Kathiresan et al., 2005). Collected sediment samples were serially diluted and inoculated on KUA medium and incubated at 36°C for 7 days. The colonies were counted and the population density has been expressed as colony forming units per gram (CFU/g) of sediment. Morphologically distinct colonies were selected and pure cultures were obtained.

2.1.3 Identification of actinobacteria

Aerial mass colour: The colour of the mature sporulating aerial mycelium was recorded in naked eye. When the aerial mass colour fell between two colours series, both the colours were recorded. If the aerial mass colour of a strain to be studied showed intermediate tints, then also, both the colour series were noted. The media used were Yeast Extract-Malt Extract Agar and Inorganic-Salt Starch Agar.

Melanoid pigments: The grouping was made on the production of melanoid pigments (i.e. greenish brown, brownish black or distinct brown, pigment modified by other colours) on the medium. The strains were grouped as melanoid pigment produced (+) and not produced (-). In a few cases, the production of melanoid pigments was delayed or weak, and therefore, it was not distinguishable. This is indicated as variable (V). This test was carried out on the media ISP-1 and ISP-7 (Appendix I), as recommended by the International Streptomyces Project (Shirling and Gottlieb, 1966).

Reverse side pigments: Reverse side pigment production of the isolate was determined on ISP7 medium. The pigment production was noted as distinctive (+) and not distinctive or none (-). In case, a colour with low chroma such as pale yellow, olive or yellowish brown occured, it was included in the latter group (-).

Soluble pigments: Soluble pigment production of isolate was observed on ISP7 medium. The diffusible pigment production other than melanin was considered positive (+) and not produced (-). The colour was recorded (red, orange, green, yellow, blue and violet).

Spore chain morphology: Spore morphological characters of the strains were studied by inoculating a loopful of one week old cultures into solidified agar medium containing sterile glass slide. The cultures were incubated at 28±2°C and examined periodically for the formation of aerial mycelium, sporophore structure and spore morphology.

2.2 Chemotaxonomical Characteristics

Hydrolysis: Hydrolysis was done for releasing amino acids. Harvested cells of each strain weighing 20 mg (fresh) were placed in an ampo bottle and 1 ml of 6 N HCl was added and sealed with alcohol blast burner. The samples were kept at 121°C for 20 h in a sand bath. The bottles were cooled by keeping them at a room temperature of 28±2°C. Hydrolysis was also done for releasing sugars. Harvested cells of each strain weighing 50 mg (fresh) were placed in an ampo bottle and 1 ml of 0.5N HCl was added and sealed with alcohol blast burner. The samples were kept at 110°C for 2 h. The bottles were then cooled by keeping them at a room temperature of 28±2°C.

Thin Layer Chromatography (TLC): Spotting of the whole cell hydrolysates was made carefully on TLC plate using a microliter pipette. Spots were of 5-10 mm in diameter. This was done by multiple applications on the same spot of very small portions of the sample, which were dried by a hand drier.

Amino acids: Each sample (3 µl) was applied on the baselines of TLC plate (20 cm x 20 cm). Adjacent to this, 1 µl of DL-diaminopimelic acid (an authentic material mixture of DAP isomers) and 1 µl of amino acetic acid (glycine) were spotted as standards. TLC plate was developed with the solvent system containing methanol: pyridine: glacial acetic acid: H2O (5: 0.5: 0.125: 2.5 v/v). It took approximately more than 4 h for development. The spots were visualized by spraying with 0.4% ninhydrin solution in water-saturated n-butanol, followed by heating at 100°C for 5 min. Spots of amino acids ran faster than DAP. The sample spots were immediately compared with the spots of the standards since spots gradually disappeared in few hours.

Whole-Cell sugars: On a cellulose TLC plate (20 cm x 20 cm), 5 µl of samples was spotted along with 3 µl of sugar solutions as standards on the same plates. Galactose, arabinose, xylose and madurose were the sugars, which were used...
as standards. TLC plate was developed with the solvent mixture containing ethyl acetate: pyridine: acetic acid: distilled water (8: 5: 1: 1.5 v/v/v). The development time was more than 4 h. Spots were visualized by spraying with aniline phthalate reagent (3.25 g of phthalic acid dissolved in 2 ml of aniline and made upto 100 ml with water saturated n-butanol). The sprayed plate was heated at 100°C for 4 min. Hexoses appeared as yellowish brown spots and pentoses, as maroon coloured spots.

**Assimilation of carbon source:** The ability of the actinobacterial strain in utilizing various carbon compounds as source of energy was studied, following the method recommended by International Streptomycetes Project (Shirling and Gottlieb, 1966). Chemically pure carbon source certified to be free of admixture with other carbohydrates and contaminating materials were used for this purpose. Carbon sources for this test were Arabinose, Xylose, Inositol, Mannitol, Fructose, Rhamnose, Sucrose and Raffinose. These carbon sources were sterilized by ether sterilization without heating. The media and plates were prepared and inoculated according to the convention of ISP project (Shirling and Gottlieb, 1966). For each of the carbon sources, utilization is expressed as positive (+), negative (-), or doubtful (±). In the 'doubtful ' strains, only a trace of growth slightly greater than that of the control was noticed.

**EPS Production and quantification:** The production of EPS from potential marine actinobacteria was estimated by the method of Sivaperumal et al., 2018. In brief, well grown actinobacteria was inoculated in yeast extract broth at room temperature. After 120hr of incubation the EPS was separated by centrifugation at 12,000 rpm for 50 min at 4°C. The pellet was suspended with 95% ice cold ethanol and allowed to stand for 24hrs at 4°C. After incubation the EPS precipitate was collected by centrifugation at 15000 rpm for 30 min at 4°C and rinsed thrice with ice cold ethanol. Then the sample was freeze dried and stored for further analysis.

**Estimation of EPS components:** Total carbohydrate in EPS was estimated by phenol sulfuric acid method with glucose as a standard (Dubois et al., 1956). The protein content was determined by bicinchoninic assay (BCA) with Bovine Serum Albumin (BSA) as a standard (Smith et al., 1985). Nucleic acid content was estimated by the method of Sheng et al., (2005).

**Total antioxidant activity:** Total antioxidant activity of the crude EPS was determined by following method: 0.3 ml of sample was prepared in different concentrations (0.5 – 3mg/ml) with 3ml of reagent solution (0.6 M sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). Reaction mixture was incubated at 95°C for 90 minutes in a water bath. Absorbance of all sample mixtures was measured at 695 nm. Total antioxidant activity has been expressed as the number of equivalents of ascorbic acid.

**DPPH Assay:** The antioxidant potential of actinobacterial EPS was determined on the basis of their scavenging activity of the stable 1,1-diphenyl-2-picryl hydrazyl (DPPH) free radical. Different concentrations (0.5-3mg/ml) of samples were mixed with 2.9ml diphenylpicrylhydrazyl (DPPH) solution (120µM) in methanol and incubated in darkness at 37°C for 30 minutes. The absorbance was recorded at 517 nm. Inhibition of free radical by DPPH in percentage (I %) was calculated for the various concentrations of the sample. Ascorbic acid was used as positive control (Kamala et al., 2015) and all the tests were carried out in triplicate.

**Semiautomatic plate assay:** The reaction mixture (3ml) containing 10 mM sodium nitroprusside and the sample (0.5-3mg/ml) in benzene: chloroform was incubated at 25°C for 150 min. After incubation, 0.5ml of the reaction mixture was mixed with 1ml of sulphanilic acid reagent (0.33% sulfanilic acid in 20% glacial acetic acid) and allowed to stand for 5 min for complete diazotization. Then, 1ml of naphthyl ethylenediamine dihydrochloride (0.1%) was added and allowed to stand for 30 min at 25°C. A pink coloured chromophore was formed and the absorbance was measured at 540 nm against the blank solution. Percentage of scavenging of NO was calculated as stated above.

Scavenging effect (%) = (A<sub>cont</sub> - A<sub>test</sub>) A<sub>cont</sub> × 100

**3. RESULTS**

The colour was found to be white with RF spore chain morphology. melanoid pigment and the
other pigments like soluble pigment and reverse side pigment were absent. Assimilation of carbon source has shown the presence of xylose and Arabinose among all the carbon compounds mentioned above. These results helped in the isolation of *streptomyces* species.

**Fig. 1.** The figure shows the *Streptomyces* culture and their spore chain morphology

**Table 1.** The table shows the cultural characteristics of Actinobacteria

| Color of aerial mycelium | White         |
|--------------------------|--------------|
| Melanoid pigment         | -            |
| Reverse side pigment     | -            |
| Soluble pigment          | -            |
| Spore chain              | RF           |
| Assimilation of carbon source |            |
| Arabinose                | +            |
| Xylose                   | +            |
| Inositol                 | -            |
| Mannitol                 | +            |
| Fructose                 | +            |
| Rhamnose                 | ±            |
| Sucrose                  | -            |
| Raffinose                | ±            |

**Table 2.** The table shows the cell wall characteristics of Actinobacteria

| Cell wall amino acids | Cell wall sugar | Cell Wall type | Index |
|-----------------------|-----------------|----------------|-------|
| LL-DAP                | MesoDAP         | Glycine        | Arabinose | Galactose | +    | -    | +    | -      | -     | l    | *Streptomyces* sp. |
Table 3. The table shows EPS components obtained from *Streptomyces* sp.

| EPS components     | %     |
|--------------------|-------|
| Carbohydrates      | 49    |
| Protein            | 27    |
| Nucleic acid       | 16    |
| Unidentified       | 8     |

Table 4. The table shows the total antioxidant activity of extracellular polymeric substances that is derived from the *Streptomyces* species. AAE- Ascorbic acid Equivalent. Ascorbic acid as a standard

| TAA      | AAE      |
|----------|----------|
| 25µg/ml  | 32.71 ± 1.217 |
| 50 µg/ml | 49.63 ± 1.302  |
| 75µg/ml  | 61.08 ± 0.812  |

Table 5. The table shows the DPPH assay of extracellular polymeric substances that are derived from marine actinobacteria *streptomyces* species

| DPPH | %     | Std |
|------|-------|-----|
| 25µg/ml | 11.39 ± 1.27 | 37.3 ± 1.27 |
| 50 µg/ml | 34.74 ± 1.31 | 62.7 ± 1.27 |
| 75µg/ml | 42.85 ± 1.28 | 78.52 ± 1.28 |
|       | 56.91 ± 0.78 | 83.59 ± 0.78 |
|       | 65.72 ± 1.26 | 92.4 ± 1.26 |
|       | 85.76 ± 1.24 | 98.6 ± 1.24 |

Table 6. The table shows the nitrogen scavenging assay of EPS obtained from *Streptomyces* sp.

| NO     | %     | Std  |
|--------|-------|------|
| 25µg/ml | 11.37 | 30.38 ± 1.127 |
| 50 µg/ml | 32.64 | 51.92 ± 1.164 |
| 75µg/ml | 47.52 | 70.34 ± 1.512 |
|       | 58.39 | 82.17 ± 1.231 |
|       | 69.57 | 90.53 ± 1.204 |
|       | 74.59 | 95.68 ± 1.168 |

LL-DAP and Glycine were found and the other cell wall amino acids mentioned above were absent and cell wall sugars i.e., arabinose and galactose were absent. The cell wall belongs to type 1. This shows an index for *streptomyces* species.

The results showed that the colour was found to be white with RF spore chain morphosity.

4. DISCUSSION

The filamentous, aerobic, soil-dwelling, gram-positive *Streptomyces* bacteria have been found residing in soil samples collected from many countries. The traditional practice regarding the isolation of *Streptomyces* from soil samples, have over the years resulted in the rediscovery of compounds which slowly exhausted the supplies of new compounds. It has been suggested that understudied ecosystems hold *Streptomyces* species which can meet the growing demand of the drug discovery and development industry [11,17,18] Researchers who made an effort to study *Streptomyces* from less explored ecosystems such as mangrove forests were able to discover novel *Streptomyces* species and *Streptomyces* strains showing potent antioxidant activities. Evidence from ear-lier animal studies have noted synthetic antioxidants as potentially unsafe for human consumption, since higher doses and prolonged exposure can induce carcinogenesis.

Nowadays, industries prefer searching for safer and better antioxidant remedies among natural sources by utilizing a variety of antioxidant assays [11,18-20]. Antioxidant activity of culture filtrate, lyophilized culture filtrate and ethyl acetate extract of *Streptomyces* species was determined by various in vitro assays such as ferric reducing power assay, phosphomolybdenum reduction, DPPH and ABTS radical scavenging activities.

The results revealed that the culture filtrate of *Streptomyces* species effectively scavenged DPP and ABTS radicals in a concentration dependent manner when compared with the IC50 values [17,21]. Production of protease from the strain of *streptomyces* was simple and it will be easy to scale up, as this actinobacteria grows on simple media with feathers as a sole source.
of carbon, nitrogen, and energy, thus allowing its enzyme production from an inexpensive substrate and a commercial potential with low productivity.

5. CONCLUSION

The present study revealed that the derived extracellular polymers from marine actinobacteria streptomyces species were found to have potent antioxidant potential activity [22-31]. Further characterisation and bio active properties should be done in the further studies, and more articles in future are yet to come in various properties of extracellular polymeric substances. Streptomyces derived from extreme and understudied ecosystems such as the mangrove forests are potential sources of biologically active and therapeutically useful compounds [4,32,33].

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

CONSENT

As per international standard or university standard, patients’ written consent has been collected and preserved by the author(s).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Salam MD. Antimicrobial Potential of Actinomycetes Isolated from Soil Samples of Punjab, India [Internet]. Journal of Microbiology & Experimentation. 2014;1. Available:http://dx.doi.org/10.15406/jmen.2014.01.00010

2. Lee H-J, Whang K-S. Streptomyces graminisoli sp. nov. and Streptomyces rhizophilus sp. nov., isolated from bamboo (Sasa borealis) rhizosphere soil [Internet]. International Journal of Systematic and Evolutionary Microbiology. 2014;64:1546–51. Available:http://dx.doi.org/10.1099/ijs.0.055210-0

3. Syed MH, Gnanakkan A, Pitchiah S. Exploration of acute toxicity, analgesic, anti-inflammatory, and anti-pyretic activities of the black tunicate, Phallusia nigra (Savigny, 1816) using mice model [Internet]. Environmental Science and Pollution Research. 2021;28:5809–21. Available:http://dx.doi.org/10.1007/s11356-020-10938-2

4. Kn R, Rakesh KN, Junaid S, Dileep N, Kekuda PTR. Antibacterial and antioxidant activities of Streptomyces species SRDP-H03 isolated from soil of Hosudi, Karnataka, India [Internet]. Journal of Drug Delivery and Therapeutics. 2013;3. Available:http://dx.doi.org/10.22270/jddt.v3i4.4568

5. Sivaperumal P, Kamala K, Rajaram R. Biosorption of long half-life radionuclide of strontium ion (Sr ) by Marine Actinobacterium Nocardiopsis sp. 13H [Internet]. Geomicrobiology Journal. 2018;35:300–10. Available:http://dx.doi.org/10.1080/0149051.2017.1350891

6. Kamala K, Sivaperumal P, Gobalakrishnan R, Swarnakumar NS, Rajaram R. Isolation and characterization of biologically active alkaloids from marine actinobacteria Nocardiopsis sp. NCS1 [Internet]. Biocatalysis and Agricultural Biotechnology. 2015;4:63–9. Available:http://dx.doi.org/10.1016/j.bcab.2014.10.005

7. A GS, Gautham SA, Mesta SC, R. O. Characterization and antimicrobial spectrum of a potent Streptomyces sp. gos2 isolated from western ghats of Karnataka, India [Internet]. International Journal of Pharmacy and Pharmaceutical Sciences. 2016;8:310. Available:http://dx.doi.org/10.22159/ijpps.2016.v8i9.1254

8. Prashith KTR, Onkarappa R, Raghavendra HL. Pharmacological activities of streptomyces Species PO-178 Isolated from Rhizosphere Soil of Agumbe, Karnataka, India [Internet]. Science, Technology and Arts Research Journal. 2013;2:83. Available:http://dx.doi.org/10.4314/star.v2i2.98892
9. Sivaperumal P, Kamala K, Rajaram R. Bioactive DOPA melanin isolated and characterised from a marine actinobacterium *Streptomyces sp*. MVC56 from Versova coast [Internet]. Natural Product Research. 2015;29:2117–21. Available: http://dx.doi.org/10.1080/147864 19.2014.988712

10. Uzma F, Chowdappa S. Antimicrobial and antioxidant potential of endophytic fungi isolated from ethnomedicinal plants of Western Ghats, Karnataka [Internet]. Journal of Pure and Applied Microbiology. 2017;11:1009–25. Available: http://dx.doi.org/10.22207/jrpm.1 2.43

11. Sivaperumal P, Kamala K, Rajaram R, Mishra SS. Melanin from marine *Streptomyces sp.* (MVC513) with potential effect against ornamental fish pathogens of *Carassius auratus* (Linnaeus, 1758) [Internet]. Biocatalysis and Agricultural Biotechnology. 2014;3:134–41. Available: http://dx.doi.org/10.1016/j.bcab.2 014.09.007

12. Chanthasena P, Nantapong N. Antimicrobial activity of *Streptomyces sp.* P90 isolated from soil in northeast thailand [Internet]. Vol. 78, Jurnal Teknologi. 2016. Available from: http://dx.doi.org/10.11113/jt.v78.8574

13. Maiti PK, Mandal S. Streptomyces cupreus sp. nov., an antimicrobial producing actinobacterium isolated from Himalayan soil [Internet]. Archives of Microbiology; 2021. Available from: http://dx.doi.org/10.1007/s00203-020- 02160-y

14. Rajeshkumar S, Venkat Kumar S, Ramaiah A, Agarwal H, Lakshmi T, Roopan SM. Biosynthesis of zinc oxide nanoparticles using *Mangifera indica* leaves and evaluation of their antioxidant and cytotoxic properties in lung cancer (A549) cells [Internet]. Enzyme and Microbial Technology. 2018;117:91–5. Available: http://dx.doi.org/10.1016/j.enzmic tec.2018.06.009

15. Gnanavel V, Roopan SM, Rajeshkumar S. Aquaculture: An overview of chemical ecology of seaweeds (food species) in natural products [Internet]. Aquaculture. 2019;507:1–6. Available: http://dx.doi.org/10.1016/j.aquac ulture.2019.04.004

16. Markov A, Thangavelu L, Aravindhan S, Zekiy AO, Jarahian M, Chartrand MS, et al. Mesenchymal stem/stromal cells as a valuable source for the treatment of immune-mediated disorders [Internet]. Stem Cell Research & Therapy. 2021;12. Available: http://dx.doi.org/10.1186/s13287-021-02265-1

17. Sowndhararajan K, Kang SC. Evaluation of *in vitro* free radical scavenging potential of *Streptomyces* sp. AM-S1 culture filtrate. Saudi J Biol Sci. 2013;20(3):227–33.

18. Desai C, Patel P, Markande AR, Kamala K, Sivaperumal P. Exploration of haloarchaea for their potential applications in food industry [Internet]. International Journal of Environmental Science and Technology. 2020;17:4455–64. Available: http://dx.doi.org/10.1007/s13762-020-02773-2

19. Kekuda PTR, Onkarappa R. Antioxidant, anthelmintic and enzyme inhibitory potential of streptomyces variabilis strain po-178 isolated from western ghat soil of Agumbe, Karnataka, India [Internet]. Journal of Biological & Scientific Opinion. 2014;2:170–6. Available: http://dx.doi.org/10.7897/2321- 6328.02239

20. Kumar P, Sivaperumal P, Manigandan V, Rajaram R, Hussain M. Assessment of potential human health risk due to heavy metal contamination in edible finfish and shellfish collected around Ennore coast, India [Internet]. Environmental Science and Pollution Research. 2021;28:8151–67. Available: http://dx.doi.org/10.1007/s11356- 020-10764-6

21. Anuswedha A. Evaluation of antibacterial activity of ethanol leaf extracts of psidium guajava linn. against clinical isolates from wound infections [Internet]. World Journal of Pharmacy and Pharmaceutical Sciences. 2017;1130–6. Available: http://dx.doi.org/10.20959/wjpps2 01705-9153

22. Rajendran R, Kunjusankaran RN, Sandhya R, Anilkumar A, Santhosh R, Patil SR. Comparative evaluation of remineralizing potential of a paste containing bioactive glass and a topical cream containing casein phosphopeptide-amorphous calcium phosphate: An *in vitro* study. Pesqui Bras Odontopediatria Clin Integr. 2019;19(0);4668.

23. Ashok BS, Ajith TA, Sivanesan S. Hypoxia-inducible factors as neuroprotective agent in Alzheimer’s disease. Clin Exp...
24. Malli SN, Selvarasu K, Jk V, Nandakumar M, Selvam D. Concentrated growth factors as an ingenious biomaterial in regeneration of bony defects after periapical surgery: A report of two cases. Case Rep Dent [Internet]. [cited 2021 Sep 15]; 2019. Available:https://pubmed.ncbi.nlm.nih.gov/30805222/

25. Mohan M, Jagannathan N. Oral field cancerization: An update on current concepts. Oncol Rev [Internet]. [cited 2021 Sep 15]. 2014;8(1). Available:https://pubmed.ncbi.nlm.nih.gov/25992232/

26. Menon S, Ks SD, RS, SR, Vk S. Selenium nanoparticles: A potent chemotherapeutic agent and an elucidation of its mechanism. Colloids Surf B Biointerfaces [Internet]. [cited 2021 Sep 15]. 2018:170. Available:https://pubmed.ncbi.nlm.nih.gov/29936381/

27. Samuel SR, Acharya S, Rao JC. School interventions-based prevention of early-childhood caries among 3-5-year-old children from very low socioeconomic status: Two-year randomized trial. J Public Health Dent [Internet]. [cited 2021 Sep 15]. 2020;80(1). Available:https://pubmed.ncbi.nlm.nih.gov/31710096

28. Praveen K, Narayanan V, Muthu Sekhar MR, Baig MF. Hypotensive anaesthesia and blood loss in orthognathic surgery: a clinical study. Br J Oral Maxillofac Surg [Internet]. [cited 2021 Sep 15]. 2001;39(2). Available:https://pubmed.ncbi.nlm.nih.gov/11286449

29. Neelakantan P, Subbarao C, Subbarao CV, De-Deus G, Zehnder M. The impact of root dentine conditioning on sealing ability and push-out bond strength of an epoxy resin root canal sealer. Int Endod J [Internet]. [cited 2021 Sep 15]. 2011;44(6). Available:https://pubmed.ncbi.nlm.nih.gov/21255047/

30. Oligonucleotide therapy: An emerging focus area for drug delivery in chronic inflammatory respiratory diseases. Chem Biol Interact. 2019;308:206–15.

31. Kumar MS, Vamsi G, Srirupi R, Sehgal PK. Expression of matrix metalloproteinases (MMP-8 and -9) in chronic periodontitis patients with and without diabetes mellitus. J. Periodontol. 2006;77(11):1803–8.

32. Kamala K, Sivaperumal P. Biomedical applications of enzymes from marine actinobacteria [Internet]. Marine Enzymes Biotechnology: Production and Industrial Applications, Part III - Application of Marine Enzymes. 2017:107–23. Available:http://dx.doi.org/10.1016/bs.afnr.2016.11.002

33. Markov A, Thangavelu L, Aravindhan S, Zekiy AO, Jarahian M, Chartrand MS, et al. Mesenchymal stem/stromal cells as a valuable source for the treatment of immune-mediated disorders [Internet]. Stem Cell Research & Therapy. 2021; 12. Available:http://dx.doi.org/10.1186/s13287-021-02265-1

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