

Isolation of Cellulose Producing Marine *Streptomyces Sp.* from Sediment Samples and their Antioxidants Properties

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ABSTRACT

Cellulase generated by cellulolytic bacteria helps to degrade the cellulose. This enzyme is considered a major industrial enzyme group and has several industrial applications. The bioconversion of renewable cellulose biomass to commodity chemicals is a potential challenge area where cellulase plays a central role. Marine *Streptomyces* species are drawing more and more attention as a promising source of new natural products. Oxidation causes Endothelial stress which is ultimately involved in endothelial dysfunction, which is obvious in adults with different cardiovascular conditions, including thalassemia. The sediment sample was collected from the Thondi area, Tamilnadu. The collected sample was sun dried for 48 hrs. and turned into fine powder by mortar and pestle. The actinobacteria was isolated and identified the marine actinobacteria with the help of aerial mass colour, melanoid pigments, reverse side pigments, soluble pigments, and spore chain morphology. Chemotaxonomic characteristics and scanning of cellulase production were done and showed potential antioxidant properties. The effect of pH and temperature on cellulase enzyme production were analysed and their potential antioxidant properties also done.

Key words:

Marine *Streptomyces*, Enzyme, Cellulase, Antioxidant activity

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INTRODUCTION

The most important agricultural waste in the world is cellulose and is an abundant natural biopolymer. This cellulosic biomass is a rich, renewable resource with a high bioconversion potential to value-added products [1]. Cellulase generated by cellulolytic bacteria helps to degrade the cellulose. This enzyme is considered a major industrial enzyme group and has several industrial applications [2]. Moreover, in the textile industry, the most important industrial application of cellulases is bi-polishing textiles and stoned denims as well as laundry detergents for home laundry in order to improve fabric softness and shine [3]. In addition, they are used in animal feed for improved nutritional quality and digestibility, fruit juice processing as well as in the baking process [4]. The bioconversion of renewable cellulose biomass to commodity chemicals is a potential challenge area where cellulase plays a central role [5].

Marine *Streptomyces species* are drawing more and more attention as a promising source of new natural products [6]. Actinomycetes bacteria of genus *Streptomyces* are one of the most promising biological sources for new natural

products and will continue, at least for the near future [7]. The intensive investigation of terrestrial actinomycetes in 1950-1970 did lead to the frequent reconstruction of bioactive compounds, attracting attention to new, ecological niches that could have been sources of new actinomycetes [8].

As the sea accounts for more than 70 percent of the surface of the world and hosts about 87 percent of the world's biodiversity, the findings of new microorganisms, including actinomycetes, appear largely undeveloped. Given the never seen diversity of marine organisms and the relatively little work done to date, more than 25000 new marine natural products have now been identified as astounding [9]. Many such compounds come from deep sea sediments, coral reefs, aquatic invertebrates and plants isolated from marine actinomycetes [10,11].

Various marine actinomycetes compounds have a significant potential to become pharmaceutical drugs. Diazepinomicin, a marine micromonospora dibenzodiazepine alkaloid, has had antibacterial and anti-tumor activities, and had glioblastoma treatment clinical studies in Phase II [12,13].

Some important papers which were done by the researchers helped in this study. Some of them are regarding the antioxidant [14,15], anti-inflammatory

[16,17] and antidiabetic properties [18-20]. Papers about anticancer were also [21,22] helpful.

As toxic and beneficial compounds, free radicals and oxidants can play a dual role, since they can be damaging or helpful for the body. The development of many human diseases has been involved. Among them, arthritis, inflammatory diseases, kidney diseases, cataracts, inflammatory bowel disease, colitis, lung dysfunction; pancreatitis are the most common [23]. Oxidation causes endothelial stress which is ultimately involved in endothelial dysfunction, which is obvious in adults with different cardiovascular conditions, including thalassemia [24]. Red blood cells are protected against oxidant damage by antioxidants and other supportive treatments [25]. Further, our team has extensive knowledge and research experience that has translated into high quality publications [26-46]. This study aims at isolation of cellulase producing marine *Streptomyces sp.* from sediment samples and their antioxidant properties.

MATERIAL AND METHOD

Sample collection and preparation

The sediment sample was collected from the Thondi area, Tamilnadu. The collected sample was sun dried for 48 hrs. and turned into fine powder by mortar and pestle.

Isolation 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 occurred, 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 contained sterile glass slide. The cultures were incubated at $28 \pm 20^\circ\text{C}$ and examined periodically for the formation of aerial mycelium, sporophore structure and spore morphology.

Chemo taxonomical 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 an 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 $28 \pm 20^\circ\text{C}$. 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 2h. The bottles were then cooled by keeping them at a room temperature of $28 \pm 20^\circ\text{C}$.

Thin Layer Chromatography (TLC)

Spotting of the whole cell hydro lysates was made carefully on TLC plate using a microliter pipette. Spots were 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 dryer.

Amino acids

Each sample (3 μl) was applied on the baselines of the 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 a 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). The developing 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 1000 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 *Streptomyces* 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.

Screening of Cellulase Production

Cellulase activity of the strains was screened qualitatively in CMC (Carboxymethyl Cellulose) agar medium. After inoculation, the plates were incubated at 37°C for 5 days. To visualize the hydrolysis zone, the plates were flooded with an aqueous solution of 0.1% Congo red for 15 min and washed with 1 M NaCl. To indicate the cellulase activity of the organisms, diameters of clear zones around colonies on CMC agar were measured.

Determination of enzyme activity

The medium was inoculated with 1 ml of spore suspension of a 7 days old culture and incubated in rotary shaker (150rpm) at ambient temperature for three days. The cell free supernatant was collected by centrifugation at 12,000rpm for 15 min. The supernatant was the enzyme source. The substrate 2% of carboxymethyl cellulose solution (CMC) was prepared with a 50mM phosphate buffer (pH 7). 1ml of crude enzyme was added with 1ml of CMC solution which incubated for 60min at (50°C) desired temperature. After incubation 2ml of 3, 5-dinitrosalicylic (DNS) reagent was added to terminate the enzyme reaction. After termination of enzyme activity all samples should be

incubated for 5min at boiling temperature then the samples transferred to an ice cold water bath. After pulp settlement, the aqueous layer was e by centrifuge (12000 rpm/min for 5min at 4°C) and the optical density was measured at 540nm. One International Unit (IU) of enzyme activity for cellulase was defined as the amount of enzyme releasing 1 μmol reducing sugar from CMC per minute using glucose as standard.

Effect of pH on enzyme production

The isolates were inoculated in CMC broth and the medium pH was adjusted from 6 – 8. The confirmation of growth was observed at 600nm. The enzyme production was quantified by the method described before.

Effect of Temperature on enzyme production

Effect of temperature on the cellulase enzyme production was analyzed by adjusting from 25, 30, 35, 40 and 50°C. The isolate was inoculated in CMC broth and incubated at various ranges of temperature. The cell growth was confirmed by the absorbance at 600nm. The enzyme production was quantified by the method described before.

RESULTS AND DISCUSSION

The rise of widespread antibiotic-resistant bacteria heightened the need to discover new antimicrobial agents. Actinomycetes, especially *Streptomyces* sp., have attracted a lot of attention because they produce a lot of useful bioactive metabolites. Isolating these species from less-explored environments may improve the chances of discovering new microbial species. Isolation of cellulase producing *Streptomyces* from marine sediments sample and effect of physical factors on enzyme production (Tables 1-5). This study isolated marine actinobacteria from a sediment sample and identified *Streptomyces* genus from the isolate using specific characteristics of the bacteria. Then the production of enzyme was confirmed by screening for cellulase and enzyme assay was carried out. The enzyme assay revealed that the Carboxymethyl cellulase agar flooded with iodine, the total activity of the enzyme was 127.41 IU/mg. The effect of temperature and pH enzyme production was studied on CMC broth. It was found that the optimum pH and temperature for maximum enzymatic activity was and respectively. It was also observed that as the temperature increased, the enzymatic activity increased. However at the highest temperature, the rate decreased again which might probably be due to enzyme degeneration. Finally, antioxidant testing was performed which showed positive results (Figures 1-2).

Table 1: Conventional Identification of marine Streptomyces.

Color of aerial mycelium	White
Melanoid pigment	-
Reverse side pigment	+

Soluble pigment	-
Spore chain	RA
Assimilation of carbon source	
Arabinose	+
Xylose	+
Inositol	+
Mannitol	+
Fructose	+
Rhamnose	+
Sucrose	+
Raffinose	+

Table 2: Chemo taxonomical characteristics of marine *Streptomyces* sp.

Cell wall amino acids			Cell wall sugar		Cell wall type	Index
LL-DAP	Meso DAP	Glycine	Arabinose	Galactose	I	Streptomyces

Table 3: Depicts the effect of pH and effect of temperature.

Effect of Temperature	IU/ml	Effect of pH	IU/ml
25	11.79 ± 2.3	6	5.29 ± 2.2
30	13.52 ± 2.7	6.5	7.24 ± 2.5
35	16.47 ± 2.5	7	9.51 ± 2.1
40	12.18 ± 2.9	7.5	12.62 ± 2.9
50	10.25 ± 2.4	8	13.58 ± 2.3

Table 4: Depicts the DPPH scavenging at different concentrations.

Concentration (µg/ml)	DPPH Scavenging	Standard
25	11.07 ± 1.5	37.3 ± 1.27
50	18.24 ± 1.9	62.7 ± 1.31
75	31.35 ± 1.4	78.52 ± 1.28
100	42.18 ± 1.8	83.59 ± 0.78
125	55.46 ± 1.4	92.4 ± 1.26
150	68.09 ± 1.7	98.6 ± 1.24

Table 5: Depicts the Nitrous oxide scavenging at different concentrations.

µg/ml	Nitrous oxide scavenging	Std
25	12.37 ± 1.4	30.38 ± 1.127
50	25.64 ± 1.8	51.92 ± 1.164
75	32.91 ± 1.4	70.34 ± 1.152

100	47.64 ± 1.9	82.17 ± 1.231
125	58.27 ± 1.7	90.53 ± 1.204
150	73.59 ± 1.4	95.68 ± 1.168



Figure 1: *Streptomyces* sp.

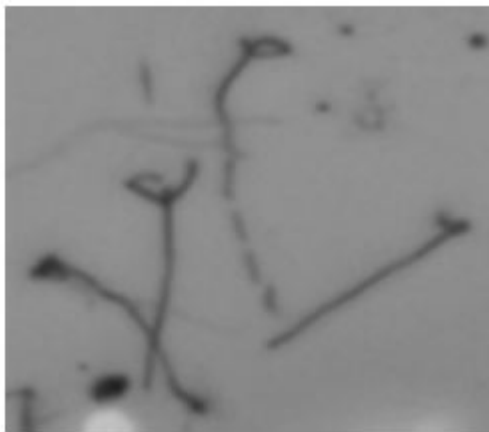


Figure 2: Spore chain morphology.

CONCLUSION

The present study was isolated and identified the marine actinobacteria of *Streptomyces* sp. From marine sediment samples. The conventional identification of chemotaxonomic characteristics was done and further scanning of cellulase production was also done. The marine actinobacterial cellulase showed potential antioxidant properties. The effect of pH and temperature on cellulase enzyme production was also analysed. The outcome of the present work concluded that marine actinobacterial enzymes can act as natural products and it could be useful in the biomedical sector.

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CONFLICT OF INTEREST

There are no conflicts of interest.

SOURCE OF INTEREST

Self.

ETHICAL CLEARANCE

Not Required.

REFERENCES

1. Dhaliwal M, More S. Optimization of cellulase production by soil bacteria using statistical design. *Int J Biol Chem Sci* 2016; 1-7.
2. Sadhu S, Maiti TK. Cellulase production by bacteria: A review. *Microbiol Res J Int* 2013; 13:235-58.
3. Hill J, Nelson E, Tilman D, et al. Environmental, economic, and energetic costs and benefits of biodiesel and ethanol biofuels. *Proc Natl Acad Sci* 2006; 103:11206-11210.
4. Lynd LR, Van Zyl WH, McBride JE, et al. Consolidated bioprocessing of cellulosic biomass: an update. *Curr Opin Biotechnol* 2005; 16:577-83.
5. Lynd LR, Currie D, Ciazza N, et al. Consolidated bioprocessing of cellulosic biomass to ethanol using thermophilic bacteria. *Bioenergy* 2008; 55-74.
6. Paulus C, Rebets Y, Tokovenko B, et al. New natural products identified by combined genomics-metabolomics profiling of marine *Streptomyces* sp. MP131-18. *Scientific Reports* 2017; 7:1-1.
7. Solecka J, Zajko J, Postek M, et al. Biologically active secondary metabolites from Actinomycetes. *Open Life Sci* 2012; 7:373-90.
8. Monciardini P, Iorio M, Maffioli S, et al. Discovering new bioactive molecules from microbial sources. *Microbiol. Biotechnol* 2014; 7:209-220.
9. Hu GP, Yuan J, Sun L, et al. Statistical research on marine natural products based on data obtained between 1985 and 2008. *Marine Drugs* 2011; 9:514-25.
10. Valli S, Suvathi SS, Aysha OS, et al. Antimicrobial potential of Actinomycetes species isolated from marine environment. *Asian Pacific J Tropical Biomed* 2012; 2:469-473.
11. Valliappan K, Sun W, Li Z. Marine actinobacteria associated with marine organisms and their potentials in producing pharmaceutical natural products. *Appl Microbiol Biotechnol* 2014; 98:7365-77.

12. Charan RD, Schlingmann G, Janso J, et al. Diazepinomicin, a new antimicrobial alkaloid from a marine *Micromonospora* sp. *J Nat Prod* 2004; 67:1431-3.
13. Mason WP, Belanger K, Nicholas G, et al. A phase II study of the Ras-MAPK signaling pathway inhibitor TLN-4601 in patients with glioblastoma at first progression. *J Neurooncol* 2012; 107:343-349.
14. Meenapriya M, Anitha R, Lakshmi T. Effect of lutein on cytochrome P450 (Isoform CYP3A4)-An in vitro Study. *Pharmacogn J* 2018; 10.
15. Devaraj E, Roy A, Veeraragavan GR, et al. β -Sitosterol attenuates carbon tetrachloride-induced oxidative stress and chronic liver injury in rats. *Naunyn Schmiedebergs Arch Pharmacol* 2020; 1-9.
16. Prathoshni SM, Anitha R, Lakshmi T. The effect of Capsicum oleoresin on nitric oxide production and nitric oxide synthase gene expression in macrophage cell line. *Pharmacogn Res* 2018; 10.
17. Cinthura C, Thangavelu L, Rajeshkumar S, et al. COX2 Inhibitory activity of *Abutilon indicum*--An In vitro Study. *Indian J Public Health Res Develop* 2019; 10.
18. Ashwini S, Anitha R. Antihyperglycemic activity of *Caralluma fimbriata*: An In vitro approach. *Pharmacogn Mag* 2017; 13:S499.
19. Leya MM, Anitha R. Anti-inflammatory effect of the aqueous fruit pulp extract of *tamarindus indica* linn in lipopolysaccharide-stimulated macrophages. *Pharmacogn J* 2019; 11.
20. Roy A, Rajagopal P, Thangavelu L. Molecular docking analysis of compounds from *Lycopersicon esculentum* with the insulin receptor to combat type 2 diabetes. *Bioinformation* 2020; 16:748-752.
21. Ashwini S, Ezhilarasan D, Anitha R. Cytotoxic effect of *Caralluma fimbriata* against human colon cancer cells. *Pharmacogn. J* 2017; 9.
22. Roy A, Rasheed A, Sleeba AV, et al. Molecular docking analysis of capsaicin with apoptotic proteins. *Bioinformation* 2020; 16:555.
23. Bendich A. Role of antioxidants in the maintenance of immune functions. *Natural antioxidants in human health and disease*. 1994; 447-67.
24. Hebbel RP, Leung A, Mohandas N. Oxidation-induced changes in microrheologic properties of the red blood cell membrane. *Blood* 1990; 76:1015-1020.
25. Shinar E, Rachmilewitz EA. Oxidative denaturation of red blood cells in thalassemia', *Seminars in hematology* 1990; 27:70-82.
26. Rajeshkumar S, Kumar SV, Ramaiah A, et al. Biosynthesis of zinc oxide nanoparticles using *Mangifera indica* leaves and evaluation of their antioxidant and cytotoxic properties in lung cancer (A549) cells. *Enzyme Microb Technol* 2018; 117:91-95.
27. Nandhini NT, Rajeshkumar S, Mythili S. 'The possible mechanism of eco-friendly synthesized nanoparticles on hazardous dyes degradation'. *Biocatal. Agric. Biotechnol* 2019; 19:101138.
28. Rajkumar PV, Prakasam A, Rajeshkumar S, et al. Green synthesis of silver nanoparticles using *Gymnema sylvestre* leaf extract and evaluation of its antibacterial activity. *S Afr J Chem Eng* 2020; 32:1-4.
29. Rajasekaran S, Damodharan D, Gopal K, et al. Collective influence of 1-decanol addition, injection pressure and EGR on diesel engine characteristics fueled with diesel/LDPE oil blends. *Fuel* 2020; 277:118166.
30. Vairavel M, Devaraj E, Shanmugam R. An eco-friendly synthesis of *Enterococcus* sp.-mediated gold nanoparticle induces cytotoxicity in human colorectal cancer cells. *Environ Sci Pollut Res* 2020; 27:8166-75.
31. Santhoshkumar J, Sowmya B, Kumar SV, et al. Toxicology evaluation and antidermatophytic activity of silver nanoparticles synthesized using leaf extract of *Passiflora caerulea* S. Afr J Chem Eng 2019; 29:17-23.
32. Raj RK. β -Sitosterol-assisted silver nanoparticles activates Nrf2 and triggers mitochondrial apoptosis via oxidative stress in human hepatocellular cancer cell line. *J Biomed Mater Res* 2020; 108:1899-908.
33. Saravanan M, Arokiyaraj S, Lakshmi T, et al. Synthesis of silver nanoparticles from *Phenerochaete chrysosporium* (MTCC-787) and their antibacterial activity against human pathogenic bacteria. *Microb Pathog* 2018; 117:68-72.
34. Gheena S, Ezhilarasan D. Syringic acid triggers reactive oxygen species-mediated cytotoxicity in HepG2 cells. *Hum Exp Toxicol* 2019; 38:694-702.
35. Ezhilarasan D, Sokal E, Najimi M. Hepatic fibrosis: It is time to go with hepatic stellate cell-specific therapeutic targets. *Hepatobiliary Pancreat Dis Int* 2018; 17:192-197.
36. Ezhilarasan D. Oxidative stress is bane in chronic liver diseases: Clinical and experimental perspective. *Arab J Gastroenterol* 2018; 19:56-64.
37. Dua K, Wadhwa R, Singhvi G, et al. The potential of siRNA based drug delivery in respiratory disorders: Recent advances and progress. *Drug Dev Res* 2019; 80:714-30.
38. Gomathi AC. Anticancer activity of silver nanoparticles synthesized using aqueous fruit shell extract of *Tamarindus indica* on MCF-7 human breast cancer cell line. *J Drug Deliv Sci Technol* 2020; 55:101376.

39. Vairavel M, Devaraj E, Shanmugam R. An eco-friendly synthesis of Enterococcus sp.-mediated gold nanoparticle induces cytotoxicity in human colorectal cancer cells. *Environ Sci Pollut Res* 2020; 27:8166–8175.
40. Ramesh A, Varghese S, Jayakumar ND, et al. Comparative estimation of sulfiredoxin levels between chronic periodontitis and healthy patients–A case-control study. *J Periodontol* 2018; 89:1241-1248.
41. Duraisamy R, Krishnan CS, Ramasubramanian H, et al. Compatibility of nonoriginal abutments with implants: Evaluation of Microgap at the implant-abutment interface, With Original Nonoriginal Abutments *Implant Dent*. 2019; 28:289-95.
42. Ezhilarasan D, Apoorva VS, Ashok Vardhan N. Syzygium cumini extract induced reactive oxygen species-mediated apoptosis in human oral squamous carcinoma cells. *J Oral Pathol Med* 2019; 48:115-21.
43. Arumugam P, George R, Jayaseelan VP. Aberrations of m6A regulators are associated with tumorigenesis and metastasis in head and neck squamous cell carcinoma. *Arch Oral Biol* 2021; 122:105030.
44. Joseph B, Prasanth CS. Is photodynamic therapy a viable antiviral weapon against COVID-19 in dentistry?. *Oral Surg Oral Med Oral Pathol Oral Radiol* 2021.
45. Gnanavel V, Roopan SM, Rajeshkumar S. Aquaculture: An overview of chemical ecology of seaweeds (food species) in natural products. *Aquaculture*. 2019;507:1-6.
46. Markov A, Thangavelu L, Aravindhan S et al. Mesenchymal stem/stromal cells as a valuable source for the treatment of immune-mediated disorders. *Stem Cell Res Ther* 2021; 12:1-30.