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Original article

Antibacterial and cytotoxicity activities of bioactive compounds from *Micrococcus* species OUS9 isolated from sea waterK. Shanthi Kumari ^{a,e}, P. Shivakrishna ^b, Atef M. Al-Attar ^{c,d}, V.S. Ramakrishna Ganduri ^{a,*}^a Department of Biotechnology, Koneru Lakshmaiah Education Foundation, Vaddeswaram, Guntur 522 502, Andhra Pradesh, India^b Lavin Laboratories, Nallakunta, Hyderabad 500 044, Telangana, India^c Princess Dr. Najla Bint Saud Al-Saud Center for Excellence Research in Biotechnology, King Abdulaziz University, Jeddah, Saudi Arabia^d Department of Biological Sciences, Faculty of Sciences, King Abdulaziz University, Jeddah, Saudi Arabia^e Department of Microbiology, Osmania University, Hyderabad 500 007, Telangana, India

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ABSTRACT

Bacterial screenings were collected from Nellore coastal regions of Bapatla and Vishakhapatnam from south India. Altogether, 29 bacterial isolates were attained from seawater and sediment samples consuming Zobel-culture media (pH = 7.3 ± 0.2) and incubated at 27 °C of temperature. Among these 29 bacterial isolates, 3 bacterial strains were shown the highest zone of inhibition producing the molecular characterization. The strains of *Micrococcus* species OUS9 was demonstrated to be potential. To characterize such activity and recognize the promising isolates, further research is required. This new natural biological product is also used in enormous sectors, pharmaceutical and food industries for preservation of natural environment. This study has confirmed that the bacteria isolates environment for halophilic bacterial growth, where there are numerous bacterial communities and many industrial applications are possible.

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1. Introduction

Marine bacteria are documented as an invaluable resource in delivering bioactive compounds and the marine natural products plays a vital role in biomedical research and pharmaceutical industry (Karbalaei-Heidari et al., 2020). For thousands of years, nature was the origin of medicinal products. There are an imposing number of contemporary drugs, primarily based on traditional medicine use, isolate from microorganisms. Microorganisms have played a growing part in antibiotic manufacturing and in another drug manufacturing in the previous century. As sources of precious bioactive metabolites, terrestrial fungi and bacteria have been extremely important for more than half a century. There has been a continuing search for fresh drug products from the ocean for over two centuries. Prior researchers have revealed as marine invertebrates are significant source for fresh biochemical compounds

and well-known fact as established through the large number of compounds present in the clinical trials, proper and reliable supplies of these compounds have proved notoriously hard to acquire from the nature. Due to these issues, (Faulkner, 2000) studies have well-documented about the avenue centered on marine microorganisms for considerable attention. Therefore, the anticipated huge biodiversity of sea organisms could have been at first sight for one of reasons (Burch-Brown and Archer, 2017); though marine derived microorganisms were not taxonomically sounded. The groundwork studies showed the wealth of microbial diversity in the oceans of the world making the capable of frontiers for discovering fresh drugs (Blunt et al., 2004).

Marine derived bacteria are defined through marine water demands in particular with sodium for growth (Fenical, 1997). Among the cases of marine associated with fungi are without ion demands and mandatory marine species are lives only sea water (Jensen and Fenical, 2000). This issue is compounded in samples near shore/estuarine, where the large proportion of resident microbes were modified for variable marine levels of exposure. All microbes may be isolated from the environmental marine for the purpose of microbial drug discovery (Gerwick and Moore, 2012). Maximum of fresh complexes have recorded from marine microorganisms which have been acquired from various species, isolated either from land, sea or both. Coherent investigation for all the microbes has been isolated from extreme sea water

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Table 1
Morphological characteristic of bacterial isolates from marine intertidal regions.

NAME OF THE INDIVIDUAL BACTERIA	COLONY CHARACTERISTICS OF ISOLATED BACTERIA	GRAMS STAINING	MOTILITY
OUS1	Yellow pigmented irregular, Opaque, Rough,	Gram positive cocci in bunch	Non-motile
OUS2	Yellow pigmented, Circular, Opaque, Rough, Slightly	Gram positive cocci	Non-motile
OUS3	Pale yellow pigmented, Circular, Opaque, Smooth, Convex	Gram positive cocci in bunch	Non-motile
OUS4	Lemon yellow pigmented, Circular, Opaque, Rough, Convex	Gram positive cocci in bunch	Non-motile
OUS5	Pale yellow pigmented Circular, Translucent, Smooth,	Gram positive rods	Non-motile
OUS6	Pale yellow pigmented, Circular, Opaque, Smooth, Convex	Gram negative rods	Motile
OUS7	Yellow pigmented, Circular, Translucent, Smooth, Convex	Gram positive short rods	Motile
OUS8	Yellow pigmented, Circular, Opaque, Smooth, Convex	Gram negative cocco bacilli	Motile
OUS9	Bright yellowish pigmented, Circular, Opaque.	Gram positive in cocci	Non-Motile
OUS10	Pale yellow pigmented, Circular, Opaque, Smooth, Flat	Gram positive cocci in bunch	Non-Motile
OUS11	Thick Yellow pigmented, Elliptical, Opaque, Smooth, Convex	Gram positive cocci in bunch	Non-motile
OUS12	Pale yellow pigmented, Circular, Opaque, Smooth, Convex	Gram positive short rods	Motile
OUS13	Light yellow pigmented, Circular, Translucent, Smooth,	Gram positive cocci in bunch	Non-motile
OUS14	Golden yellow pigmented, Congregated, Opaque, Smooth,	Gram positive cocci in bunch	Non-motile
OUS15	Yellow pigmented, Circular, Opaque, Smooth, Convex	Gram positive cocci	Non-motile
OUS16	Lemon yellow pigmented, Circular, Opaque, Smooth, Convex	Gram positive cocci in bunch	Non-Motile
OUS17	Light yellow pigmented, Irregular, Translucent, Smooth, Flat	Gram negative rods	Motile
OUS18	Lemon yellow pigmented, Circular, Opaque, Smooth, Convex	Gram positive cocci	Non-motile
OUS19	Yellow pigmented, Circular, Opaque, Rough, Convex	Gram positive cocci	Non-motile
OUS20	Lemon yellow pigmented, Circular, Opaque, Rough, Convex	Gram positive cocci	Non-motile
OUS21	Bright yellow pigmented, Circular, Opaque, Smooth,	Gram positive cocci	Non-motile
OUS22	Yellow pigmented, Circular, Translucent, Smooth, Convex	Gram positive cocci	Non-motile
OUS23	Light yellow pigmented, Circular, Translucent, Smooth,	Gram positive cocci	Non-motile
OUS24	Pale yellow pigmented, Circular, Opaque, Smooth, Convex	Gram positive cocci	Non-motile
OUS25	Pale yellow pigmented, Circular, Opaque, Smooth, Convex	Gram negative rods	Motile
OUS26	Yellow pigmented, Circular, Opaque, Rough,	Gram positive cocci	Non-motile
OUS27	Pale yellow pigmented, Circular, Opaque, Smooth, Convex	Gram positive cocci in bunch	Non-motile
OUS28	Lemon yellow pigmented, Circular, Opaque, Rough, Convex	Gram positive cocci in bunch	Non-motile
OUS29	Pale yellow pigmented Circular, Translucent, Smooth,	Gram positive rods	Non-motile

environment for the purpose of microbial drug discovery. Based on different studies on species, the majority of new bioactive metabolites from sea associate microorganisms reports were derived from species which can be isolated. While, these optional species of marine constitute obvious sources of new metabolites, their environmental functions and adaptation levels for marine environment are not known (Garcia and Rosenberg, 2010). Survey carried on marine bacteria were separated from marine algae and invertebrates which showed as antimicrobial metabolites are produced in a large proportion (Burgess et al., 1999). The production of non-bacterial bioactive metabolites which inhibit the setting of potential competitors has also been shown to cause by epiphytic bacteria present in marine combines with rich algal surface and invertebrates (Petersen et al., 2020).

The novel cyclical decapeptide antibiotic Loloatin B, which prevent development by M-resistant *S. aureus* MRSA (*Staphylococcus aureus* methicillin resistant), and VRE (Vancomycin *Enterococcus* resistant) was made by species *Bacillus* in Papua-Nova Guinea (Calfee, 2012). Thiomarinol, eminent antibiotics has discovered to produce marine bacterium *Alteromonas* rava. Marine microorganisms, including *Bacillus loloatins*, have been recorded as antibiotics. Ascomycin through *ascomyceticus* var. *Streptomyces hygrosopicus*, Rhamnase from *Saccharopolyspora spinosa* (Madduri et al., 2001), pyrones from *Pseudomonas* (Singh et al., 2003), Kanglemycin from *Nocardia mediterranei* var. *kanglemycin*, Zorbamycin from *Streptomyces flavoviridis*. Therefore, current study aims to explore to isolate bioactive potential bacteria with seawater for an antibacterial activity.

2. Materials and methods

2.1. Sampling site and collection

Both seawater and sample soils were collected from Nellore coastal region premises in south India. The water samples were

collected in 500 mL of sterilized and autoclavable collection bottles. The sedimentation samples were collected into sterile plastic polythene bags and sealed. The samples were collected under aseptic conditions and were placed on sterile icepacks until further process. These samples were inoculated with 1–2 h after collection (Jayaprakashvel et al., 2010).

Table 2
Antagonistic activity of bacterial isolates from marine intertidal regions.

Isolates	Gram positive	Gram negative
OUS1	ND	ND
OUS2	ND	ND
OUS3	ND	ND
OUS4	ND	ND
OUS5	ND	ND
OUS6	ND	ND
OUS7	ND	ND
OUS8	ND	ND
OUS9	6	9
OUS10	ND	ND
OUS11	ND	ND
OUS12	ND	ND
OUS13	ND	ND
OUS14	ND	ND
OUS15	1	ND
OUS16	ND	3
OUS17	ND	ND
OUS18	ND	ND
OUS19	ND	ND
OUS20	ND	ND
OUS21	ND	ND
OUS22	ND	ND
OUS23	2	ND
OUS24	ND	ND
OUS25	ND	ND
OUS26	ND	ND
OUS27	ND	ND
OUS28	ND	ND
OUS29	ND	ND



Fig. 1. Pigmentation of isolated bacteria from marine intertidal regions.

2.2. Isolation of marine bacteria

All the collected samples were marked according to the locations collected, from each sample 60 μL of water were shredded over the Zobells agar plates purchased from Hi-Media and incubated at 28 $^{\circ}\text{C}$ for 24 and 48 h in incubator. After the incubation, different colonies were transferred to the fresh sterile slants for further use (Jayadev and Lekshmi, 2016).

2.3. Screening of for bioactive compounds producing bacterial

2.3.1. Antagonism assay

Strahl et al (2002) has standardized the *invitro* antagonism assay method against bacterial strains like *Escherichia coli* and *Staphylococcus aureus*. Lawn culture was performed through utilizing sterilized cotton swab and incubates for 1 min. Ten micro-liter of bacterial culture was transferred into wells and petri dishes were incubated at 30 $^{\circ}\text{C}$ for overnight. Antagonistic interactions were scored based on presence and appearance of inhibition zones. One of the isolates strains which scored higher inhibition zone was selected for further characterization.

2.3.2. Molecular-based characterization

The 16S rRNA sequence was used in bacterial strains which showed best inhibition against the opted pathogens for molecular identification. The bacterial culture DNA sample, which was amplified using the 16S rRNA primers mainly forward (5'-TCACGGAGTTT-GATCCTG-3') and reverse (5'-GCGGCTGCACGTA GTT-3') primers were used to sequence 16S rRNA gene-sequence. Genotyping was performed as per the documented studies (Khan et al., 2019). A phylogenetic tree was acquired with maximum probability demonstrating the evolutionary association with the selected sequence.

2.3.3. Fermentation process

Using 250 mL of Erlenmeyer flasks, fermentation was performed with the selected active bacterial strains consists of 100 mL of Zobell-broth medium. Pure selected bacteria strains were inoculated with 1 mL of culture suspension for the sterilized fermented broth. Using rotary shaking incubator at 250 rpm, inoculated flasks were incubated at 28 $^{\circ}\text{C}$ for 5 days. The fermented media was centrifuged for crude extract preparation at 10,000 rpm or 20 mins after incubation.

2.3.4. Solvent stabilization

Selection of appropriate solvent to extract the bacterial pigment, initially the solvent capable of supporting maximum pigment output was standardized using various solvents such as ethanol, acetone, methanol, ethyl acetate, petroleum ether, chloroform, hexane, distilled water and diethyl ether (Mahato et al., 2019).

2.3.5. Antibacterial activity

Agar technique was implemented for the antibacterial activity of extracts with crude pigments and evaluated against numerous bacterial pathogens such as *Salmonella* species (PM-08), *Staphylococcus aureus* (PM-14), *E.coli* (PM-04), *B. subtilis*, procured from M/s. Pure microbes, Pune. Plates were incubated at 37 $^{\circ}\text{C}$ of room temperature for 18–24 h and the diameter of inhibition area (mm) were assessed in the final of the experiment and the activity index calculation was performed. The measurement took three distinct, set instructions and recorded average values (Fatima et al., 2019).

2.3.6. Antioxidant activity

DPPH scavenging activity of crude extracts was evaluated. In short, 10 μL of each sample was mixed in methanol (1.0 mL) with 90 μmolL^{-1} DPPH; prepared with methanol at final quantity of 4 mL. These mixtures were vigorously shaken at 37 $^{\circ}\text{C}$ and

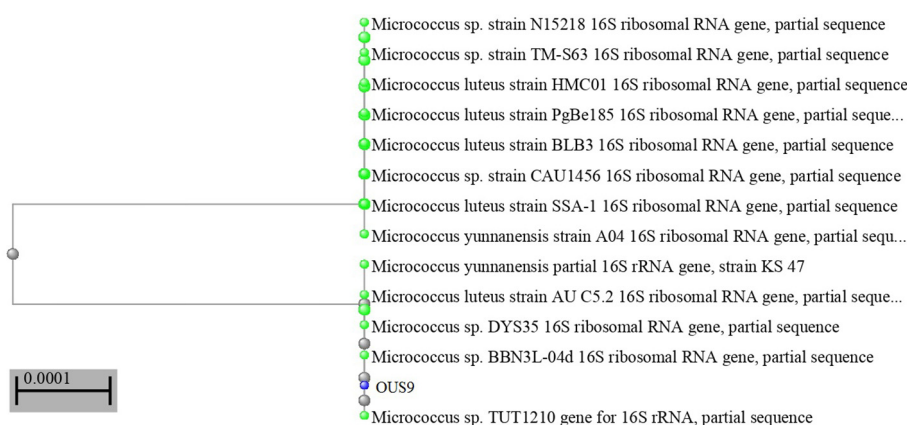


Fig. 2. Phylogenetic tree of *Micrococcus* OUS9 bacterial isolate from marine intertidal regions.

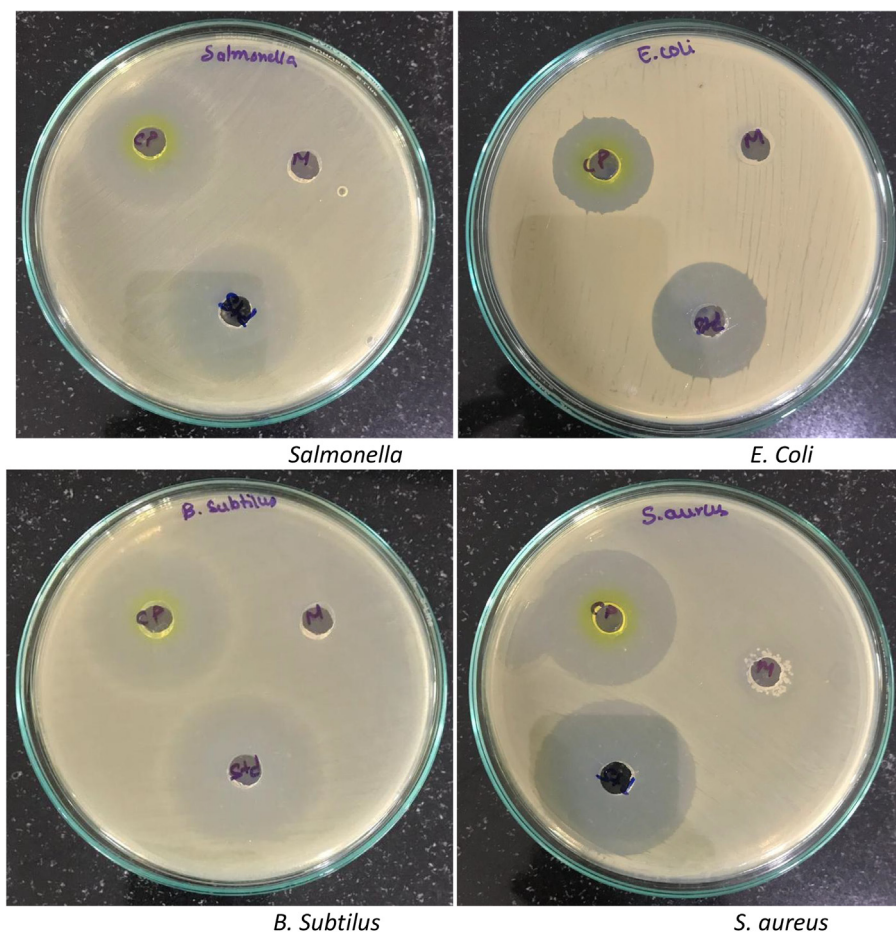


Fig. 3. Antibacterial activity of Methanol crude extracts from *Micrococcus* species OUS9.

incubated in the dark mode for 1hr. Resulting solution absorbance was appraised at 517 nm. Total reactions were carried out in triplicates with Butylated hydroxy anisole as positive control.

2.3.7. Extraction of secondary metabolites

Cells were separated from broth by centrifuging at 6000 rpm at 10 °C for 30 mins. The metabolites were extracted from the supernatant with liquid–liquid extraction technique with equal amount of ethyl acetate and concentration of crude extracts through rotary evaporation (Ming et al., 2018). Cells were washed and separated with methanol. Next, methanolic fraction was obtained and concentrated with yield of crude extracts.

2.3.8. Isolation and purification of bioactive compounds

Davies-Bolorunduro et al (2019) method was adopted from Kwon et al (2009) studies and we have applied in this study to isolate and purify the bioactive compounds. Flash column chromatography was performed.

2.3.9. Anticancer activity

The MTT assay is used to determine the cellular viability or metabolic activity in microcapsules which determines the cell proliferation and cytotoxicity with colorimetric assay based on Yellow-colored, water-soluble tetrazolium dye; MTT which lowers in formazan crystals. Mitochondrial lactate dehydrogenase is produced through liver cells lowers in MTT to insoluble formazan crystals which exhibit purple color when dissolved into the suitable solvent and intensity of number of viable cells can be recorded

at 570 nm spectrophotometrically (Moorthy and Rangappan, 2016).

3. Results and discussion

The composition of the marine ecosystem and biogeochemistry of the oceans play a crucial role. The great variety of microbes in our waters continues therefore vital to be understood. In order to capture rare, less abundantly organisms, efforts are required to investigate regions containing the biggest biodiversity, such as aquatic settings (Salihoglu et al., 2013). The notable variety of marine micro-organisms is due to their initial development, speed of generations and micro-environmental heterogeneity (Staley, 2006). The ocean is heterogenous owing to its nutrient patches and microscale incline, which can help into various inches (Kassen and Rainey, 2004).

In the present investigation the total 29 different bacterial cultures were isolated from different locations of Nellore district regions the bacterial colonies present on agar plates with morphologically different pigment producing has been identified. The selected colonies were sub-cultured in individual testtubes and observed for morphological characterization shown in the (Table 1). The chosen colonies were screened by well diffusion for antagonistic activity. Process against two pathogenic bacteria out of 29 selected bacterial cultures OUS9 have shown efficient (Table 2) antagonistic activity, the results showed that the selected isolate have good growth and ability of high pigment producing capacity as shown in the (Fig. 1). In one of studies of two human-pathogenic bacteria have discovered that marine isolates

Table 3
Biochemical characteristics of selected bacterial strain.

Gram Staining	Gr+
Shape and growth	Cocci
Motility stab	Non-motile
Growth and macconkey plates	+
Tellurite reduction	
Oxidase Production	-
Catalase Production	+
Indole Production	-
O/F test	
ONPG Hydrolysis	
H ₂ S Production	-
Growth at 4 °c	
Growth at 40 °c	
Production of extra-cellular enzymes	
Amylase	
Gelatinase	+
Cellulase	
urease	-
Caesinase	
Acid from	
D-Arabinose	-
D-Inositol	-
D-Lactose	+
D-Maltose	-
D-Rhamnose	-
D-Sorbitol	
D-Glucose	-
D-Mannose	+
D-Trehalose	-/+
D-Xylose	+
Maltotriose	+
Acetate	+
Lactic acid	+
Malic acid	+
Propionic acid	+
Pyruvic acid	+
Glycerol	+
Alpha Hydroxybutyrate	+
Beta Hydroxybutyrate	+
Serine	+
Glutamate	-
Sorbitol	+
Glucose	
Growth in NaCl	
0%	+
0.5%	+
1%	+
Tween 40	+
Tween 80	+
0%	+
0.5%	+
1%	+

are more efficacious than bacteria which live freely living bacteria (Nair and Simidu, 1987). Studies investigating the frequencies of marine bacterial isolates; antagonistic interactions discovered between 5 and 8 percent of isolates expressing activity level (Krasilnikova, 1961). Although these trials concentrated on marine isolates as prospective antibiotic producers, the target species were

non-marine bacteria, often human pathogens. Similarly, more than our understanding of antibiotics producing and understanding of environmental antibiotic sensitivity is restricted. Kanamycin and penicillin antibiotics have diverse form of concentration for inhibition of marine bacteria.

Carlos et al (2013) has evaluated the 16S rDNA for the bacterial diversity for the similar samples of coral settings and for large abundance of proteobacteria. For different weavy softs, stony corals, sponges and sea water of this phylum was recorded earlier in an elevated abundance (Porporato et al., 2013). These strains were further reconfirming through ribotyping molecular technique using 16S rRNA gene sequence and this partial sequence of 16S rRNA gene was obtained with the blast which was documented in *Micrococcus species* OUS9 (Fig. 2) and further the sequence was deposited in GenBank (Accession number MN108086). Microorganisms living in the rhizospheres of diversity of crops are probable due to the abundance of substrate shown from the roots when compared with non-rhizosphere soils to synthesize and release auxin as secondary metabolites (Ahmad et al., 2005). Nair and Simidu (1987) studies have documented this antibacterial compound are not only inhibits terrestrial bacteria also, for ecological importance to native bacterial strains. Yellow pigment extracted from isolated bacteria for high content of pigments was investigated. High contents of pigment were obtained from methanol solvent when compared with ethanol, hexane and petroleum benzene solvents.

Many new species are acknowledged as promising source for fresh bio-active compounds in taxonomic terms (Huang et al., 2012). Wealthy cause for secondary metabolism is with the novel structures at outstanding biological activities has been found in marine bacteria extracted from deep sea sediments. A strain AB 18-032 termed as actinomycete *Verrucosisspora* has been extracted from the sediment sample obtained at 289 m of depth from Japanese Sea (Bister et al., 2004). Abyssomicin C (8) has shown methicillin-resistant antibiotic activity and *S. Aureus* (MRSA) and S-resistant to vancomycin had 4 and 13 µg/mL of MIC values. A representative class-I spirotetron is regarded as Abyssomicin C. This biosynthesis is initially recognized and obtained over bacterial source which inhibits para aminobenzoic acid (PABA) biosynthesis. Abyssomicin C and its atropisomeric inhibits PABA respectively. Further, discovering the marine environment is to be promising strategy for active compounds and for development of resistant therapies for many microorganisms, which indicates the requirement of new drugs are required. Precisely, for anti-fungal, anti-bacterial, anti-viral and anti-protozoal activities.

The present study was carried out with crude pigments were verified for antimicrobial activity with pathogenic bacteria has also been observed that out of Chloroform, Ethanol, Methanol and Acetone extracts, with standard drug reference and Methanol crude pigment showed zone of inhibition greater against *Bacillus subtilis* 15 mm, followed by *S. aureus* with 12 mm, *Salmonella* 11 mm, *Escherichia coli* 09 mm, *P. aeruginosa* 06 mm and lowest zone was observed to *K. pneumoniae* 02 mm (Fig. 3, Table 3). This confirms as crude Yellow pigment of *Micrococcus species* isolates were well

Table 4
Antibacterial activity of Chloroform, Ethanol, Methanol and Acetone extracts from *Micrococcus species* OUS9.

Bacteria Pathogens	Zone of inhibition in mm				Standard
	Chloroform extract	Ethanol extract	Methanol extract	Acetone extract	
<i>Bacillus subtilis</i>	-	-	15	-	19
<i>Salmonella</i>	-	-	11	-	16
<i>Escherichia coli</i>	-	-	09	02	12
<i>P. aeruginosa</i>	05	-	06	-	08
<i>K. pneumoniae</i>	-	-	02	-	14
<i>S. aureus</i>	-	01	12	04	15

Table 5
Antioxidant activity of crude pigment isolated from *Micrococcus* species OUS9.

Concentration (mg/ml)	Antioxidant activity of methanol extracted crude pigment isolated from <i>Micrococcus</i> sp			
	Standard	ABTS	NO	DPPH
100	56.21 ± 4.9	44.14 ± 2.9	42.13 ± 3.4	48.44 ± 6.2
75	42.27 ± 1.9	32.95 ± 3.9	39.26 ± 4.2	43.19 ± 5.7
25	32.19 ± 1.3	20.24 ± 2.9	25.75 ± 1.4	31.47 ± 2.9

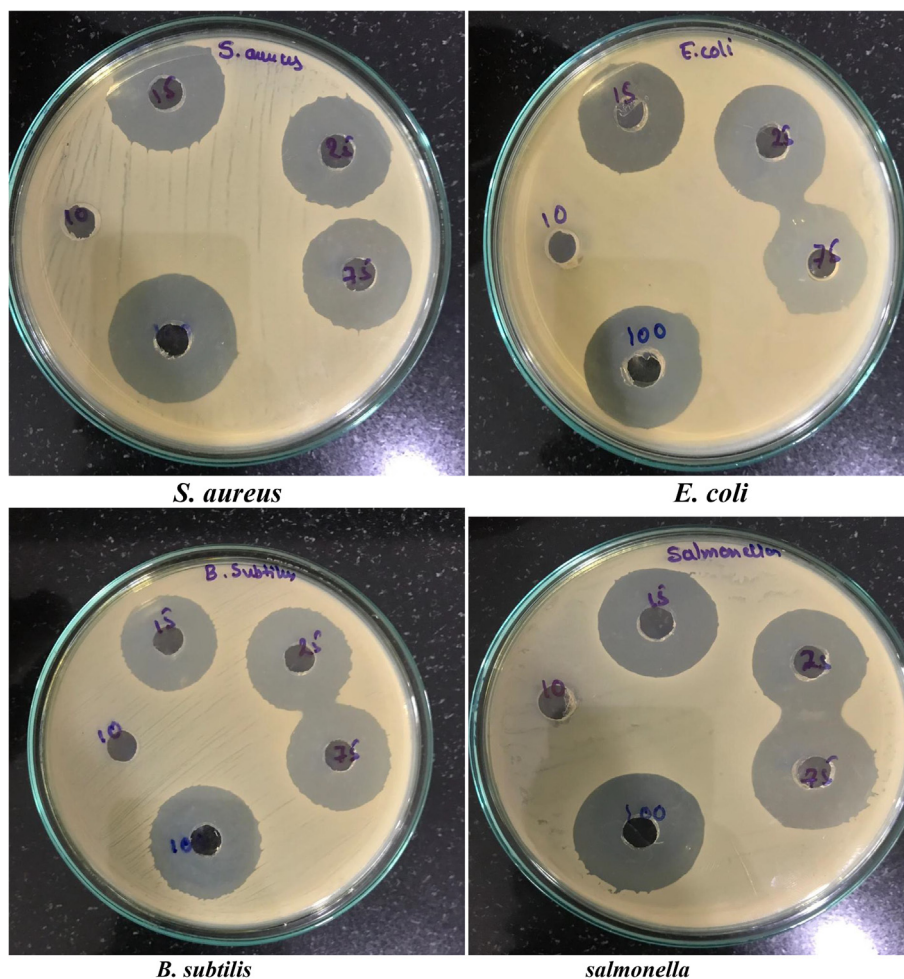


Fig. 4. Antibacterial activity (MIC) of pure compound (13th Yellow fraction) isolated from *Micrococcus* species OUS9.

and active against both gram positive and negative bacteria. For their natural product contents such as algae, ascidians, corals, microorganisms and sponges which is known to be total forms of marines were investigated (Arif et al., 2004). Throughout thousands of years, ecological pressures like space competition, symbiosis, predation and tide variations have led to the biosynthesis of complicated of secondary metabolite species, which in turn enable them for adopting the competitive and hostile environment (Da Silva et al., 2006). Different marine organisms such as algae and invertebrates in sea surface or cavum has been found to be more nutritive than inanimate and marine water and may have large number of bacteria among them (Bonugli-Santos et al., 2015). These few species of bacteria are usually non-symbiotic of host which can be allied bacteria (Bultel-Ponce et al., 1999) and are compelled to create antibiotic resistance secreted by their host.

In the present investigational study, carried out the free radical scavenging activity of *Micrococcus* species OUS9 extract with concentration range from 25, 75 to 100 mg/ml and ascorbic acid

standard used as a standard. The result of DPPH, ABTS and NO scavenging activity indicates *Micrococcus* species OUS9 extract are potentially active. As shown clearly in the Table 4 that the extract shows 48.44 ± 6.2% inhibition as compared to standard 56.21 ± 4.9 in DPPH activity followed by 44.14 ± 2.9 in ABTS and 42.13 ± 3.4% inhibition in NO with 100 (mg/ml) (Table 5). With promising antioxidant activity, the inhibition effect increased in higher levels. It has a maximum of 176 of 95 per cent. Species of reactive oxygen plays important role to protect human body from damage (Govindarajan et al., 2005). In addition, past epidemiological surveys have shown a reduction in consumption of natural antioxidants in cancer and other oxidatively damaged illnesses (Rietveld and Wiseman, 2003).

Extraction of crude through solvent extraction is a significant phenomenon for opting the appropriate solvent for extracting high yields of bioactive compounds. Previous studies have confirmed the strong antimicrobial potential for ethyl acetate extraction

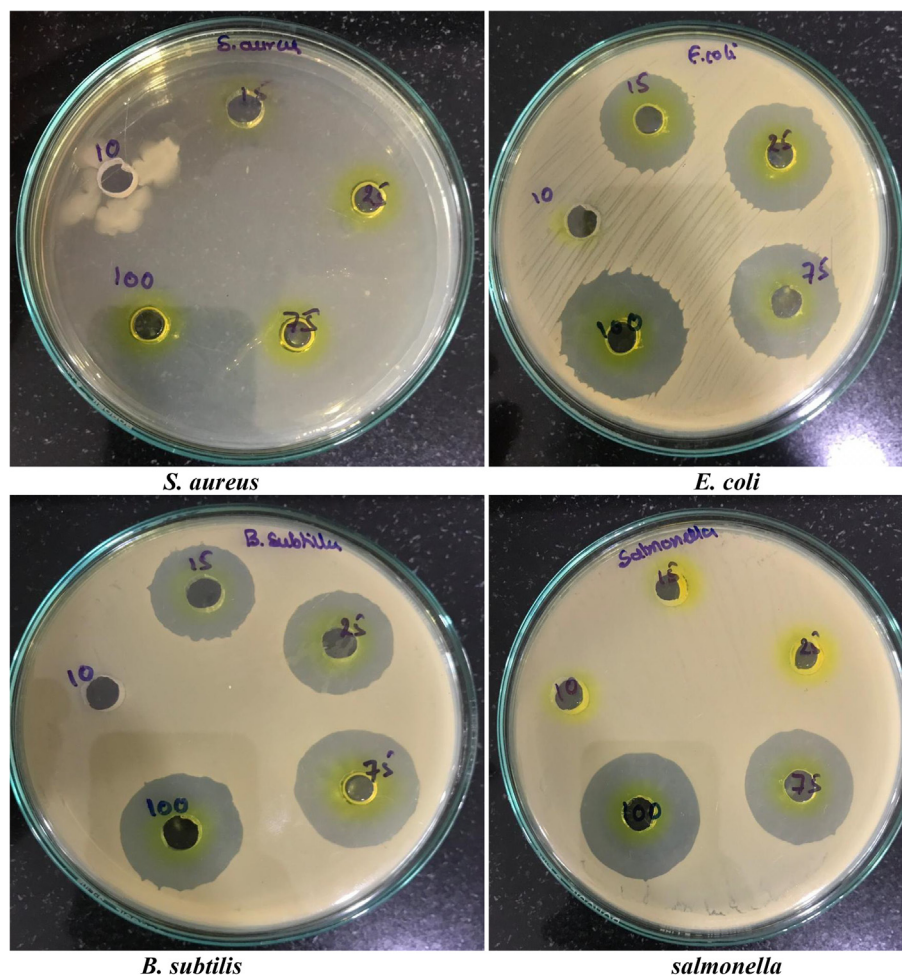


Fig. 5. Antibacterial activity (MIC) of pure compound (10th Yellow fraction) isolated from *Micrococcus* species OUS9.

against the pathogens of fungi and bacteria. Here from the eluted fractions with column chromatography is tested for antimicrobial study and the best fraction was further identified. KLUF-10 and KLUF-13 with molecular weight 568.9 and KLUF-13 the molecular weight with 263.38. On the basis of these observations the purified compound is proposed to be KLUF-10 as 3-Hydroxy- β , ϵ -caroten-3'-one, KLUF-13 1-(1-(4-methoxyphenyl)-2-(methyl amino) ethyl) cyclohexan-1-ol and which was isolated from *Micrococcus* species OUS9 for the first time and is to appear that novel compound with good antimicrobial activity as shown in the Figs. 4 and 5 supplementary table 1). The most promising results for anticancer activity were obtained with KLUF-10 and KLUF-13 fractions of the *Micrococcus* sp. OUS9 strain. The inhibitory effect of pure compounds treated cells was observed after 24 h of incubation. The results of cytotoxicity study performed by MTT assay suggest that the given test compound, KLUF-10 and KLUF-13 are anticancer in nature against the both HeLa and MCF-7 Cell lines and the IC50 concentrations are 34.5ug/ml and 34.95ug/ml respectively IC50 concentrations are HeLa 28.9 ug/ml and MCF-7 26.6ug/ml (Supplementary Table 2; Supplementary Figs. 1-2). In multiple microbial metabolites, the role of pigment is to act as coloring agent in food processing and cosmetic industries and also own anti-oxidant, anti-inflammatory, anti-cancer, and anti-microbial activities. The current results of this study suggest that the coastal region's ability as an origin of marine bacteria to generate antibacterial compounds which can and must be explored for many pharmaceutical applications.

4. Conclusion

In the marine environment, bioactive compounds that produce microorganisms are widely distributed. Bioactive metabolites from marine bacteria which acts as an anchor for survival between pathogenic bacteria and their environment. This research is a step towards understanding and testing bioactive metabolites generating marine bacteria in the region around the shore of Nellore, Andhra Pradesh, India. Screening of these bacteria by screening techniques for the manufacturing of bioactive metabolites. The strain of *Micrococcus* species OUS9 demonstrated potential. To characterize such activity and recognize the promising isolates, further research is required. This new natural biological product is also used in various sectors such as the pharmaceutical industry, the food industry and the preservation of the natural environment

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jksus.2020.07.003>.

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