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Research Article

ISOLATION OF ACTINOMYCETES FROM THE SEDIMENTS OF PICHAVARAM MANGROVE FOREST, SOUTH INDIA AND ANALYSING THEIR ANTIBACTERIAL EFFICACY

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ABSTRACT

Objective: The aim of the present investigation is to isolate actinomycetes from the sediments of Pichavaram mangrove forest, South India, and to screen for their antibacterial efficiency.

Methods: Actinomycetes were isolated by culturing the samples in Starch Casein Agar medium; they were screened primarily for their antibacterial efficiency against Gram-positive and Gram-negative bacterial organisms. Solvent extraction was done with 50% (percentage) ethyl acetate, crude extracts of actinomycetes were prepared at different concentrations using dimethyl sulfoxide and treated against the bacterial organisms. Antibacterial assay was done in Mueller–Hinton agar medium.

Results: Thirteen actinomycetes were isolated; among them, four actinomycete isolates (Pichavaram mangrove actinomycete 2 [PMA2], PMA6, PMA9, and PMA13) exhibited antibacterial activity.

Conclusion: Isolate PMA2 exhibited very strong antibacterial activity and isolate PMA13 is weakly active against the tested bacterial organisms.

Keywords: Actinomycetes, Starch casein agar medium, Salinity, Crude extract, Antibacterial efficiency, Antibacterial assay.

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INTRODUCTION

Coastal region is an important region for human beings since the beginning of time. Coastal ecosystem supports coral reefs, seagrasses, marine biota, and the growth of mangrove forest. Mangroves are the most important ecosystems of coastal and marine region. Mangrove forests provide direct and indirect contributions to human beings and natural habitats in the ocean. Soil microbes are important sources for displaying great biological activity against several pathogens. Bioactive molecules are capable of modulating metabolic process and they exhibit beneficial effects such as antioxidant activity, inhibition or induction of enzymes, inhibition of receptor activities, and induction and inhibition of gene expression. They are also useful for the protection mechanism [1]. Actinomycetes have more value both economically and biotechnologically which can produce 80 % of total available antibiotics in the world. The most important genera for the production of antibiotics are *Streptomyces* and *Micromonospora* [2]. Actinomycetes exist in various places of environment, including soil, freshwater, and marine water environments. The biologically active terrestrial compounds are overexploited, so the search for the new compounds has been increased toward the marine ecosystem. Marine actinomycetes are different from terrestrial actinomycetes both phylogenetically and physiologically [3]. The marine sediments are capable of synthesizing bioactive secondary metabolites [4,5]. In 2014, the World Health Organization (WHO) reported that the resistance toward antimicrobials has developed much and it is the major challenge to overcome this problem. The WHO also promotes the medicinal sources from the traditional medicinal system [6]. Hence, the study was aimed to screen antibacterial efficiency by producing bioactive natural compounds from the actinomycetes isolated from the sediments of Pichavaram Mangrove Forest, South India.

METHODS

Sample collection

The water and sediment samples were collected from the mangrove forest in Thandavarayan Sholagan Pet, Chidambaram Taluk, Cuddalore district, Tamil Nadu, South India. The geographical location is shown in Fig. 1.

The field is situated in 11.41° N (North) latitude and 79.79° E (East) longitude at an altitude of above +5.25 M (meter) mean sea level. The water samples were collected in polypropylene tubes and the sediment samples were collected in plastic bags at five different locations at a depth of one feet each [7,8]. The samples were collected at 7.00 a.m. (ante meridian) during winter season. During sample collection, the sample temperature and pH were tested. Then, the samples were brought to the laboratory and stored at 4°C [9].

Salinity test (total dissolved salts [TDS])

To find TDS, 100 ml of water sample was evaporated in a hot air oven. Then, the salt settled at the bottom was measured. The tests were done twice and the average value has been taken [10].

Isolation of actinomycetes

The samples were diluted serially in the water brought from the mangrove forest and 10^{-4} diluted sample was plated on starch casein agar medium [10]. The media were supplemented with cycloheximide (25 µg/ml) and nalidixic acid (25 µg/ml) for the inhibition of fungi and Gram-negative bacteria, respectively. The plates were incubated for 7 days at 30°C [9]. The actinomycetes were used against Gram-positive and Gram-negative bacterial organisms to screen their antibacterial efficiency [7].

Test bacterial organisms

Antibacterial susceptibility was detected against ten bacterial strains of the Microbial Type Culture Collection and Gene Bank and listed in Table 1 [11]. Among ten bacterial organisms, each five bacterial organisms are Gram-positive and Gram-negative organisms [12-14].

Screening for the antibacterial efficiency

The antibacterial efficiencies of actinomycete isolates were determined against ten bacterial strains. The actinomycete sample

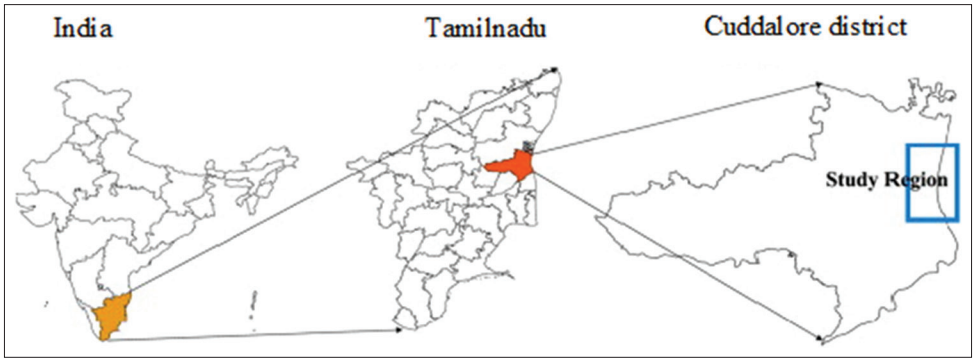

Fig. 1: Geographical location of Thandavarayan Sholagan Pet, South India. Tamil Nadu state has been marked in India; it has been maximized to show its districts. In Tamil Nadu state, Cuddalore district has been shaded and maximized to show the study region Thandavarayan Sholagan Pet (▢ blue color box)

Table 1: List of bacterial organisms used for screening antibacterial efficacy

Type of bacterial organism	Name of the organism	Abbreviation	MTCC number
Gram-positive organisms	*Bacillus subtilis*	*Bs*	MTCC 1133
	B. megaterium	*Bm*	MTCC 2949
	B. cereus	*Bc*	MTCC 430
	Staphylococcus aureus	*Sa*	MTCC 3160
	S. epidermidis	*Se*	MTCC 3382
Gram-negative organisms	*Escherichia coli*	*Ec*	MTCC 1692
	Salmonella typhi	*Sti*	MTCC 3216
	Salmonella typhimurium	*Stm*	MTCC 3214
	Pseudomonas aeruginosa	*Pa*	MTCC 2581
	Klebsiella pneumoniae	*Kp*	MTCC 2653

MTCC: Microbial type culture collection

was streaked linearly on the surface of nutrient agar medium exactly at the center and incubated for 7 days at 37°C. The bacterial organisms were inoculated on both sides perpendicularly to the actinomycetes at the distance of 5 mm (millimeter) from the actinomycete [15]. The Gram-negative organisms were streaked on one side and the Gram-positive organisms were on the other side and incubated for 48 h at 37°C [7,13].

Antibacterial assay

Solvent extraction was done with 50% ethyl acetate and diluted in dimethyl sulfoxide for the preparation of different concentrated crude extracts of all the four actinomycetes [12]. The antibacterial activities of the actinomycetes were assessed against ten bacterial organisms using well diffusion assay. Mueller-Hinton agar plates were prepared and swabbed with the bacterial organisms. Four wells were made in 6 mm diameter each [16]. The different concentrated crude extract was loaded in all wells in 100 µl volume. The plates were incubated at 37°C for overnight. The antibacterial activity was then recorded as growth free inhibition zones around the well [17]. The experiments were repeated up to 3 times to find the mean value.

RESULTS

Sample collection

The water and sediment samples were collected from the mangrove forest in Thandavarayan Sholagan Pet, South India. During sample collection, the temperature and pH were verified. The temperature was 28°C and the pH was 7.2.

Salinity test

To find TDS, 100 ml of water sample was evaporated in a hot air oven. The salt settled at the bottom was weighed. It was 2.3 g; therefore, TDS of the water was calculated as 2.3%.

Isolation of actinomycetes

A total of 13 actinomycetes were isolated based on different colony morphology from the samples collected at five different locations. The actinomycetes were named as isolate Pichavaram mangrove actinomycete 1 (PMA1) to isolate PMA13. The colony morphology of the actinomycete isolates was listed in Table 2.

Screening for the antibacterial efficiency

Isolate PMA2 has strong activity on *Bacillus megaterium*, mild activity on *Bacillus subtilis, Bacillus cereus*, *Staphylococcus aureus, Staphylococcus epidermidis*, *Escherichia coli*, *Salmonella typhi*, *Salmonella typhimurium*, and *Pseudomonas aeruginosa.* Isolate PMA2 has no activity on *Klebsiella pneumoniae.*

Strong activity was shown by isolate PMA6 on *B. megaterium, S. aureus, Salmonella typhi*, and *Salmonella typhimurium.* Mild activity was shown on *S. epidermidis.* Mild or nil activity was shown on *B. cereus.* There was no activity against *B. subtilis, E. coli*, *P. aeruginosa,* and *K. pneumoniae.*

Isolate PMA9 has shown strong antibacterial activity on *S. aureus*, mild activity on *B. megaterium*, *B. cereus*, *S. epidermidis*, *P. aeruginosa*, *Salmonella typhi*, and *Salmonella typhimurium.* Isolate PMA9 was not shown the activity against *B. subtilis, E. coli*, and *K. pneumoniae.*

Isolate PMA13 was showing mild activity on *S. epidermidis.* The rest of the bacterial organisms were resistant toward the isolate PMA13. The antibacterial efficiency of actinomycetes is shown in Table 3.

Antibacterial assay

Isolate PMA2

Isolate PMA2 has produced 17±0.62 mm diameter zone of inhibition on *B. megaterium* with 1.43% of crude extract and 25±0.7 mm diameter zone of inhibition with the same organism at the concentration of 2.86 %. It also produced 15±0.66 mm, 17±0.99 mm, and 19±0.7 mm diameter zone of inhibition on *S. aureus* at the concentrations of 1.43%, 2.145%, and 2.86%, respectively.

Even at the concentration of <1% (0.715%), isolate PMA2 produced 15±0.65 mm and 14±0.53 mm diameter zone of inhibition on *E. coli* and *Salmonella typhi*, respectively. Furthermore, it has produced 17±0.74 mm, 20±0.5 mm, and 24±0.98 mm diameter zone of inhibition on *E. coli* at the concentrations of 1.43%, 2.145%, and 2.86 %, respectively. It also produced 22±0.45 mm, 23±0.37 mm, and 26±0.71 mm diameter zone of inhibition on *Salmonella typhi* at the concentrations of 1.43%, 2.145%, and 2.86%, respectively. Isolate

Table 2: Colony morphology of the actinomycetes from soil sediments of Pichavaram mangrove forest, South India

Isolate	Morphology
PMA1	Slightly white with small colonies
PMA2	Yellow with small colonies
PMA3	Ash color
PMA4	Slightly black and slightly pink
PMA5	Ash color
PMA6	Slightly yellow
PMA7	Light pink
PMA8	Light green
PMA9	Slightly yellow with small colonies
PMA10	Slightly pink
PMA11	Light yellow with small colonies
PMA12	Pale white with large colonies
PMA13	Slightly black with large colonies

PMA: Pichavaram mangrove actinomycete

Table 3: Antibacterial efficiency of actinomycetes from soil sediments of Pichavaram mangrove forest, South India

Actinomycetes	Bacterial organisms									
	Gram-positive organisms					Gram-negative organisms				
	Bs	*Bm*	*Bc*	*Sa*	*Se*	*Ec*	*Pa*	*Sti*	*Stm*	*Kp*
PMA1	–	–	–	–	–	–	–	–	–	–
PMA2	+	++	+	+	+	+	+	+	+	–
PMA3	–	–	–	–	–	–	–	–	–	–
PMA4	–	–	–	–	–	–	–	–	–	–
PMA5	–	–	–	–	–	–	–	–	–	–
PMA6	–	++	–/+	++	+	–	–	++	++	–
PMA7	–	–	–	–	–	–	–	–	–	–
PMA8	–	–	–	–	–	–	–	–	–	–
PMA9	–	+	+	++	+	–	+	+	+	–
PMA10	–	–	–	–	–	–	–	–	–	–
PMA11	–	–	–	–	–	–	–	–	–	–
PMA12	–	–	–	–	–	–	–	–	–	–
PMA13	–	–	–	–	+	–	–	–	–	–

–: Not detected, +: Mild activity, ++: Strong activity, /: or The actinomycetes PMA2, PMA6, PMA9, and PMA13 were actively performing antibacterial activity and these four actinomycetes were taken for further studies. PMA: Pichavaram mangrove actinomycete

PMA2 produced 16±0.81 mm and 19±0.43 mm diameter zone of inhibition on *Salmonella typhimurium* and 10±0.7 mm and 12±0.88 mm diameter zone of inhibition on *K. pneumonia* at the concentrations of 2.145% and 2.86%, respectively. The diameter of the zone of inhibition produced by isolate PMA2 is shown in Fig. 2.

Isolate PMA2 has higher inhibitory activity on the bacterial organisms *Salmonella typhi*, *B. megaterium*, *E. coli*, *S. aureus*, and *Salmonella typhimurium.* It has mild inhibitory activity on *K. pneumoniae.* It has not produced any zone of inhibition on *B. subtilis*, *B. cereus*, *S. epidermis*, and *P. aeruginosa.*

Isolate PMA6

Isolate PMA6 has strong antibacterial activity on *B. megaterium* (18±1.16 mm), *B. cereus* (19±0.82 mm), *S. aureus* (18±0.62 mm), and *Salmonella typhi* (19±0.33 mm). Mild activity was shown against *Salmonella typhimurium* (13±0.9 mm), *P. aeruginosa* (12±0.5 mm), and *K. pneumonia* (10±0.57 mm). The activity was not detected against *B. subtilis, S. epidermidis*, and *E. coli.* These activities were shown at its higher concentrated (3.52 %) crude extracts. At the concentration of 2.64% crude extract, 16±0.7 mm, 17±0.36 mm, 15±0.73 mm, 19±0.7 mm, and 10±0.56 mm diameter zone of inhibitions were recorded against *B. megaterium*, *B. cereus*, *S. aureus*, *Salmonella typhi*, and *Salmonella typhimurium*, respectively. There was no zone of inhibition against the rest of the bacterial organisms at 2.64% crude extract. 16±0.56 mm, 13±0.31 mm, and 12±0.21 mm diameter zone of inhibitions were recorded against *B. cereus*, *S. aureus*, and *Salmonella typhi* at 1.76% of crude extract. The rest of the bacterial organisms were not inhibited at 1.76% of crude extract. Even at the Concentration of <1% (0.88 %), isolate PMA6 could able to produce a zone of inhibitions in the diameter of 12±0.6 mm and 11±0.4 mm against *B. cereus* and *S. aureus*, respectively. The diameter of the zone of inhibition produced by the isolate PMA6 is given as Fig. 3.

Isolate PMA9

Isolate PMA9 has shown highest zone of inhibition against *B. megaterium,* they were recorded as 10±0.42 mm, 16±0.56 mm, and 18±0.56 mm at the concentrations of 1.76 %, 2.64 %, and 3.52 %, respectively. The mild activity was shown against *S. aureus*, the zones of inhibitions were recorded as 10±0.56 mm, 10±0.66 mm, 12±0.59 mm, and 12±0.61 mm at the concentrations of 0.88%, 1.76%, 2.64%, and 3.52%, respectively. At the concentrations of 3.52% and 2.64%, the zones of inhibitions were recorded as 10±0.45 mm and 8±0.7 mm against *S. epidermidis*

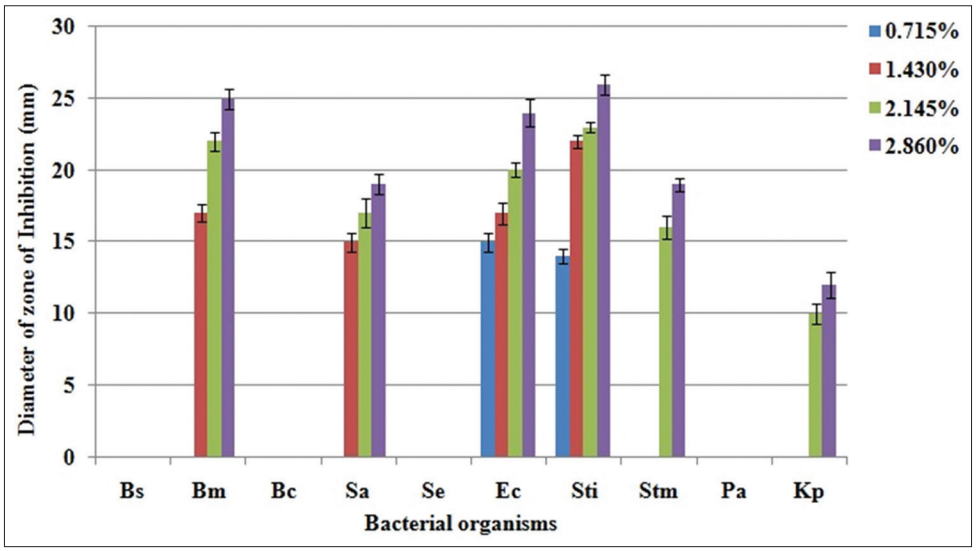

Fig. 2: Graphical representation of antibacterial assay of isolate Pichavaram mangrove actinomycete 2 isolated from soil sediments of Pichavaram mangrove forest. All the experiments were performed in triplicates and mean value was calculated by the standard deviation (SD) (mean value±SD)

and 15±0.74 mm and 11±0.36 mm against *Salmonella typhimurium*. The antibacterial activity was not detected against *B. subtilis, B. cereus, E. coli, Salmonella typhi*, *P. aeruginosa*, and *K. pneumonia.* The diameter of the zone of inhibition produced by PMA9 is shown in Fig. 4.

Isolate PMA13

Isolate PMA13 has Mild antibacterial activity against *B. megaterium*, the zones of inhibitions were recorded as 10±0.22 mm, 10±0.49 mm, 15±0.6 mm and 14±0.79 mm at the concentrations of 0.175%, 1.43%, 2.145%, and 2.86%. At the concentration of 2.86%, 8±0.56 mm zone of inhibition was recorded against *B.* cereus. Isolate PMA13 was active against *S. aureus,* produced 8±0.57 mm and 8±0.19 mm zones of inhibitions at the concentrations of 2.145 % and 2.86 %, respectively. 12±0.38 mm and 8±0.33 mm zones of inhibitions were recorded against *E. coli* at the concentrations of 2.145% and 2.86%, respectively. The activity was not detected against *B. subtilis, S. epidermidis, Salmonella typhi*, *Salmonella typhimurium, P. aeruginosa,* and *K. pneumonia.* The diameter of the zone of inhibition produced by PMA13 is shown in Fig. 5.

DISCUSSION

Actinomycetes exist in most of the places of nature and they possess the ability of synthesizing several biologically active compounds such as antibacterial, antifungal, antiviral, antiparasitic, herbicides, pesticides, antioxidant, and anti-tumor [1]. We examined the ability of actinomycetes from the sediments of Pichavaram mangrove forest to for their antibacterial efficacy.

In our study, a total of 13 actinomycetes were isolated from five different locations of Pichavaram mangrove forest and perpendicular streaking was done to find out the antibacterial efficiency against ten bacterial organisms. One hundred six actinomycetes were isolated from five different soil samples. These actinomycetes were cross streaked against microbial pathogens [1].

Plant sources contain good bioactive molecules and it acts as a source of antimicrobial and antioxidant agents [18-21]. *Allium cepa* possess bioactive natural products and might be used for the treatment of infectious diseases of bacteria [22]. The growth of *Salmonella* bacteria Thy 1 was inhibited *in vivo* and it has proved that manila extract has the

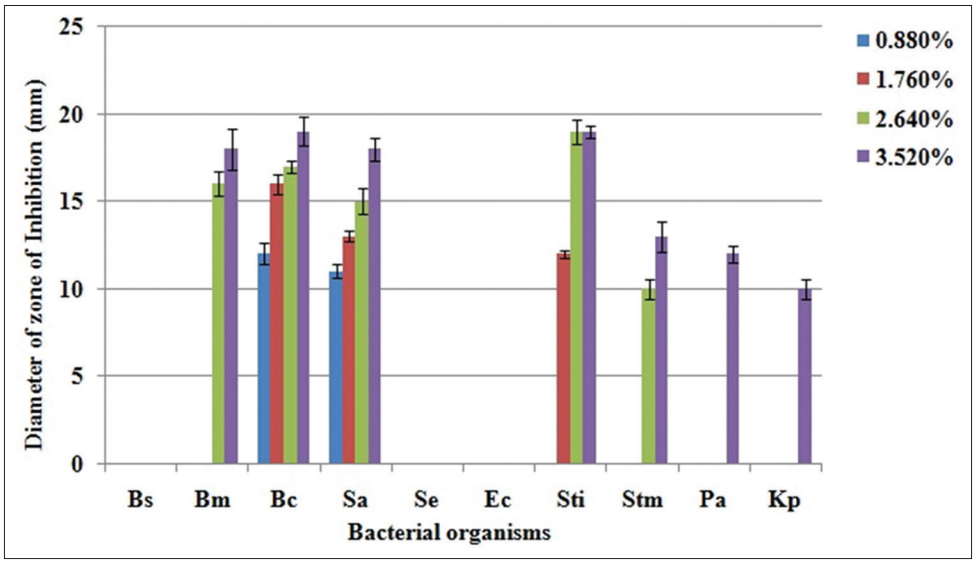

Fig. 3: Graphical representation of antibacterial assay of isolate Pichavaram mangrove actinomycete 6 isolated from soil sediments of Pichavaram mangrove forest. All the experiments were performed in triplicates and mean value was calculated by the standard deviation (SD) (mean value±SD)

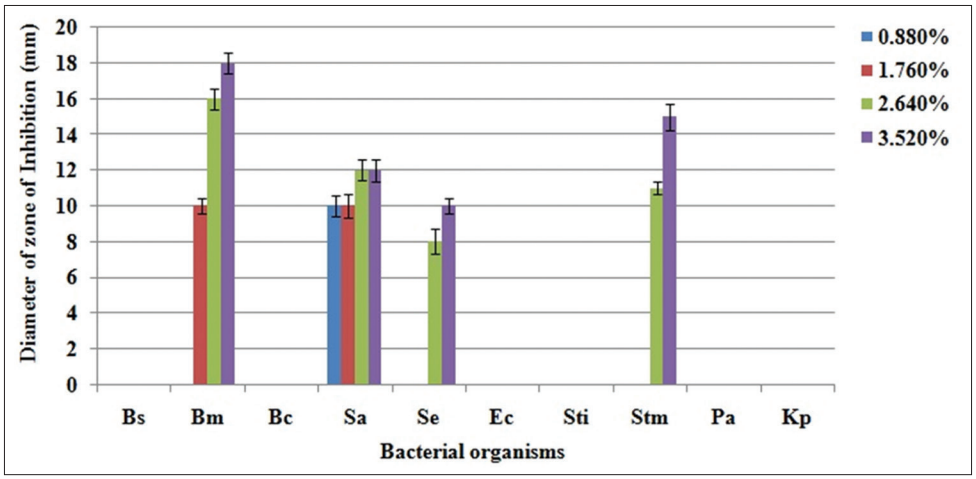

Fig. 4: Graphical representation of antibacterial assay of isolate Pichavaram Mangrove Actinomycete 9 isolated from soil sediments of Pichavaram mangrove forest. All the experiments were performed in triplicates and mean value was calculated by the standard deviation (Mean value±SD)

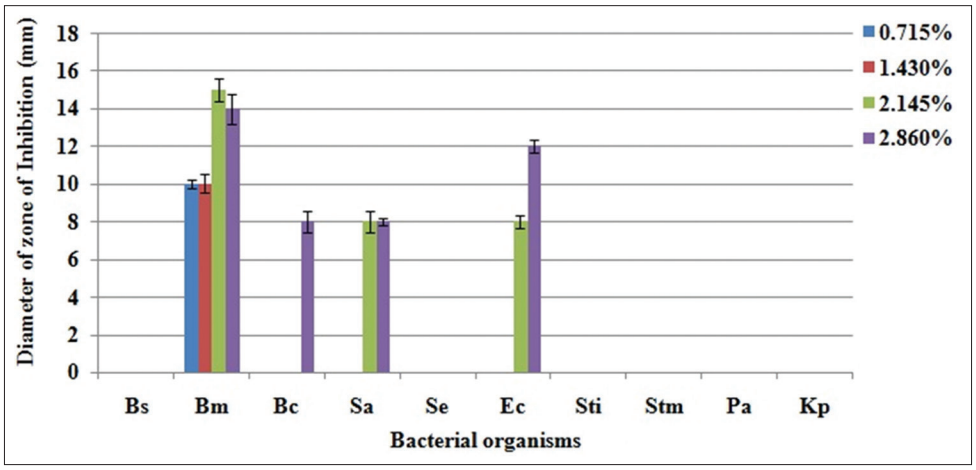

Fig. 5: Graphical representation of antibacterial assay of isolate Pichavaram mangrove actinomycete 13 isolated from soil sediments of Pichavaram mangrove forest. All the experiments were performed in triplicates and mean value was calculated by the standard deviation (SD) (mean value±SD)

capability for the inhibition [23]. Ethanol extract of turmeric, cinnamon, and clove was tested against *E. coli* and *S. aureus*. These two bacterial organisms are sensitive to all three plants; cinnamon is more active than the other two plants [24].

The primary screening for the antagonistic activity of actinomycetes showed antibacterial activity against both Gram-positive and Gram-negative bacterial organisms [3]. In the same way, the actinomycetes were streaked perpendicularly and they acted against Gram-positive and Gram-negative bacterial organisms. This revealed that the actinomycetes produce antibacterial compound against both Gram-positive and Gram-negative bacterial organisms.

Out of 13 actinomycetes isolates, only 4 (31%) actinomycetes were able to produce antibacterial compound. Thirty actinomycetes were examined, of this, only 53.3% showed a positive result against the test bacteria [25]. Twenty isolates (36.4%) of 55 were potent against the test bacterial organisms. Thirteen isolates showed a positive result to more than one genus, only one isolate showed positive result against all four species of *Shigella*, seven isolates showed antibacterial activity against three species of *Shigella* and not with *Shigella sonnei* [26]. Even, our actinomycetes could produce the antibacterials at less percentage; they work even at very low concentrations.

India is one of the richest wealth of long seacoast and a good source of marine wealth [27]. Marine sponge-associated bacterial isolates have been evidenced as the sources for the production bioactive compounds [28]. Antimicrobial agents contribute themselves significantly in 20th century, especially therapeutics.

Ethyl acetate extract of the actinomycetes was used in this study, ethyl acetate extract of *Bergenia ciliata* showed the highest zone of inhibition (7.5 mm) against *B. megaterium*, moderate activities against *Nocardia tenerifensis* and *B. subtilis* with 6.2 mm and 5.5 mm zone of inhibitions, respectively [29]. Ethyl acetate extract of endophytic fungi *Fusarium* sp. showed the best result against *E. coli* (microbial type culture collection [MTCC] 443) by generating 20.66±0.57 mm diameter zone of inhibition. The same extract created 15.66±0.57 mm, 14.66±0.57 mm, 13.66±0.57 mm, and 12.66±0.57 mm diameter zone of inhibition against *P. aeruginosa* (MTCC 424), *K. pneumoniae* (MTCC 452), *Sphingomonas paucimobilis* (MTCC 6363), and *Proteus vulgaris* (MTCC 426) [30]. Ethyl acetate extract is more potent and highly active on the bacterial organisms.

Among all four actinomycetes, the highest zone of inhibition was recorded by isolate PMA2 against *Salmonella typhi* (26±0.71 mm). Lowest zones of inhibitions were recorded by PMA2 against *K. pneumonia* (12±0.88 mm), isolate PMA6 against *K. pneumonia* (10±0.57 mm), 10±0.45 mm against *S. epidermidis* by the isolate PMA9, 8±0.56 mm and 8±0.19 mm against *B. cereus* and *S. aureus*, respectively, by the isolate PMA13. *Kocuria kristinae* produced highest zone of inhibition against *B. cereus* (10.2 mm), whereas *Streptomyces flaveolus* produced a very low zone of inhibition (2.5 mm) against *K. pneumonia* [2]. *K. pneumonia* is more sensitive to isolate PMA2 and isolate PMA6 than *S. flaveolus*.

B. subtilis was not inhibited by all four organisms and *B. megaterium* and *S. aureus* were inhibited by all four organisms. *P. aeruginosa* was inhibited only by the isolate PMA6 and *S. epidermidis* was inhibited only by isolate PMA9. The leaves and twigs extracts of *Capparis cartilaginea* Decne had weaker antibacterial activity against *S. aureus* and no activity against *E. coli* [31]. *E. coli* was not inhibited by all 12 strains, moderate to high activity was recorded by all 12 strains against *B. cereus* and only one strain can able to inhibit *K. pneumonia* [2], whereas *E. coli* is inhibited by isolate PMA2 and isolate PMA13. Isolate PMA2 could inhibit *E. coli* even at low concentration and high concentration of isolate PMA13 is needed to inhibit the same bacterial organism.

India like developing countries identifies the new drugs from natural sources. Few antibiotics like tetracycline are extracted from soil actinomycetes [32]. Microorganisms are the good source of enzymes, antimicrobials and they are helpful for the production of various industrial products [8]. It is vital to develop alternative drugs for the treatment of infectious diseases [33].

The sediments of Pichavaram mangroves possess certain important chemical compounds and serve as nutraceuticals, pharmaceuticals, and antimicrobials. These antimicrobials have been recommended to treat various diseases. Natural antimicrobials have greater potential applications and contribute a significant impact on health-care system of human beings and to prevent various diseases [34].

CONCLUSION

The present findings of the study gives a scientific application of the mangrove sediment as antimicrobials and commonly used for various microbial based diseases. Further, we concluded that these natural antimicrobials are the alternatives for synthetic antimicrobial drugs.

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AUTHORS' CONTRIBUTIONS

M. Aarthi working as an Assistant Professor in the Department of Biotechnology, K. S. Rangasamy College of Arts and Science, Tiruchengode, Tamil Nadu, designed and performed the experiments. Dr. V. Balakrishnan, Assistant Professor in the Department of Botany, Arignar Anna Government Arts College, Namakkal, supervise work and manuscript work and review process. Dr. D. Kamalanathan, Assistant Professor in the department of Biotechology, K S Rangasamy College of Arts and Science, Tiruchengode, Tamilnadu. Helped to analyze the data and to write the paper. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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