**ORIGINAL PAPER**

# Evaluation of antimicrobial activity of the extract of *Streptomyces euryhalinus* isolated from the Indian Sundarbans

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## Abstract

The discovery of new antimicrobials is the prime target in the fight against antimicrobial resistance. The continuous search for new lead compounds from bacteria of untapped and extreme ecosystems such as mangroves is currently being undertaken. This study describes the metabolite profiling of the *Streptomyces euryhalinus* culture extract. Previously, *Streptomyces euryhalinus* was isolated from the mangrove forest of Indian Sundarbans as a novel microorganism. The antimicrobial mechanism of action of *Streptomyces euryhalinus* culture extract against bacteria and fungi has been analyzed in this study. The gas chromatography–mass spectrometry profile of the ethyl acetate extract bacterial culture displayed the presence of several bioactive compounds with antibacterial, antifungal and antioxidant properties. The bacterial extract showed significant antimicrobial activity in terms of zone of inhibition, minimum inhibitory concentration, minimum bactericidal concentration, and minimum fungicidal concentration. Moreover, substantial capacity to alter or damage the inner membrane as well as the outer membrane of the gram-positive and gram-negative bacteria was exhibited by the bacterial extract. This membrane alteration or damaging potential of the extract is the mechanism of action. Biofilm formation inhibition property of the extract also signified its antimicrobial action, and possible use against resistant bacteria. The extract has shown notable activity against the virulence factors like prevention of hemolysis in bacteria and inhibition of secreted aspartyl proteinase in fungi. These functions of the bacterial extract have revealed the extent of its action in the prevention of infection by terminating the secretory virulence factors and by damaging the tissue.

**Keywords** Antibacterial · Anti-biofilm · Antifungal · Mangrove · Metabolite profiling

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## Introduction

Microbial infections are a grave concern to the world and their treatments are posing a serious threat to human life due to multidrug resistance. Methicillin-resistant *Staphylococcus aureus* (MRSA) has caused the spread of community-associated infections with increased multidrug resistance (Thomas 1994). In a review, Lord Jim O'Neill (2014) has warned that antibiotic resistance can cause the death of 10 million people by 2050. To reverse the situation or to prevent death from multidrug-resistant pathogens, new antibiotics are the need of the hour. Presently the discoveries of new antibiotics are limited due to the exit of the pharmaceutical companies from this area of research and lack of interest from the other research communities (Cooper and Shlaes 2011). Investigations on terrestrial microorganisms or microorganisms from traditional habitats for the discovery of new antibiotics have proved to be constrained options as it frequently leads to the rediscovery of old molecules and appeared to be less

effective to treat multidrug-resistant infections (Xu 2012; Debbab et al. 2011). Currently, the search of new antibiotics has shifted from traditional habitats to new or extreme environment or ecology like marine, desert, or mangroves (Xu 2012, 2014). Mangroves are the locus of rich microbial diversity due to their distinctive characters like high rainfall, tidal flooding, light, salinity, and temperature (Feller et al. 2010; Andreote et al. 2012). Mangrove forests encompass numerous microorganisms and plants which could be the potential sources of new bioactive molecules owing to their distinct ecological and environmental attributes (Huang et al. 2012; Arumugam et al. 2010; Xu et al. 2014). Several novel bacteria and fungi have already been identified and reported from different mangrove forests (Biswas et al. 2017; Wei et al. 2011).

The Sundarbans is the largest tidal halophytic mangrove forest in the world and covers nearly 10,200 sq. km. in Bangladesh and India (Gopal and Chauhan 2006). The estuarine habitats of the Sundarbans harbor diverse biological entities with a constant supply of nutrients and biogeochemical cycling (Meire et al. 2005). Spatial differences and seasonal variations in biogeochemical processes help the region to maintain and produce the high biodiversity in this ecosystem. Though the Sundarbans is a detritus-based rich ecosystem, minimal data are available concerning its microbial diversity and abundance (Ghosh et al. 2010). Limited researches were carried out to date on bacterial identification and isolation of bioactive compounds from the Indian Sundarbans. However, few studies have highlighted the potential of the Sundarbans to yield novel bacteria and bioactive compounds (Arumugam et al. 2010, 2011; Biswas et al. 2017). The search for bacterial diversity and their novel bioactive metabolites from the Indian Sundarbans can address the global crisis of multidrug-resistant infections.

Actinomycetes are well-known producers of different secondary metabolites like antibiotics. Xiamycin, Salinosporamide A, Chalcomycin B, Antimycin A18, etc., are examples of the molecules produced by mangrove-derived actinomycetes (Xu et al. 2014). *Streptomyces euryhalinus* sp. nov. was isolated from the sediment sample of the Lothian Island of the Indian Sundarbans. It is a Gram-positive, aerobic bacteria which can grow with 0–20% of sodium chloride and is resistant to penicillin (Biswas et al. 2017). The present research is focused on the metabolite profiling and assessment of antibacterial and antifungal properties of *Streptomyces euryhalinus* culture extract. This study also analyzed the activities of the culture derived metabolites on the permeability of the bacterial membrane along with the termination of secretion of virulence factors by bacteria and fungi.

## Methods

### Collection of bacteria

*Streptomyces euryhalinus* (Biswas et al. 2017) was isolated from the sediment soil of the Lothian Island (Lat. 20° 50′ N, Long. 88° 19′ E) of the Indian Sundarbans (Saha et al. 2005). It was grown on storage medium (units: g/L: starch (potato) 10.0, casein (protein rich) 3.0, peptone 1.0, malt 1.0, yeast extract 1.0, dipotassium phosphate 0.5, distilled water 500 ml and artificial sea water 500 ml, agar 2%, pH 7.2) described by Biswas et al. (2017). The strain was incubated at 28 °C for 4 days for optimum growth. It is available in the Leibniz Institute DSMZ–German Collection of Microorganisms and Cell Cultures (DSM 103378$^T$) and Chinese Center for Industrial Culture Collection (CICC 11032$^T$). For lab storage, cultures of the strain were preserved in glycerol stocks (10–15%) in − 80 °C and − 20 °C freezers as well as in lyophilized powder form.

### Fermentation and extraction

Production of the antimicrobial component from the strain *S. euryhalinus* was carried out with the shake flask method as described by Saha et al. (2005) using a modified production medium (MPM: glucose 2.0, starch 2.0, yeast extract 0.5, soybean meal 2.0, $CaCO_3$ 0.32, NaCl 0.25, $CuSO_4$ 0.005, $ZnSO_4$ 0.005, $MnCl_2$ 0.005, distilled water 1000 ml, pH 7.2, units expressed in g/L) and incubated for 72–96 h at 30 °C. The filtered broth was extracted thrice using equal volume of ethyl acetate as solvent. Finally, the ethyl acetate extract was concentrated at 45 °C using rotary vacuum evaporator to get the crude extract.

### GC–MS analysis of *S. euryhalinus* extract

Gas chromatography (GC) analysis of culture extract obtained from *S. euryhalinus* was performed using Perkin Elmer Clarus SQ8C gas chromatograph equipped with DB 5 MS capillary standard nonpolar column (30 m × 0.25 mm × 0.25 μm film thickness). Helium at a flow rate of 1 mL/min was selected as a carrier gas and the split mode ratio was kept as 1:12. The injector temperature was fixed at 250 °C and the obtained peaks were identified by NIST library data.

### Antimicrobial activity

The antimicrobial activity of the ethyl acetate extract of *S. euryhalinus* was primarily evaluated by the agar well diffusion method (Hriduyatulla et al. 2018). About 20 μl of

exponential phase cultures of *Staphylococcus aureus* MTCC 2940, *E. coli* MTCC-1195 and *Candida albicans* MTCC 227 were spread onto the nutrient agar and Sarbouraud dextrose agar plates. Different concentrations of crude extract were placed into the wells. The plates were then placed overnight in an incubator at 37 °C and the clearance zones were noted as an indicator of antimicrobial activity.

## Determination of minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC)

The MIC values of the bacterial extract were evaluated against *S. aureus* MTCC 2940, *E. coli* MTCC-1195, and *Candida albicans* MTCC 227 following micro broth dilution method in 96-well microtiter plates (Sarker et al. 2007; Weigand et al. 2008). The bacterial and fungal strains were cultivated in Muller–Hinton Broth (MHB) and Sabouraud dextrose broth (SDB), respectively and incubated at 37 °C for 18 h. The turbidity for the test microbial cultures was adjusted to $10^6$ CFU per ml. Total 50 µl of MHB or SDB, 50 µl of extracts, and 10 µl of microbial cultures were added to each well making the final concentration of $5 \times 10^5$ CFU per ml. Resazurin (10 µl stock solution prepared by dissolving 270-mg tablet in 40-ml sterile distilled water) was added to the each well of microtiter plates after 18 h of incubation at 37 °C. The change of colour was observed and the lowest concentration with no change in color was marked as the MIC value. Minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) were determined by spreading cultures from the wells onto the corresponding plates. MBC or MFC of the samples was determined by observing the plates with a minimum concentration of the extract having no colony growth (Devi et al. 2010; Sundararaman et al. 2013).

## Activity of *S. euryhalinus* extract on bacterial membrane permeability

### Bacterial membrane integrity assay

The effect of the extract on bacterial cell membrane integrity was studied by recording the discharge of internal material which shows maximum absorbance at 260 nm (Li et al. 2014). Bacterial strains *S. aureus* MTCC 2940 and *E. coli* MTCC 1195 were used as test organisms. The bacterial cultures grown in nutrient broth were centrifuged at 8000 rpm for 10 min, cell pellets were washed and suspended in 0.5% NaCl. Specific concentrations (½ MIC, MIC, 2MIC) of *S. euryhalinus* extracts were added to the cell suspension and release of internal material was determined using a UV–visible spectrophotometer at 260 nm (Lambda 25 UV/visible spectrophotometer; Perkin Elmer, USA) for a period of 12 h.

Cell suspensions of test organisms not treated with the bacterial extract were considered as controls.

## Crystal violet assay for membrane permeability

Membrane permeability alternation capacity of the bacterial extract was evaluated using crystal violet assay (Li et al. 2014) with *S. aureus* MTCC 2940 and *E. coli* MTCC 1195. The bacterial strains were grown in nutrient broth and centrifuged at 9300*g* for 5 min to collect the cell masses. The bacterial cell pellets were washed and suspensions were prepared using 0.5% NaCl solution. Bacterial extract of ½ MIC, MIC, and 2 MIC doses were added in the cell suspension and incubated for 6 h at 37 °C. Centrifugation of the treated cell suspensions was carried out at 9300*g* for 5 min to collect the cell masses. The bacterial cells were suspended into 0.5% NaCl solution containing crystal violet (10 µg/ml), and finally incubated at 37 °C for 10 min. Finally, the optical density of the supernatant obtained after centrifugation of treated bacterial suspension at 13,400*g* for 15 min was measured using UV–Visible spectrophotometer at 590 nm. The crystal violet uptake of samples (as a percentage) was calculated as:

(Sample OD value)/(crystal violet solutions OD value) × 100.

## Bacterial inner membrane permeability assay

Discharge of cytoplasmic β-galactosidase in the culture medium by *E. coli* MTCC-1195 and *S. aureus* MTCC 2940 which was complemented with substrate o-nitrophenyl –β-D-galactoside (ONPG) after treating with bacterial extract was determined as inner membrane permeabilization (Ibrahim et al. 2000). Bacterial culture has grown in Luria–Bertani (LB) broth supplemented with 2% lactose was collected at logarithmic phase and centrifuged for 10 min at 11,000 g, and suspended in 0.5% NaCl solution after washing. Cell suspension (200 µl), ONPG (10 µl), and bacterial extract of different concentrations (1/2 MIC, MIC, 2 MIC) were taken in a 96-well plate. Release of o-nitrophenol was recorded using a spectrophotometer (Lambda 25 UV/visible spectrophotometer; PerkinElmer, USA) at 415 nm. Bacterial culture suspension including extract was considered as control.

## Evaluation for antibiofilm capacity

Inhibition of biofilm formation assay of the extract was carried out at different concentrations in 96-well microtiter plates. Cultures of *S. aureus* MTCC 2940 and *E. Coli* MTCC1195 were grown overnight in Luria broth and different concentrations (1/2MIC, MIC, 2MIC) of extract were added in 96-well microtiter plates which were incubated in a

static position for 24 h at 37 °C. Thereafter the free-floating cells along with excess broth were discarded and dried for 30 min at 60 °C after washing with phosphate buffer solution (PBS) (pH 7.2). Crystal violet solution (1%) was used to stain the biofilm produced in the wells for 15 min. After washing with distilled water, crystal violet bound to the biofilm was resolubilized with 200 µl of ethanol and the absorbance was recorded at 595 nm with the help of a microplate reader (Spectra Max M5, Molecular Device, USA) (Wu et al. 2015, 2016).

## Effects of *S. euryhalinus* extract on virulence factors produced by *S. aureus* MTCC 2940 and *E. coli* MTCC 1195

### Hemolysis assay

Hemolytic toxicity of the bacterial extract was evaluated using murine erythrocytes. About 10 ml of murine blood was centrifuged for 5 min at 2500 rpm, and pellets were cleansed using PBS. Erythrocyte suspension (3%) was prepared using PBS. Different concentrations (1/2 MIC, MIC and 2MIC) of bacterial extract were added to the erythrocyte suspension (10 µl). The supernatant was collected after centrifugation of the mixture at 2500 rpm for 5 min and the optical density was recorded spectrophotometrically (Lambda 25 UV/visible spectrophotometer; PerkinElmer, USA) at 540 nm for the determination of hemoglobin released (Kaur et al. 2017). Erythrocyte suspension along with sterile distilled water was considered as blank.

### Secreted aspartyl proteinase (Sap) inhibition assay using *C. albicans*

Candidal Sap inhibition activity of the bacterial extract was evaluated by following the method of Sundararaman et al. (2013). Mixture of *C. albicans* culture (100 µl), Sap induction medium (20 ml) (2.0 g yeast extract, 23.4 g yeast carbon base, and 4.0 g BSA in 1 L distilled H$_2$O, pH 5.0) was treated with different concentrations (1/2 MIC, MIC, and 2MIC) of bacterial extract under the incubation at 37 °C for 48 h with shaking. Thereafter, culture supernatant (0.1 ml) was added to 0.9 ml medium which comprised of citrate buffer (0.1 M, pH 3.2) and BSA (0.2% w/v) which was incubated for 1 h at 37 °C for evaluation of proteinase inhibition. About 1 ml of 5% (w/v) ice-cold TCA was added into the mixture to cease the reaction. Centrifugation of the mixture was carried out for ten minutes at 1500$g$ and optical density of the supernatant was recorded at 280 nm using a UV–visible spectrophotometer (Lambda 25 UV/visible spectrophotometer; PerkinElmer, USA). Sterile distilled water was considered as control.

## Statistical analysis

Experiments were performed in triplicate, and results are presented as mean ± SD.

## Results and discussion

### GC–MS profile of extract

GC–MS study revealed the presence of several bioactive compounds in the ethyl acetate extract of *S. euryhalinus.* Different known compounds like triclosan, 2,4-di-tert-butyl-phenol, dibutyl phthalate, phenanthrene, 16-methyl-, methyl ester, 17-pentatriacontene, 7,9-di-tert-butyl -1-oxaspiro (4,5) deca-6,9-diene-2,8-dione, benzenepropanoic acid 3,5-bis (1,1–148 dimethylethyl)-4-hydroxy- methyl ester, heptadecanoic, etc., were present which are known for their antioxidant, antibacterial, antimicrobial, antibiofilm and antifungal activities (Table 1) (Kovács et al. 2008; Padmavathia et al. 2015; Varsha et al. 2015). Compounds with the percentage of an area more than 0.284 (%) in the GC–MS spectrogram of the bacterial extract were selected for determination. The presence of unknown or new compounds in the extract cannot be ruled out though it is not indicated in the result after GC–MS.

### MIC, MBC and MFC of the extract

Zone of inhibition of extract against *S. aureus* MTCC 2940*, E. coli* MTCC 1195, and *Candida albicans* MTCC 227 in agar well diffusion method was found to be 25 mm, 20 mm and 14 mm, respectively. MIC of the extract against *S. aureus* MTCC 2940*, E. coli* MTCC-1195, and *C. albicans* MTCC 227 was recorded as 1 µg/ ml, 2 µg/ ml and 2 µg/ ml. MBC of the extract determined is 4 µg/ml and 8 µg/ ml against *S. aureus* MTCC 2940 and *E. coli* MTCC 1195, respectively. MFC of the extract against *C. albicans* MTCC 227 was 6 µg/ml.

### Effects of *S. euryhalinus* extract on bacterial cell membrane integrity

Disfigurement of the bacterial membrane was evaluated by the release of UV absorbing intracellular components. The *S. euryhalinus* extract showed the highest membrane damage activity at 2 MIC concentration against the test bacteria. The OD value increased from 0.081 to 0.412 at 2MIC concentration and at MIC concentration it was increased up to 0.379 in case of *E. coli*. In case of *S. aureus,* OD value reached 0.496 from 0.194 at 2MIC concentration whereas at MIC concentration OD value reached 0.412. (Fig. 1a, b). Results indicated that the presence of *S. euryhalinus* extract decreases

**Table 1** Presence of compounds in extract explored using GC–MS

| RT | Compound | Theoretical Formula | MW (g/mol) |
|---|---|---|---|
| 11.987 | 2,4-Di-tert-butylphenol | $C_{14}H_{22}O$ | 206.32 |
| 15.949 | 2-Propenoic acid, tridecyl ester | $C_{16}H_{30}O_2$ | 254.41 |
| 17.644 | Dibenzo [b,E][1,4]dioxin, 2,7-dichloro- | $C_{12}H_6Cl_2O_2$ | 253.08 |
| 17.804 | Phenanthrene | $C_{14}H_{10}$ | 178.23 |
| 19.375 | Phthalic acid, decyl isobutyl ester | $C_{22}H_{34}O_4$ | 362.51 |
| 19.695 | 8-Pentadecanone | $C15H30O$ | 226.40 |
| 20.240 | 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione | $C_{17}H_{24}O_3$ | 276.37 |
| 20.766 | Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester | $C_{18}H_{28}O_3$ | 292.41 |
| 21.271 | Dibutyl phthalate | $C16H22O4$ | 278.34 |
| 21.736 | Triclosan | $C_{12}H_7Cl_3O_2$ | 289.53 |
| 22.696 | à-D-Galactopyranoside, methyl 2,6-bis-O-(trimethylsilyl)-, cyclic butylboronate | $C_{17}H_{37}BO_6Si_2$ | 404.45 |
| 23.817 | Hexadecen-1-ol, trans-9- | $C_{16}H_{32}O$ | 240.43 |
| 24.087 | 16-Octadecenoic acid, methyl este | $C_{19}H_{36}O_2$ | 296.5 |
| 24.607 | Heptadecanoic acid, 16-methyl-, methyl ester | $C_{19}H_{38}O_2$ | 298.51 |
| 26.138 | 17-Pentatriacontene | $C_{35}H_{70}$ | 490.94 |
| 28.659 | [1,1'-Biphenyl]-2,3'-diol, 3,4',5,6'-tetrakis(1,1-dimethylethyl)- | $C_{28}H_{42}O_2$ | 410.63 |
| 29.259 | Hexanedioic acid, dioctyl ester | $C_{22}H_{42}O_4$ | 370.57 |
| 31.325 | Phthalic acid, di(2-propylpentyl) ester | $C_{24}H_{38}O_4$ | 390.56 |
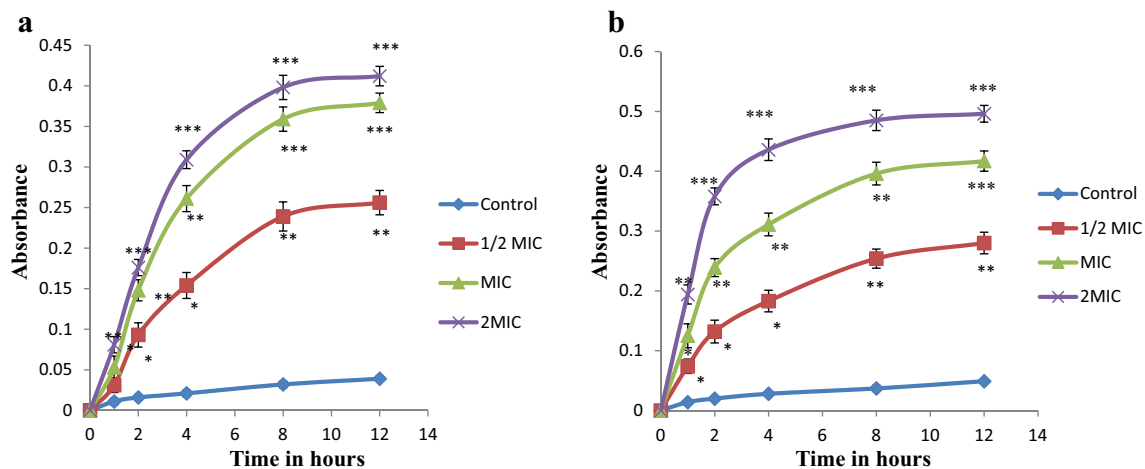


**Fig. 1 a** Cell membrane permeability effect of *S. euryhalinus* extract on *E. coli* MTCC 1195. Error bars are displaying the $\pm$ SD of three replicates. **b** Cell membrane permeability effect of *S. euryhalinus* extract on *S. aureus* MTCC 2940. Error bars are displaying the $\pm$ SD of three replicates *$p$ value $< 0.05$, **$p$ value $< 0.005$, ***$p$ value $< 0.001$

the rise of absorbance and it remains nearly constant over a prolonged time in case of Gram-negative bacteria. Contrarily, the absorbance changed abruptly and increased slowly after a long period in case of Gram-positive bacteria. This occurred as a result of dissimilarities in the membrane structure of gram-positive and gram-negative bacteria. Gram-positive bacteria have peptidoglycan layer with several pores which facilitate the entry of antimicrobial molecules into cell and thereby cause cellular damage (Tao et al. 2011). Results also corroborated damage of the cell membrane by

the *S. euryhalinus* extract after interaction with cytoplasmic membrane (Denyer 1990). Damage of cell membrane causes release of $K^+$, $PO^{4-}$, DNA, RNA, etc. (Li et al. 2014).

## Crystal violet assay of *S. euryhalinus* extract on bacterial membrane permeability

Crystal violet assay is helpful to evaluate the alteration in the bacterial membrane by the antimicrobial agents. In case of an altered or damaged membrane, crystal violet penetrates

the membrane easily whereas it fails to penetrate if the membrane is intact. The *S. euryhalinus* extract at 2 MIC concentration exhibited an increment of crystal violet uptake from 18.59 (control) to 74.49%, in case of *E. coli*, and in case of *S. aureus,* it is increased from 19.09 (control) to 79.63% (Fig. 2). Results exhibited by *S. euryhalinus* extract are comparable with studies conducted by Li et al. (2014), Sana et al. (2018). Extract showed higher membrane alteration property at MIC dose in comparison with carvacrol and ciprofloxacin (Khan et al. 2017; Sana et al. 2018). This result displayed the significant alteration in bacterial membrane permeability and membrane damage property of *S. euryhalinus* extract.

## Activity of *S. euryhalinus* extract on the permeability of the bacterial inner membrane

The capacity of the antimicrobials for permeability or penetration of bacterial inner membrane is manifested by the
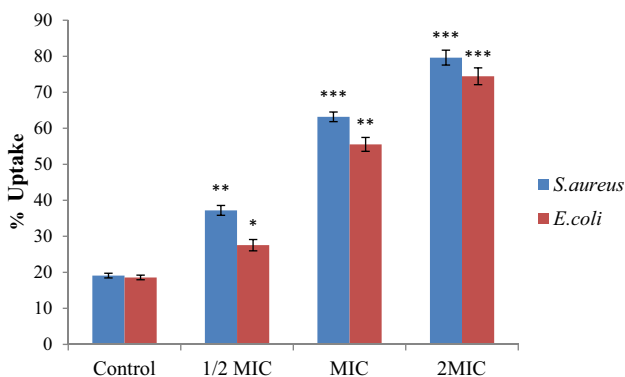
release of cytoplasmic beta-galactosidase in both gram-positive and gram-negative bacteria. The obtained results revealed discharge of cytoplasmic beta-galactosidase is dose and time-dependent (Fig. 3a, b). Greater absorbance was found at 2MIC concentration than MIC concentration which signifies that *S. euryhalinus* extract showed a greater inner membrane permeability effect at dose of 2MIC. The obtained results showed a sharp increase of absorbance up to 80 min for gram-positive and gram-negative bacteria, followed by steady-state. This result is significant and comparable with the research conducted by Je and Kim (2006) and Ibrahim et al. (2000). These empirical values indicate occurrences of changes or damage of the inner membrane of bacteria by the extract. Therefore, *S. euryhalinus* extract has the potential of causing both inner as well as outer membrane alterations of the targeted bacterial strains.

## Inhibition of biofilm formation by *S. euryhalinus* extract

Biofilm formation by the microbes or microbial communities is directly involved with antibiotic resistance (Michalska and Wolf 2015; Simones et al. 2009). *S. euryhalinus* extract demonstrated notable biofilm inhibition activity in a dose-dependent manner. In case of *E. coli*, inhibition of biofilm formation at MIC and 2 MIC dose was observed as 53.64% (±0.60) and 67.90% (±0.027), respectively (Fig. 4a). 2 MIC doses of the extract displayed 78. 49% (±0.037) inhibition of biofilm formation against *S. aureus* whereas 62.99% (±0.049) inhibition was observed at MIC concentration (Fig. 4b). The antibiofilm potential of *S. euryhalinus* extract can be compared with earlier researches (Kaur et al. 2017; Yadav et al. 2015). Extract with significant antibiofilm activity could be a potential agent to fight against the biofilm forming bacterial pathogens with antibiotic resistance.



**Fig. 2** Alteration in uptake of crystal violet in *S. aureus* MTCC 2940 *and E. coli* MTCC 1195 after treatment with *S. euryhalinus* extract. Error bars are displaying the ± SD of three replicates *$p$ value < 0.05, **$p$ value < 0.005, ***$p$ value < 0.001
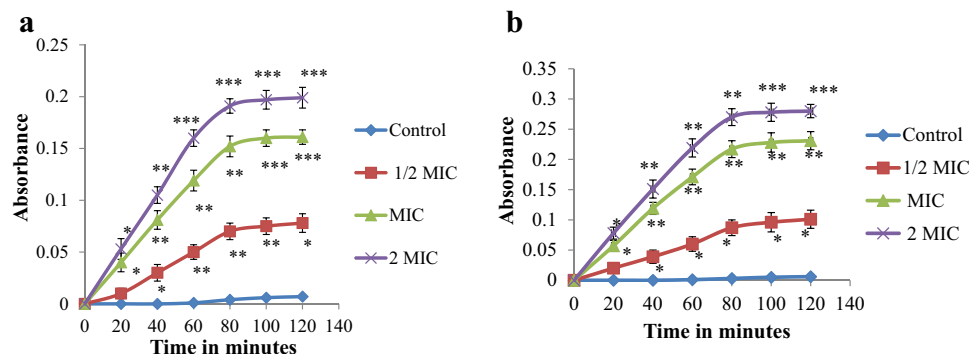


**Fig. 3** **a** Evaluation of permeability of inner cell membrane recorded through release of cytoplasmic β- galactosidase from *E. coli* MTCC 1195 upon treatment with *S. euryhalinus* extract. Error bars are displaying the ± SD of three replicates. **b** Evaluation of permeability

of inner cell membrane recorded through release of cytoplasmic β-galactosidase from *S. aureus* MTCC 2940 upon treatment with *S. euryhalinus* extract. Error bars are displaying the ± SD of three replicates *$p$ value < 0 .05, **$p$ value < 0.005, ***$p$ value < 0.001

**Fig. 4** **a** The inhibition of *E. coli* MTCC 1195 biofilm formation by *S. euryhalinus* extract recorded at 595 nm. Error bars are displaying the ± SD of three replicates. **b** The inhibition of *S. aureus* MTCC 2940 biofilm formation by *S. euryhalinus* extract recorded at 595 nm. Error bars are displaying the ± SD of three replicates, \*$p < 0.005$, \*\*$p < 0.001$
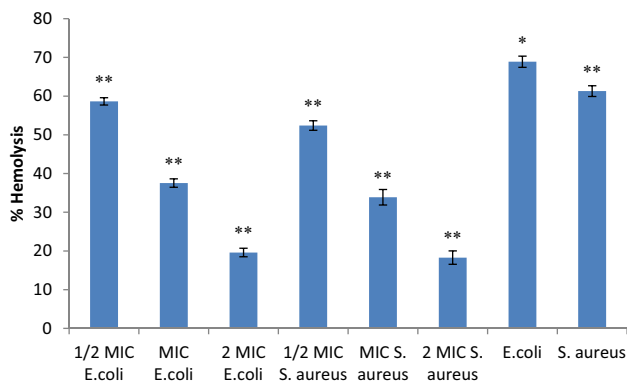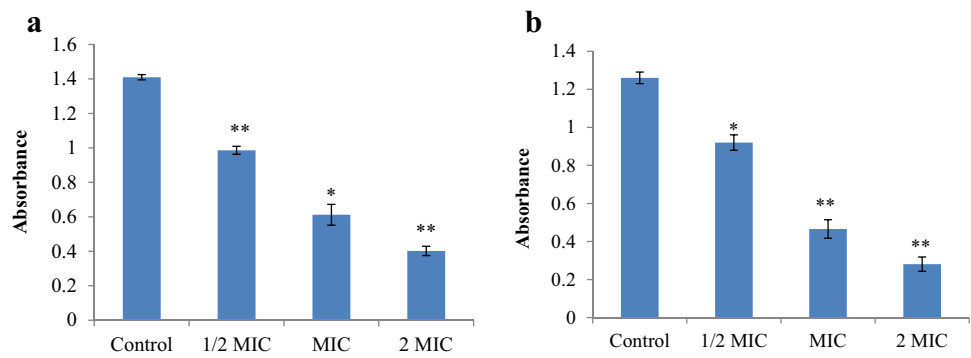




**Fig. 5** Inhibition of hemolysis of erythrocyte by *S. euryhalinus* extract on *Staphylococcus aureus* MTCC 2940 and *E. coli* MTCC 1195. Error bars are displaying the ± SD of three replicates, \*\*$p$ value < 0.005

**Fig. 6** Inhibition of secreted aspartyl proteinase (Sap) by *S. euryhalinus* extract on *Candida albicans* MTCC 227. Error bars are displaying the ± SD of three replicates, \*$p$ value < 0.05, \*\*$p$ value < 0.005, \*\*\*$p$ value < 0.001

## Hemolysis activity of *S. euryhalinus* extract

Reduction of hemolysis was displayed by extract at 2 MIC dose from 61.29% (± 0.0009) to 18.28% (± 0.0002) in case of *S. aureus* (Fig. 5). In case of *E. coli*, hemolysis is reduced from 68.88% (± 0.002) to 19.6% (± 0.001) at 2 MIC concentration (Fig. 5). Hemolysin toxin is a virulence factor produced by the pathogenic bacteria which helps to expedite the infection (Kupferwasser et al. 2003). This result may be attributed to the capacity of *S. euryhalinus* extract for inhibition of bacterial virulence factor.

## Action of extract on secreted aspartyl proteinase (Sap) inhibition

The *S. euryhalinus* extract showed a gradual drop of Sap inhibition activity in *C. albicans*. The 2 MIC dose of the extract was found to exhibit the highest inhibition activity among other concentrations. The effective concentration of the extract was comparatively lower than that of standard drugs (Fig. 6). The observed results were found to be comparable with previous studies (Schaller et al. 2003;
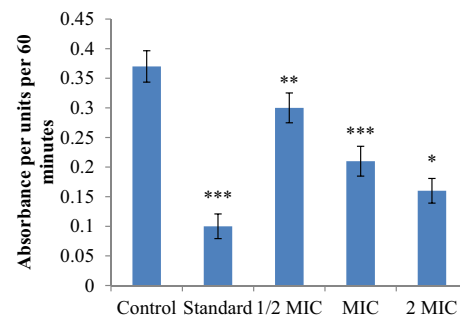
Sundararaman et al. 2013). Proteinase activity of *C. albicans* corresponded to the virulence factor MacDonald and Odds 1983), the tissue invasion, and damage capacity (Schaller et al. 1999; Borg and Rüchel 1988). Therefore *S. euryhalinus* extract at 2 MIC dose could suppress virulence factor and tissue damage capacity of *C. albicans*.

## Conclusion

New or novel antibiotics are indispensable to fight against antibiotic resistance. Actinomycetes are a promising source of bioactive metabolites. Mangrove being a unique ecology, may serve as the potential supplier of new or novel bacteria and their secondary metabolites. GC–MS profile of ethyl acetate extract obtained from *S. euryhalinus* culture has shown the presence of several important metabolites which are biologically active. The *S. euryhalinus* extract exhibited significant MIC, MBC and MFC values against both bacteria and fungi. This study showed that ethyl acetate extract can alter or damage the inner membrane as well as the outer membrane of gram-negative and gram-positive bacteria. 2 MIC concentration of the extract has shown the highest activity in bacterial membrane damage. The membrane

damaging properties of *S. euryhalinus* extract were found more potent against gram-positive bacteria than gram-negative bacteria due to dissimilarities in their cell membrane structure. Antibiofilm formation capacity of the extract validated antimicrobial activity in relation to antibiotic resistance. Hemolysis prevention and Sap inhibition activities of the bacterial extract have strengthened the evidence of prevention or termination of bacterial and fungal virulence factors. This bioassay guided comprehensive study indicated that the ethyl acetate extract obtained from *S. euryhalinus* has potent antimicrobial activity expressed through cell membrane destruction mechanism.

**Author contributions** KB conceptualized and performed all experiments, and framed the article, DB supported to perform the cell membrane related experiments, interpretation of data and reviewed the article, MS helped to analyses the data and reviewed the article, JM conceptualized the study, supervised the study, and reviewed the article, SK supported in antibiofilm assay and hemolysis assay, and reviewed the article.

## Declarations

**Conflict of interest** We declare no conflict of interest.

**Accession numbers** Chinese Centre for Industrial Culture Collection 11032$^T$, Leibniz Institute DSMZ–German Collection of Microorganisms and Cell Cultures DSM 103378$^T$.

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