



Research Article

Phytochemical profiling, antioxidant potentiality and antibacterial activity of the ethanolic extracts of *Rosenvingea* sp. of Bay of Bengal

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ABSTRACT

The objective of the present study was to explore the phytochemical profiling, antioxidant and antimicrobial activity of 50% ethanolic extract of *Rosenvingea* sp. found in the Bay of Bengal of Bangladesh. Seven phytochemicals were tested from the ethanolic extract of *Rosenvingea*, where four phytochemicals, namely steroid, glycosides, alkaloids, and tannins were present. However, ethanol extract exhibited low antioxidant activity compared to standard ascorbic acid as measured by DPPH-Free radical scavenging assay. In Brine Shrimp lethality bioassay, 50% ethanolic extract showed an LC₅₀ value of 10.88 mg/mL, whereas positive control (K₂Cr₂O₇) showed LC₅₀ 59.97 µg/mL suggesting the less toxic property of the ethanolic extract. *In-vitro* antimicrobial activity of ethanolic extract of *Rosenvingea* sp. was investigated against gram-positive and gram-negative bacteria species (*Staphylococcus*, *Bacillus*, *Pseudomonas*, *Salmonella*, and *Klebsiella*) by agar disc diffusion method. The highest antibacterial activity was noticed against *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Staphylococcus hominis* with a zone of inhibition of 5.00±1.00 mm, 3.66±0.57 mm, and 3.33±0.57 mm, respectively. This is the first study on *Rosenvingea* sp. from the Bay of Bengal, reporting its phytochemical, antioxidant, and antimicrobial potentiality. However, more study is required to elucidate its commercial viability in the food and medicine industries.

Introduction

In recent years, many reports have focused on structurally new and biologically active metabolites found in marine sources. The brown seaweeds of *Rosenvingea* (*Scytosiphonaceae*, *Phaeophyceae*) are a tropical, subtropical marine plant distinguished by their cylindrical to somewhat compressed, dichotomous, or alternately branched, erect, hollow thalli with plurangia forming surface sori. Currently, nine known

Rosenvingea species (*R. sanctaerucis* Børgesen, *R. fastigate* Børgesen, *R. intricata* Børgesen, *R. orientalis* Børgesen, *R. floridana*, *R. nhatrangensis*, *R. antillarum*, *R. australis* and *Rosenvingea stellata* Børgesen) are found worldwide (Santiañez and West, 2019; *World Register of Marine Species*, 2020). The genus *Rosenvingea* (*Phaeophyceae*, *Scytosiphonaceae*) was first discovered by Børgesen (1914), which

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included several species, and molecular phylogeny of these species were recorded by modern scientific approaches (Jo et al., 2006). Though the taxonomic description of *Rosenvingea sp.* and their different habitat at Arabian seas available (Huisman et al., 2018; Aisha and Shameel, 2016). However, no such information is available for *Rosenvingea sp.* of Bay of Bengal.

The culture and asexual life cycle of brown algae from pacific Mexico have been well studied (West et al., 2010), and their commercial applications in food and medicine have been characterized by molecular techniques (Klochkova et al., 2017). Plants-associated microorganisms play a role in plant seed germination, growth, roots, shoots development by increasing photosynthetic capacity (Tapia et al., 2016). Marine macroalgae-associated bacteria also have represented a broad spectrum of antibacterial activity and potential therapeutic agents to develop pharmaceuticals (Kizhakkekalam and Chakraborty, 2019). In the last few years, brown algae have been considered a rich source of bioactive compounds like phloroglucinol. Its derivatives have shown anti-inflammatory, antiproliferative, antioxidants, and antidiabetics antiallergic, antiaging effects (Abdel Hamid et al., 2018). Health benefits like neuroprotective potential, different antifungal activity, including fluconazole-resistant strain, and anticancer activity on specific cancer cell lines have also been reported (Martins et al., 2018).

Diabetes mellitus or commonly known as diabetes is considered one of the significant risk factors for mortality worldwide, and *Rosenvingea sp.* has shown to have substantial antidiabetic activity (Yuvaraj et al., 2017). This Marine algae also reported

antiprotozoal activity and found it very effective against Leishmaniasis, a vector-borne neglected tropical disease caused by protozoan parasites of the *Leishmania* genus and transmitted by the female *Phlebotomus* and *Lutzomyia* sand flies (Yamthe et al., 2017). On the other hand, sulfated polysaccharide H3-a1 isolated from edible brown seaweed significantly affected the growth of human acute promyelocytic leukemia cells (HL-60), human breast carcinoma (MCF-7), and human hepatocellular carcinoma cancer cell lines (Wang et al., 2010).

Seaweeds serve as a reservoir of the mass number of macronutrients and micronutrients as an alternative to vegetables, a potential source of natural antioxidants, and high quality of PUFA and minerals (Kumar et al., 2011). They are also used as a rich essential fatty acid source for mammals (Nogueira et al., 2017). Other than these, seaweed liquid fertilizer has been getting its popularity as it possesses important plant growth factors like auxin, cytokinin, and gibberellins which are important for plant growth, seed germination, and photosynthetic pigmentation (Thirumaran et al., 2009).

To date, 193 seaweed species have been identified in Bangladesh; out of them, 19 species have been reported to have commercial importance, associated with 94 genera (Sarkar et al., 2016). The present study has shown the qualitative phytochemical analysis, potential antioxidant activity, cytotoxic and antibacterial activity of the *Rosenvingea* ethanol extract found in St. Martin Island of Bay of Bengal for the first time. However, further investigation is necessary to perceive their commercial importance.

Materials and Methods

Sample Collection and Processing

Rosenvingea samples were collected from the southernmost tip of the country, St. Martin Island of Bangladesh, roughly between 20°37'16.9.32" N and 267°41'11.256" W and separated by a channel from the mainland about 13 km (Figure 1). Water temperature and salinity of the collection area fluctuated from 22-29°C and 21.0-33.5 PSU respectively, and average turbidity was 1.5 m to 8.0 m in shoreline (Khan et al., 2016).



Fig. 1. Collected *Rosenvingea* sample from Bay of Bengal

The samples were collected from this place because of its abundance in that area. The sample was washed with fresh seawater to remove epiphyte, debris, and other wastes. It was then rinsed with distilled water and preserved in a two-liter light protective jar containing 50% ethanol. The collected sample was identified by the World Register of Marine Species, Macroalgal Herbarium Portal & Algae Base (*World Register of Marine Species*, 2020). The sample was taken out from preservation and washed again with sterile distilled water and cut into small pieces for drying at 37°C in a shade dryer. The dried samples were then powdered with mortar pestle and stored at room temperature or -20°C until further use.

Extract preparations

1g powder was transferred to a 250 mL conical flask containing 10 gL 50% Ethanol and kept in a shaking incubator with 150 rpm at 25°C for 4 weeks after maceration samples were filtered by double-layer filter paper. (Whatman® qualitative filter paper, Grade 1 circles, diam.15 mm) (Gul et al., 2017).

Phytochemical screening

The qualitative phytochemical analysis of phenolic compounds, tannins, alkaloids, steroids, steroidal glycosides, flavonoids, and saponins was determined using different methods described previously, i.e., phenolic compounds with lead acetate test, tannins with ferric chloride test, and alkaloids with Mayer's test, steroids, and glycosides with Salkowski's test, flavonoids with alkaline reagent test and saponins with the frothing test. (Vimalkumar et al., 2014; Daisy et al., 2016; Gul et al., 2017; Dahanayake et al., 2019; Ahsan et al., 2020; Islam et al., 2020; Rahman et al., 2021)

DPPH Scavenging Activity

DPPH scavenging activity was determined using a method described previously by Ahsan et al., 2020. In brief, 0.04 mg/mL of DPPH (2, 2-diphenyl-1-picrylhydrazyl) dissolved in 95% methanol at the total volume of 100 mL solution. 1.5 mL of sample (50 µL, 100 µL, 150 µL, 200 µL, 250 µL, 300 µL of sample added to 1450 µL, 1400 µL, 1300 µL, 1250 µL, 1200 µL of sample solvents respectively) mixed with 1.5 mL of DPPH solution and stored in darkness with room temperature for 1 hour. Then absorbance was measured with 517 nm wavelength using UV-VIS spectrophotometer.

DPPH free radical scavenging activity was calculated in the following formula (Blois et al., 1958)

$$\% \text{ inhibition} = \{1 - (\text{A sample} - \text{A blank}) / (\text{A control} - \text{A blank})\} \times 100$$

Where A sample= Absorbance of the sample (Sample dilution + DPPH solution)

A blank= Absorbance of blank for each sample dilution (sample dilution+DPPH solvent)

A control= Absorbance of control reaction (sample solvent + DPPH solution)

L-ascorbic acid was used as a positive control. All experiments were performed in triplicate.

Brine Shrimp Lethality Assay (BSLA)

Brine shrimp lethality assay was used to test the cytotoxicity activity of the 50% ethanolic extract using the previously mentioned method (Ahsan et al., 2020; Alam et al., 2011a,b). 200 mg of brine shrimp (*Artemiasalina*) eggs were hatched in 1.5 liters of seawater with continued aeration in a suitable jar illuminated by a 60 watts incandescent bulb for 24 hours. Air-dried crude extract was added to 5ml of seawater to achieve the final concentration of 2.5, 5, 7.5, 10, 20, and 40 mg/mL, respectively, and 10 nauplii were transferred in each test tube. After 24 hours, the number of dead nauplii was calculated in this formula.

$$\text{Mortality (\%)} = (\text{number of dead nauplii}) / (\text{number of dead nauplii} + \text{number of alive nauplii}) \times 100$$

270, 225, 180, 135, 90, 45 $\mu\text{g/mL}$ concentration of ($\text{K}_2\text{Cr}_2\text{O}_7$) potassium dichromate used as a positive control. All experiments were done in triplicate.

Antimicrobial Activity

The antimicrobial activity of the *Roseningea* extract was determined by the agar well diffusion method against gram-positive and gram-negative bacteria (Aguree and Onilimor, 2019). Bacterial strains were cultured in LB broth and incubated at 37°C for 18 hours in an incubator. All strains were diluted with sterile distilled water after incubation, and prior to using all inoculums were incubated for 30 minutes at 37°C. 70 μL of 50% ethanolic extract of different concentrations were loaded in respective wells. Commercial antibiotic disc, Tetracycline 30, Kanamycin 30, Ciprofloxacin 5, Ampicillin 10 were obtained from Bio Maxima S.A, Lublin, Poland and were used as a positive control, and sample solvents were used as a negative control. For the diffusion of the extracts, the Petri dishes were left for an hour with closed lids. The Petri dishes were incubated overnight at 37°C or 18 hours, and the zone of inhibition around the well was measured with the help of a ruler and recorded for further calculation. All tested were done in triplicate.

Determination of Minimal Inhibitory Concentration (MIC)

The minimal inhibitory concentration was determined by the agar well diffusion method with slight modification (Okeke et al., 2001). 70 μL of the volume was applied with 6.25, 12.5, 25, 50 and 100 mg/mL of concentration where the commercially antibiotic disc Tetracycline 30, Kanamycin 30, Ciprofloxacin 5, Ampicillin 10 used as a positive control, and 10x, 5x, 1x dilution of 50% Ethanol used as a negative control. All plates were incubated at

37°C for 24 hours. The least concentration of *Rosenvingea* ethanol extract was taken as MIC, which was compared with the commercial antibiotic disc. All tests were done in triplicate.

Statistical Analysis

Using the ANOVA test, two samples t-test, IC₅₀ calculated by linear regression, LC₅₀ was calculated by Probit analysis. Mean±SD was used for triplicate data and p values <0.05 for significance.

Results and Discussion

Phytochemical screening

The first step in determining the existence of bioactive compounds is to conduct phytochemical screening. Due to the qualitative and quantitative differences in the existence of bioactive compounds in the same organisms as a result of different solvents, storage conditions, and ultimately biodiversity, we attempted to do the phytochemical screening of this seaweed, according to some other

published research (Rahman et al., 2021). A phytochemical study was carried out on 50% ethanolic extract of *Rosenvingea*. Here, the four weeks macerated sample showed Alkaloids, Steroids, Glycosides, and Tannins (Table 1).

UV-Vis Spectrum

UV Visible Spectral analysis of 50% Ethanol extract of *Rosenvingea* was selected from 1100 nm to 190 nm wavelength for the preliminary detection by their wavelength and absorbance. The absorbance profile reflects the presence of different phyto-chemicals at different wavelengths ranging from 190 nm to 1100 nm. Absorption spectra are distinctive for flavonoids and their derivatives, and here in this study, flavonoids gave absorbance at 195.5 nm. Thus, these extract spectral position and their intensities provide valuable information for flavonoids from the brown algae of *Rosenvingea*. (Rajeswari and Jayaparakash, 2019) (Table 2).

Table 1. Qualitative test of the phytochemicals of 50% ethanolic extract of *Rosenvingea*.

Samples	Steroids	Glycosides	Flavonoids	Alkaloids	Tannins	Phenolic	Saponins
50% EtOH ethanolic extract of <i>Rosenvingea</i>	+	++	-	+	+	-	-

(-): not detectable, (+): low quantities, (++) : moderate quantities.

Table 2. Wavelength and Absorbance of 50% EtOH of *Rosenvingea* extract were selected in a dual-beam spectrophotometer.

UV-Spectroscopy			
No	Solvents	Wavelength (nm)	Absorbance
1	50% EtOH ethanolic extract of <i>Rosenvingea</i>	195.50	0.646

UV-Spectroscopy: 190 nm to 1100 nm selected.

Antioxidants, DPPH Scavenging Activity

Due to various metabolic activities, lots of free radicals are generated in the human body, which is compensated through free radical scavengers, antioxidants as a part of homeostasis (Tlili and Sarikurku, 2020). It is well known that seaweeds are a rich source of antioxidants (Cahyana et al., 1992; Alam, 2020). To find out the possible role of this seaweed, DPPH radical scavenging assay was done (Fig. 2). The highest scavenging activity ($27.61 \pm 16.59\%$ inhibition) in 30 minutes of 50% ethanol extract at 300 μ l (30 mg) was found, out of 100 mg/mL of stock concentration. The IC_{50} value was determined by linear regression of extract, and two-tailed t-tests were determined.

In our phytochemical screening study, we confirmed the presence of tannin, and alkaloids, which would account for the antioxidant activity based on the previous report (Osawa, 1994; Khandare, 2012; Ponnaniakamideen et al., 2014). So, the antioxidant compounds in the ethanolic extract are established by the IC_{50} value, which was 109246.7 μ g/mL at 30 minutes, where the IC_{50}

for standard ascorbic acid was 8.45 μ g/mL (Table 3). However, this value also indicates the possibility of having more antioxidant activities if the extract is prepared with other organic solvents like chloroform, methanol, etc.

Cytotoxicity Assay

The mortality of $86.66 \pm 5.77\%$ was found with 50% Ethanol extract, where the stock concentration was 50 mg/mL. LC_{50} values calculated by Probit regression analysis and two-tailed t-test was determined with every group (Figure 3). The median lethal concentration (LC_{50}) for 50% ethanolic extract was 10.88 mg/mL, where for the positive control ($K_2Cr_2O_7$), it was 59.97 μ g/mL. The toxicity level was determined by Clarkson's toxicity index, where 100-500 μ g/mL is considered highly toxic (Clarkson et al., 2004). To determine median lethal concentration, Meyer's toxicity index was followed where <1000 μ g/mL is considered toxic (Meyer et al., 1982). So, the ethanolic extract of *Rosenvingea* is less toxic compared to potassium dichromate (Fig. 3).

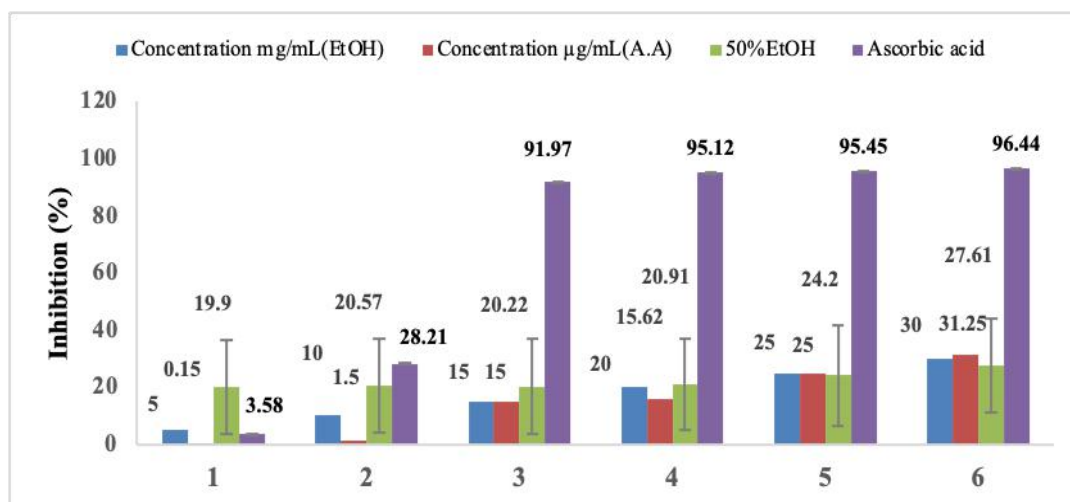


Fig. 2. Antioxidant activity of 50% ethanolic extract of *Rosenvingea* measured by DPPH assay. Ascorbic acid was used as positive control and percentage of inhibition was calculated.

Table 3. DPPH scavenging activity test.

NO	Sample	Concentration	Inhibition %	IC 50 $\mu\text{g/mL}$
1	50% EtOH extract of <i>Rosenvingea</i>	5 mg/mL	19.90 \pm 16.42	109246.7
		10 mg/mL	20.57 \pm 16.35	
		15 mg/mL	20.22 \pm 16.52	
		20 mg/mL	20.91 \pm 15.90	
		25 mg/mL	24.20 \pm 17.63	
		30 mg/mL	27.61 \pm 16.59	
2	Ascorbic acid	0.15 $\mu\text{g/mL}$	3.58 \pm 0.10	8.45539826
		1.5 $\mu\text{g/mL}$	28.21 \pm 0.15	
		15 $\mu\text{g/mL}$	91.97 \pm 0.11	
		15.625 $\mu\text{g/mL}$	95.12 \pm 0.04	
		25 $\mu\text{g/mL}$	95.45 \pm 0.11	
		31.25 $\mu\text{g/mL}$	96.44 \pm 0.13	

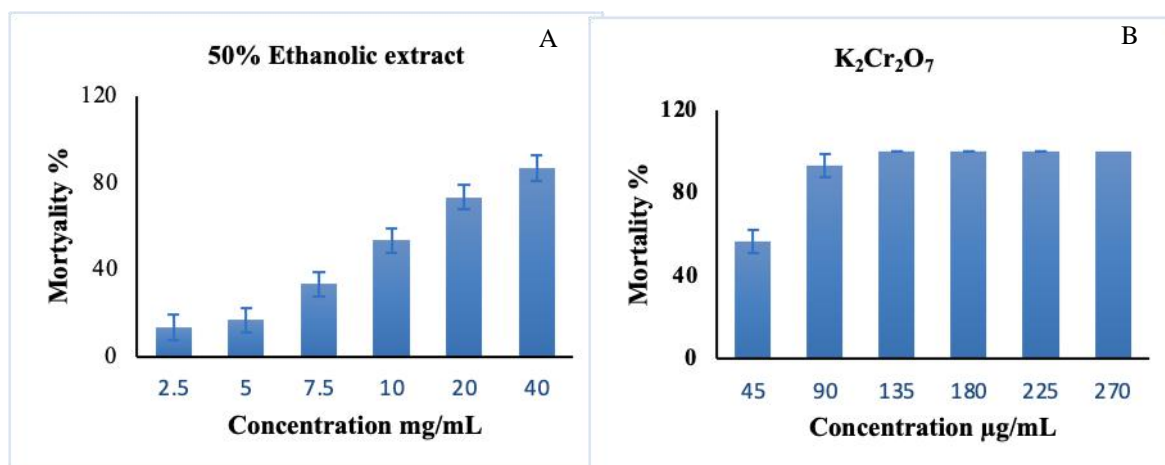


Fig. 3. (A) LC₅₀ value of 50% ethanolic extract of *Rosenvingea*. (B) Positive control ($\text{K}_2\text{Cr}_2\text{O}_7$).

Antibacterial activity

An ethanolic extract used *in vitro* against three gram-positive and four gram-negative bacteria. The highest zone of inhibition was found in one gram-positive and two gram-negative bacteria. *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Staphylococcus hominis* with inhibition of 5.00 \pm 1.00 mm, 3.66 \pm 0.57 mm & 3.33 \pm 0.57 mm, respectively, whereas commercial ampicillin was resistant in gram-negative, Tetracycline, Ampicillin,

Ciprofloxacin, Kanamycin used in positive control and sample solvent used as negative control (Table 4).

So, it is evident from the comparative study that gram-negative bacteria were better sensitive than gram-positive bacteria against the ethanolic extract suggesting its good candidature for antimicrobial research in the future. Based on our phytochemical screening

Table 4. Zone of Inhibition with different concentrations of 50% EtOH extract of *Rosenvingea*.

No.	Indicator Strain	Concentration mg/ml	Zone of Inhibition (Excluding negative control)	(+) Ve control	
				50% EtOH extract of <i>Rosenvingea</i>	Commercial disc Zone of inhibition (Mean±SD)
1	<i>Staphylococcus aureus</i>	2.5 mg/mL	-	Tetracycline (30)	21.00±1.00 mm
		5.0 mg/mL	-	Ampicillin (10)	2.66±0.57 mm
		10.0 mg/mL	-	Ciprofloxacin (5)	22.00±1.00 mm
				Kanamycin (30)	15.66±0.57 mm
2	<i>Bacillus cereus</i>	2.5 mg/mL	-	Tetracycline (30)	7.00±1.00 mm
		5.0 mg/mL	-	Ampicillin (10)	Resistance
		10.0 mg/mL	-	Ciprofloxacin (5)	19.33±0.57 mm
				Kanamycin (30)	15.00±1.00 mm
3	<i>Staphylococcus hominies</i>	2.5 mg/mL	-	Tetracycline (30)	21.00±1.00 mm
		5.0 mg/mL	-	Ampicillin (10)	3.66±0.57 mm
		10.0 mg/mL	3.33±0.57 mm	Ciprofloxacin (5)	22.00±1.00 mm
				Kanamycin (30)	18.66±1.15 mm
4	<i>Pseudomonas aeruginosa</i>	2.5 mg/mL	-	Tetracycline (30)	13.33±0.57 mm
		5.0 mg/mL	-	Ampicillin (10)	Resistance
		10.0 mg/mL	3.66±0.57 mm	Ciprofloxacin (5)	31.00±1.00 mm
				Kanamycin (30)	28.66±1.15 mm
5	<i>Salmonell Typhimurium</i>	2.5 mg/mL	-	Tetracycline (30)	2.33±0.57 mm
		5.0 mg/mL	-	Ampicillin (10)	Resistance
		10.0 mg/mL	-	Ciprofloxacin (5)	4.66±0.57 mm
				Kanamycin (30)	17.33±1.15 mm
6	<i>Salmonella typhi</i>	2.5 mg/mL	-	Tetracycline (30)	20.00±1.00 mm
		5.0 mg/mL	-	Ampicillin (10)	14.66±1.15 mm
		10.0 mg/mL	-	Ciprofloxacin (5)	14.33±0.57 mm
				Kanamycin (30)	17.33±0.57 mm
7	<i>Klebsiella pneumonia</i>	2.5 mg/mL	-	Tetracycline (30)	7.33±0.57 mm
		5.0 mg/mL	-	Ampicillin (10)	Resistance
		10.0 mg/mL	5.00±1 mm	Ciprofloxacin (5)	11.33±1.15 mm
				Kanamycin (30)	10.66±1.15 mm

Each value is presented as mean ± SD, (-) = no zone of inhibition.

and previous report, it can be said that the presence of alkaloids would account for the antimicrobial activity, which would possibly perturb bacterial FtsZ-Z ring formation and inhibits bacterial cytokinesis leading to its bactericidal activity (Beuria et al., 2005).

Minimum Inhibitory Concentration (MIC)

MIC (Minimum Inhibitory Concentration) of the *Rosenvingea* ethanolic extract was found to be greater than conventional resistance antibiotic disc with *Klebsiella pneumonia*. However, *Rosenvingea* ethanolic extract did not show considerable MIC with bacterial strains (Table 5). Usually, the antimicrobial compound is more bacteriostatic when the

MIC inhibits the zone with the lowest concentration. So, *Rosenvingea* ethanol extract is likely more bacteriostatic comparing to the traditional resistance antibiotic and in different conditions.

Effect of temperature on antimicrobial potency

The *Rosenvingea* 50% ethanol extract was pre-incubated in the water bath for 30 minutes from 30°C and 90°C to investigate the effect of temperature on antimicrobial potency. This treated extract clearly showed a zone of inhibition with *Klebsiella pneumonia* (Table 6), suggesting its capacity to retain antimicrobial potency even at high temperatures. However, the extract lost its antimicrobial potency at 90°C.

Table 5. MIC of 50% *Rosenvingea* ethanol extract.

Indicator strain	MIC according to zone of inhibition
	50% EtOH extract of <i>Rosenvingea</i>
<i>Klebsiella pneumonia</i>	3.5 mg/mL
<i>Staphylococcus aureus</i>	Nil
<i>Bacillus cereus</i>	Nil
<i>Staphylococcus hominies</i>	7.0 mg/mL
<i>Pseudomonas aeruginosa</i>	7.0 mg/mL
<i>Salmonellatyphimurium</i>	Nil
<i>Salmonella typhi</i>	Nil

Table 6. Effect of temperature on the antimicrobial potency of *Rosenvingea* ethanolic extract.

Temperature Celsius)	Antimicrobial potency
30	+
60	+
90	-

+ Antimicrobial Potency, - no antimicrobial potency

Conclusion

This study has explored the various phytochemicals, including steroids, glycosides, alkaloids, and tannins, in the brown seaweeds of *Rosenvingea* for the first time. The antioxidant property of ethanolic extract being slightly lower than standard ascorbic acid. So, this seaweed may have promising antioxidant potential, but further elucidation is necessary to prove its commercial viability. This brown seaweed of *Rosenvingea* also showed very low cytotoxicity when it was compared with the positive control ($K_2Cr_2O_7$), which suggests its possibility to be used as a potential candidate in developing anti-proliferative agents or cancer research. Another notable feature of this extract is the antimicrobial activity against gram-positive and gram-negative human pathogenic bacteria. This extract also showed its capacity to retain antimicrobial potency at high temperatures and in ampicillin resistance strain. So, that it can be a promising antibacterial candidate in developing next-generation antibiotics and drugs, in this study, data obtained regarding the effects of the *Rosenvingea* extract in vitro were promising and highlighted their potentiality for commercial applications in the future.

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