Extraction isolation purification and Analysis of Antimicrobial Properties in the Extracts of Gracillaria corticata

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ABSTRACT
10g of raw materials taken in 50 ml of Methanol, kept for 4hr extraction on water bath at 50oC, Filtered using Whatmann Filter paper No. 1, Methanol filtrate was kept for evaporation on water bath at 50 degrees Celsius, based on yield Red algae methanol crude (RMC) was taken for liquid-liquid extraction using Chloroform and methanol among which Fraction RMLLMF (Red Methanol L-L Methanol fraction) was taken up for further purification using column chromatography and the fraction RCF2 with purity 97.89% in HPLC was subjected to Anti-microbial analysis using well- diffusion method. Over all, the compound showed best inhibitory activity against Staphylococcus aureus, Bacillus subtilis and Candida albicans.
Keywords: Antimicrobial, MTT, Gracillaria corticata, Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli, Aspergillus niger, Candida albicans
Abbreviations: RCF1 (Red column fraction 1), RCF2 (Red column fraction 2), RCF3 (Red column fraction 3), RCF4 (Red column fraction 4), RMLLMF (Red Methanol liquid- liquid methanol Fraction), TLC (Thin Layer Chromatography), HPLC (High-Performance Liquid Chromatography), BMC (Brown algae methanol crude), RMC (Red algae methanol crude)
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INTRODUCTION

The significance of marine organisms, particularly macroalgae (seaweeds), as a rich source of natural products with potential therapeutic applications is being analysed worldwide. Seaweeds contain bioactive compounds with diverse biological activities, such as antibacterial, antifungal, and anti-inflammatory properties. Approximately 2400 natural products have been isolated from macroalgae, with potential applications in developing new drugs against cancer, microbial infections, and inflammations. Seaweeds are rich in essential nutrients like vitamins, iodine, potassium, and minerals. The ocean covers 70% of the Earth's surface and is home to a vast array of biological diversity. Marine organisms, including seaweeds, produce unique natural products with potential therapeutic applications. Seaweeds are eukaryotic organisms that live in salty water and are a potential source of bioactive natural products. They contain compounds like sterols, terpenoids, and brominated phenolic compounds that show bioactivity against microorganisms. These compounds have been found to have a range of biological activities, including Antibacterial, Antifungal, Antiviral, Anti-inflammatory, Antitumor, Cytotoxic (toxic to cells), Antimitotic (inhibiting cell division). The bioactive compounds found in seaweeds have potential applications in the development of new drugs against various diseases, including Cancer, Microbial infections, Inflammations (Seenivasan et al., 2010). Seaweeds are also a rich source of essential nutrients like vitamins, iodine, potassium, and minerals. Red algae, in particular, are known to synthesize halogenated metabolites, particularly bromine and iodine. These compounds have been found to have bioactive properties. Bioactive substances are compounds that have an effect on living cells or tissues. These substances can have beneficial or adverse effects, depending on their properties and the context in which they are used. Microbiologists and pharmacologists are interested in finding new bioactive substances that can be used to develop new drugs or treatments (Sudhakar et al., 2023).

Enteric pathogens, such as *Escherichia coli* and *Salmonella typhi*, are a major cause of diarrheal illness; these pathogens can cause severe dehydration, electrolyte imbalances, and even death, particularly in children under five years old. Seaweeds have been found to exhibit antimicrobial activity against a range of microorganisms,

including bacteria, fungi, and viruses, this activity is due to the presence of bioactive compounds that can inhibit the growth of microorganisms or kill them outright. The antimicrobial activity of seaweeds has been attributed to a range of compounds, including, Terpenoids, Phlorotannins, Steroids, Phenolic compounds, Halogenated ketones and alkanes, cyclic sulfides and Fatty acids. The bioactive compounds found in seaweeds have potential applications in the development of new antimicrobial agents. These agents could be used to treat a range of infections, including diarrheal illness. Seaweed-derived compounds could also be used in the development of new pharmaceuticals, food preservatives, and cosmetics (Sasikala C et al., 2017). This study aims to evaluate the antimicrobial activity of the red algae *Gracilaria corticata* against 4 bacterial strains and 2 fungal strains. This research has the potential to contribute to the development of new antibacterial agents from natural sources.

1. Materials & Methods

Gracilaria corticata and *sargassum crassifolium* were dried powdered and kept for 4hr extraction on water bath at 50ºC, Filter using Whatmann Filter paper No. 1, Methanol filtrate was kept for evaporation on water bath at 50ºC After crude extraction in methanol, Red algae methanol crude (RMC) and Brown algae methanol crude (BMC) further taken for L-L extraction.

2.1Liquid-Liquid extraction

Brown algae methanol crude (BMC) and Red algae methanol crude (RMC) were dissolved in 10mL of methanol (500mg/10ml) and were used for liquid-liquid partition chromatography. Chloroform, Methanol and hexane soluble fractions were separated using separating funnel.

L-L fractions were kept for evaporation on water bath at 50ºC and Yield were noted and coded as per table 3.1**,** Based on yield RMLLMF (Red Methanol liquid-liquid methanol fraction) was taken for column chromatography and also RMLLMF analyzed by TLC and HPLC to check the purity (Table 3.2 and 3.3).

2.2 TLC for crude and L-L fractions

10mg/ml samples were prepared (based on the solubility, table 3.1), 2.5 µl of samples were spotted on TLC plate and allowed to dry. A TLC plate is made up of a thin layer of Silica gel 0.25mm with fluorescent indicator F_{254} with Solvent system Chloroform: methanol (9.5:0.5) was used for TLC analysis. The strip or plate is then placed with this end dipping in to the solvent mixture, taking care that the sample spot/zone is not immersed in the solvent. As the solvent moves towards the other end of the strip, the test mixture separates into various components. This is called as the development of TLC plates. The separation depends on several factors the plate is removed after an optimal development time and dried and the spots/zones are detected using UV chamber and Rf value is calculated using

Rf = Distance moved by compound /distance moved by solvent

2.3 HPLC for crude and L-L fractions

Samples: Test samples (20mg/mL) were prepared from stock with HPLC grade Methanol and Chloroform (based on solubility, table 3.1) and used for HPLC analysis.

HPLC condition:

I. Instrument: Shimadzhu LC- Prominence 20AT
II. Column: C18 column 250 mm x 4.6 mm, 5u particle
III. Mobile Phase: Linear
A: HPLC grade Methanol (50%)
B: HPLC grade Water (50%)
IV. Flow Rate: 1ml/min
V. Injection volume: 20ul
VI. Absorbance: 254nm

2.4Column fractionation

Preparation of extract for column chromatography

500mg of RMLLMF (Red Methanol liquid- liquid methanol Fraction) + 1.5ml of Methanol.

Column chromatography

Prepare 4g silica gel bed in chromatographic column (300mm x 15mm) in chloroform

Prepared sample was loaded on the column, Eluted with 50 ml 5% Methanol chloroform mixture, Approx.8-10ml of fractions are collected in each test tubes based on color in test tube no.1-6, Eluted with 20 ml 10% Methanol chloroform mixture, Approx.8-10ml of fractions are collected in each test tubes based on color in test tube no.7-12, Eluted with 20 ml 20% Methanol chloroform mixture, 10ml of fractions are collected in test tube no.13, Eluted with 10 ml 30% Methanol chloroform mixture, Collected in test tube no.14, Eluted with 10 ml 40% Methanol chloroform mixture, Collected in test tube no.15, Eluted with 10 ml 50% Methanol chloroform mixture, Collected in test tube no.16, Eluted with 10 ml 60% Methanol chloroform mixture, Collected in test tube no.17, Eluted with 10 ml 70% Methanol chloroform mixture, Collected in test tube no.18, Eluted with 20 ml 100% Methanol collected in test tube no.19-32, Fractions were analysed by TLC. Fractions with similar pigment were pooled based on the TLC. Column fractions were kept for evaporation (Figure1.1-1.3 and table 1-2), Among RCF1, RCF2, RCF3 and RCF4 Fractions, The sample RCF2 analysed by HPLC to check percent purity, Column Fraction RCF2 (Red column fraction 2) is having 97.89% purity (Table 3.4) and was subjected to anti-microbial analysis.

2.5 TLC for column fractions

10mg/ml samples were prepared (based on solubility), 2.5 µl of samples were spotted on TLC plate and allowed to dry. A TLC plate is made up of a thin layer of Silica gel 0.25mm with fluorescent indicator F_{254} with Solvent system Chloroform: methanol (9.5:0.5) was used for TLC analysis. The

strip or plate is then placed with this end dipping in to the solvent mixture, taking care that the sample spot/zone is not immersed in the solvent. As the solvent moves towards the other end of the strip, the test mixture separates into various components. This is called as the development of TLC plates. The separation depends on several factors the plate is removed after an optimal development time and dried and the spots/zones are detected using UV chamber and Rf value is calculated using

Rf = Distance moved by compound /distance moved by solvent

2.6 HPLC for column fractions Samples:

Test samples (20mg/mL) were prepared from stock with HPLC grade Methanol and used for HPLC analysis.

HPLC condition:

I. Instrument: Shimadzhu LC- Prominence 20AT
II. Column: C18 column 250 mm x 4.6 mm, 5u particle
III. Mobile Phase: Linear
A: HPLC grade Methanol (50%)
B: HPLC grade Water (50%)
IV. Flow Rate: 1 ml/min
V. Injection volume: 20ul
VI. Absorbance: 254nm

2.7 Anti-microbial studies

Media and reagents

Sl.No	Particulars	Source	Catalogue No
1	Potato Dextrose Agar	HiMedia	MH096-500G
2	Sabouraud Dextrose agar	HiMedia	SMH063D
3	Nutrient broth	HiMedia	M001500G
4	LB broth	SRL	46502

Test organisms:

Test Microorganisms used bacteria- *Bacillus subtillis* MTCC 12, *Staphylococcus aureus*-MTCC96, *E. coli*-MTCC 443 and *Pseudomonas areuginosa* -MTCC 424 and fungi- *Candida albicans*-ATCC-18804 and *Aspergillus niger*-ATCC 6275

Inoculum:

All *cell* suspensions were prepared from cultures grown on respective broth (Respective broth mentioned in section 2.4) for 16-18hr at 37°C for bacteria and at room Temperature for 48-72 hrs for fungi. Cell density is adjusted to 1-2 x 10^5 cells/ml using 0.5 McFarland Standard.

Growth conditions

I. *Bacillus subtillis MTCC 121: Nutrient broth and agar; Aerobic*
II. *Staphylococcus aureus-MTCC96: Nutrient broth and agar; Aerobic*
III. *E.coli-MTCC 443: LB broth and agar; Aerobic*
IV. *Pseudomonas areuginosa -MTCC 424: Nutrient broth and agar; Aerobic*
V. *Candida albicans-ATCC-18804: S*abouraud Dextrose broth and Sabouraud Dextrose agar; *Aerobic*
VI. *Aspergillus niger- ATCC 6275-Potato dextrose broth and agar; Aerobic*

Standards and controls

i. **Standard**: Ciprofloxacin for bacteria and Itraconazole for fungi.
ii. **Controls**: Methanol

Test compound:

Stocks of samples were prepared in solvents as summarized below

Sample	Solvent	Stock	Test Concentration
Compound	Methanol	100 mg/ml	100mg/ml (20µl;2mg/well) 100mg/ml (10µl;1mg/well)
Itraconazole	DMSO	5mg/ml	5mg/ml (10µl;0.05mg/well)
Ciprofloxacin	Water	0.1mg/ml	0.1mg/ml (10µl;0.001mg/well)

Procedure: Determination of Antimicrobial activity by well diffusion method

Cell suspensions of test organisms (*Bacillus subtillis, Staphylococcus aureus, E.coli, Pseudomonas aeruginosa ,Candida albicans and Aspergillus niger)* adjusted to cell density of 1-2 x 10^5 cells/ mL were inoculated (100µL per plate) on agar medium (Respective agar mentioned in section 2.4) uniformly spread using sterile Glass L spreader. Agar wells (5mm) were made on the inoculated agar plate with sterile cork borer and numbered from the backside of the plate. Test samples (10 and 20µl) from the stocks mentioned in section 2.6 to achieve final test concentrations of 1 and 2mg /well, Standard Itraconazole (10µl from 5 mg/mL) and Ciprofloxacin (10µl from 0.1mg/ml) appropriate controls (20µl respective solvents mentioned in section 2.6) were added premarked wells on agar plates. Test samples loaded plates were kept at 2-8°C in refrigerator for 20 minutes for diffusion of test

samples from the well and later bacterial plates incubated at 37^0C for 16-24hrs and fungal plates were incubated at room temperature for 24-72hrs.. The plates were observed for zone of inhibition and tabulated (Barry, A. L., and L. D. Sab h. 1974).

3 - Results

Table 3.1: Yield after L-L extraction

Red Algae					
Sample name	**Sample taken for extraction**	**Sample code**	**Solvent fraction**	**Yield**	**Yield%**
Red algae methanol crude (RMC)	515mg	RMLLMF(Red Methanol liquid-liquid methanol fraction)	Methanol	178mg	34.56%
		RMLLCF(Red Methanol liquid-liquid chloroform fraction)	Chloroform	6mg	1.16%
		RMLLHF(Red Methanol liquid-liquid hexane fraction)	Hexane	-	-

Brown Algae					
Sample name	**Sample taken for extraction**	**Sample code**	**Solubility**	**Yield**	**Yield%**
Brown algae methanol crude (BMC)	513mg	BMLLMF(Brown Methanol liquid-liquid methanol fraction)	Methanol	166mg	32.35%
		BMLLCF(Brown Methanol liquid-liquid chloroform fraction)	Chloroform	-	-
		BMLLHF(Brown Methanol liquid-liquid hexane fraction)	Hexane	7mg	1.36%

Table 3.2 : TLC characteristic for crude and liquid-liquid fractions (Red Algae).

Sample	**TLC band at 254nm**	**Retention factor**	**TLC Profile Characteristics**		
			366nm	**254nm**	**Visible light**
(RMC) Red algae methanol crude	6	1	Green	Dark brown	Forest green
		0.96	Pink	Brown	Sea foam green
		0.58	Green	Brown	Green
		0.35	-	Brown	Green
		0.32	Brown	Brown	Orange
		0.17	-	Brown	Green
RMLLMF (Red Methanol liquid-liquid methanol fraction)	8	1	-	Dark brown	Forest green
		0.96	Pink	Brown	Sea foam green
		0.58	-	Brown	Green
		0.35	-	Brown	-
		0.32	-	Brown	Green
		0.17	Green	Brown	Orange
		0.10	-	Brown	Green

RMLLCF (Red Methanol liquid-liquid chloroform fraction)	4	0.48	-	Violet	-
		0.41	-	Violet	-
		0.32	-	Violet	-
		0.21	-	Violet	-

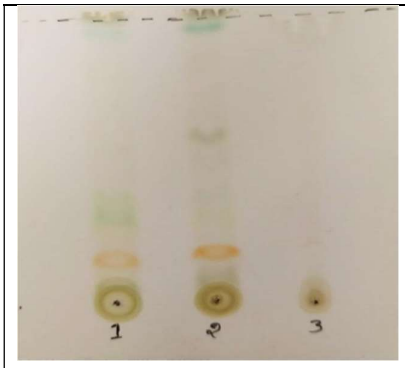


Fig 3.1 TLC Chromatogram at Visible light
(1-Methanol crude, 2- MLLMF, 3-MLLCF)

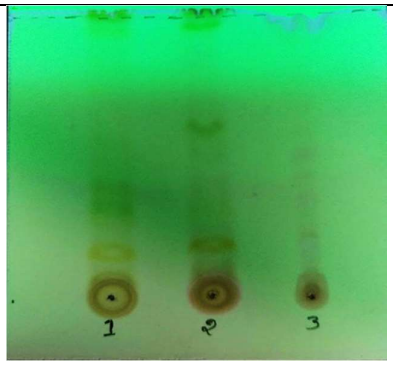


Fig 3.2 TLC Chromatogram at Short wave
UV 254nm (1-Methanol crude, 2-MLLMF, 3-MLLCF)

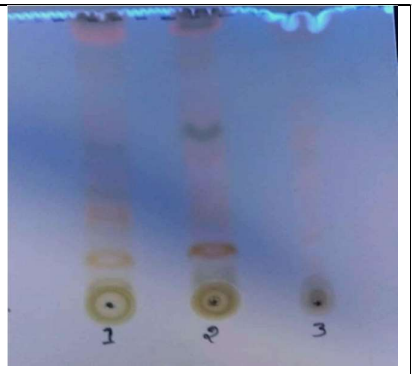


Fig 3.3 TLC Chromatogram at long wave
UV 366nm (1-Methanol crude, 2-MLLMF, 3-MLLCF)

Table 3.3: Crude and LL fractions HPLC summary

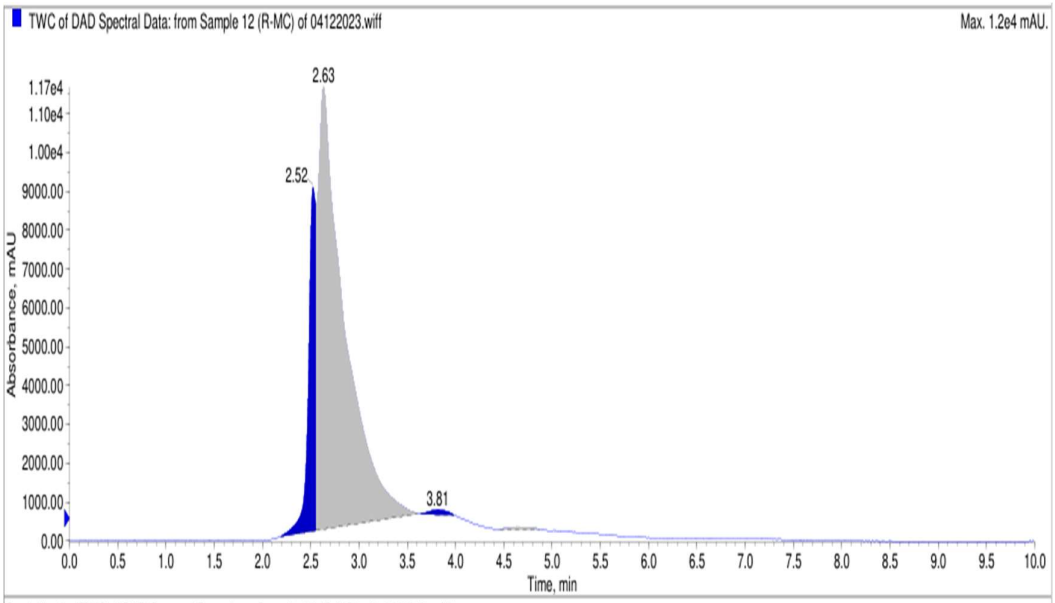
Crude:

Sample	**Area (%)**	**Retention time (min)**	**Peak no.**	**Yield**	**Chromatogram no.**
(RMC) Red algae methanol crude	81.83	2.63	2	515mg	Fig 1.4

Column fraction code	Area (%)	Retention time (min)	Peak no.	**Yield**	Chromatogram no.
RMLLMF (Red Methanol liquid-liquid methanol fraction)	90.30	3.02	3	178mg	Fig 1.5
	4.17	2.60	2		
	3.69	2.52	1		
RMLLCF (Red Methanol liquid-liquid chloroform fraction)	48.60	2.78	4	6mg	Fig 1.6
	23.20	5.38	6		
	21.50	1.52	1		

	17.25	2.52	1		

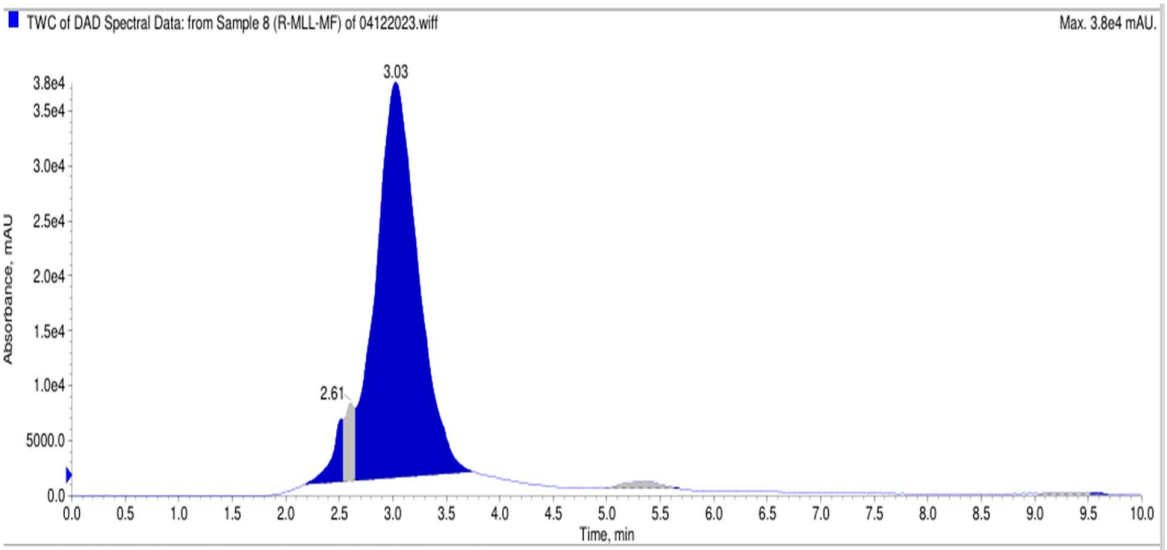
L-L fractions:



Peak List for "TWC of DAD Spectral Data: from Sample 12 (R-MC) of 04122023.wiff"

	Time (min)	Area (mAU x min)	%Area	Height (mAU)	%Height	Width (min)	Baseline Type
1	2.5234	4.6110e4	17.2522	8858.7927	43.3419	0.3640	Valley
2	2.6328	2.1873e5	81.8380	1.1394e4	55.7470	1.0440	Valley
3	3.8131	1517.4058	0.5677	127.4846	0.6237	0.3400	Base to Base
4	4.6339	914.0940	0.3420	58.7438	0.2874	0.4080	Base to Base

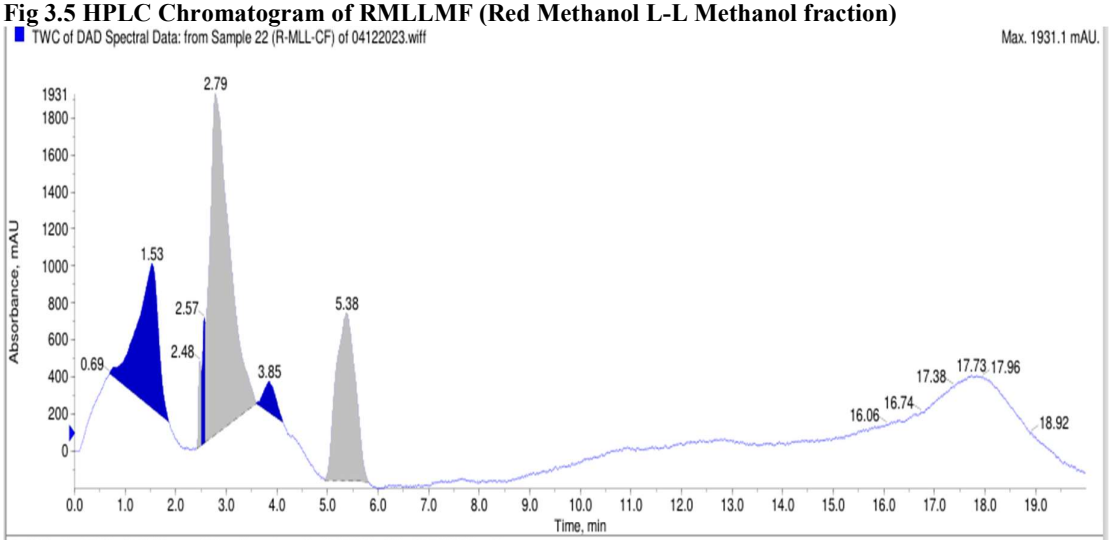
Fig 3.4 HPLC Chromatogram of RMC (Red- Methanol crude)



Peak List for "TWC of DAD Spectral Data: from Sample 8 (R-MLL-MF) of 04122023.wiff"

	Time (min)	Area (mAU x min)	%Area	Height (mAU)	%Height	Width (min)	Baseline Type
1	2.5214	3.9996e4	3.6961	5689.0183	11.4247	0.3480	Valley
2	2.6081	4.5168e4	4.1741	7096.9708	14.2521	0.1160	Valley
3	3.0273	9.7716e5	90.3017	3.5948e4	72.1900	1.0880	Valley
4	5.3632	1.6236e4	1.5004	724.4074	1.4548	0.6480	Valley
5	5.6462	201.9416	0.0187	108.6138	0.2181	0.0440	Valley
6	9.2590	2632.6220	0.2433	122.6638	0.2463	0.4840	Valley
7	9.5353	710.9446	0.0657	106.5701	0.2140	0.1640	Valley

Fig 3.5 HPLC Chromatogram of RMLLMF (Red Methanol L-L Methanol fraction)



Peak List for "TWC of DAD Spectral Data: from Sample 22 (R-MLL-CF) of 04122023.wiff"

	Time (min)	Area (mAU x min)	%Area	Height (mAU)	%Height	Width (min)	Baseline Type
1	1.5283	2.2066e4	21.5037	778.2963	16.1082	1.1560	Base to Base
2	2.4796	1640.7926	1.5990	458.6701	9.4930	0.0920	Valley
3	2.5670	2570.9686	2.5054	674.2540	13.9549	0.0760	Valley
4	2.7859	4.9879e4	48.6078	1843.3438	38.1513	1.0120	Valley
5	3.8535	2650.0931	2.5825	168.9267	3.4962	0.5120	Base to Base
6	5.3844	2.3809e4	23.2016	908.1755	18.7963	0.8560	Base to Base

Fig 3.6 HPLC Chromatogram of RMLLCF (Red Methanol L-L Chloroform fraction)

Table 3.4 : Yield after column chromatography

Test tube pooled	Code	Yield (mg)
Test tube 1-7	RCF1 (Red column fraction 1)	-
Test tube 8 to 12	RCF2 (Red column fraction 2)	105mg
Test tube 13	RCF3 (Red column fraction 3)	16mg
Test tube 14 to 32	RCF4 (Red column fraction 4)	37mg

Table 3.5 : TLC characteristic for column fractions

Sample	TLC Band	Retention factor	TLC Profile Characteristics		
			366nm	254nm	Visible light
Test tube 1	-	-	-	-	-
Test tube 2	-	-	-	-	-
Test tube 3	-	-	-	-	-
Test tube 4	-	-	-	-	-
Test tube 5	-	-	-	-	-
Test tube 6	-	-	-	-	-
Test tube 7	-	-	-	-	-
Test tube 8	4	0.94	Blue	Green	Grey
		0.80	Blue	Green	Grey
		0.29	Pink	Green	yellow
		0.23	Blue	Green	Grey
		011	Brown	Green	Grey
Test tube 9	2	0.23	Blue	Green	Grey
		011	Brown	Green	Grey
Test tube 10	2	0.23	-	Green	-
		011	-	Green	-
Test tube 11	-	-	-	-	-
Test tube 12	-	-	-	-	-
Test tube 13	1	0.00	Pink	Green	-
Test tube 14	1	0.00	-	Blue	-
Test tube 15	1	0.00	-	Blue	-
Test tube 16	1	0.00	-	Blue	-
Test tube 17	1	0.00	-	Blue	-
Test tube 18	1	0.00	-	Blue	-

Test tube 19	1	0.00	-	Blue	-
Test tube 20	1	0.00	-	Blue	-
Test tube 21	1	0.00	-	Blue	-
Test tube 22	1	0.00	-	Blue	-
Test tube 23	-	-	-	-	-
Test tube 24	-	-	-	-	-
Test tube 25	-	-	-	-	-
Test tube 26	-	-	-	-	-
Test tube 27	-	-	-	-	-
Test tube 28	-	-	-	-	-
Test tube 29	-	-	-	-	-
Test tube 30	-	-	-	-	-
Test tube 31	-	-	-	-	-
Test tube 32	-	-	-	-	-

Note: Where: Test tubes 1-32 are column fractions before pooling. Based on TLC, 1-32 test tubes were pooled and named as RCF1, RCF2, RCF3 and RCF4 Fractions (Table3.5)

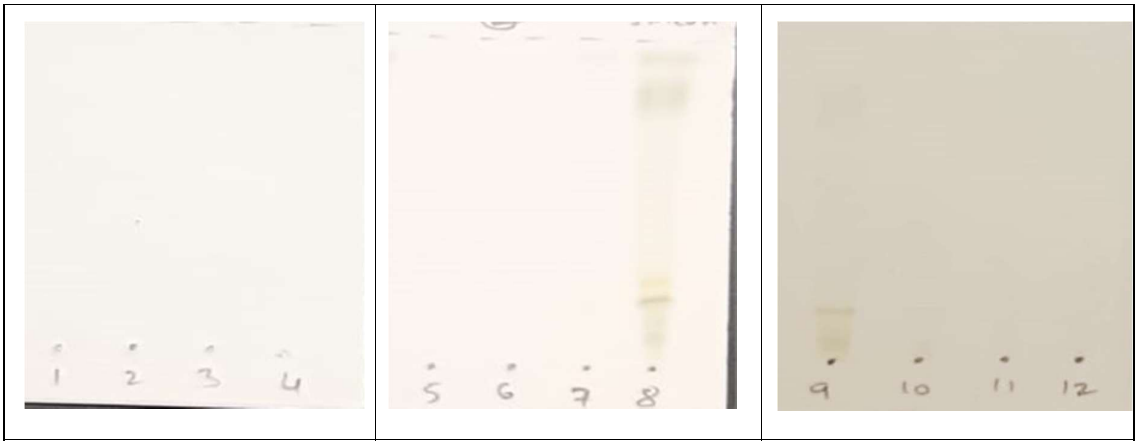


Fig 3.7 TLC Chromatogram at Visible light (Test tube no.1-32)

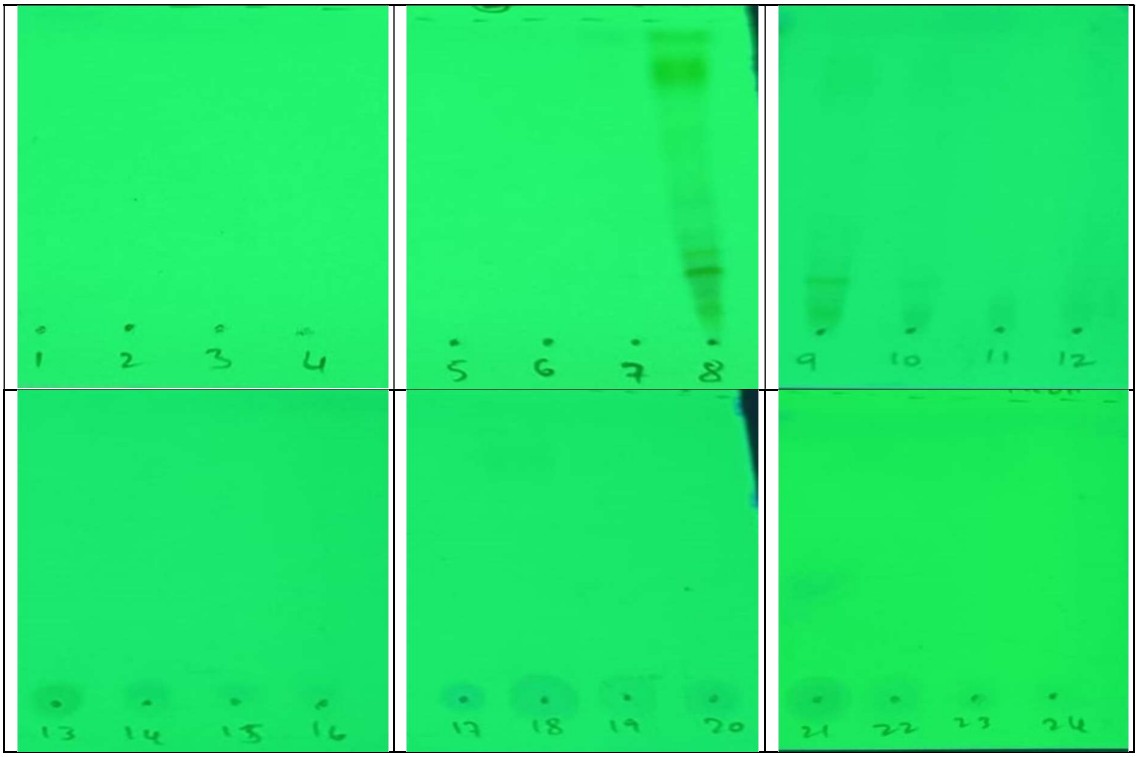


Fig 3.8 TLC Chromatogram at Short wavelength (Test tube no.1-32)

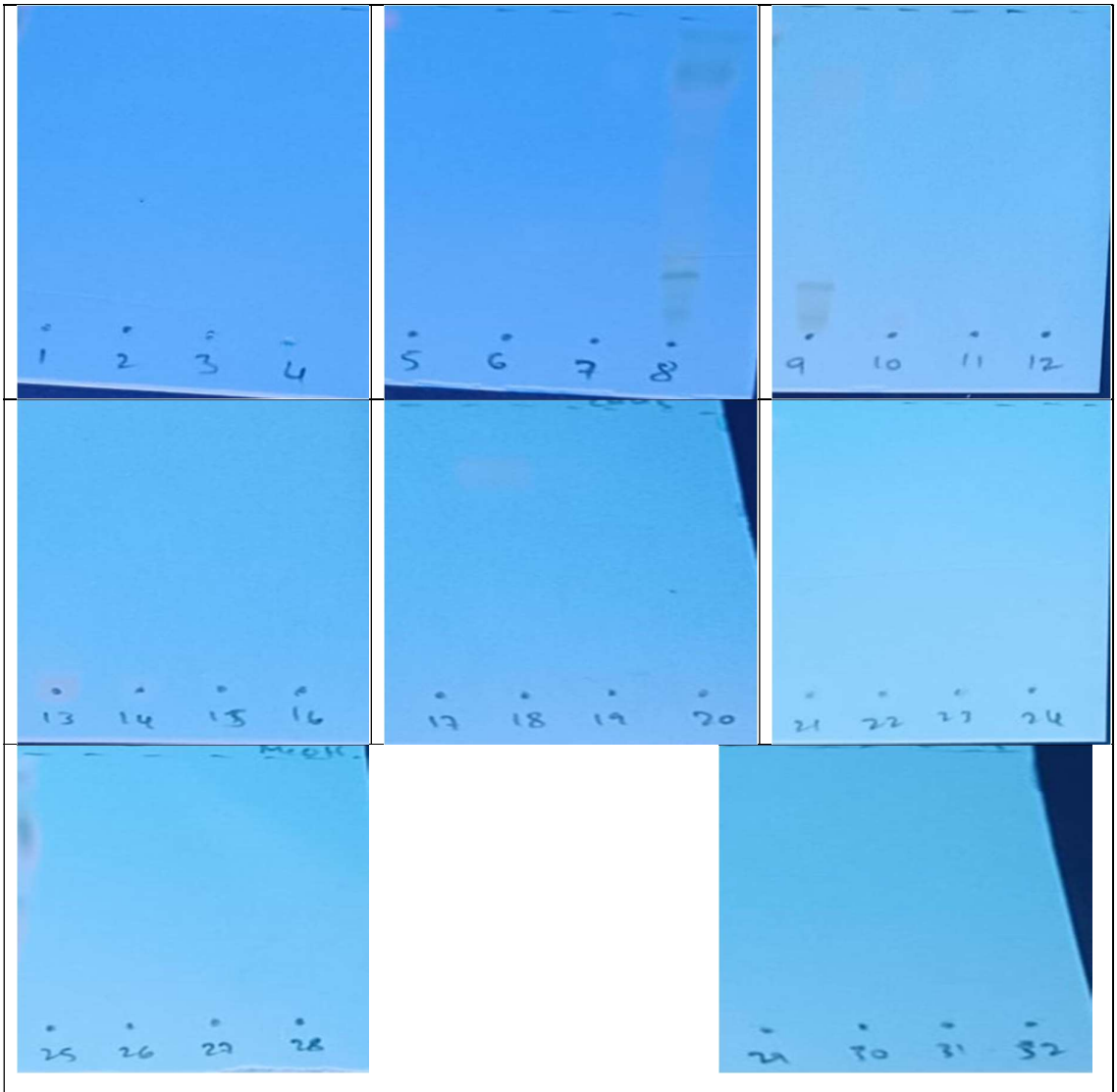
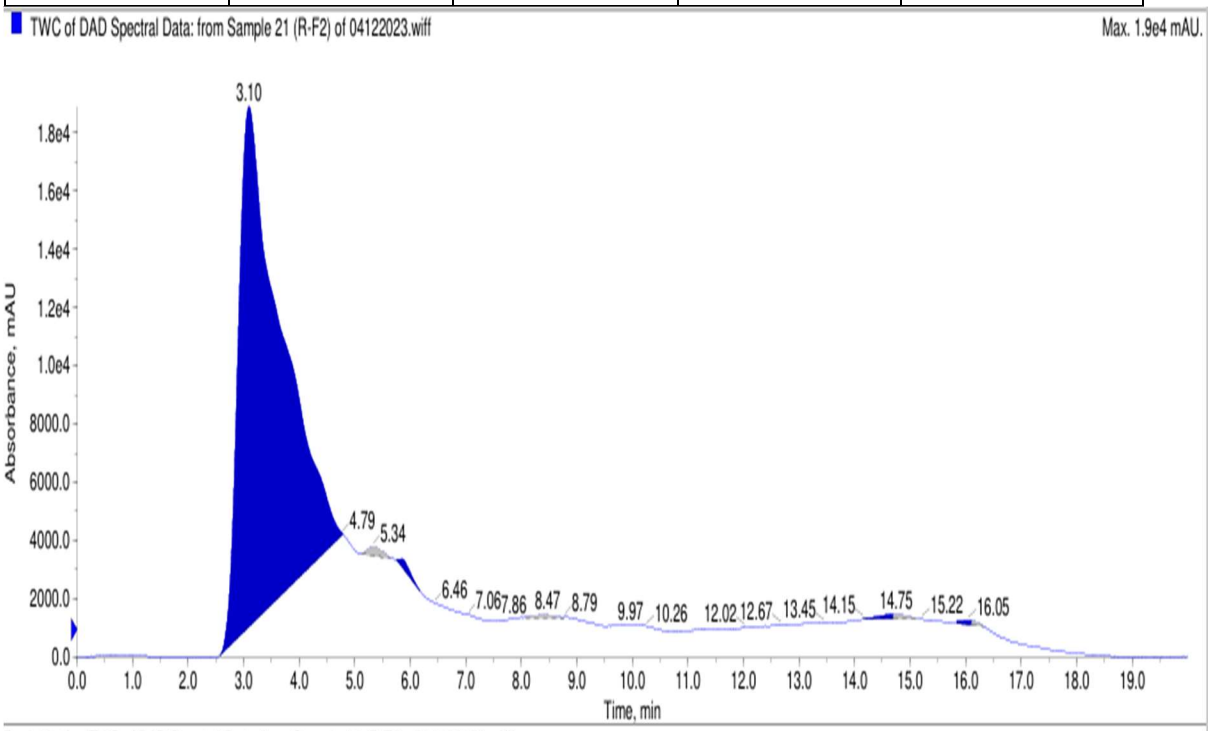


Fig 3.9 TLC Chromatogram at Long wavelength (Test tube no.1-32)

Table 3.6: HPLC summary Report of column fractions.

Solubility	Sample	Yield (mg)	Purity (%)
Methanol	RCF2	105mg	97.89

Column fraction Code	Area (%)	Retention time (min)	Peak No.	Chromatogram no.
RCF2 (Red column fraction 2)	97.89	3.09	1	Fig 2
	0.58	5.34	2	
	0.41	5.84	3	



Peak List for "TWC of DAD Spectral Data: from Sample 21 (R-F2) of 04122023.wiff"

	Time (min)	Area (mAU x min)	%Area	Height (mAU)	%Height	Width (min)	Baseline Type
1	3.0957	9.5695e5	97.8971	1.7859e4	93.2347	2.2040	Base to Base
2	5.3443	5687.5311	0.5818	324.3014	1.6930	0.5240	Base to Base
3	5.8469	4094.4092	0.4189	265.2840	1.3849	0.4160	Base to Base
4	8.4715	2532.5238	0.2591	105.6799	0.5517	0.7000	Valley
5	14.6993	2784.6159	0.2849	157.9087	0.8244	0.5440	Valley
6	14.7458	2653.2771	0.2714	163.1677	0.8518	0.4720	Valley
7	16.0525	1719.8836	0.1759	138.9703	0.7255	0.2720	Valley
8	16.1309	1083.7748	0.1109	140.5873	0.7339	0.2040	Valley

Fig 3.10 HPLC Chromatogram of RCF2

Table 3.7 : HPLC summary Report of column fractions.

Table 3.8: Inhibitory activity of test compounds against *Staphylococcus aureus, Bacillus subtillis, E. coli, Pseudomonas aeruginosa, Candida albicans and Aspergillus niger*

Sample name	**Micro organism**	**Concentration (µg/well)**	**Zone of inhibition(mm)**	**Reference figure**	**Method**
Ciprofloxacin	*Staphylococcus aureus*	1µg/well	18.00± 0.00	Fig-1	Agar diffusion method
Control (Methanol)		20µl/well	-		
Compound		2mg/well	07.5 ± 0.71		
		1mg/well	-		
Ciprofloxacin	*Bacillus subtilis*	1µg/well	16.50+0.71	Fig-2	
Control (Methanol)		20µl/well	-		
Compound		2mg/well	08.00 ± 0.00		
		1mg/well	-		
Ciprofloxacin	*Pseudomonas aeruginosa*	1µg/well	15.00 ±0.00	Fig-3	
Control (Methanol)		20µl/well	-		
Compound		2mg/well	-		
		1mg/well	-		
Ciprofloxacin	*Escherichia coli*	1µg/well	28.00 ±0.00	Fig-4	
Control (Methanol)		20µl/well	-		
Compound		2mg/well	-		
		1mg/well	-		
Itraconazole	*Aspergillus niger*	50µg/well	18.50 ±2.12	Fig-5	
Control (Methanol)		20µl/well	-		
Compound		2mg/well	-		
		1mg/well	-		
Itraconazole	*Candida albicans*	50µg/well	16.00 ±0.00	Fig-6	
Control (Methanol)		20µl/well	-		
Compound		2mg/well	-		
		1mg/well	-		

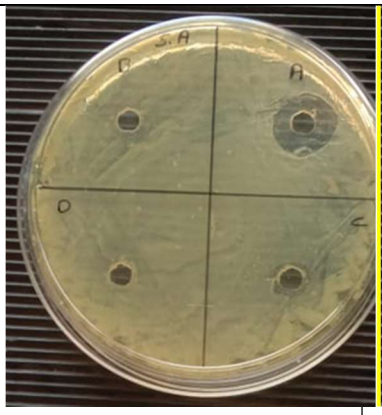


Fig-1*: Staphylococcus aureus*: A- Standard (Ciprofloxacin; 1µg/well) B- Control

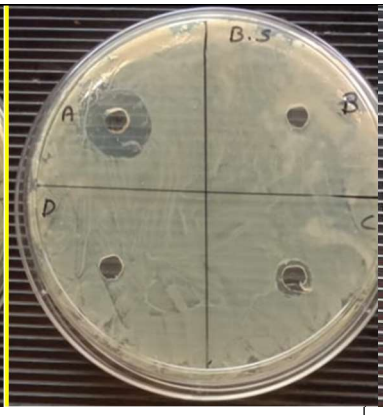


Fig-2*: Bacillus subtilis*: , A- Standard (Ciprofloxacin; 1µg/well), B- Control

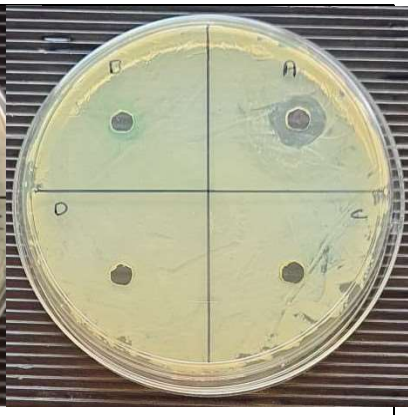


Fig-3*: Pseudomonas aeruginosa*: A- Standard (Ciprofloxacin; 1µg/well), B- Control (Methanol),

(Methanol), C- Sample (2mg /well), D-Sample (1mg /well).	(Methanol), C- Sample (2mg /well), D-Sample (1mg /well).	C- Sample (2mg /well), D-Sample (1mg /well).

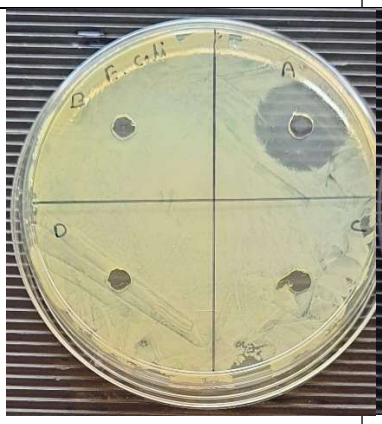
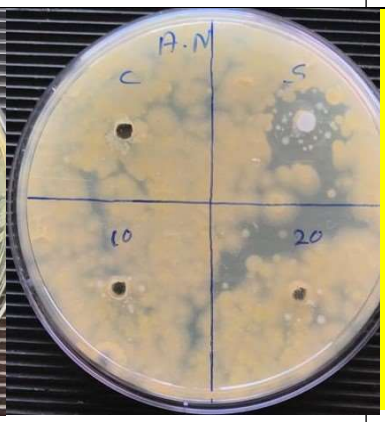

	

Fig-4*: Escherichia coli:* A-Standard (Ciprofloxacin; 1µg/well), B- Control (Methanol), C- Sample (2mg /well), D-Sample (1mg /well).	Fig-5*: Aspergillus niger:* A-Standard (itracanazole;50µg/well), B-Control (Methanol), C- Sample (2mg /well), D-Sample (1mg /well).	Fig-6*: Candida albicans:* A-Standard (itracanazole;50µg/well), B- Control (Methanol), C- Sample (2mg /well), D-Sample (1mg /well).
Plate-1 Inhibitory activity of test compounds against *Staphylococcus aureus, Bacillus subtillis, E. coli, Pseudomonas aeruginosa, Candida albicans and Aspergillus niger*		

4. Discussion

The crude extracts Brown algae methanol crude (BMC) and red algae methanol crude (RMC) were taken for liquid-liquid extraction. In L-L extraction the percentage yield for RMLLMF (Red Methanol liquid-liquid methanol fraction) is 34.56%, RMLLCF (Red Methanol liquid-liquid chloroform fraction) is 1.16%, no yield in RMLLHF (Red Methanol liquid-liquid hexane fraction, BMLLMF (Brown Methanol liquid-liquid methanol fraction) is 32.35%, no yield in BMLLCF (Brown Methanol liquid-liquid chloroform fraction) and BMLLHF (Brown Methanol liquid-liquid hexane fraction) is 1.36%. RMLLMF (Red Methanol liquid-liquid methanol fraction) was taken for column chromatography and also RMLLMF analyzed by TLC and HPLC to check the purity further in coloumn Chromatography Fraction RCF2 (Red column fraction 2) having purity about 97.89% (based on HPLC) was subjected to antimicrobial studies Similar to the findings of Thi-Van Hoang, et al, 2024 which highlights the potential of marine red macroalgae, specifically *Gracilaria corticata*, *Chondrus ocellatus*, and *Posphyra perforata*, as a source of bioactive compounds with antimicrobial and anticancer properties. And in the study conducted by Sudhakar et al., 2024 phycoerythrin expressed less antimicrobial activity against pathogens Methanol extracts showed more potent antimicrobial activity compared to hexane extracts. Both methanol and hexane extracts demonstrated dose-dependent cytotoxic effects on cancer cells. The purified compound of *G. corticata* Methanolic Extract showed mild inhibitory effect on *Staphylococcus aureus* and *Bacillus subtilis* recording a 07.5± 0.71 mm and 0.8± 0.00 mm zone of inhibition at 2 mg/ well concentration and the standard drug Ciprofloxacin giving a 18.00± 0.00 and 16.50± 0.71mm zone of inhibition at 1µg/ well concentration Respectively. Whereas, the compound was not effective against *P. aeruginosa, E. coli, A. niger and C. albicans.*

Conclusion

The findings of the study indicate that the extracts of *Gracilaria corticata* have important phytochemicals, though the antimicrobial potential is limited to few organisms at higher dose, further studies on more organisms is necessary to draw deeper and precise conclusions.

Declarations Author contributions

Suma K.M. :

collection and processing of plant material, methodology, formal analysis and experimenting, writing — original draft preparation. Dr. Parameshwar Naik T.: conceptualization, methodology, review, editing, and supervision.

Conflicts of interest

The authors declare that they have no personal or financial interests in the present study or there is no funding body that can potentially influence the results.

The authors were not involved in the editorial review or the decision-making body to publish the article.

Ethics approval

Not applicable

Availability of data and material

Not applicable

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