Uttar Pradesh Journal of Zoology

Volume 44, Issue 24, Page 343-362, 2023; Article no.UPJOZ.3140
ISSN: 0256-971X (P)

Antibacterial Activity of Some Selected Marine Macro-algae from Rameswaram Coastal Region, India

Nandhini Selvaraj [a], Mishel Francis [a], Pavithra Senthilkumar [a], Aravinth Annamalai [b] and Saroja Ramasubbu Sivakumar [a*]

[a] Department of Botany, School of Life Science, Bharathidasan University, Tiruchirappalli- 620024, India.
[b] Department of Marine Science, Bharathidasan University, Tiruchirappalli- 620024, India.

Authors' contributions

This work was carried out in collaboration among all authors. Author NS did the methodology, Investigation, conceptualization, resources, data curation, formal analysis and writing - original draft of the manuscript. Authors MF and PS did the data curation, formal analysis of the study. Author AA did the mapping and data curation, formal analysis, review & editing of the study. Author SRS did the conceptualization, review & editing, supervision of the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.56557/UPJOZ/2023/v44i243847

Editor(s):
(1) Prof. Juan Carlos Troiano, University of Buenos Aires, Buenos Aires, Argentina.
Reviewers:
(1) Natalija Atanasova-Pancevska, University Sts Cyril and Methodius, North Macedonia.
(2) Rahman, Pattimura University, Indonesia.

Original research Article

Received: 22/10/2023
Accepted: 28/12/2023
Published: 30/12/2023

ABSTRACT

Marine algae are known as source of bioactive secondary metabolites. Twenty macroalgae were collected from Mandapam cost, Gulf of Mannar, Rameswaram were tested against two-gram negative bacteria's using agar well diffusion method. The following species were used in the current study include seven species in *Chlorophyta* (*Caulerpa peltata, Ulva lactuca, Halimeda gracilis,*

**Corresponding author: Email: sivakumar.sr@bdu.ac.in, drsrsivakumar@gmail.com;*

Chaetomorpha aerea, Cladophora vagabunda, Enteromorpha flexuosa, Halimida macroloba), seven in Phaeophyta (*Sargassum cinereum, Padina boergesenii, Sargassum cristaefolium, Turbinaria decurrens, Turbinaria conoides, Hydroclathrus clathratus*, *Lobophora variegate*) and six in *Rhodophyta* (*Hypnea spicefera, Solieria robusta, Porphyra indica, Ceramium rubrum, Gelidiella acerosa Gracilaria edulis*). They were air-dried thoroughly and powdered using a grinder. Powdered algae were extracted using solvents such as water, ethanol and methanol. In the present study ethanol extract was found to be excellent antibacterial activity from macroalgae. Among the selected Twenty different macroalgae the red algae (*Rhodophyta*) showed the higher antibacterial activity followed by brown (Phaeophyta) and green (*Chlorophyta*) against the tested two selective negative bacteria (*Pseudomonas aeruginosa* and *Klebsiella pneumoniae).* This finding supports the view that algal extracts are a reliable source of bioactive compounds for upcoming medication development.

Keywords: Macroalgae; secondary metabolites; Pseudomonas aeruginosa; Klebsiella pneumoniae; antibacterial activity.

1. INTRODUCTION

There is more interest in marine species as a result of an increase in demand for testing new therapeutic medications made from natural resources. Seaweeds are also known as marine macroalgae, are plant-like organisms that often are alive and adhering to rock or other hard surfaces in coastal environments [1,2]. They belong to three major groups *Chlorophyta* (green), Phaeophyta (Brown) and *Rhodophyta* (Red). In the past 40 years, marine macroalgae (seaweeds) generated over 3000 novel chemical structures that are physiologically active, the majority of which were isolated from the phylum *Rhodophyta* [3,4]. A never-ending supply of raw materials for the culinary, pharmaceutical, food and cosmetic sectors comes from marine algae [5]. Substances like alginate, carrageenan and agar were obtained from marine macroalgae are used in the field of medicine and pharmacy for decades [6,7]. Several seaweeds have bioactive components that stop some Gram positive and Gram-negative bacterial pathogens from growing [8].

Although little research has been done to establish the ecological function of these chemicals, macroalgae constitute a rich source of naturally occurring bioactive molecules [9] and the use of marine natural products with the ability to prevent bacterial growth offers significant pharmacological potential [10]. The antibacterial properties of marine algae against various diseases have received particular interest [11].

Pseudomonas aeruginosa gram-negative bacteria which belong to the family Pseudomonadaceae and in clinics, *P. aeruginosa* has emerged as an opportunistic pathogen [12]. To build an infection, *P. aeruginosa* takes advantage of gaps in the host's defence. *P. aeruginosa* is the poster child for an opportunistic human infection, in fact. The bacterium seldom infects healthy tissues, but it can enter any tissue that is suffering from immunodeficiency [13]. *Klebsiella pneumonia* is a gram-negative bacterium which belongs to Enterobacteriaceae and it has the ability of colonizing invading and causing infection to the human body [14]. The opportunistic bacterial pathogen *Klebsiella pneumoniae* is renowned for producing antibiotic resistance genes with a high frequency and variety [15] An increasing public health concern for developing countries like Bangladesh, where resources are limited, is the global spread of multi-drug resistant (MDR) strains of *Klebsiella pneumoniae* [16,17].

In human population and aquaculture organism the bacterial infection causes high morality [18]. Due to widespread diseases and the widespread indiscriminate use of antibiotics, the usage of antibiotics has substantially increased in the modern era [19]. It has been demonstrated that the extracts and active components of certain marine algae have antibacterial action against Gram positive and Gram-negative bacteria [20]. One of the most potent and effective developments in contemporary science & technology for the prevention and treatment of infectious diseases is the discovery and development of antibiotics [21]. In this study we aim to screen aqueous, methanol and ethanol of Twenty marine macro algae belongs to three different seaweed groups to identify and assess the antibacterial activity against *Pseudomonas aeruginosa* and *Klebsiella pneumoniae.*

2. MATERIALS AND METHODS

2.1 Collection of Macroalgae

The fresh macroalgae where collected in the region of Mandapam Coast, Gulf of Mannar, Rameswaram, Tamil Nādu, India (Fig. 1). Totally there are Twenty species of macroalgae were collected that includes seven species in *Chlorophyta* (Green) are *Caulerpa peltata, Ulva lactuca, Halimeda gracilis , Chaetomorpha aerea, Cladophora vagabunda, Enteromorpha flexuosa* and *Halimida macroloba,* seven species in Phaeophyta (Brown) that includes *Sargassum cinereum, Padina boergesenii, Sargassum cristaefolium, Turbinaria decurrens, Turbinaria conoides, Hydroclathrus clathratus* and *Lobophora variegata* and six species in *Rhodophyta* (Red) includes *Hypnea spicefera, Solieria robusta, Porphyra indica, Ceramium rubrum, Gelidiella acerosa* and *Gracilaria edulis*. The collected macroalgal species were identified by the experts in these fields using standard literature and taxonomic keys and online database [22,23], WoRMS; AlgaeBase). The algae were thoroughly washed with tap water to remove any contaminants, then washed thrice with distilled water and shade dried at room temperature (2 weeks). Then, the shade-dried algae were ground well into a fine powder by using an electrical blender and used for later extractions.

2.2 Preparation of Macroalgal Extract

The Macroalgae were taken to make into a fine powder. All the powdered Macroalgal samples were stored in air tight glass containers. The 100 g of each powdered seaweed was taken in the separate conical flask and 300 mL of each solvent aqueous, methanol and ethanol was added into it [24-27]. Then it is kept in shaker for 7 days in room temperature 28^0C. After filtration through Whatman No. 1 filter paper, the extract was collected and all the obtained extracts were concentrated under a reduced pressure using a rotary evaporator and dried in air (in a petri dish) to obtain a paste extract. The obtained dried paste extract was then stored at 4 °C for further bioassay.

2.3 Collection of Bacterial Strain

The antibacterial activity of seaweed extract was tested against two selective negative bacterial strains. The bacterial strains of *Pseudomonas aeruginosa* and *Klebsiella pneumonia* was obtained from MTCC, Chandigarh, India. Each bacterial strain was subculture overnight at 35^0C in nutrient agar slant.

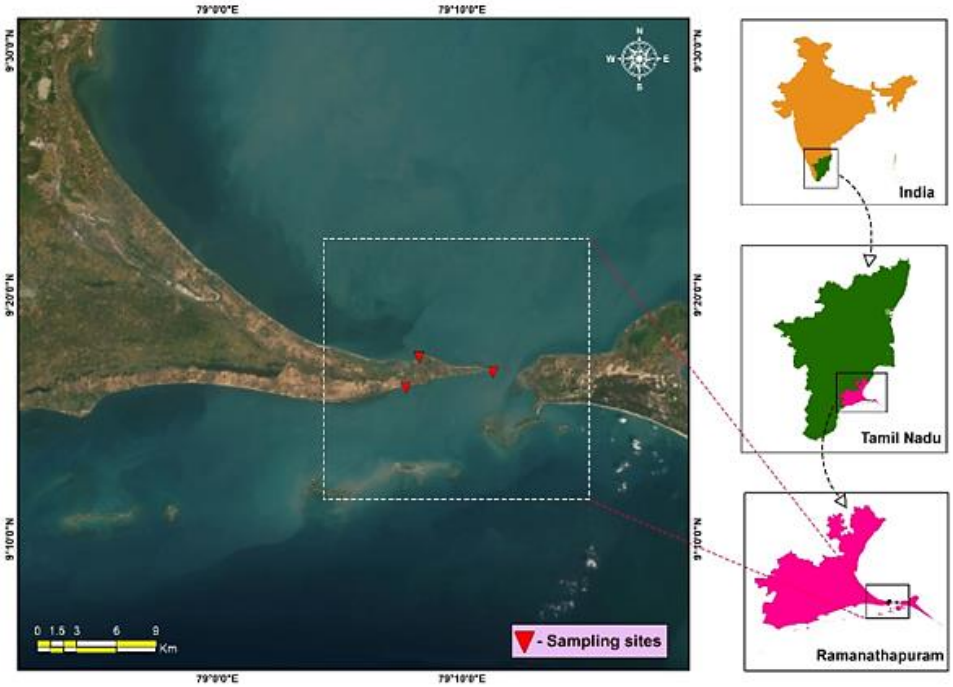


Fig. 1. Macroalgae sampling sites along the Palk Bay and Gulf of Mannar

2.4 Agar Well Diffusion Method

The agar well diffusion method was used for antibacterial assay of different solvent extracts. The Muller Hilton agar was used for this assay. An aliquot of culture was evenly swabbed by using sterile cotton swab on the solidified agar and the wells were made in the diameter of 6 mm by using sterile cork borer. The crude extract obtained from macroalgae was inoculated (µg/mL) in the each well and incubated for 24 hours at 37^0C. Here, the Azithromycin is used as positive control. After the incubation period the zone inhibition of each extract was measured by zone scale. Triplicate was done to confirm the antibacterial activity of each extract against two selective negative bacteria. The data from the triplicates were transformed into mean (M) and standard deviation (SD) values using the MS Excel tool (SD) [28].

3. RESULTS AND DISCUSSION

The results show the antibacterial activity of the Macroalgal extract against two selective negative bacteria *Pseudomonas aeruginosa* and *Klebsiella Pneumonia* by using three different solvents such as aqueous, methanol and ethanol. The antibacterial activity of three different solvents were shown in the Table 1. Among the three different solvents ethanol and Methanol show the highest antibacterial activity against two selective negative bacteria (Tables 3 & 4). Among the 20 species of macroalgae the maximum zone of inhibition (14.67 ± 0.58 mm) was noted in ethanolic extract of *Turbinaria conoides* and *Soleiria robusta* against *Klebsiella pneumonia* (Table 4; Fig. 6). Based on the results of the all the macroalgae the ethanolic extract obtained from *Hypenea spicefera (*13.67 ± 0.58 mm)*, Caulerpa peltate* (13.33 ± 0.58 mm & 13.67 ± 0.58 mm)*, Gracilaria edulis (*12.67 ± 0.58 mm & 11 ± 1 mm)*, Halimida macroloba* (14.67 ± 0.58 mm & 12 ± 1 mm) and *Ulva lactuca (*12.67 ± 0.58 mm & 12.33 ± 0.58 mm) shows maximum zone of inhibition against the tested two selective negative bacteria *Pseudomonas aeruginosa* and *Klebsiella Pneumonia* (Fig. 6 & 9). And also, the results shows that the Methanol extract obtained from *Caulerpa peltate* (12 ± 1mm)*, Ulva lactuca* (13.67 ± 0.58 mm)*, Sargassum cristaefolium (*13.67 ± 0.58 mm) *and soleiria robusta* (12 ± 1 mm) shows the maximum of zone of inhibition activity against *Klebsiella pneumoniae* and also the Methanol extract obtained from *Porphyra indica* (13.67 ± 0.58 mm) and *Solieria robusta* (10.33 ± 0.58 mm) shows maximum zone of inhibition against *Pseudomonas aeruginosa* (Table 3; Figs. 5 & 8). The water extract obtained from *Caulerpa peltate, Ulva lactuca (*13 ± 1 mm)*, Chetomorpha area* (12 ± 1 mm)*, Turbinaria decurrens* (13.33 ± 0.58 mm)*, Hydroclathrus clathratus (*12.67 ± 0.58 mm) *and Solieria robusta* (11.33 ± 0.58 mm) results the maximum zone of inhibition activity against *Klebsiella pneumoniae* (Table 2; Figs. 7&10) and also the water extract of *Caulerpa peltate (*13 ± 1 mm) and *Turbinaria decurrens* (12.67 ± 1.53 mm) showed maximum zone of inhibition against *Pseudomonas aeruginosa*. There is no zone of inhibition noted in *Cheatomorpha area, Gelidiella acerosa,* and *Hypnea spicefera.* Among the selected 20 different macroalgae the red algae (*Rhodophyta*) showed the higher antibacterial activity followed by brown (Phaeophyta) and green (*Chlorophyta*) against the tested two selective negative bacteria. Similarly, Anne Bansemir et al. [29] reported the antibacterial activity of Dichloromethane, methanol and water extracts of 26 species of cultivated seaweeds were screened for their antibacterial activities against five fish pathogenic bacteria strains (*Aeromonas salmonicida* ssp. *salmonicida, Aeromonas hydrophila* ssp. *hydrophila, Pseudomonas anguilliseptic*a, *Vibrio anguillarum*, *Yersinia ruckeri*). The dichloromethane extracts of *Asparagopsis armata*, *Ceramium rubrum*, *Drachiella minuta*, *Falkenbergia rufolanosa, Gracilaria cornea* and *Halopitys incurvus* showed strong antibacterial activities. *V. anguillarum* and *P. anguilliseptica* were the two most susceptible bacteria strains.

3.1 Antibacterial activity of Red Macroalgae (*Rhodophyta*)

The initial study of antibacterial activity of red algae was shown in Fig. 2. The maximum antibacterial activity (14.67 ± 1 mm) was observed in *Solieria robusta* and *Hypnea spicifera* (13.67 ± 0.58 mm) against *Klebsiella pneumonia* and in *Pseudomonas aeruginosa* the maximum zone of inhibition was noted in *Gracilaria edulis* (12.67 ± 0.58 mm) and *Hypnea spicifera* (13 ± 1 mm). The minimum zone of inhibition (9.67 ± 0.58 mm) was noted in *Solieria robusta* against *Pseudomonas aeruginosa*. There is no zone of inhibition was noted in *Gelidiella acerosa* and *Porphyra indica*. In Methanol extract of red algae the maximum zone of inhibition was observed in *Porphyra indica* (13.68 ± 0.58 mm) and *Soleiria robusta* (10.68 ± 0.58 mm) against *Pseudomonas aeruginosa* and

Solieria robusta (12 ± 1 mm) and *Porphyra indica* (10.67 ± 0.58 mm) shows maximum zone of inhibition against *Klebsiella pneumonia.* The minimum zone of inhibition was observed in *Gelidiella acerosa* and *Hypnea spicifera.* In water extract of red algae, the maximum zone of inhibition observed in *Solieria robusta* (9.33 ± 0.58 mm) and *Hypnea spicefera* (9.33 ± 0.58 mm) against two selective negative bacteria. There is no zone was recorded in *Gelidiella acerosa, Gracilaria edulis, Porphyra indica* and *Ceramium rubrum*. Similarly, Güner et al. [30] reported the activity of *Ceramium rubrum* methanol extract against *E. coli*, *Enterococcus faecalis* and *S. aureus.* Previously, Manilal et al. [31] studied the antibacterial activity of *Gelidium pusillum* methanol extract against *V. harveyi*, *V. alginolyticus*, *V. vulnificus*, *V. parahaemolyticus* and *V. alcaligenes.* Similarly, Munoz-ochoa et al. [32] noted the two strains *S. aureus* and *S. pyogenes* were studied against ethanol extract of *Gelidium robustum*. Additionally, Amorim et al. [33] tested the aqueous extract of *Gracilaria ornata* against *E. coli* and also Manilal et al. [31] studied the antibacterial activity of *Portieria horemanii* from methanol extract against V. *harveyi, V. alginolyticus* and *V. vulnificus*.

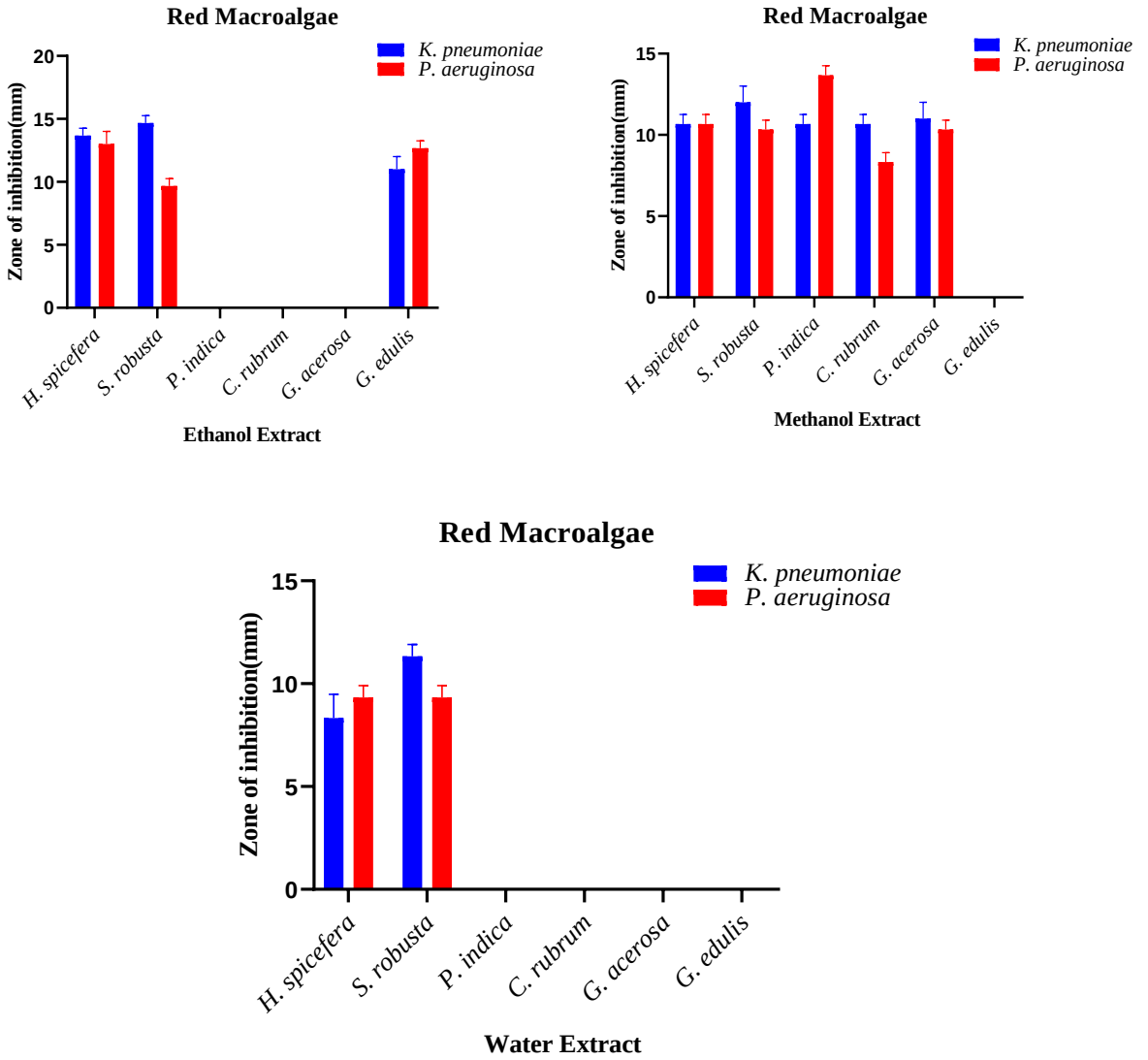


Fig. 2. Diameter averages (mm) of the zones of inhibition of Ethanol, Methanol and Water extracts of red macroalgae

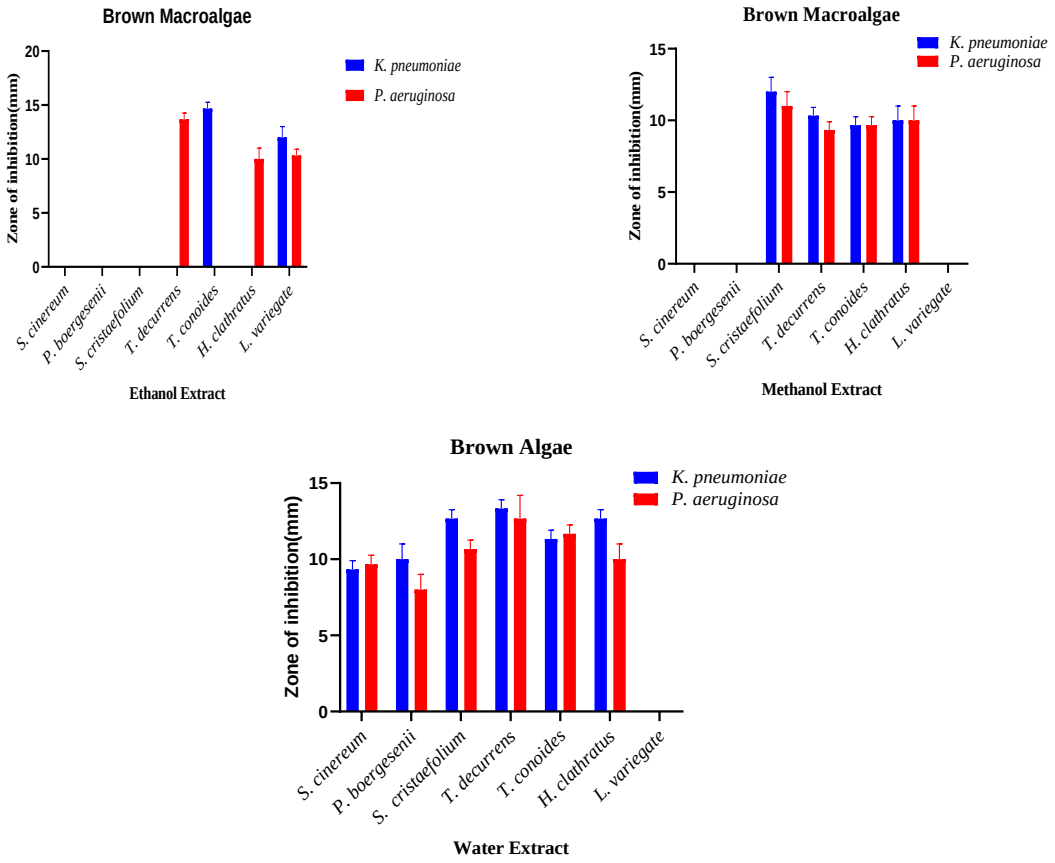


Fig. 3. Diameter averages (mm) of the zones of inhibition of Ethanol, Methanol and Water extracts of brown macroalgae

3.2 Antibacterial Activity of Brown Algae (Phaeophyta)

In the ethanol extract of brown algae, the maximum zone of inhibition was noted (Fig. 3) in the extract of *Turbinaria decurrens* (14.67 ± 0.58 mm) and *Lobophora varigata* (12 ± 1 mm) against *Klebsiella pneumonia*. For *Pseudomonas aeruginosa* the maximum zone was recorded in *Turbinaria conoides* (13.67 ± 0.58 mm) and the minimum zone of inhibition was recorded in *Hydroclathrus clathratus* (10 ± 1 mm) against *Pseudomonas aeruginosa*. There is no zone of inhibition was observed in *Turbinaria decurrens* and *Sargassum cristaefolium.* The result of brown algae in Methanol extract showed that the maximum zone of inhibition was noted in *Sargassum cristaefolium* (12 ± 1 mm) against *Klebsiella pneumoniae* and (11 ± 1 mm) inhibition was against *Pseudomonas aeruginosa*. The minimum zone of inhibition was showed in *Turbinaria decurrens* and *Turbinaria conoides* against both the selected negative bacteria. The result of brown algae in water extract showed that the maximum zone of inhibition (12.67 ± 1.53 mm) was recorded in *Turbinaria decurrens* (12.67 ± 1.53), *Turbinaria conoides* (11.67 ± 0.58 mm) against *Pseudomonas aeruginosa* and the maximum zone of inhibtion (13.33 ± 0.58) in *Turbinaria decurrens* against bacteria *Pseudomonas Klebsiella pneumonia*. The minimum zone (10 ± 1 mm) was recoded in *Hydroclanthrus clathratus* against *Pseudomonas aeruginosa* and 9.33 ± 0.58 mm observed in *Sargassum cinereum* against *Klebsiella pneumonia.* Similarly, Taskin et al. [34] noted the zone of inhibition in the Methanolic extract of *Padiana pavoniva* against *E. coli*. Also, Jaswir et al. [35] reported in their study that Methanol, acetone and ethyl acetate extracts from *Sargassum flavellum* against *B. substils*. Similarly, *Hydroclathrus clathratus*, *Padina concrescens* and *Padina Mexicana* extracts obtained from ethanol showed the activity against *S. aures* and *S. pyogenes* [32]. Additionally, Vijayabaskar et al. [36] observed the antibacterial activity from the methanol extract of *Turbinaria ornate and Sargassum wightii* against *S. aureus*, *B. subtilis, E. coli* and *P. aeruginosa.*

Table 1. Anti-bacterial activity of Marine macroalgal species

		Water		Ethanol		Methanol	
Group	**Macroalgae**	***P. aeruginosa***	***K.pneumoniae***	***P. aeruginosa***	***K.pneumoniae***	***P. aeruginosa***	***K.pneumoniae***
Chlorophyta	*Caulerpa peltate*	**	**	**	**	*	**
	Ulva lactuca	**	**	**	**	**	**
	Halimeda macroloba	*	*	**	**	**	**
	Chaetomorpha aerea	**	**	-	-	-	-
	Cladophora vagabunda	-	*	**	**	**	**
	Enteromorpha flexuosa	-	-	**	**	*	*
	Halimeda gracilis	*	*	-	-	**	-
Phaeophyta	*Sargassum cinereum*	*	*	-	-	-	-
	Padina boergesenii	*	*	-	-	-	-
	Sargassum cristaefolium	**	**	-	-	**	**
	Turbinaria decurrens	**	**	**	-	*	*
	Turbinaria conoides	**	**	-	**	*	*
	Hydroclathrus clathratus	**	*	*	-	*	*
	Lobophora variegate	-	-	*	**	-	-
Rhodophyta	*Hypnea spicefera*	-	*	**	**	**	**
	Solieria robusta	*	**	*	**	*	*
	Porphyra indica	-	-	-	-	**	**
	Ceramium rubrum	-	-	-	-	*	**
	Gelidiella acerosa	-	-	-	-	*	**
	Gracilaria edulis	-	-	**	**	-	-

*Notes: *1-10 mm and ** 10-20 mm (Zone of inhibition)*

Table 2. Antibacterial activities of Water extract of Marine macroalgal species

		Water Extract	
Group	**Macroalgae**	***K. pneumoniae***	***P. aeruginosa***
Chlorophyta	*Caulerpa peltate*	13±1	13±1
	Ulva lactuca	11.67±0.58	11.67±0.58
	Halimeda macroloba	10.33±0.58	8.33±0.58
	Chaetomorpha aerea	12±1	11.67±0.58
	Cladophora vagabunda	-	10±1
	Enteromorpha flexuosa	-	-
	Halimeda gracilis	9±1	10.33±0.58
Phaeophyta	*Sargassum cinereum*	9.33±0.58	9.67±0.58
	Padina boergesenii	10±1	8±1
	Sargassum cristaefolium	12.67±0.58	10.67±0.58
	Turbinaria decurrens	13.33±0.58	12.67±1.53
	Turbinaria conoides	11.33±0.58	11.67±0.58
	Hydroclathrus clathratus	12.67±0.58	10±1
	Lobophora variegate	-	-
Rhodophyta	*Hypnea spicefera*	-	9.33±0.58
	Solieria robusta	11.33±0.58	9.33±0.58
	Porphyra indica	-	-
	Ceramium rubrum	-	-
	Gelidiella acerosa	-	-

Table 3. Antibacterial activities of methanol extract of Marine macroalgal species

		Methanol Extract	
Group	**Macroalgae**	***K. pneumoniae***	***P. aeruginosa***
Chlorophyta	*Caulerpa peltate*	12±1	8.33±0.58
	Ulva lactuca	13.67±0.58	12±1
	Halimeda macroloba	10.67±0.58	11.33±0.58
	Chaetomorpha aerea	-	-
	Cladophora vagabunda	-	-
	Enteromorpha flexuosa	10.33±0.58	10±1
	Halimeda gracilis	-	11±1
Phaeophyta	*Sargassum cinereum*	-	-
	Padina boergesenii	-	-
	Sargassum cristaefolium	12±1	11±1
	Turbinaria decurrens	10.33±0.58	9.33±0.58
	Turbinaria conoides	9.67±0.58	9.67±0.58
	Hydroclathrus clathratus	10±1	10±1
	Lobophora variegate	-	-
Rhodophyta	*Hypnea spicefera*	10.67±0.58	10.67±0.58
	Solieria robusta	12±1	10.33±0.58
	Porphyra indica	10.67±0.58	13.67±0.58
	Ceramium rubrum	10.67±0.58	8.33±0.58
	Gelidiella acerosa	11±1	10.33±0.58
	Gracilaria edulis	-	-

Table 4. Antibacterial activities of Ethanol extract of the seaweed's species

		Ethanol Extract	
Group	**Species**	***K. pneumoniae***	***P. aeruginosa***
Chlorophyta	*Caulerpa peltate*	13.67±0.58	13.33±0.58
	Ulva lactuca	12.33±0.58	12.67±0.58
	Halimeda macroloba	12±1	14.67±0.58
	Chaetomorpha aerea	-	-
	Cladophora vagabunda	14±67	11.33±0.58
	Enteromorpha flexuosa	12±67	10.67±0.58
	Halimeda gracilis	-	-
Phaeophyta	*Sargassum cinereum*	-	-
	Padina boergesenii	-	-
	Sargassum cristaefolium	-	-
	Turbinaria decurrens	-	13.67±0.58
	Turbinaria conoides	14.67±0.58	-
	Hydroclathrus clathratus	-	10±1
	Lobophora variegate	12±1	10.33±0.58
Rhodophyta	*Hypnea spicefera*	13.67±0.58	13±1
	Solieria robusta	14.67±0.58	9.67±0.58
	Porphyra indica	-	-
	Ceramium rubrum	-	-
	Gelidiella acerosa	-	-
	Gracilaria edulis	11±1	12.67±0.58

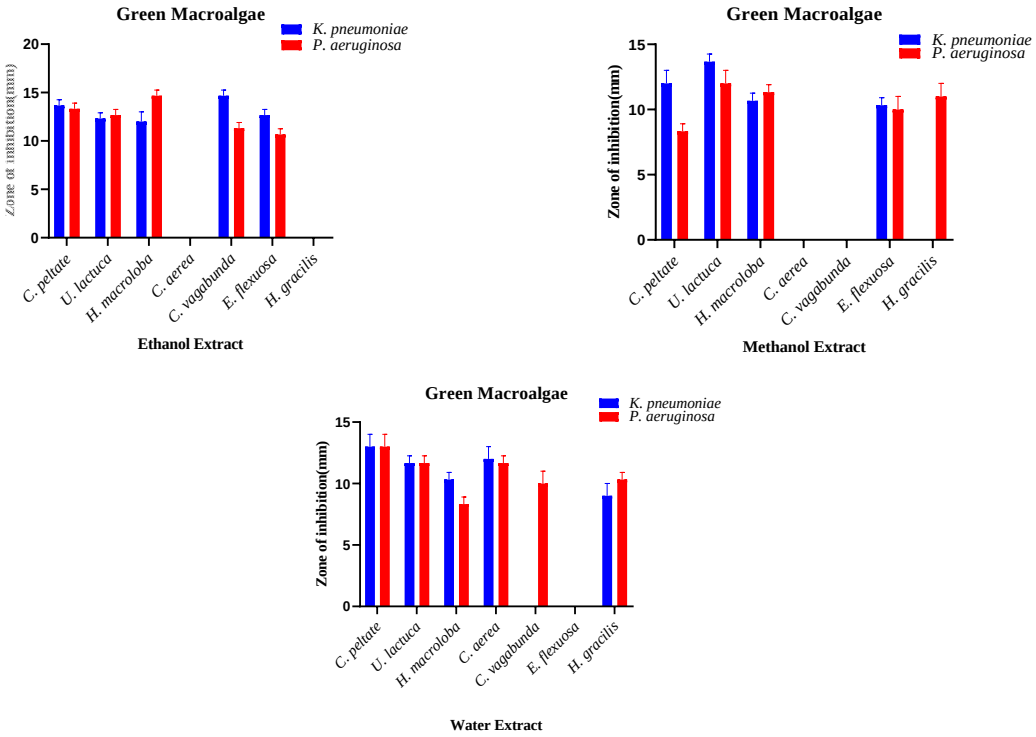
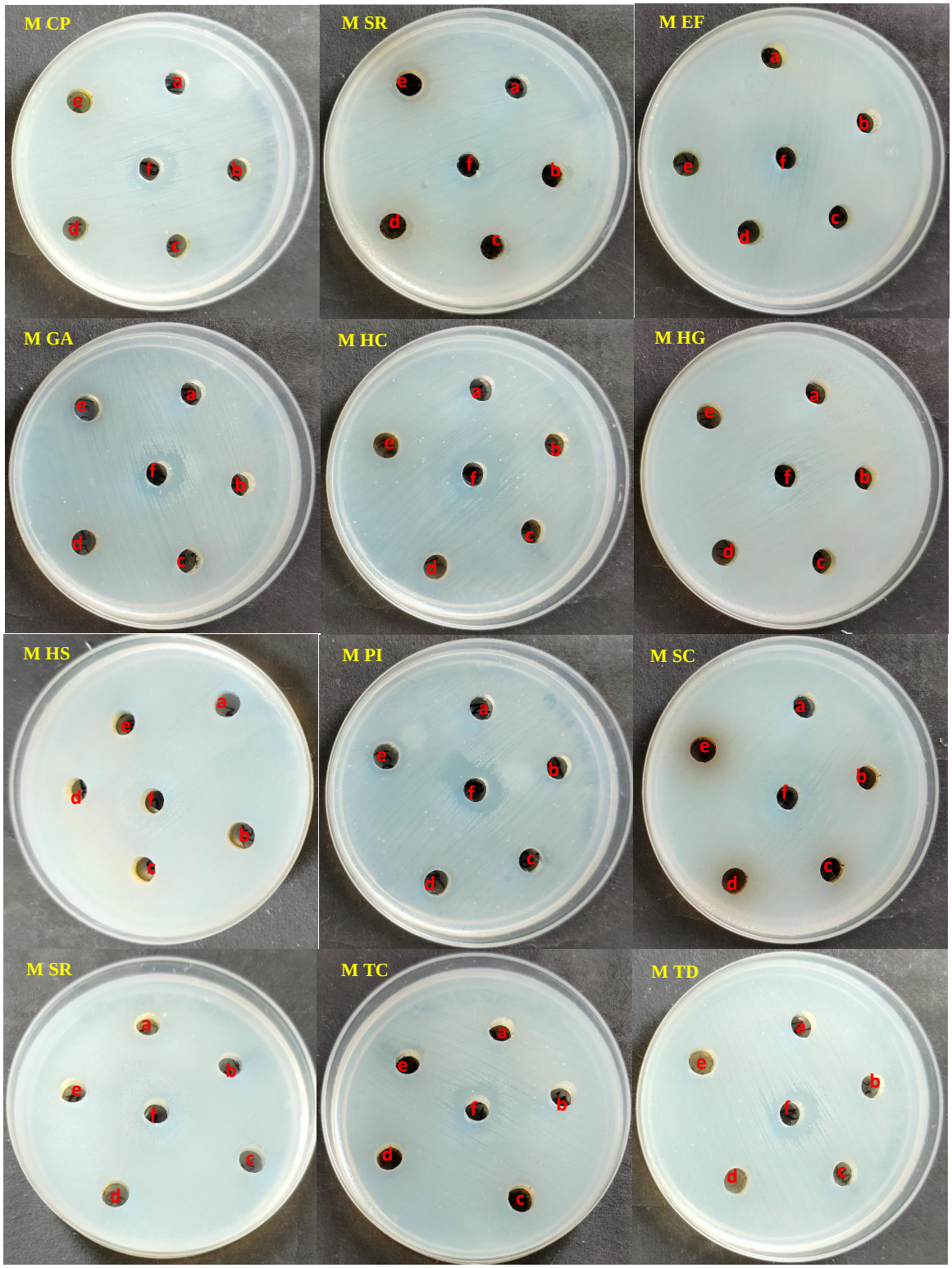


Fig. 4. Diameter averages (mm) of the zones of inhibition of Ethanol, Methanol and Water extracts of green macroalgae

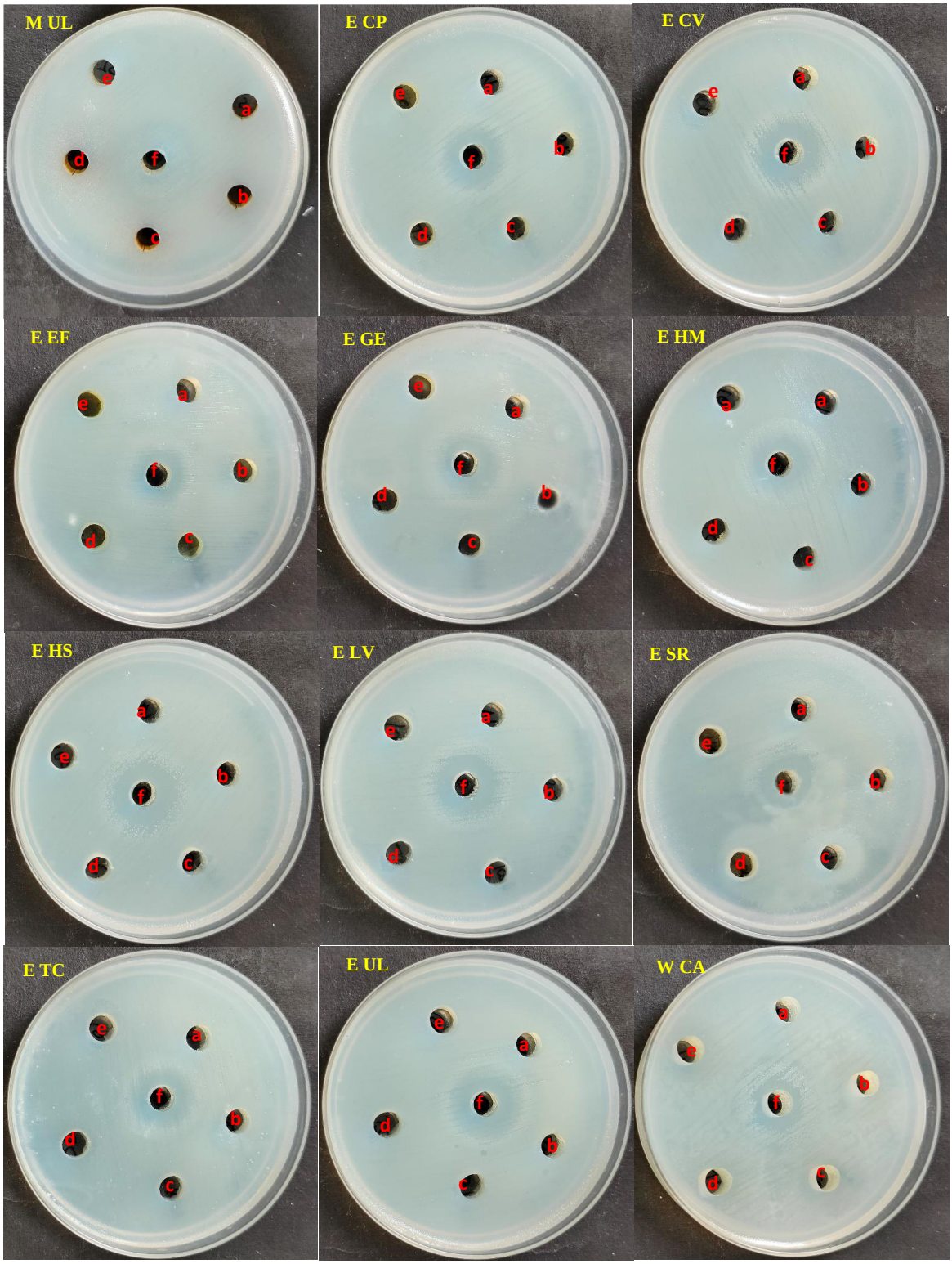
3.3 Antibacterial Activity of Green algae (*Chlorophyta*)

The results of green algae in ethanol expresses (Fig. 4) the maximum zone of inhibition (14.67 ± 67 mm) in the extract obtained from *Cladophora vagabunda against Klebsiella pneumoniae,* (12.67 ± 0.58 mm) observed in the extract of *Enteromorpha flexuosa* and 12.33 ± 1 mm in *Ulva lactuca* against *Klebsiella pneumonia* and also 13.33 ± 0.58 mm zone of inhibition was observed in the extract of *Caulerpa peltate* against both *Pseudomonas aeruginosa* and *Klebsiella pneumonia.* The minimum zone 10.67 ± 0.58 mm was noted in the extract of *Enteromorpha flexuosa* against *Pseudomonas aeruginosa* and 12 ± 1 mm zone was noted in the extract of *Halimeda macroloba* against *Klebsiella pneumoniae*. There is no zone of inhibition was noted in *Cheatomorpha aerea* against the selected pathogens. The results of green algae in Methanol extract expresses the maximum zone of inhibition 13.67 ± 0.58 mm and 12 ± 1mm in the extract obtained from *Ulva lactuca* and *Caulerpa peltata* against *Klebsiella pneumoniae*. For *Pseudomonas aeruginosa* the maximum zone 12 ± 1 mm shown in the extract of *Ulva lactuca*. The minimum zone of inhibition 10.33 ± 0.58 mm was recorded in the extract obtained in the extract of *Enteromopha flexuosa* against *Klebsiella pneumonia*. There is no zone of inhibition was recorded in the extract obtained from *Ulva lactuca, Halimeda gracilis, Enteromorpha flexuosa* and *Chetomorpha aerea* against *Pseudomonas aeruginosa*. The results of green algae in water extract shows that the maximum zone of inhibition 13 ± 1 mm was observed in the extract obtained from *Caulerpa peltate*, 12 ± 1 mm in the extract obtained from *Ulva lactuca* and 11.67 ± 0.58 mm in the extract obtained from *Cheatomorpha aerea* against *Pseudomonas aeruginosa* and 13 ± 1 mm was recorded in the extract of *Caulerpa peltate* against *Klebsiella pneumonia*. The minimum zone of inhibition 10 ± 1 mm was noted in the extract obtained from *Halimeda gracilis* against both *Pseudomonas aeruginosa* and *Klebsiella pneumonia*. Previously Vallinayagam et al. [37] was recorded from the extract of *U. lactuca* against *P. aeruginosa* (12 mm). Similarly, Pierre et al. [38] reported the antibacterial activity from water extract of *Chetomorpha aerea* against *Bacilus subtilis*, *Micrococcus luteus* and *S. aureus*. *Caulerpa lentillifera* and *Caulerpa racemosa* extract from methanol, ethyl acetate and water show the activity against *E. coli, Staphylococcus aureus, Streptococcus sp.,* and *Salmonella sp.* [39].



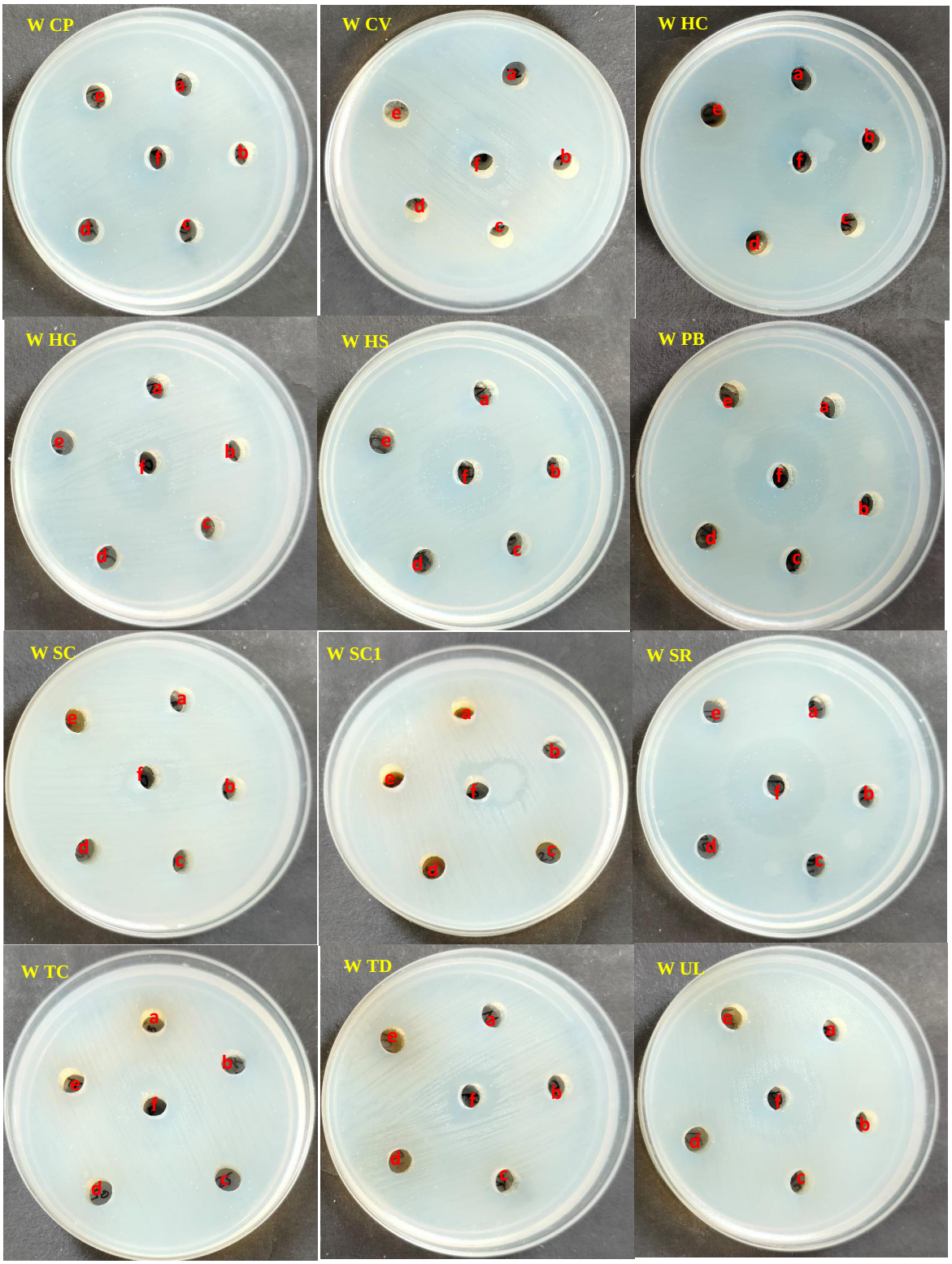
a: 0 µg/mL; b: 50 µg/mL; c: 100 µg/mL; d: 150 µg/mL; e: 200 µg/mL; f: Azithromycin (30 µg/mL)
M- Methanol , CP- *C. peltate*, *SR-S. robusta*, *EF- E. flexuosa* , *GA- G. edulis*, *HC- H. clathratus*, *HG-H. gracilis*, *HS-H. spicifera*, *PI-P. indica*, *SC-S. cinereum*, *TC-T. conoides*, *TD- T. decurrens*, *UL-U.lactuca*

Fig. 5. Antibacterial activity of Methanol extract against *Klebsiella pneumoniae*



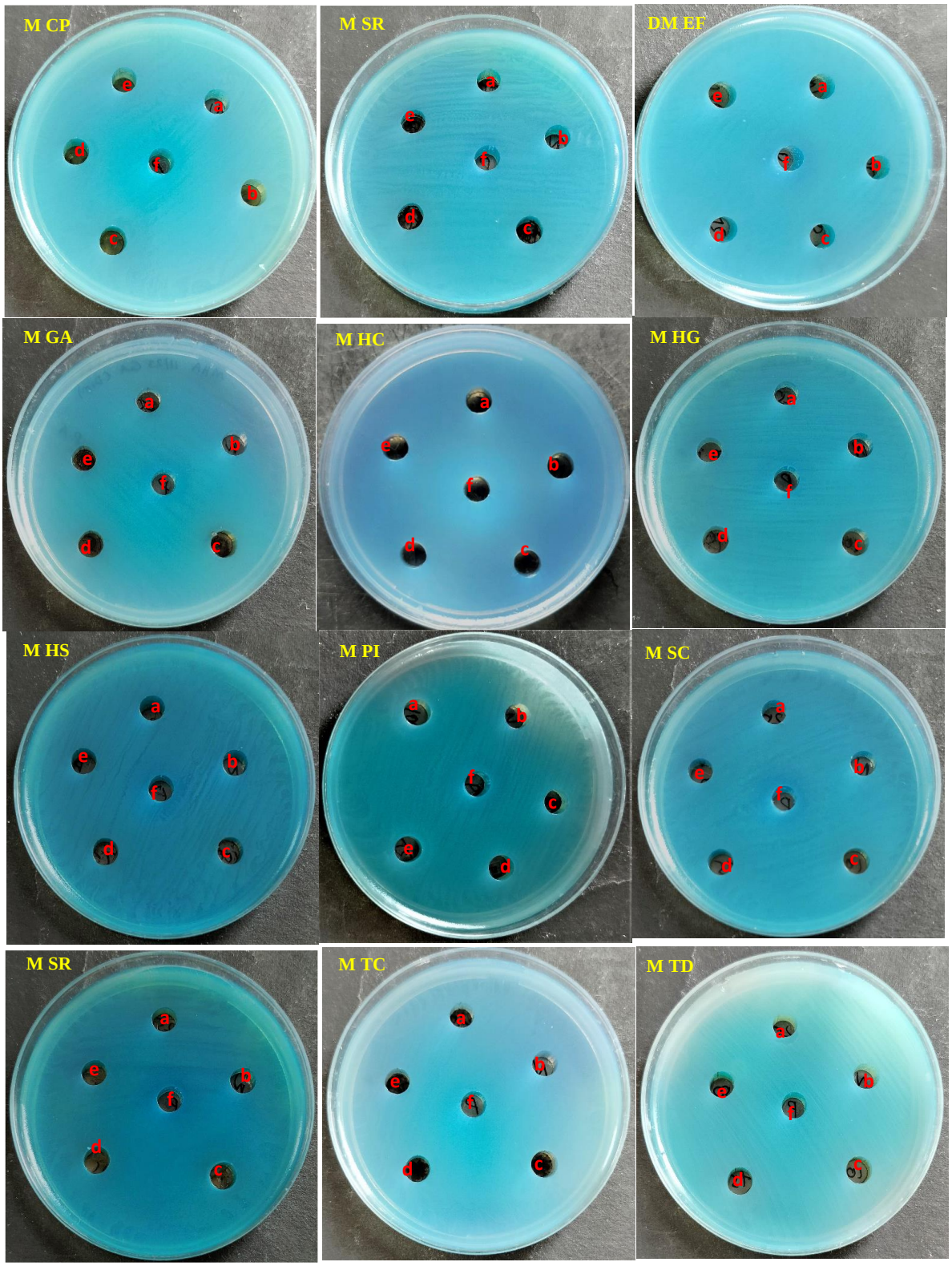
a: 0 µg/mL; b: 50 µg/mL; c: 100 µg/mL; d: 150 µg/mL; e: 200 µg/mL; f: Azithromycin (30 µg/mL)
E- Ethanol extract *CP- C. peltate, SR-S. robusta, EF- E. flexuosa , GA- G. edulis, HC- H. clathratus, HG-H. gracilis, HS- H. spicifera, PI-P. indica, SC-S. cinereum, TC-T. conoides, TD- T. decurrens, UL-U.lactuca, LV-L. varigeata, HM-H. macroloba*

Fig. 6. Antibacterial activity of Ethanol extract against *Klebsiella pneumoniae*



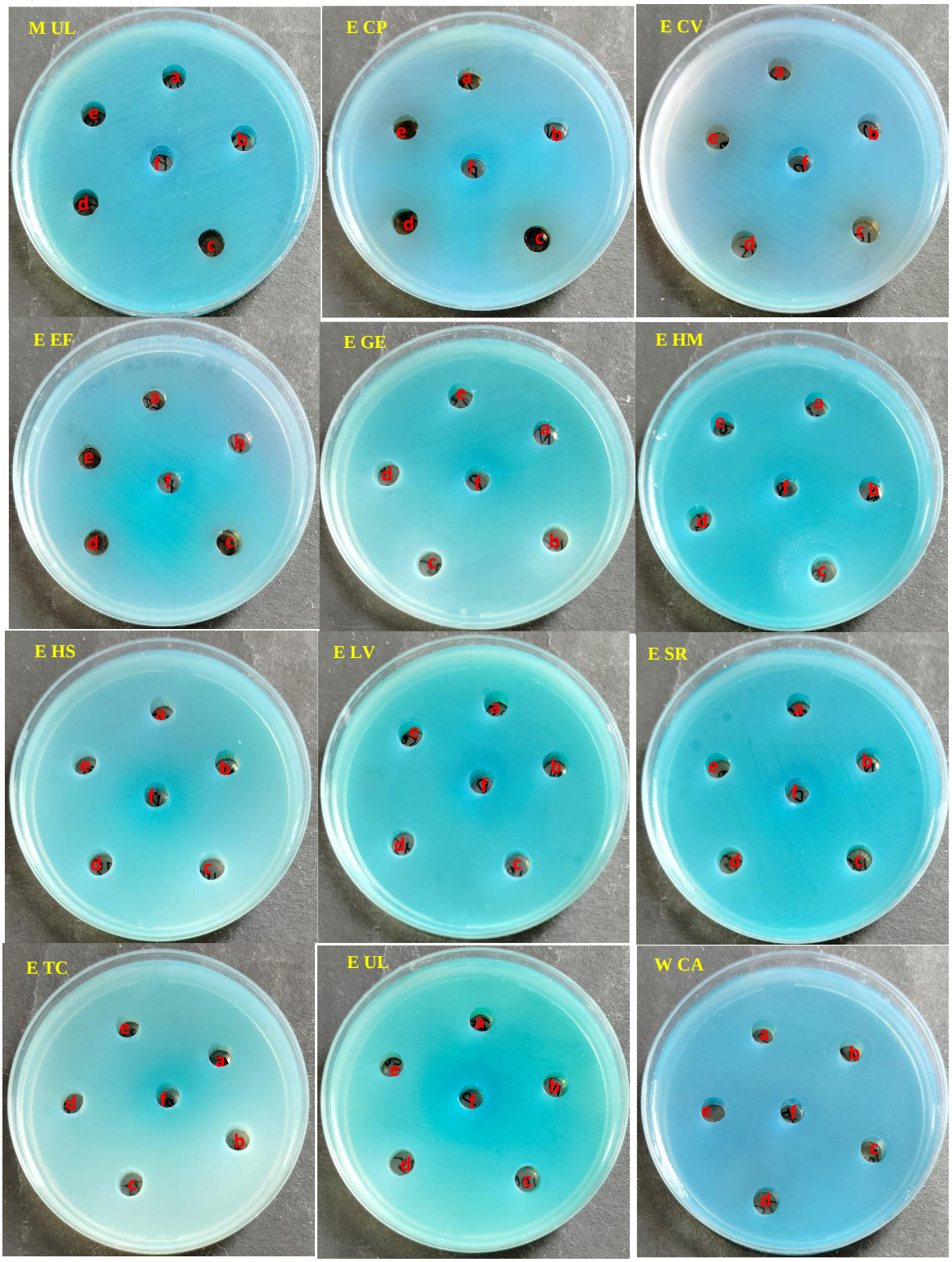
a: 0 µg/mL; b: 50 µg/mL; c: 100 µg/mL; d: 150 µg/mL; e: 200 µg/mL; f: Azithromycin (30 µg/mL)
W- Water extract, *CP- C. peltate, SR-S. robusta, EF- E. flexuosa , GA- G. edulis, HC- H. clathratus, HG-H. gracilis, HS- H. spicifera, PI-P. indica, SC-S. cinereum, TC-T. conoides, TD- T. decurrens, UL-U.lactuca, LV-L. varigeata, HM-H. macroloba*

Fig. 7. Antibacterial activity of Water extract against *Klebsiella pneumoniae*



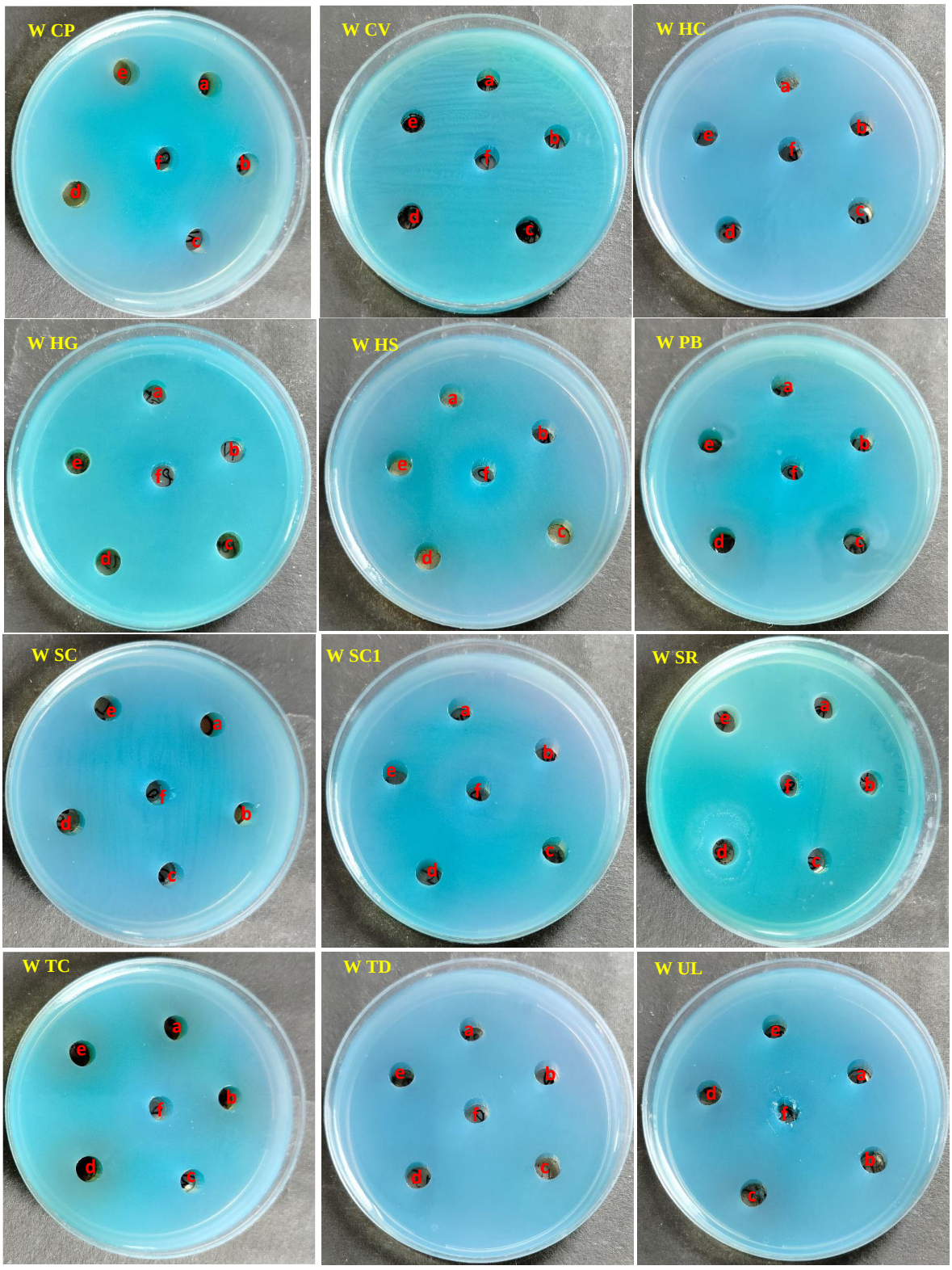
a: 0 µg/mL; b: 50 µg/mL; c: 100 µg/mL; d: 150 µg/mL; e: 200 µg/mL; f: Azithromycin (30 µg/mL)
M- Methanol , CP- *C. peltate*, *SR-S. robusta*, *EF- E. flexuosa* , *GA- G. edulis*, *HC- H. clathratus*, *HG-H. gracilis*, *HS- H. spicifera*, *PI-P. indica*, *SC-S. cinereum*, *TC-T. conoides*, *TD- T. decurrens*, *UL-U.lactuca*

Fig. 8. Antibacterial activity of Methanol extract against *Pseudomonas aeruginosa*



a: 0 µg/mL; b: 50 µg/mL; c: 100 µg/mL; d: 150 µg/mL; e: 200 µg/mL; f: Azithromycin (30 µg/mL) E- Ethanol extract W- Water extract, *CP- C. peltate, SR-S. robusta, EF- E. flexuosa , GA- G. edulis, HC- H. clathratus, HG-H. gracilis, HS- H. spicifera, PI-P. indica, SC-S. cinereum, TC-T. conoides, TD- T. decurrens, UL-U.lactuca, LV-L. varigeata, HM-H. macroloba*

Fig. 9. Antibacterial activity of Ethanol extract against *Pseudomonas aeruginosa*



a: 0 µg/mL; b: 50 µg/mL; c: 100 µg/mL; d: 150 µg/mL; e: 200 µg/mL; f: Azithromycin (30 µg/mL)
W- Water extract, *CP- C. peltate, SR-S. robusta, EF- E. flexuosa , GA- G. edulis, HC- H. clathratus, HG-H. gracilis, HS- H. spicifera, PI-P. indica, SC-S. cinereum, TC-T. conoides, TD- T. decurrens, UL-U.lactuca, LV-L. varigeata, HM-H. macroloba SC1- S. cristaefolium*

Fig. 10. Antibacterial activity of water extract against *Pseudomonas aeruginosa*

4. CONCLUSION

This work proved that marine macroalgae have the potential to be a source of bioactive compounds that are resistant to bacteria. This study concludes that marine macroalgae have potent bioactive compounds for the selected negative bacteria. The findings clearly demonstrate that seaweeds are a fascinating source of biologically active chemicals that might be used in addition to or instead of conventional antibiotics for the prophylactic and treatment of bacterial human diseases. Further, to find the most effective new compounds against bacterial pathogens, however, more purification and chemical characterization research is required.

DATA AVAILABILITY STATEMENT

The data that support the findings of the study are available from the corresponding author upon responsible request

ACKNOWLEDGEMENT

The authors are thankful to Bharathidasan University and RUSA 2.0, Biological sciences for providing facilities and also thank RUSA 2.0, Biological sciences, for the financial support.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Bukhari NTM, Rawi NFM, Hassan NAA, Saharudin NI, Kassim MHM. Seaweed polysaccharide nanocomposite films: a review. International Journal of Biological Macromolecules. 2023;125486.
2. Sivakumar SR, Annamalai A, Ramasam M, Sarbudeen M. Isolation and characterization of l-asparaginase from seaweeds collected from rameswaram coast, southeast india. Vegetos. 2023:1-10.
3. Aravinth A, Dhanasundaram S, Perumal P, Vengateshwaran TD, Thavamurugan S, Rajaram R. Biological activities of the brown seaweed dictyota ciliolata with special reference to the human diseases transmitting aedes aegypti's larvae. Biomass Conversion and Biorefinery. 2023;1-17.
4. Fakee J, Bolton JJ, Le Roes-Hill M, Durrell KA, Antunes E. Beukes DR. Antimicrobial activity of the secondary metabolites isolated from a south african red seaweed. Laurencia corymbosa. Molecules. 2023; 28(5):2063.
5. Bennett JP, Robinson LF, Gomez LD. Valorisation strategies for brown seaweed biomass production in a european context. Algal Research. 2023;103248.
6. Lomartire S, Gonçalves AM. Algal phycocolloids: bioactivities and pharmaceutical applications. marine drugs. 2023;21(7):384.
7. Perumal P, Dhanasundaram S, Aravinth A, Amutha V, Santhanam P. Larvicidal property of the extracts of the seaweeds; Sargassum wightii, S. ilicifolium and Gelidiella acerosa against Anopheles stephensi, Aedes aegypti and Culex quinquefasciatus. Biocatalysis and Agricultural Biotechnology. 2022 ;1;43:102436.
8. Vinodkumar M, Packirisamy ASB. Exploration of bioactive compounds from sargassum myriocystum; a novel approach on catalytic inhibition against free radical formation and glucose elevation. Topics in Catalysis. 2023;1-14.
9. Kaur M, Saini KC, Mallick A, Bast F. Seaweed-associated epiphytic bacteria: diversity, ecological and economic implications. Aquatic Botany. 2023; 103698.
10. Labes A. Marine resources offer new compounds and strategies for the treatment of skin and soft tissue infections. Marine Drugs. 2023;21(7):387.
11. Priyanka KR, Rajaram R. Preliminary screening on anticandidal properties of marine macroalgae collected from palk bay and gulf of mannar, southeast coast of india. Biomass Conversion and Biorefinery. 2022;1-10.
12. Wood SJ, Kuzel TM, Shafikhani SH. Pseudomonas aeruginosa: infections, animal modeling, and therapeutics. Cells. 2023;12(1):199.
13. Cláudia-Ferreira A, Barbosa DJ, Saegeman V, Fernández-Rodríguez A, Dinis-Oliveira RJ, Freitas AR. ESCMID Study group of forensic and post-mortem microbiology (esgfor). the future is now: unraveling the expanding potential of human (necro) microbiome in forensic investigations. Microorganisms. 2023; 11(10):2509.
14. Maleki NS, Babazadeh F, Arzanlou M, Teimourpour R, Dogaheh HP. Serotyping

and molecular profiles of virulence-associated genes among klebsiella pneumoniae isolates from teaching hospitals of ardabil, iran: a cross-sectional study. Health Science Reports. 2023;6(9):1557.
15. Pu D, Zhao J, Chang K, Zhuo X, Cao B. Superbugs with hypervirulence and carbapenem resistance in klebsiella pneumoniae: the rise of such emerging nosocomial pathogens in China. Science Bulletin; 2023.
16. Ayukekbong JA, Ntemgwa M, Atabe AN. The threat of antimicrobial resistance in developing countries: causes and control strategies. Antimicrobial Resistance & Infection Control. 2017;6(1):1-8.
17. Effah CY, Sun T, Liu S, Wu Y. Klebsiella pneumoniae: an increasing threat to public health. Annals Of Clinical Microbiology And Antimicrobials. 2020;19(1):1-9.
18. Gnanavel V, Roopan SM, Rajeshkumar S. Aquaculture: an overview of chemical ecology of seaweeds (food species) in natural products. Aquaculture. 2019;507:1-6.
19. Velev V, Pavlova M, Alexandrova E, Popov M, Lutakov I, Tcherveniakova T, Angelova A, Hristozova E, Kalchev Y, Ivanov I. Study on patients with clostridioides difficile infection during the covid-19 pandemic in bulgaria. Biotechnology & Biotechnological Equipment. 2023;37(1):188-193.
20. Sukhikh S, Prosekov A, Ivanova S, Maslennikov P, Andreeva A, Budenkova E, Babich O. Identification of metabolites with antibacterial activities by analyzing the ftir spectra of microalgae. Life. 2022;12(9): 1395.
21. Chinemerem Nwobodo D, Ugwu MC, Oliseloke Anie C, Al-Ouqaili MT, Chinedu Ikem J, Victor Chigozie U, Saki M. Antibiotic resistance: the challenges and some emerging strategies for tackling a global menace. Journal of clinical laboratory analysis. 2022;36(9): 24655.
22. Balaji S, Edward JKP, Samuel VD. Coastal and marine biodiversity of gulf of mannar, southeastern india–a comprehensive updated species list. Gulf of Mannar Biosphere Reserve Trust, Publication. 2012;22:128.
23. Thirumalaiselvan S, Rajkumar M, Vinothkumar R, Remya L, Batcha SM. Seagrass, seaweed and mangrove ecosystem of gulf of mannar and palk bay region; 2020.
24. Ferreira RL, Da Silva BC, Rezende GS, Nakamura-Silva R, Pitondo-Silva A, Campanini EB, Brito MC, da Silva EM, Freire CCDM, Cunha AFD, Pranchevicius MCDS. High prevalence of multidrug-resistant *klebsiella pneumoniae* harboring several virulence and β-lactamase encoding genes in a brazilian intensive care unit. Frontiers in microbiology. 2019; 9:3198.
25. Manilal A, Selvin J, Thajuddin N, Sujith S, Panikkar M, Idhayadhulla A, Kumar RS. Biopotentials of marine alga, *lobophora variegata* collected from the south indian littoral. Thalassas; 2012;28(1):47-54.
26. Paul G, Yusuf S, Sharma S. Unmasking of the Brugada syndrome phenotype during the acute phase of amiodarone infusion. Circulation. 2006;114(11);489-491.
27. van Wyk AS, Prinsloo G. Health, safety and quality concerns of plant-based traditional medicines and herbal remedies. South African Journal of Botany. 2020; 133:54-62.
28. Mohan PK, Krishna TA, Thirumurugan A, Kumar TS, Kumari BR. Chemical profiling and in vitro antiurolithiatic activity of pleurolobus gangeticus (l.) j. st.-hil. ex h. ohashi & k. ohashi along with its antioxidant and antibacterial properties. Applied Biochemistry and Biotechnology. 2022;194(11):5037-5059.
29. Bansemir A, Blume M, Schröder S, Lindequist U. Screening of cultivated seaweeds for antibacterial activity against fish pathogenic bacteria. Aquaculture. 2006;252(1):79-84.
30. Güner A, Yavaşoğlu NÜK. Evaluation of antioxidant, antimicrobial and antimutagenic activity with irritation effects of ceramium rubrum (red algae) extract. International Journal of Secondary Metabolite. 2018;5(4):279-287.
31. Manilal A, Sugathan S, Joseph S, George KS, Shakir C, Lipton AP. Antimicrobial potential of marine organisms collected from the southwest coast of india against multiresistant human and shrimp pathogens. Scientia marina. 2010;74(2): 287-296.
32. Muñoz-Ochoa M, Murillo-Álvarez JI, Zermeño-Cervantes LA, Martínez-Díaz S, Rodríguez-Riosmena R. Screening of extracts of algae from baja california sur, mexico as reversers of the antibiotic

resistance of some pathogenic bacteria. Eur. Rev. Med. Pharmacol. Sci. 2010; 14(9):739-747.

33. Amorim RDNDS, Rodrigues JAG, Holanda ML, Quinderé ALG, Paula RCMD, Melo VMM, Benevides NMB. Antimicrobial effect of a crude sulfated polysaccharide from the red seaweed gracilaria ornata. Brazilian Archives of Biology and Technology. 2012; 55:171-181.
34. Taskin E, Caki Z, Ozturk M. Assessment of in vitro antitumoral and antimicrobial activities of marine algae harvested from the eastern mediterranean sea. African Journal of Biotechnology. 2010;9(27): 4272-4277.
35. Jaswir I, Tope AHT, Raus RA, Monsur HA, Ramli N. Study on anti-bacterial potentials of some malaysian brown seaweeds. Food Hydrocolloids. 2014;42: 275-279.
36. Vijayabaskar P, Shiyamala V. Antibacterial activities of brown marine algae (sargassum wightii and turbinaria ornata) from the gulf of mannar biosphere reserve. Advances in Biological Research. 2011; 5(2):99-102.
37. Vallinayagam K, Arumugam R, Kannan RRR, Thirumaran G, Anantharaman P. Antibacterial activity of some selected seaweeds from pudumadam coastal regions. Global Journal of Pharmacology. 2009;3(1):50-52.
38. Pierre G, Sopena V, Juin C, Mastouri A, Graber M, Maugard T. antibacterial activity of a sulfated galactan extracted from the marine alga *chaetomorpha aerea* against *staphylococcus aureus*. Biotechnology and Bioprocess Engineering. 2011;16: 937-945.
39. Nagappan T, Vairappan CS. Nutritional and bioactive properties of three edible species of green algae, genus caulerpa (caulerpaceae). Journal of Applied Phycology. 2014;26:1019-1027.