Antibacterial Activity of Chlorophyta, Phaeophyta, and Rhodophyta Using Various Antibiotics as Positive Controls: A Systematic Review and Meta-Analysis

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ABSTRACT

Different species of marine macroalgae have been reported to demonstrate antimicrobial activities against numerous bacteria, with varying results. According to the studies, other antibiotics have been used as positive controls. This study evaluated the effect of crude extracts and sulphated polysaccharides from Chlorophyta, Phaeophyta, and Rhodophyta in inhibiting bacterial growth in terms of the diameter of inhibition zones (DIZ) using a systematic review and meta-analysis approach. A total of 835 data, extracted from 23 selected articles, were analyzed using OpenMee software by comparing the standardized mean difference (SMD) and 95% confidence interval (CI). The largest DIZ that Chlorophyta showed was 35 mm, Phaeophyta was 27.3 mm, and Rhodophyta was 25.66 mm, which was categorized as a very strong activity. The crude extract revealed a better inhibitory activity than the sulphated polysaccharides. The overall effect size for crude extracts was with SMD = -1.72 (CI = -1.96 to -1.48, I^2 = 84.65%, $p < 0.000$) and for sulphated polysaccharides with SMD = -13.07 (CI = -16.00 to -10.14, I^2 = 85.8%, $p < 0.000$), respectively. Subgroup analysis showed that when ciprofloxacin was used, the SMD value was -12.88 (CI = -14.50 to -11.25), whereas if ampicillin was used, the SMD value was 1.81 (CI = 1.27 to 2.35). This study proved that Chlorophyta, Phaeophyta, and Rhodophyta revealed promising antibacterial activities. However, the overall effect size was affected by the antibiotic used when comparing the SMD of the DIZ using a meta-analysis approach. Other factors, such as extraction methods and bacterial strains that likely affect the overall effect size, are subjected to further analysis in the next study.

Keywords: Crude extracts, the diameter of inhibition zone, marine macroalgae, sulphated polysaccharide

Introduction

Marine macroalgae are known as natural coastal terrestrial resources rich in nutrition and bioactive compounds. Marine macroalgae are plant like protists that generally can be classified into three divisions: green macroalgae (Chlorophyta), brown macroalgae (Phaeophyta), and red macroalgae (Rhodophyta). It has been suggested that 1,800 species of Chlorophyta, 1,800 species of Phaeophyta, and 6200 species of Rhodophyta have been reported (Pereira, 2021). The pigment responsible for the green color of Chlorophyta is, e.g., chlorophyll a and b, fucoxanthin is responsible for the brown color of Phaeophyta, and phycobilin for the red color of Rhodophyta. The unique bioactive compounds from macroalgae have gained increasing interest for their potentially beneficial health effects. The bioactive compounds of macroalgae, however, are highly variable depending on species, geographic origin, environmental conditions, and season of harvest (Øverland et al*.,* 2019). Bioactive compounds of macroalgae, such as polysaccharides, carotenoids, vitamins, phenolics, and phycobiliproteins, appear to exhibit activities as antioxidants, antibacterials, antifungals, antivirals, and anticancer agents (Overland et al., 2019).

The antibacterial activity of macroalgae has been widely reported, including proteins, polyphenols, polysaccharides, pigments (chlorophyll and carotenoids), and polyunsaturated fatty acids (PUFAs) (Imbs & Zvyagintseva 2018; Øverland et al*.,* 2019; Silva et al*.,* 2020). For example, protein extracts from *Caulerpa occidentalis* interfered with the growth of

Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, and *Enterococcus faecalis* (Silva et al., 2020). Polyphenols from brown macroalgae extracted from *Fucus vesiculosus* actively inhibited both Gram-positive and Gram-negative bacteria (Imbs & Zvyagintseva 2018). In addition, macroalgae pigments, namely fucoxanthin, attacked both Gram-positive and Gram-negative bacteria and lipid extracts from green macroalgae species *Chaetomorpha linum* actively inhibited *Vibrio ordalii* and *Vibrio vulnificus* (Alves et al., 2020; Cardoso et al., 2019; Gomes-Dias et al., 2022; Liu et al., 2020).

During the antibacterial testing of a bioactive compound, it is common to use antibiotics as a positive control, such as amoxicillin, ampicillin, chloramphenicol, ciprofloxacin, and streptomycin. By including antibiotics, it can be determined whether the compound being tested exhibits low, moderate, or strong activity. According to the studies, different antibiotics have been used as positive controls. This recent study used a systematic literature review and meta-analysis (SR-MA) approach to evaluate the effect of crude extracts and sulphated polysaccharides of Chlorophyta, Phaeophyta, and Rhodophyta in inhibiting the bacterial growth, using different antibiotics as a positive control.

Material and Methods

Materials

This study used reputable national and international journal articles from ScienceDirect and Google Scholar data sources available from 5 August 2023 to 5 January 2024. Other platforms, such as ResearchGate, Semantic Scholar, and Academic Journal, were used to provide full-text articles. The tools used were Mendeley Reference Manager for Desktop software, Microsoft Excel version 2016, and OpenMEE version 2010.

Search Strategy and Selection

The collection of articles was conducted using PICO criteria (Population, Intervention, Comparison, Outcome) and inclusion-exclusion criteria (Tawfik et al., 2019). The research questions included antibacterial macroalgae as a population, extraction method, antibacterial form, macroalgae division, and test bacteria as intervention; controls (e.g., streptomycin, ampicillin) as a comparison; and macroalgae antibacterial potential as outcome. The journal article search used the keywords 'antibacterial,' 'activity,' 'Chlorophyta,' 'Phaeophyta,' 'Rhodophyta,' and 'macroalgae,' with Boolean operator provisions OR, AND, and NOT.

Journal articles were selected using the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA 2020) (Page et al., 2021), which included identification, selection, and suitability and eligibility for selected journal articles for meta-analysis. The selected journal articles are then tabulated in Microsoft Excel. The inclusion criteria were (1) Articles discussing the antibacterial activity of macroalgae measuring the diameter of inhibition zone (DIZ); (2) No country restrictions; (3) Original articles in Indonesian and English with complete relevant data; (4) National and international articles from reputable data sources; and (5) Include controls, number of samples (N), mean/mean (X), and standard deviation/ Standard deviation (SD). The exclusion criteria were (1) Articles with incomplete data; (2) Gray literature (data in the form of government reports, theses, and dissertations that have not been published); and (3) Articles that were as results of symposiums or conferences which were not accredited/indexed and reviewed.

Data Extraction

The information from the selected journal articles was tabulated in Microsoft Excel, including the author's name, year of publication, article origin, article index, macroalgae division, the antibacterial form (crude extract or sulphated polysaccharide), extraction method, test bacteria, control, solvent, experimental repetition, the average value of the inhibitory zone (mm) and standard deviation. In addition, the inhibitory zone as a measure of the inhibitory power of antibacterial compounds was grouped into four categories, namely weak (< 5 mm), medium (5 – 10 mm), strong (10 – 20 mm), and very strong (> 20 mm) (Roza et al., 2022).

Statistical Analysis

During analysis, the DIZ of crude extract and sulphated polysaccharides from macroalgae were included in the experimental group (E), while the DIZ of antibiotics as controls was included in the control group (C). Weighting analysis using Hedges'd (Standard Mean Difference / SMD) as the statistical analysis was processed using the OpenMEE application.

The average, standard deviation, and number of experimental repetitions were extracted from selected journal articles. The collected data were calculated for the standard deviation (SD), the correction factor for sample size (J), and the effect size value (*d*) (Goulet-Pelletier & Cousineau, 2018). The effect size value was calculated by the formula:

$$d = \frac{\underline{X}^E - \underline{X}^C}{S} J$$

Where $\underline{X}^E$ is the mean value of the experimental group, and $\underline{X}^C$ is the mean value of the control group. J is the correction factor of a small sample size. The value of J was calculated by the formula:

$$J = 1 - \frac{3}{(4(N^C + N^E - 2) - 1}$$

Next, S represents the pooled standard deviation, which is defined as:

$$S = \sqrt{\frac{(N^E - 1)(s^E)^2 + (N^C - 1)(s^C)^2}{(N^E + N^C - 2)}}$$

Where N^C is the sample size of the experimental group, S^E is the sample size of the control group, S^E is the standard deviation of the experimental group. The variance of Hedges' d (V_d) is described as:

$$v_d = \frac{(N^C + N^E)}{N^C N^E} + \frac{d^2}{(2(N^C + N^E))}$$

$$S_d = \sqrt{v_d}$$

The cumulative effect size (d_{++}) is calculated by the formula:

$$d_{++} = \frac{(\sum_{i=1}^{n} w_i \, d_i)}{(\sum_{i=1}^{n} w_i)}$$

Where W_i is the inverse of the sampling variance: $w_i = \frac{1}{v_d}$. The accuracy of the effect size is explained using a 95% confidence interval (CI), which is d $\pm$ (1.95 $\times S_d$). The % weight value is calculated by the formula:

$$\%w = \frac{Wd}{\sum Wd}$$

Then the value of I^2 can be obtained by the formula:

$$I^2 = \left(\frac{Q - df}{Q}\right) \times 100$$

$$Q = \sum Wd \, . \, d^2 - \left(\frac{\sum Wd \, . \, d^2}{\sum Wd}\right)$$

The results of the confidence interval and effect size calculations are then interpreted in the form of a forest plot. The effect size of macroalgae antibacterial activity is expressed in the form of Standard Mean Difference (SMD), heterogeneity assessment is expressed in the form of percentage I^2 and significance assessment in the form of p value. The SMD value is useful for measuring the effect size of two different independent groups with a certain intensity. The effect size value is statistically significant if the CI does not cross the zero value of the SMD. The SMD value with a 95% confidence interval calculation is divided into three intensities, namely small (d" 0,2), medium (± 0,5) and large (e" 0,8). The heterogeneity of the study is shown in the form of an I^2 index, where I^2 > 50% indicates heterogeneity (Afandi et al., 2021). The value of heterogeneity (I^2) is useful for expressing variation between studies. The percentage of heterogeneity is divided into three ranges, namely low (25%), medium (50%), and high (75%). The p-value is useful for expressing the significance of the calculations that have been made. A significant p-value is expressed by p <0,001 (Andrade, 2020).

Results and Discussion

Selected Studies

The studies that were identified, screened, and selected based on the PRISMA approach, are shown in Figure 1. Using the determined keywords, 2,591 journal articles were identified. However, after screening and selection according to the inclusion and exclusion criteria, 23 selected articles were finally obtained for systematic review and meta-analysis study.

In this study, the studies selection for systematic review as well as for meta-analysis were based on the availability of the control data, although for the systematic reviews, the control data were not necessarily included. A broad range of studies have been found with a focus on macroalgae since macroalgae are found in almost every aquatic environment in all geographical areas and are rich in beneficial components for humans. Hence, the role of health-promoting effects, including antioxidant, anti-inflammatory, antimicrobial, and anti-cancer, has been widely explored in phytochemicals and unique polysaccharides of marine macroalgae (Ravi et al., 2019; Saeed et al., 2020).

The selected journal articles, with certain information such as location, division, number of data studies, genus, and form of potential antibacterial activity, are presented in Table 1. From the 23 selected articles, a total of 835 data sets of studies were obtained. The studies were carried out in 12 countries which comprised antibacterial activity data set on Chlorophyta (n=159), Phaeophyta (n = 476), Rhodophyta (n = 200).

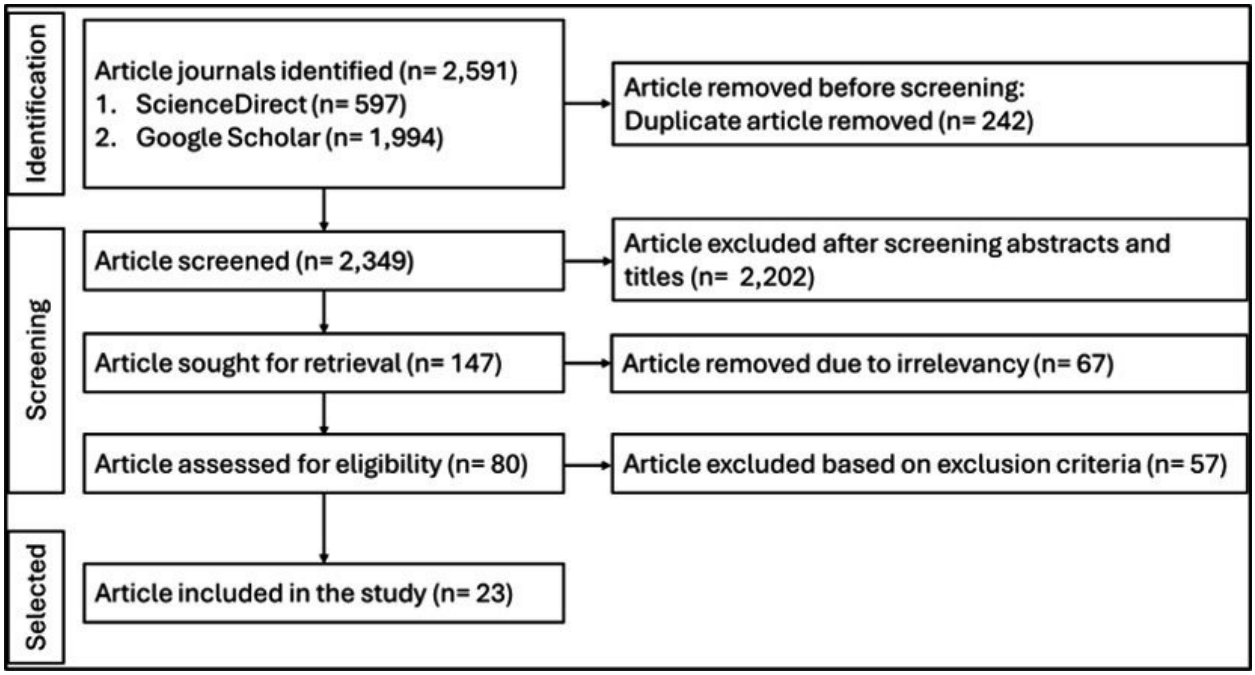


Figure 1. Journal selection results using the PRISMA approach

Table 1. List of studies used in the systematic review and meta-analysis

No.	Reference	Location	Division	n	Genus	Form
1.	Al Khazan et al. (2016)	Saudi Arabia	Chlorophyta	48	Ulva	Crude extract
2.	Albratty et al. (2023)	Saudi Arabia	Phaeophyta	24	Sargassum	Crude extract
3.	Assaw et al. (2018)	Malaysia	Rhodophyta	21	Gracilaria	Crude extract
4.	Avila-Romero et al. (2023)	Saudi Arabia	Chlorophyta	17	Caulerpa, Ulva, Cymopolia, Dictyosphaeria	Crude extract
			Phaeophyta	2	Sargassum	
			Rhodophyta	20	Compsothamnion, Tricleocarpa, Galaxaura, Laurencia, Titanophycus, Hypnea, Amphiroa	
5.	Capillo et al. (2018)	Switzerland	Rhodophyta	15	Gracilaria	Crude extract
6.	El Nur et al. (2021)	Pakistan	Chlorophyta	6	Halimeda	Crude extract
			Phaeophyta	5	Turbinaria	
			Rhodophyta	5	Jania	
7.	El-Manawy et al. (2019)	Egypt	Chlorophyta	2	Caulerpa	Crude extract
			Phaeophyta	12	Hormophysa, Polycladia, Padina	
			Rhodophyta	2	Digenea	
8.	El-Sheekh et al. (2020)	Romania	Phaeophyta	305	Cystoseira, Padina, Sargassum	Crude extract

9.	Kandhasamy & Arunachalam (2008)	Kenya	Chlorophyta Phaeophyta Rhodophyta	16 24 16	Ulva, Caulerpa Padina, Sargassum Gracilaria, Hypnea	Crude extract
10.	Karthick et al. (2019)	India	Rhodophyta	21	Amphiroa	Crude extract
11.	Li et al. (2018)	Switzerland	Chlorophyta Phaeophyta Rhodophyta	5 7 5	Ulva, Gracilariiopsis Sargassum, Ishige Gloiopeltis	Crude extract
12.	Marfuah et al. (2018)	Indonesia	Chlorophyta	6	Caulerpa	Crude extract
13.	Marudhupandi & Kumar (2013)	India	Phaeophyta	8	Sargassum	Sulphated polysaccharide
14.	Mashjoor et al. (2016)	Netherlands	Chlorophyta Phaeophyta	15 21	Ulva Padina	Crude extract
15.	Pakingking et al. (2022)	Iran	Chlorophyta	15	Ulva	Crude extract
16.	Palani et al. (2022)	United States	Rhodophyta	9	Hypnea	Crude extract
17.	Pierre et al. (2011)	South Korea	Chlorophyta	5	Chaetomorpha	Sulphated polysaccharide
18.	Priya et al. (2018)	India	Rhodophyta	11	Grateloupia	Crude extract
19.	Ravi et al. (2019)	India	Rhodophyta	61	Jania	Crude extract
20.	Rizzo et al. (2017)	India	Chlorophyta Phaeophyta Rhodophyta	3 10 4	Chaetomorpha, Ulva Cystoseira, Dictyopteris, Fucus, Sargassum, Undaria Gracilaria, Hypnea	Sulphated polysaccharide
21.	Saeed et al. (2020)	Egypt	Chlorophyta Rhodophyta	11 7	Ulva, Enteromorpha Jania, Gelidium	Crude extract
22.	Salem et al. (2011)	Nigeria	Chlorophyta Phaeophyta Rhodophyta	16 49 8	Codium, Caulerpa Padina, Sargassum, Cystoesira Actinotrichia	Crude extract
23.	Vijayabaskar et al. (2012)	Netherlands	Phaeophyta	9	Sargassum	Sulphated polysaccharide

Four of 23 journal articles reported the antibacterial activity in the form of sulphated polysaccharides, with 30 data sets of study, while the other journals reported the activity in the form of crude extracts of macroalgae, with 805 data sets of study. Sulphated polysaccharides are present in the cell wall of macroalgae, comprised mainly of cellulose and hemicellulose. They belong to negatively charged polysaccharides due to the cross-linkage of sulphate group ions with complex molecules of polysaccharides (Muthukumar et al., 2021). The crude extracts contain different bioactive compounds depending on the solvent and procedure of extraction.

Antibacterial Activity among the Macroalgae Genus

Thirty-two macroalgae genera demonstrated antibacterial activities within the selected studies (Figure 1). The four main genera that were widely explored were *Sargassum* (28.13%), *Ulva* (25%), and *Caulerpa* and *Padina* (each 15.63%). The genus *Sargassum* has been the object of interest in different countries, such as Indonesia, India, Saudi Arabia, Kenya, Nigeria, Romania, Switzerland, and the Netherlands. *Sargassum* belongs to the brown macroalgae (Phaeophyta), comprising numerous species, which are distributed throughout the temperate and tropical oceans and are generally found in shallow water and on coral reefs. The genus *Ulva has* also received considerable attention worldwide due to its macroalgal properties with antibacterial activities. *Ulva* belongs to the green macroalgae and is generally found in vegetated coastal environments (Qie et al., 2023)

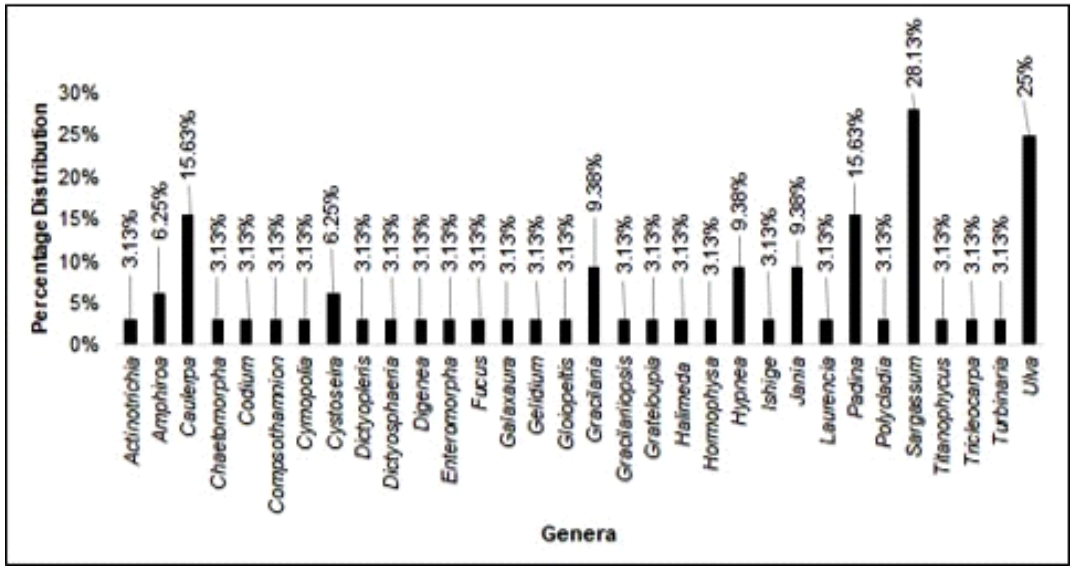


Figure 2. Percentage distribution of macroalga genera under the selected articles (n=23).

The antibacterial activity of the macroalgae division (Chlorophyta, Phaeophyta, Rhodophyta) in this study was indicated by the formation of a diameter of inhibitory zone (DIZ) (Tables 2,3 and 4). A total of eight genera under the Chlorophyta showed good antibacterial activities against 19 different bacteria (Table 2) with DIZ in a range from 6 mm to 35 mm. The three genera with the largest DIZ were *Ulva* (6 – 35 mm), *Caulerpa* (6 – 19.8 mm), and *Halimeda* (12 – 17 mm). The largest DIZ was demonstrated by a crude extract of *Ulva reticulata*, which formed 35 mm of inhibition zone towards methicillin-resistant *S. aureus* (MRSA) (Al Khazan et al., 2016).

Furthermore, the crude extract of seven of the eight Chlorophyta genera actively inhibited S. aureus, a pathogenic Gram-positive bacterium. This result supported the finding that Chlorophyta was more active in inhibiting Gram-positive bacteria in comparison to Gram-negative bacteria (Kandhasamy and Arunachalam 2008). The crude extracts of macroalgae exhibit antibacterial activities, likely due to their bioactive compounds, including phenolic compounds, alkaloids, fatty acids, and others (Michalak and Chojnacka 2015; Hakim and Patel 2020). The sulphated polysaccharide also showed antimicrobial activities against *S. aureus* (Pierre et al., 2011; Rizzo et al., 2017), although it was not as strong as the crude extracts.

Table 2. The inhibition zone of crude extract and sulphated polysaccharide of genera belonging to Chlorophyta

Genus	n	Tested Bacteria	DIZ (mm)	Form	Reference
Caulerpa	31	*Bacillus cereus, Bacillus subtilis, Enterobacter aerogenes, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Micrococcus luteus, Pseudomonas aeruginosa, Salmonella sp., Salmonella* Typhi*, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus faecalis*	6 – 19.8	Crude extract	Avila-Romero et al. 2023; El-manawy et al. 2019; Kandhasamy & Arunachalam, 2008; Marfuah et al. 2018; Salem et al. 2011
Chaetomorpha	5	*Staphylococcus aureus*	10 - 13	Sulphated polysaccharide	Pierre et al. 2011 and Rizzo et al. 2017
Codium	6	*Bacillus cereus, Enterococcus faecalis, Escherichia coli, Pseudomonas aeruginosa, Salmonella sp., Staphylococcus aureus*	9 – 11.8	Crude extract	Salem et al. 2011
Cymopolia	3	*Salmonella* Typhi*, Staphylococcus aureus, Staphylococcus epidermidis*	6	Crude extract	Avila-Romero et al. 2023
Dictyosphaeria	3	*Salmonella* Typhi*, Staphylococcus aureus, Staphylococcus epidermidis*	*6*	Crude extract	Avila-Romero et al. 2023

Enteromorpha	2	*Pseudomonas mirabilis, Klebsiella pneumoniae*	11 - 15	Crude extract	Saeed et al. 2020
Halimeda	6	*Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus*	12 - 17	Crude extract	El Nur et al. 2021
Ulva	102	*Aeromonas hydrophila, Bacillus pumilus, Bacillus subtilis, Enterobacter aerogenes, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Micrococcus luteus, Pseudomonas aeruginosa, Pseudomonas mirabilis, Salmonella* Typhi, *Staphylococcus aureus, Staphylococcus aureus (MRSA), Staphylococcus epidermidis, Streptococcus agalactiae, Streptococcus faecalis*	6 - 35	Crude extract	Al khazan et al. 2016; Avila-Romero et al. 2023; Kandhasamy & Arunachalam, 2008; Li et al. 2018; Mashjoor et al. 2016
	1	*Photobacterium damselae subsp. damselae*	8	Sulphated polysaccharide	Rizzo et al. 2017

Note: DIZ= Diameter of Inhibition Zone

Among the genera under the Phaeophyta, nine genera have been studied and showed significant DIZ towards 27 different target bacteria (Table 3). The three genera with the highest inhibition zone diameter were *Hormophysa* (19 – 27.3 mm), *Padina* (7 – 25 mm), and *Sargassum* (6 – 22.8 mm). Six of the eight Phaeophyta genera are able to inhibit *S. aureus*. Crude extract of *Hormophysa cuneiformis* showed the largest DIZ (27.3) mm towards *S. aureus*. *H. cuneiformis* is an abundant brown macroalga that grows on the coral reefs of the Red Sea and South East Asia (El-Manawy et al., 2019).

The crude extract of *H. cuneiformis* possessed a broad-spectrum antimicrobial effect through the growth suppression of *E. faecalis, S. aureus, and P. aeruginosa* in a comparable manner to commercial antibiotics (El-Manawy et al., 2019). The biggest DIZ obtained by sulphated polysaccharide was showed by *Sargassum swartzii* with 22 mm zone of inhibition towards *B. subtilis* (Vijayabaskar et al., 2012).

Table 3. The inhibition zone of crude extract and sulphated polysaccharide of genera belong to Phaeophyta

Genus	n	Tested Bacteria	DIZ (mm)	Form	Reference
Cystoseira	121	*Bacillus cereus, Enterobacter aerogenes, Enterococcus faecalis, Escherichia coli, Pseudomonas aeruginosa, Salmonella sp., Salmonella* Typhimurium, *Shigella flexneri, Staphylococcus aureus, Staphylococcus aureus (MRSA), Streptococcus pyogenes*	6.5 - 17	Crude extract	El-sheekh et al. 2020; Salem et al. 2011
Dictyopleris	2	*Salmonella sp.*	8	Sulphated polysaccharide	Rizzo et al. 2017
Fucus	3	*Photobacterium damselae subsp. damselae, Salmonella sp.*	10 -13	Sulphated polysaccharide	Rizzo et al. 2017
Hormophysa	6	*Enterococcus faecalis, Pseudomonas aeruginosa, Staphylococcus aureus*	19 – 27.3	Crude extract	El-manawy et al. 2019
Ishige	3	*Aeromonas hydrophila, Escherichia coli, Staphylococcus aureus*	7.33 – 9.75	Crude extract	Li et al. 2018
Padina	144	*Bacillus cereus, Bacillus pumilus, Bacillus subtilis, Enterobacter aerogenes, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Micrococcus luteus, Pseudomonas aeruginosa, Salmonella sp., Salmonella* Typhimurium, *Shigella flexneri, Staphylococcus aureus, Staphylococcus aureus (MRSA), Staphylococcus epidermidis, Streptococcus pyogenes*	7 - 25	Crude extract	El-manawy et al. 2019; El-sheekh et al. 2020; Kandhasamy & Arunachalam, 2008; Mashjoor et al. 2016; Salem et al. 2011
Polycladia	4	*Pseudomonas aeruginosa*	15.2 – 20.3	Crude extract	El-manawy et al. 2019

Sargassum	169	*Aeromonas hydrophila, Bacillus cereus, Bacillus subtilis, Enterobacter aerogenes, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Micrococcus luteus, Pseudomonas aeruginosa, Salmonella sp., Salmonella* Typhi*, Salmonella* Typhimurium*, Shigella flexneri, Staphylococcus aureus, Staphylococcus aureus (MRSA), Staphylococcus epidermidis, Staphylococcus pyogenes, Streptococcus faecalis, Streptococcus pyogenes*	6 – 22.8	Crude extract	Albratty et al. 2023; Avila-Romero et al. 2023; El-sheekh et al. 2020; Kandhasamy & Arunachalam, 2008; Li et al. 2018; Salem et al. 2011
	19	*Aeromonas hydrophila, Bacillus subtilis, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Klebsiella sp., Proteus, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella sp., Salmonella* Typhi*, Shigella flexneri, Shigella sonnei, Staphylococcus aureus, Vibrio cholerae*	8 - 22	Sulphated polysaccharide	Vijayabaskar et al. 2012; Marudhupandi and Kumar 2013; Rizzo et al. 2017
Turbinaria	5	*Escherichia coli, Staphylococcus aureus*	13 - 16	Crude extract	El Nur et al. 2021

Note: DIZ= Diameter of Inhibition Zone

Macroalga under the Rhodophyta division also showed good activity by forming an inhibition zone (Table 4), although the largest DIZ in general was less than those formed Phaeophyta and Chlorophyta. Fifteen genera were studied, and the crude extract of *Jania showed the largest DIZ (24.66 mm) Rubens* against *Aeromonas hydrophila* (Ravi et al., 2019). *Jania rubens* is a type of red macroalgae found in marine waters worldwide. *Gracilaria* sp., a member of Rhodophyta, also exhibited good antibacterial activity, with the largest diameter of inhibition zone (DIZ) of 19 mm against *B. subtilis* (Capillo et al., 2018). *Gracillaria* is an important source of phycocolloids, such as agar, alginate, and carrageenan (Assaw et al., 2018).

Table 4. The inhibition zone of crude extract and sulphated polysaccharide of genera belong to Rhodophyta

Genus	n	Tested Bacteria	DIZ (mm)	Form	Reference
Actinotrichia	8	*Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa, Salmonella sp., Staphylococcus aureus*	7.8 - 12	Crude extract	Salem et al. 2011
Amphiroa	23	*Enterococcus faecalis, Escherichia coli, Proteus mirabilis, Pseudomonas fluorescens, Salmonella* Typhi*, Staphylococcus epidermidis, Streptococcus pneumoniae, Vibrio alginolyticus, Vibrio parahaemolyticus*	6.28 – 13.25	Crude extract	Avila-Romero et al. 2023 and Karthick et al. 2019
Compsothamnion	3	*Salmonella* Typhi, *Staphylococcus aureus, Staphylococcus epidermidis*	6	Crude extract	Avila-Romero et al. 2023
Digenea	2	*Pseudomonas aeruginosa*	14.3	Crude extract	El-manawy et al. 2019
Galaxaura	1	*Salmonella* Typhi	6	Crude extract	Avila-Romero et al. 2023
Gelidium	3	*Pseudomonas mirabilis, Klebsiella pneumoniae*	9 - 12	Crude extract	Saeed et al. 2020
Gloiopeltis	3	*Aeromonas hydrophila, Escherichia coli, Staphylococcus aureus*	7 -10.83	Crude extract	Li et al. 2018

Gracilaria	43	*Bacillus subtilis, Enterobacter aerogenes, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Micrococcus luteus, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus faecalis, Vibrio cholerae*	7.6 - 19	Crude extract	Assaw et al. 2018; Capillo et al. 2018; Kandhasamy & Arunachalam, 2008; Rizzo et al. 2017
Gracilariopsis	2	*Aeromonas hydrophila, Staphylococcus aureus*	8.5 – 12.5	Crude extract	Li et al. 2018
Grateloupia	11	*Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus*	11 - 16	Crude extract	Priya et al. 2018
Hypnea	20	*Bacillus subtilis, Enterobacter aerogenes, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Micrococcus luteus, Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus faecalis*	6.36 - 14	Crude extract	Avila-Romero et al. 2023; Kandhasamy & Arunachalam, 2008; Palani et al. 2022; Rizzo et al. 2017
Jania	70	*Aeromonas hydrophila, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Pseudomonas mirabilis, Staphylococcus aureus, Vibrio vulnificus*	7 – 24.66	Crude extract	El Nur et al. 2021; Ravi et al. 2019; Saeed et al. 2020
Laurencia	7	*Escherichia coli, Salmonella* Typhi, *Staphylococcus aureus, Staphylococcus epidermidis*	6 - 9	Crude extract	Avila-Romero et al. 2023
Titanophycus	1	*Salmonella* Typhi	6	Crude extract	Avila-Romero et al. 2023
Tricleocarpa	3	*Salmonella* Typhi, *Staphylococcus aureus, Staphylococcus epidermidis*	6 -7	Crude extract	Avila-Romero et al. 2023

Note: DIZ= Diameter of Inhibition Zone

Furthermore, as shown in Table 5, the antibacterial activities of the macroalgae can be categorized as weak, medium, strong, and very strong based on their DIZ. Based on these results, the macroalgae under Phaeophyta was found to be the most promising source of antibacterial compounds, although Chlorophyta and Rhodophyta were also potential. With an abundant dataset for study, it was revealed that 35.33% of the antibacterial testing resulted in very strong and strong antibacterial activities. However, due to high variability in antibacterial activity, a meta-analysis will be important to determine the significance of the study.

Table 5. Inhibitory zone category of macroalgae against bacteria

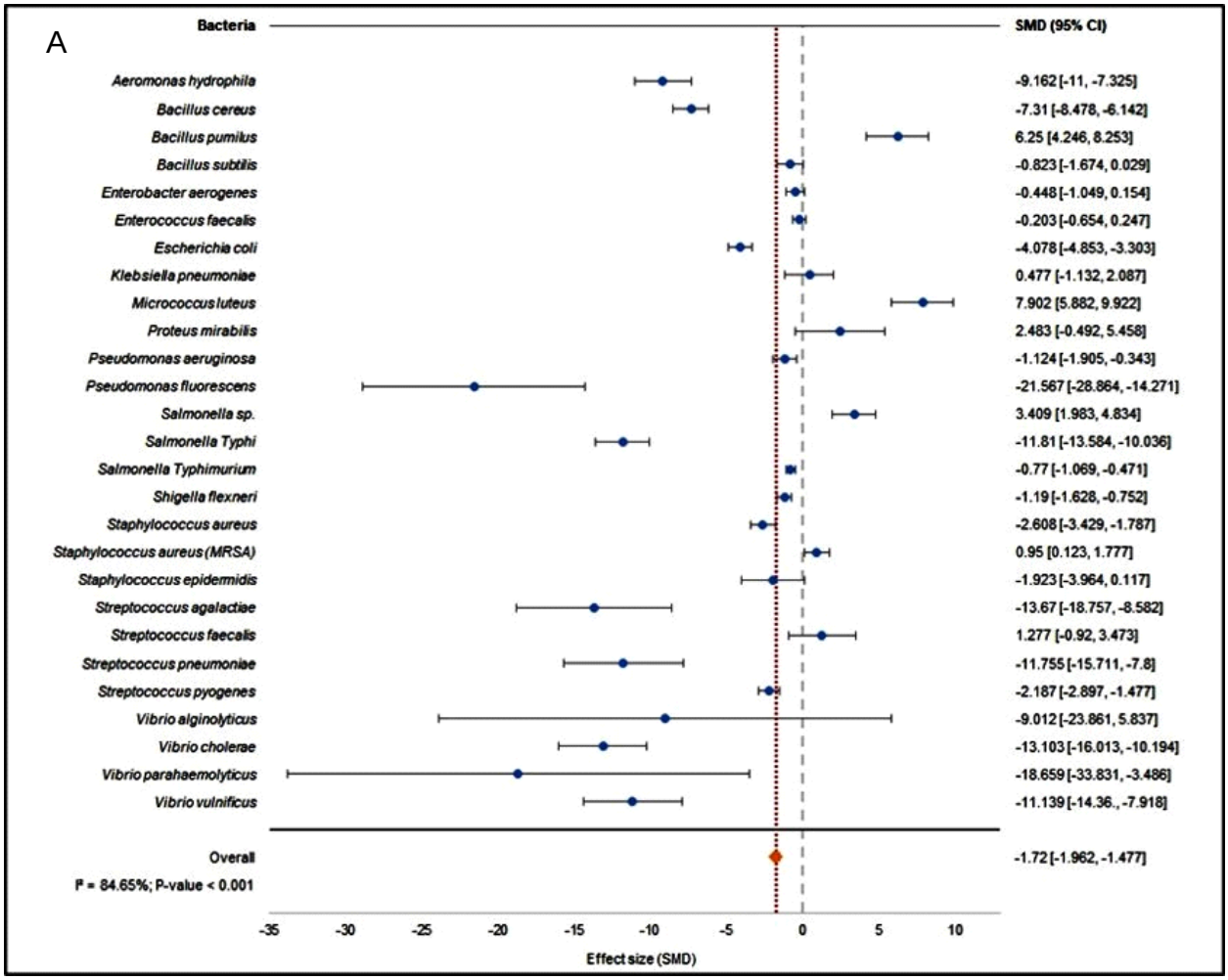
Division	Inhibitory Zone Category		
	Very Strong (>20 mm)	Strong (10-20 mm)	Medium (5-10 mm)
Chlorophyta (n= 159)	2.04%	13.29%	4.31%
Phaeophyta (n= 476)	3.11%	32.22%	21.68%
Rhodophyta (n= 200)	0.36%	14.01%	9.58%

The overall effect size of the antibacterial activity of Chlorophyta, Phaeophyta, and Rhodophyta on selected bacteria

The significant effect of crude extracts and sulphated polysaccharides from Chlorophyta, Phaeophyta, and Rhodophyta in inhibiting bacterial growth was analyzed based on the standardized mean difference (SMD) and 95% confidence interval of the DIZ (mm) and summarized in a forest plot (Figure 3). In general, although by systematic review there were found that different extracts and sulphated polysaccharides of Chlorophyta, Phaeophyta, and Rhodophyta showed good antibacterial activities, the

overall effect size of crude extracts (n= 805) and sulphated polysaccharides (n = 30) on the target bacteria was with SMD = -1.72 with CI = -1.96 to -1.48 (I^2 = 84.65%, and p < 0.000) and SMD = -13.07 with CI = -16.00 to -10.14 (I^2 = 85.8%, and p < 0.000), respectively.

These cumulative results indicated that the demonstrated antimicrobial activity was low. This might be due to the fact that, when calculating the SMD for each study, positive controls were involved, which may have varying strengths of antimicrobial activity towards particular bacteria. For example, streptomycin showed DIZ of 21-25 mm (Ravi et al., 2019), whereas ampicillin showed 12-15 mm DIZ (Al Khazan et al., 2016; Mashjoor et al., 2016) against *E. coli.* Hence, when a very strong antibiotic is used with a DIZ that was greater than that indicated by the crude extract, the SMD will tend to have a negative value. However, the individual results for crude extracts on particular bacteria showed a significant positive effect, such as against *B. pumilus* (SMD = 6.25 [CI = 4.25 to 8.25]), *M. luteus* (SMD = 7.90 [CI = 5.88 to 9.92]), and *Salmonella* sp. (SMD = 3.41 [CI = 1.98 to 4.83]). These results demonstrate that using a meta-analysis approach, several studies have achieved statistical significance in inhibiting bacterial growth despite the overall cumulative value showing insignificant results.



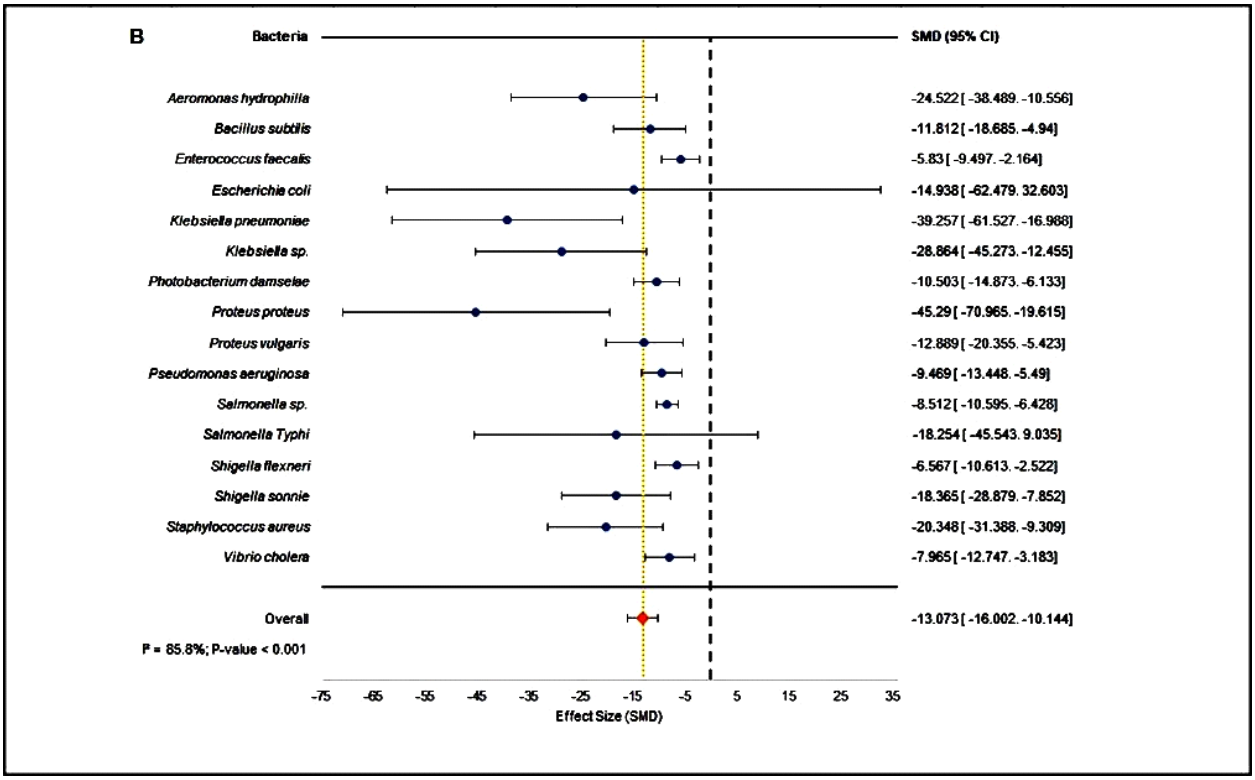


Figure 3. The effect size of macroalgae antibacterial activity (Diameter Inhibitory Zone in mm) against different bacteria by crude extract (A) and sulphated polysaccharides (B). The value to the right of the x=0 line indicates that the intensity of antimicrobial activity in the experimental group is higher than that in the control group and vice versa.

Many factors could influence the SMD value, considering that I^2 was also high (>50%), which indicates a high level of heterogeneity was found. Apart from the use of different positive controls, the use of various extraction solvents could also affect the antimicrobial activity of macroalgae. Subgroup analysis in future studies will be conducted to examine further the significant factors that can significantly influence the overall effect size using a meta-analysis approach.

Overall Effect Size of Macroalgae Inhibition Zone Associated with Different Controls

Under the 23 studies analyzed, six different antibiotics were used as controls, namely amoxicillin, ampicillin, chloramphenicol, ciprofloxacin, streptomycin, and tetracycline (Table 5). The most frequently used control in antibacterial activity testing was chloramphenicol, while the least frequently used control was amoxicillin.

Table 5. Diameter of inhibitory zone of tested bacteria using different antibiotic

Form	n	Tested Bacteria	DIZ (mm)	Reference
Crude Extract				
Amoxicillin	38	*Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Staphylococcus aureus, Streptococcus agalactiae*	7 - 36.11	Marfuah et al. 2018, Pakingking et al. 2022, Saeed et al. 2020
Ampicillin	99	*Bacillus pumilus, Bacillus subtilis, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus aureus (MRSA), Staphylococcus epidermidis*	11 - 19	Al khazan et al. 2016, Capillo et al. 2018, Mashjoor et al. 2016

Chloramphenicol	435	*Aeromonas hydrophila, Bacillus subtilis, Enterobacter aerogenes, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Micrococcus luteus, Pseudomonas aeruginosa, Salmonella* Typhi*, Salmonella* Typhimurium*, Shigella flexneri, Staphylococcus aureus, Staphylococcus aureus (MRSA), Staphylococcus epidermidis, Streptococcus faecalis*	8.5 - 32.32	Avila-Romero et al. 2023, El-manawy et al. 2019, El-sheekh et al. 2020, Kandhasamy et al. 2008, Li et al. 2018, Salem et al. 2011
Ciprofloxacin	82	*Aeromonas hydrophila, Enterococcus faecalis, Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Pseudomonas fluorescens, Streptococcus pneumoniae, Vibrio alginolyticus, Vibrio parahaemolyticus, Vibrio vulnificus*	9.12 - 29	Karthick et al. 2019, Ravi et al. 2019
Streptomycin	68	*Bacillus subtilis, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pyogenes*	0 - 37	Albratty et al. 2023, El Nur et al. 2021, El-manawy et al. 2019, Palani et al. 2022, Priya et al. 2018
Tetracycline	83	*Bacillus cereus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Salmonella sp., Staphylococcus aureus, Vibrio cholerae*	7.5 - 31.3	Assaw et al. 2018, Salem et al. 2011
Sulphated Polysaccharide				
Ampicillin	14	*Aeromonas hydrophila, Bacillus subtilis, Enterococcus faecalis, Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella* Typhi*, Shigella flexneri, Staphylococcus aureus*	17 - 41	Pierre et al. 2011, Vijayabaskar et al. 2012
Chloramphenicol	3	*Salmonella sp.*	20	Rizzo et al. 2017
Tetracycline	13	*Escherichia coli, Klebsiella pneumoniae, Klebsiella sp., Photobacterium damselae subsp. damselae, Proteus, Pseudomonas aeruginosa, Salmonella sp., Salmonella* Typhi*, Shigella sonnei, Vibrio cholerae*	19 - 37	Marudhupandi and Kumar 2013, Rizzo et al. 2017

Note: DIZ= Diameter of Inhibition Zone

The largest DIZ was demonstrated by ampicillin against *S. aureus* (Pierre et al., 2011). Surprisingly, no inhibition (DIZ = 0 mm) was showed by streptomycin against *P. aeruginosa* (Palani et al., 2022). However, in other studies, streptomycin showed strong inhibition against *P. aeruginosa,* with a diameter of inhibition zone (DIZ) of 25.3 mm (Albratty et al., 2023) and a DIZ of 20.4 mm (El-Manawy et al., 2019).

The overall effect size on inhibitory zones of crude extract and sulphated polysaccharide of macroalgae on the test bacteria associated with the positive controls used is presented in Figure 4. As indicated in Figure 4, the choice of antibiotics as control greatly affected the overall effect size.

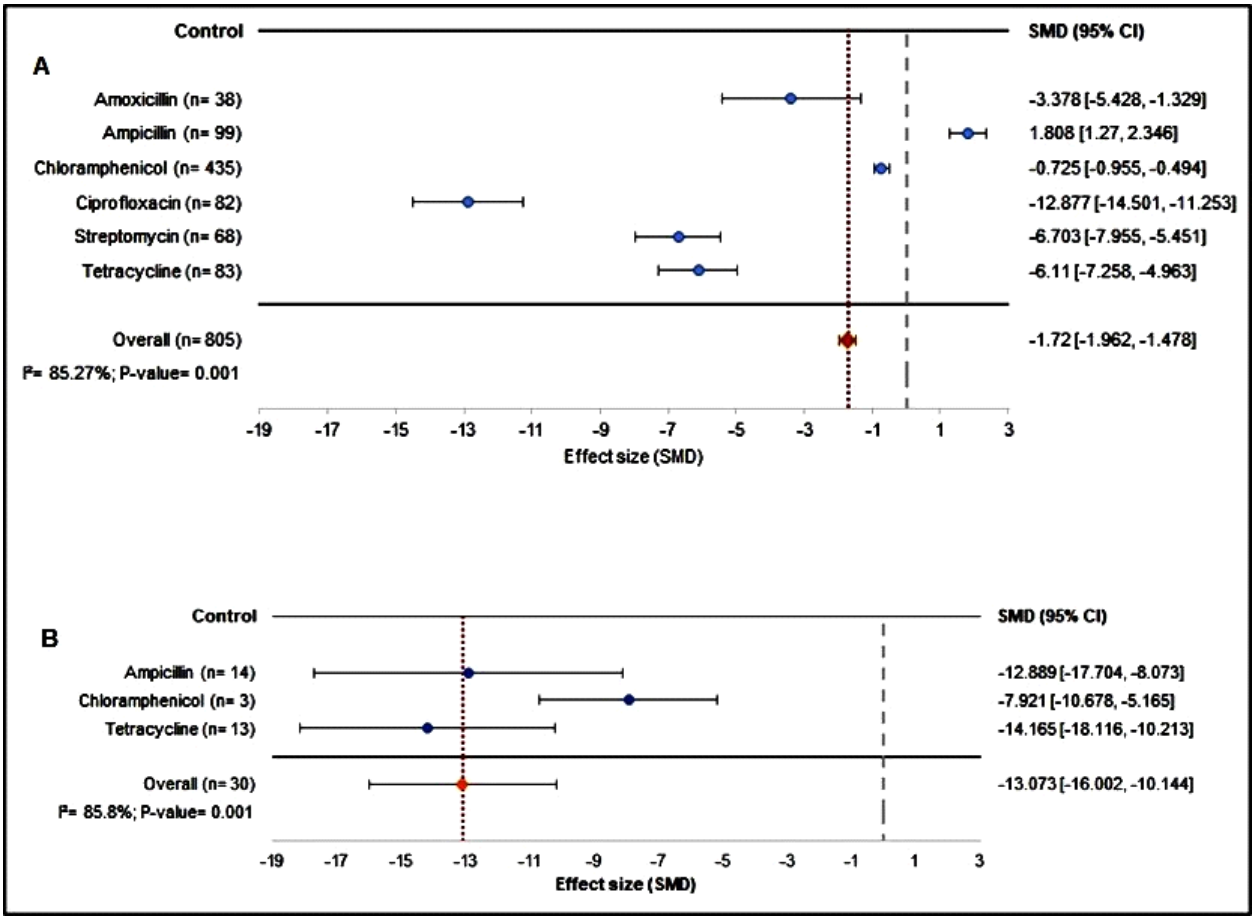


Figure 4. The effect size of macroalgae antibacterial activity (Diameter Inhibitory Zone in mm) against different bacteria by crude extract (A) and sulphated polysaccharides (B) based on the antibiotic used as control. The value to the right of the x=0 line indicates that the intensity of the antimicrobial activity of the experimental group is higher than that of the control group and vice versa.

Except for the studies with ampicillin (n= 99) as positive control, other studies resulted in negative overall side effects. The most positive result on SMD was shown by antibacterial testing of the crude extract using ampicillin (n = 99) as a positive control, with SMD = 1.81 (CI = 1.27 to 2.35). This result indicated that the crude extract in the respective studies showed a larger DIZ than that shown by ampicillin. A study by Al Khazan et al. (2016) reported that the crude extract of *U. reticulata* (n = 48) formed DIZ in a range of 12-35 mm, whereas ampicillin formed DIZ in a range of 13-18 mm against various bacteria. Furthermore, Mashjoor et al. (2016) reported that the crude extract of *U. flexuosa* (n=12) formed DIZ in a range of 12-28 mm; the crude extract of *Padina boergesenii* (n=12) formed DIZ in a range of 13-25 mm; and the crude extract of *Padina antillarum* (n=12) formed DIZ in a range of 12-25 mm whereas ampicillin formed DIZ in a range of 11-19 mm towards different bacteria.

Furthermore, the study on sulphated polysaccharide showed a negative SMD, indicating that the antibacterial activity of sulphated polysaccharides was lower than that shown by antibiotics. The greatest value is chloramphenicol of -10.775 (I2 = 20.89%; p value < 0.001). Hence, the value of the overall effect size of the antibacterial activity was greatly influenced by the control used when significance was assessed using meta-analysis.

Publication Bias

A funnel plot is a graphic representation of the studies in a meta-analysis conducted to check for potential publication bias visually. The dots scattered to the left of the abscissa are the small or negative effect size range, while the right side of the abscissa is the opposite effect size range. The presence of a point that is further down the funnel indicates a larger standard error value. Studies with good precision have smaller standard errors; additionally, the potential for publication bias is indicated by examining the symmetry of the patterns formed (Dowdy et al., 2022).

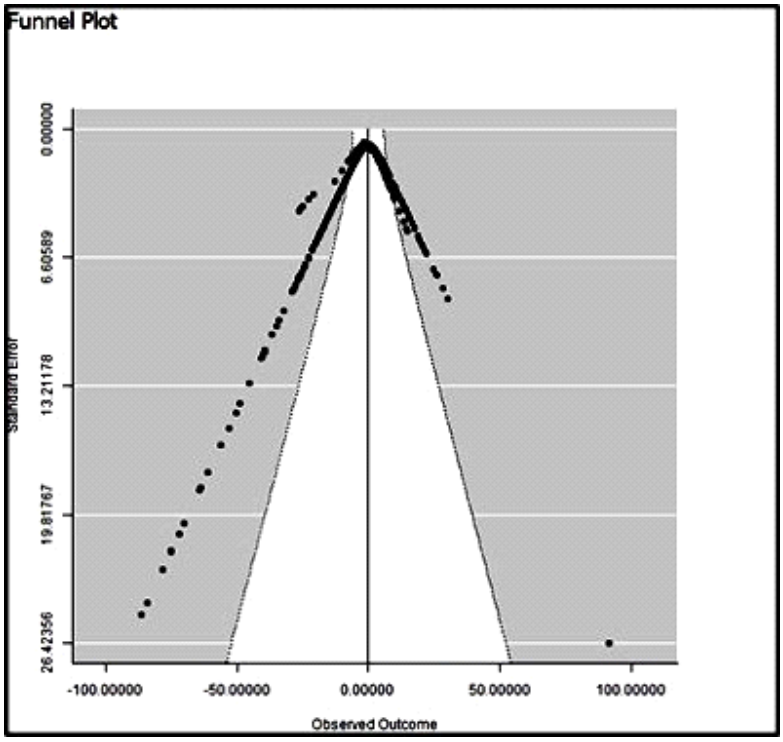


Figure 5. Funnel plot for publication bias analysis

The results of this meta-analysis show good study precision, but the pattern formed is not symmetrical. This can be caused by publication bias, heterogeneity, and interconnected methodology. According to Aisbett et al. (2023) points outside the triangle area indicated publication bias. The interpretation of the funnel plot is considered subjective because it relies solely on visual assessment and, therefore, cannot be used as strong evidence to determine whether the funnel plot results are symmetrical or asymmetrical. Furthermore, the Fail-Safe N approach was used to overcome publication bias by providing how large a number it is to be able to conclude that the conclusions of the meta-analysis results are robust to the problem of publication bias. There are tolerance categories for Fail-Safe N numbers, namely weak (Fail-Safe N < 5k +10), medium (Fail-Safe N = 5k + 10), and strong Fail-Safe N > 5k +10) (Retnawati et al., 2018). The information k is the number of studies used, namely 835 studies. Table 6 shows the fail-safe number of the study, which is categorized as robust.

Table 6. Fail-safe number

Forest Plot	*Fail-Safe N*	Category
Crude extract	259.12	Robust
Sulphated polysaccharide	4.22	Robust
Total	329.82	Robust

Conclusion

There is increasing interest in the potential antimicrobial activities of the crude extract and sulphated polysaccharide compounds in macroalgae Chlorophyta, Phaeophyta, and Rhodophyta. These macroalgae revealed promising antibacterial activities, evidenced by very strong and strong activities in inhibiting various bacterial growth by systematic review study. The largest inhibition zone of crude extracts of Chlorophyta was 35 mm, by Phaeophyta was 27.3 mm, and by Rhodophyta was 25.66 mm. The largest inhibition zone shown by sulphated polysaccharides of Chlorophyta was 13 mm, by Phaeophyta was 22 mm, while by Rhodophyta has not been reported. Furthermore, when analyzing the overall effect size by meta-analysis approach on those antibacterial activities, the choice of antibiotic as positive control greatly affected the results on standardized means of difference (SMD). Using ciprofloxacin, the SMD value was found = -12.88 (CI = -14.50 to -11.25), whereas if ampicillin was used, the SMD value was 1.81 (CI = 1.27 to 2.35). Other factors, such as extraction methods and bacterial strains, which are also likely to affect the overall effect size, will be subjected to further analysis in the next study.

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Supplementary Materials

Supplementary material is not available for this article.

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