The effectiveness of potential marine plants as pathogen bacterial inhibitory compounds

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ABSTRACT. With the development of research and technology, marine plants can be a bio-prospective for secondary metabolites product. The content of marine plant as a source of secondary metabolites product has an important role as a source of antibacterial pathogen compounds. The study aimed to utilize extracts of Ipomoea pes-caprae, Sonneratia alba, Gracilaria debilis and Dictyoshaeria versluysii on the antibacterial formation of pathogenic bacteria. The research was conducted in laboratory following several steps procedure i.e. extraction of samples with ethyl acetate and alcohol as solvent and then diffusely testing of antibacterial activity. All experiment was done with two replications. Pathogen bacteria used in this experiment are Escherichia coli, Salmonella typhimurium, Enterococci, Staphylococcus aureus, Clostridium perfringens, and Pseudomonas. The result showed that extract ethyl acetate performs better than extract of alcohol to inhibit pathogen bacteria. The extract of ethyl acetate created inhibitory zone activity against E. coli was 2.9 ± 0.20 mm (I. pes-caprae), 2.8 ± 0.12 mm (Sonneratia alba), 26.0 ± 0.16 mm (G. debilis), and 32.0 ± 0.08 mm (D. versluysii). Result of inhibitory zone activity against Salmonella were 30.5 ± 0.10 mm (I. pes-caprae), 2.5 ± 0.10 mm (S. alba), 27.5 ± 0.16 mm (G. debilis), and 29.18 ± 0.18 mm (D. versluysii). Inhibitory activity zone for Enterococci were 2.5 ± 0.10 mm (I. pes-caprae), 3.4 ± 0.30 mm (S. alba), 38.0 ± 0.10 mm (G. debilis), and 42.0 ± 0.18 mm (D. versluysii), whilst for S. aureus the inhibitory activity zone were 2.5 ± 0.1 mm, 3.4 ± 0.10 mm, 30.0 ± 0.12 mm, and 31.0 ± 0.1 mm for I. pescaprae, S. alba, G. debilis and D. versluysii respectively. Results of inhibitory activity zone for C. perfringens were 2.9 ± 0.2 mm, 2.7 ± 0.12 mm, 26.5 ± 0.14 mm and 32.0 ± 0.1 mm for I. pes-caprae, S. alba, G. debilis and D. versluysii while for Pseudomonas were 2.7 ± 0.2 mm, 2.4 ±0.14 mm, 28.0 ± 0.10 mm and 26.0 ± 0.22 mm.

1. Introduction
Epidemiological evidence suggests regular seaweed consumption may protect against a range of diseases of modernity [1]. The antimicrobial properties of seaweed extracts have been well documented over the years [2]. The abundant potential of biological marine plant resources is now being seen, studied, developed and made into various products for industrial purposes including food, chemical, cosmetic and pharmaceutical industries. Priorities of marine biological resources that have a positive effect on antibacterial for human health generally contain primary metabolites known as hydrocolloid which have high economic value.

Hydrocolloid compounds have been used as industrial ingredients, including agar-agar, carrageenan, and alginate. In addition to primary metabolite products, secondary metabolite products from marine plants began to be studied. Among secondary metabolites which have been widely studied are bioactive substances which have the potential to be developed for antimicrobials which include antibacterial, antifungal, and anti-virus [3].
Some of the marine plants that contain secondary metabolites such as Ipomoea pes-caprae, Sonneratia alba, Gracilaria debilis and Silpau (Dictyosphaeria versluysii). Secondary metabolites of D. versluysii with ethanol treatment (alkaloids, sterols, quinone, streptonoid and saponin), 8 secondary metabolites with ethyl acetate treatment (alkaloids, flavonoids, phenols, steroids, quinone, tannin, streptonoid and saponyn, and secondary metabolite content of D. versluysii with hexane treatment (streptonoid and saponin) [4]. These plants have the potential to produce diverse bioactive metabolites with activities that are useful as antibacterial, antiviral, antifungal and cytostatic [5].

The development of bacterial resistance in pathogenicity infection bacterial to humans is a new problem at the moment. The research for alternative uses of new drug compounds, therefore, is very important in annihilating this pathogenic infection. Bioactive compounds from I. pes-caprae, S. alba, G. debilis and D. versluysii are some of the new antibacterial sources obtained from marine natural resources. Most secondary metabolites are biosynthesized from many primary metabolites such as amino acids, acetyl co-A, mevalonic acid, and methanolate between. In addition, some natural antimicrobial compounds derived from plants include phytoalexin, organic acids, essential oils (volatile), phenolics and some groups of plant pigments or similar compounds [6, 7].

The use of synthetic chemicals as a controller of bacterial growth in foodstuffs at the present time can have adverse effects on health. For that reason, natural control ingredients are needed which do not cause adverse effects on health. This can be done, for example, through the extraction of seaweed like G. debilis as an effective antibacterial material towards Escherichia coli bacteria, Salmonella, Enterococci, Clostridium perfringens, Staphylococcus aureus, and Pseudomonas aeruginosa. Inactivation of bacteria is a result of the interaction of an antibacterial compound with certain parts of bacterial cells. These antibacterial compound interactions can cause a number of changes or damage to bacterial cells and can ultimately affect the function of cell metabolism and at a certain level can destroy bacterial cells [6].

With the vast possibility of developing an antibacterial from marine plant, a search for new compounds as a secondary metabolites from marine plant extracts as antibacterial, antifungal and antimicrobial is widely open. Tortosa (2001) Research testing in the activity of antimicrobial ingredients in vitro can be done in two ways i.e. dilution method and agar diffusion method. Dilution methods are commonly used to determine the MIC (minimum inhibitory concentration) and MLC (minimum lethal concentration) of antimicrobial ingredients [8].

Test of Gracilaria sp, as antibacterial against Escherichia coli and Staphylococcus aureus shows that bacterial growth inhibition of E.coli was 14.33 ± 3.22 mm and S.aureus was 12.67 ± 2.08 mm with MIC values of 0.05% (Melki et al., 2011)[9] Other study also shown the potential of red algae Gelidium sp. as an antibacterial again oral bacteria [10] and also from some seaweeds as an antibacterial against skin diseases arise from Pseudomonas aeruginosa, Staphylococcus epidermis and Micrococcus [11].

With the development of research in antibacterial compound and large opportunity to explore the potency of marine plant as bioactive, this study was aimed to utilize the extracts of some marine plant i.e. Ipomea pes-caprea, Sonneratia alba, G. debilis and D. versluysii on antibacterial formation of pathogenic bacteria E.coli, Salmonella, Enterococci, S. aureus, C. perfringens and P. aerogiossa.

2. Materials and Method

2.1. Materials

Antibacterial substances extracts used in this study are horse tread, Gracilaria sp, G. debilis, and D. versluysii, while other supporting materials used are ethyl acetate, 70% alcohol, filter paper, nutrient agar (NA), nutrient broth (NB), paper discs, cultures of E. coli, Salmonella, Enterococci, S.aureus, C.perfringens and P. aerogiossa.

The equipment used is analytic balance sheets, glass jars, funnels, rotary vacuum, evaporator brands Buchi, Petri dish, test tubes, cotton, media bottles, osse needles, tweezers, incubators, hot plates, autoclave, bunsen burner, micropipettes, calipers, colony counter, filter paper, and centrifuge.
2.2. Method
The method used in this study is an experimental method according to Melki et al [9] with the step of implementation as follows: cleaning of raw materials, extraction, antibacterial activity test of *E.coli*, *Salmonella*, *Enterococcus sp.*, *S. aureus*, *C. perfergens*, and *P. aerogiossa*. The parameter used in this experiment is an inhibitory zone of each antibacterial extract obtained from the marine plant. The susceptibility of each bacterium was measured in diameter of inhibition zone [10, 12].

3. Results and Discussion
The results of extraction of marine plants using ethyl acetate and ethanol and their effect on the inhibition zone of 6 bacteria tested can be seen graphically in figure 1, 2, and 3. Figure 1 shows an extract of marine plant and their inhibition zone against *E. coli* (A) and *Salmonella* (B). In general, marine plant extracted with ethyl acetate has wider inhibition zone compared to alcohol both for *E. coli* and *Salmonella* sp. The smallest mean diameter of inhibition zone was range between 2.2 mm to 2.9 mm and was found at *I. pas-caprea* and *S. alba* both from ethyl acetate and alcohol.

When the inhibition zone size diameter was compared to a standardized chart to give a result whether it sensitive, resistant, or intermediate [12, 13], the result shows that *G. debilis* with ethyl acetate solvent have potential as antibacterial towards *E. coli* since the minimum inhibition zone (MIZ) is > 18 mm whilst *D. versluysii* only susceptible for ethyl acetate solvent. In the meantime, all marine plants extracted with ethyl acetate solvent have potential as an antibacterial against *Salmonella* except *Gracilaria* sp (Figure 1B).

Figure 1. Inhibition zone diameter (mm) of all four marine plants extracted with ethyl acetate and alcohol towards *E. coli* and *Salmonella*

Figure 2 shows the minimum inhibition zone diameter of four marine plants extracted with ethyl acetate and alcohol solvent against *Enterococci* (A) and *S. aureus bacteria* (B). When these value compared to a standardized chart, it was found that only *G. debilis* and *D. versluysii* has potential as antimicrobial towards *Enterococci* both from ethyl acetate and an alcohol solvent, whilst test on *S. aureus* bacteria, only *G. debilis* and *D. versluysii* from ethyl acetate that has susceptible towards these two bacteria.
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Figure 2. Inhibition zone diameter (mm) of all four marine plants extracted with ethyl acetate and alcohol towards Enterococci and S. aureus.

Test of four marine plants as an antimicrobial on pathogen bacteria C. perfringens and P. aeruginosa is shown in Figure 3. From all inhibition zone produced only G. debilis and D. verslusii extracted with ethyl acetate have higher inhibition zone both for C. perfringens (Figure 3A) and P. aeruginosa (Figure 3B) bacteria compared to I. pes-caprea and S. alba. This result suggests that extraction of G. debilis and D. verslusii with ethyl acetate has a resistant effect towards those two pathogenic bacteria.

Figure 3. Inhibition zone diameter (mm) of all four marine plants extracted with ethyl acetate and alcohol towards C. perfringens and P. aeruginosa.

The ability of marine plant extract to inhibit bacteria depends on the metabolites contained in these marine plants. D. verslusii seaweed generally has the biggest inhibition zone compared to I. pes-caprea and G. debilis. The response of bacterial and microbial growth inhibition power produced is influenced by the content of active compounds contained in natural substances such as alkaloids, steroids, flavonoids, phenols, saponins, terpenoids and quinones [14].

The four marine plants mentioned above have bacteriocidal properties with different inhibitory level sensitivity against each of pathogen bacteria tested. It is stated that antibacterial activity is said to be the best when the same concentration test will produce better antibacterial activity [15]. This result shows that only G. debilis extracted with ethyl alcohol (ethylac) have a higher inhibitory zone for all pathogens bacterial tested whilst when extracted
with alcohol effective only for *E. coli* (ϕ = 18.34 mm) and at Enterococci (ϕ = 29.75 mm). This result also shows that the marine plant *I. pes-caprea* produced the smallest inhibition zone for all bacteria tested. Figure 1 to Figure 3 shows the complete result of 4 marine plant inhibitory zones produced towards all pathogen bacteria tested.

Seaweeds have been one of the richest and most promising sources of bioactive primary and secondary metabolites [16] and their discovery has significantly expanded in the past three decades [17]. Some of the seaweeds from the genus *Gracilaria* used as antibacterial compounds using various different extraction media and have an active inhibition activity against bacteria are *G. cervicornis*, *G. debilis*, *G. domingensis*, *G. pygmea*, *G. sjoestedii*, *G. tikvahiae* [16]. The zone of inhibition of *G. edulis* methanol extract against bacteria was maximum against Gram-positive cocci *Streptococcus pyogenes*, followed by *Bacillus subtilis*, *S. aureus*, *Streptococcus epidermis*, and *B. cereus*. For Gram-negative bacteria, the maximum zone of inhibition was recorded in the methanol extract of *G. edulis* against *Klebsiella pneumoniae* followed by *Enterobacter aerogenes*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *E. coli* and *Vibrio cholera* [18].

Study on antimicrobial activity of *S. alba* shows that the n-hexane and chloroform extract exhibited no activity against the tested microorganisms at a concentration of 400μg/disc. The carbon tetrachloride extract showed moderate inhibitory activity against various Gram-positive bacteria such as (10 mm), *Bacillus subtilis* (11mm), *Sarcina lutea* (12mm) and Gram-negative bacteria such as *Pseudomonas aeruginosa* (10mm) and *Shigella dysenteriae* (12mm) [19]. Another study on *S. alba* extract against fish pathogen *Aphanomyces invadans* shows that methanol extract of *S. alba* effectively inhibited the mycelial growth of *A. invadans* at a minimum concentration of 1000 ppm for both the agar and filter paper diffusion experiments. The study suggests that *S. alba* methanol extract with a concentration of 1000 ppm may be used as an alternative to controlling the invasive growth of the EUS causative agent, *A. invadans* [20]. Study on in-vitro antimicrobial activity of mangrove plant *S. alba* shows that antimicrobial activities were observed against the gram-positive bacteria *S. aureus* and *B. cereus*, the gram-negative *E. coli* and the yeast *Cryptococcus neoformans*. It was concluded that *S. alba* exhibit antimicrobial activity against certain microorganisms [20].

Study on the antimicrobial compound from *I. per-caprea* shows that methanol extraction exhibited remarkable antibacterial activity on four human pathogenic bacteria tested viz. *E. coli*, *Klebsiella pneumoniae*, *Vibrio parahemlyticus*, and *Proteus mirabilis*. The extract was ineffective in *S. typhi* [21]. Another study on the antibacterial potential of *I. pes-caprea* shows that ethyl acetate and acetone extracts produced a large zone of inhibition against *Arthrobacter protophormiae*, *Rhodococcus rhodochrous*, *Bacillus subtilis*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Proteus mirabilis*, *Proteus vulgaris*, *Enterobacter aerogenes* than the standard antibiotic. It was concluded that *I. pes-caprae* has bioactive content and promising natural antimicrobial agents and also natural antioxidants that can be harnessed as potential antibacterial and antioxidants [21].

4. Conlusion

The magnitude of the zone of resistance of different bacteria is due to the ability of each bacterium to fight antibacterial activity. The ability depends on the thickness and composition of the cell wall. The present study shows differences in inhibition zone produced by each marine plant used at different extract solvent. Extracts of sea plants *I. pes-caprea*, *S. alba*, *G. debilis* and *D. versluisii* with the extraction of ethyl acetate solvents is more effective in inhibiting *E.coli*, *Salmonella sp*, *Enterococci*, *S. aureus*, *C. perfringens*, and *P. aerogiosa* bacteria growth.

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