Antibacterial potential of seaweeds: Insights from a comparative study

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Abstract. Seaweeds are an abundant and sustainable source of bioactive compounds, with growing interest in their potential as natural antibacterial agents. This study investigates the antibacterial properties of three distinct seaweed species: *Ulva intestinalis* Linnaeus, 1753*, Galaxaura rugosa* (J. Ellis & Solander) J.V.Lamouroux, 1816*,* and *Halimeda opuntia* (Linnaeus) J.V.Lamouroux, 1816*.* Comparative analysis revealed that the species had different levels of antibacterial activity. The chloroform and methanol extracts of *Galaxaura rugosa* were the most significant against *Staphylococcus aureus, Bacillus subtilis,* and *Vibrio eltor,* with inhibition zones ranging from 8 mm to 10.5 mm, followed by the methanol extract of *Ulva intestinalis,* which ranged from 8.1 mm to 9.1 mm. The methanol extract of *Halimeda opuntia* only showed an 8.7 mm inhibition zone against *Vibrio eltor.* The findings highlight the potential of these seaweeds as sources of natural antibacterial agents and provide insights for further exploration of their applications in pharmaceutical and biotechnological fields.
Key Words: antibacterial, seaweed, *Halimeda opuntia, Galaxaura rugosa, Ulva intestinalis,* West Nusa Tenggara.

Introduction. The increasing frequency of antibiotic-resistant infections has necessitated an urgent quest for new antibacterial medicines (Shannon & Abu-Ghannam 2016; World Health Organization 2022; Tania et al 2023; Belluz 2024). Natural sources, particularly marine habitats, are progressively investigated for bioactive compounds. Seaweeds have considerable ecological and commercial importance. Located in global coastal waters, they are recognized for generating a variety of bioactive substances, such as polysaccharides, phenolics, terpenoids, and flavonoids, which demonstrate antibacterial characteristics (Shannon & Abu-Ghannam 2016; Rocha et al 2018; Cotas et al 2020; Afzal et al 2023; Bouzenad et al 2024).

Seaweeds are classified into three primary groups according to coloration and biochemical composition: green (Chlorophyta), brown (Phaeophyta), and red (Rhodophyta) (El-Beltagi et al 2022; Baghel et al 2023) Each class generates distinct bioactive metabolites that enhance its antibacterial characteristics. Green seaweeds are abundant in chlorophyll and fatty acids, whereas brown seaweeds possess phlorotannin and fucoidans, and their sulfated polysaccharides distinguish red seaweeds. These compounds shield seaweeds from microbial invasion in marine ecosystems and exhibit potential as therapeutic agents against bacterial illnesses in people and animals (Cardoso et al 2019; Cermeno et al 2020; Yang et al 2021). Researchers have already shown that seaweed extracts can kill Gram-positive and Gram-negative bacteria, including types used in medicine, like *S. aureus* and *E. coli.* The action methods are diverse, encompassing the rupture of bacterial cell membranes and the suppression of protein and DNA synthesis. The findings indicate that seaweeds may provide a substantial source of antibacterial agents, potentially overcoming the limits of traditional antibiotics (Perez et al 2016; Shannon & Abu-Ghannam 2016). Nevertheless, comparative studies on various seaweed species and their antibacterial effectiveness remain scarce, warranting additional study (Kumar & Granesan 2018).

This study aims to provide insight into the antibacterial potential of three distinct seaweed species, *U. intestinalis, G. rugosa,* and *H. opuntia,* collected from West Nusa Tenggara waters, Indonesia, through a comparative study of their bioactivity. The study results are expected to provide insights into the prospective application of the studied seaweed as an alternative antibacterial source.

Material and Method

***Sample collection*.** Three selected seaweed species, *U. intestinalis, G. rugosa,* and *H. opuntia,* were gathered from the coastal waters of West Nusa Tenggara in Indonesia during low tide. The seaweed was manually collected and promptly cleaned with seawater to remove debris, sand, epiphytes, and extraneous materials adhering to the thalli. Subsequently, they were conveyed to the laboratory. The samples were rinsed with distilled water to eliminate surface salts. The material was indirectly sun-dried for four days and powdered in a blender. Then, it was placed in a dark container and maintained at ambient temperature for later examination.

***Extraction process*.** Fifty grams of each seaweed powder were macerated with 500 mL of methanol at ambient temperature for three days, with daily agitation. The procedure was reiterated using fresh methanol until the filtrate turned colorless. The filtrate was further evaporated at 40°C using a rotary evaporator until a viscous extract was obtained (Abu et al 2017). The same procedure uses hexane, diethyl ether, and ethanol as solvents.

***Antibacterial test*.** This study tested *B. subtilis, S. aureus, E. coli,* and *V. eltor* for antibacterial activity. In this method, 20 μL of nutrition broth medium with 10 μL of test bacteria in an incubator shaker at 200 rpm for 24 hours at 30°C. Then, up to 20 μL of nutrition solution containing bacteria tested was poured into each Petri plate and allowed to settle. Each paper disk received 10 μL substance at 1000 μg mL^{-1} concentration. Similarly, using ampicillin (15 μg mL^{-1}) established a positive control. The inhibition zones that appeared surrounding each paper disk were measured in millimeters with a transparent ruler (Hudzicki 2016).

Results and Discussion

The rising interest in seaweed as an antibacterial agent corresponds with the escalating need for sustainable and friendly solutions (Kim & Wijesekaran 2010; Pereira & Cotas 2024; Teixeira et al 2024). In contrast to synthetic antibiotics, which can include energy-intensive manufacturing methods and may result in environmental pollution, seaweed-derived compounds are renewable and biodegradable. Additionally, the utilization of seaweeds is consistent with the tenets of blue biotechnology, which promotes the sustainable exploitation of marine resources for human advantage (Kim & Wijesekaran 2010).

The current study investigated the efficacy of extracts from three seaweed species, each exhibiting a unique activity profile. The methanol extract of *U. intestinalis* and *G. rugosa* slowed the growth of three reference bacteria, namely *B. subtilis, S. aureus,* and *V. eltor.* The clear zone measurement results ranged from 8.1 mm to 10 mm (Figure 1, Figure 2, and Figure 3). Simultaneously, the chloroform extract of *G. rugosa* exhibited action against three reference bacteria, resulting in a clear zone diameter between 8.2 mm and 10.5 mm. The diethyl ether extract of *G. rugosa* showed 8.7 mm inhibition zones for *B. subtilis* and 8.6 mm inhibition zones for *V. eltor* (Figure 3). The methanol extract of *H. opuntia* worked well against *B. subtilis,* creating an 8.7 mm-wide clear zone (Figure 2). All extracts examined in this study presented no activity against *E. coli* (Figure 4).

The findings of this study are close to those of Fayzi et al (2020). This study involved the collection of four seaweed species, namely *Bifurcaria bifurcata, Coralina officinalis, Ulva fasciata,* and *C. elongata,* from the Moroccan Atlantic coast. Both *B. bifurcata* and *U. fasciata* had clear zones of 8.33 mm to 14.33 mm for *S. aureus, B. subtilis,* and *E. coli* in their methanolic extracts, but only *B. subtilis* with a clear zone of 9.66 mm was seen in *C. officinalis.* All the extracts have no activity against *P. aeruginosa,* while *C. elongata* has no activity against all the bacteria tested. Similarly, Kolanjinathan et al (2009) reported research results on three types of seaweed, *Gracilaria edulis, Caulerpa chemnitzia,* and *Hydroclathrus* sp., collected from Mandapam waters, India. The ethanol extracts of the three seaweeds inhibited the growth of all tested bacteria except *B. subtilis* and *Enterobacter aerogenes.* The size of the inhibition zone for the three ethanol extracts was against the bacteria *E. coli* (6.8-11.9 mm), *S. aureus* (9.2-13.7 mm), *Streptococcus faecalis* (8.5-14.9 mm), and *P. aeruginosa* (7.8-14.9 mm).

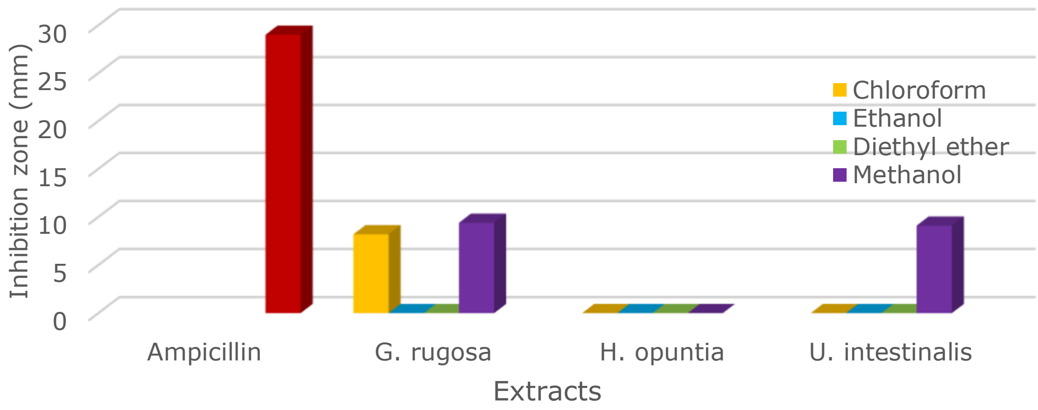

Figure 1. The antibacterial activity of seaweed extracts against *Staphylococcus aureus.*

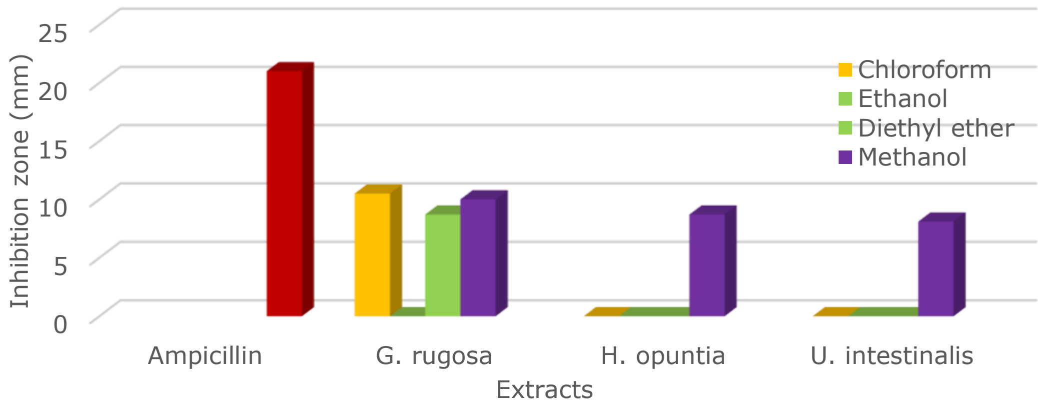

Figure 2. The antibacterial activity of seaweed extracts against *Bacillus subtilis.*

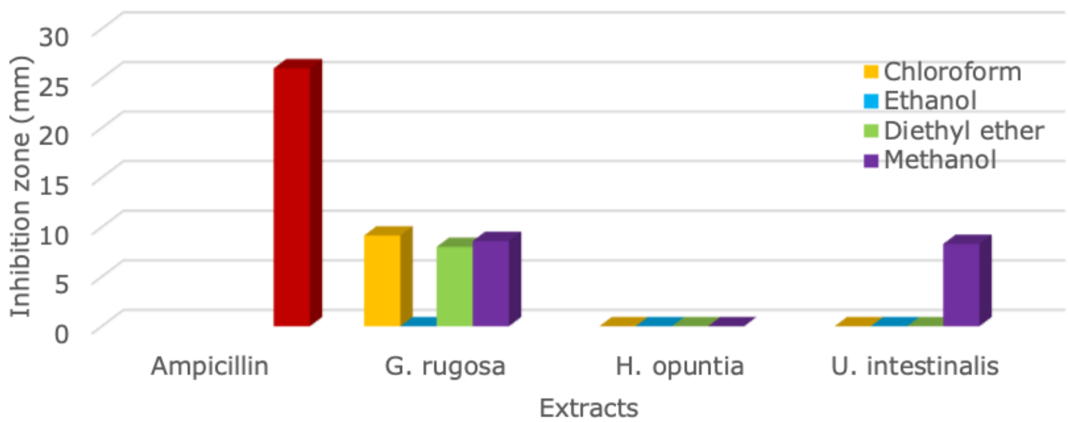

Figure 3. The antibacterial activity of seaweed extracts against *Vibrio eltor.*

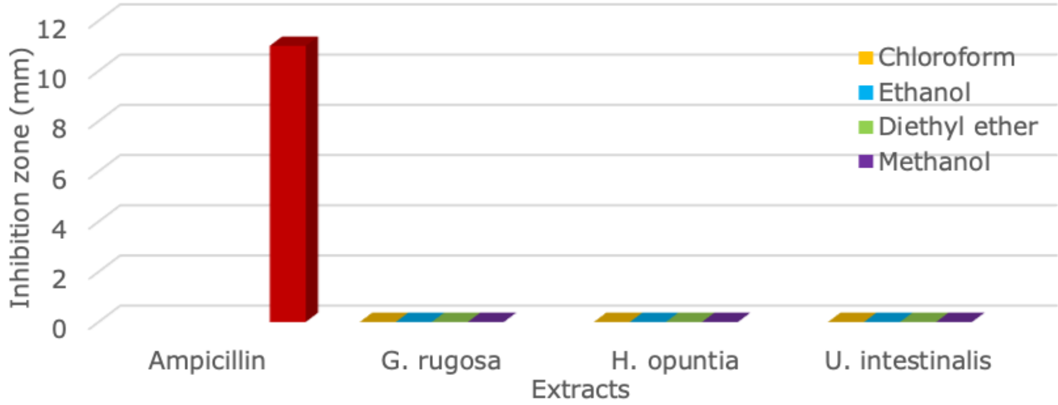

Figure 4. The antibacterial activity of seaweed extracts against *Escherichia coli.*

Madkour et al (2019) examined the antibacterial potential of three brown seaweeds, namely *Cystoseira myrica, Turbinaria ornate,* and *P. pavonica,* which were gathered from the Red Sea coastline of Egypt. The ethanol and isopropanol extract of *T. ornata* inhibited the growth of *S. aureus* in 15 mm and 14 mm zones, respectively. The acetone extract of *T. ornata* inhibited the growth of *P. aeruginosa* by 14.5 mm, the isopropyl alcohol extract of *C. myrica* by 15.5 mm, and the isopropyl alcohol extract of *T. ornata* by 14.75 mm.

Srikong et al (2015) investigated the antibacterial activity of two seaweed species, *U. intestinalis,* and *Gracilaria fisheri,* which were collected from the coastline of Pattani, Thailand. The study discovered that *U. intestinalis* in hexane extract exhibited a significant inhibition zone (16.45 mm) against MRSA, followed by *E. faecalis* and *S. aureus,* which showed an inhibition zone was 13.88 mm and 13.72 mm, respectively. However, it gave only a little activity against *V. alginolyticus* and *V. harveyi,* with the inhibition zone around 6.82 mm and 6.78 mm, respectively. Moreover, the hexane extract of *G. fishery* gave an inhibition zone against MRSA (13.47 mm) and *S. aureus* (13.27 mm).

Li et al (2018) reported six species of seaweeds were gathered from the Zhejiang coast, namely *U. prolifer, U. pertusa, Gloipeltis furcata, Gracilariopsis lemaneiformis, Ishige okamurae,* and *S. fusiforme.* All seaweeds showed antibacterial activity against *Aeromonas hydrophila* (7.50-12.25 mm) and *S aureus* (7.33-10.83 mm). *Ulva pertusa, S. fusiforme, G. lemaneiformis,* and *G. furcata* showed zones of inhibition to *E. coli* ranging from 7.00 to 10.00 mm, while *U. prolifera* and *G. lemaneiformis* showed zone inhibition to *V. alginolyticus* at 9.00 and 10.33 mm, respectively. On the other hand, *S. fusiforme* showed activity against *P. aeruginosa* (7.75 mm) and did not show antibacterial activity against the other four tested bacteria.

Conversely, the methanolic extract of brown seaweed *Hydroclathrus clathratus* taken from the Gulf of Mannar exhibited strong activity against *S. aureus* (21 mm), *B. subtilis* (18.33 mm), *P. aeruginosa* (26.33 mm), *E. coli* (19.32 mm), and *Klebsiella pneumoniae* (18.14 cm) (Vimala & Poonghuzhali 2017).

Omar et al (2012) evaluated the antibacterial activity of seven types of seaweed harvested from the southern coast of Jeddah, Saudi Arabia. These species were *Enteromorpha prolifera, Ulva reticulata, Cystosiera myrica, Padina pavonica, Turbinaria triquetra, Sargassum portieriatum,* and *Garcilaria multipartite*. It was shown that petroleum ether extracts of *U. reticulata* could inhibit *K. pneumoniae, E. coli,* and methicillin-resistant *Staphylococcus aureus* (MRSA) bacteria with 23 mm, 25 mm, and 20 mm clear zones. The ethyl acetate extract of *E. prolifera* inhibited the growth of MRSA with an inhibition zone of 25 mm. Several extracts of *U. reticulata* simultaneously significantly suppressed *B. subtilis* (17 to 21 mm). *Padina pavonica* and *C. myrica* showed inhibition zones varied from 11 to 35 mm and 11 to 23 mm, respectively. The highest inhibiting MRSA were the methanol and diethyl ether extracts of *C. myrica* (22 mm and 23 mm), respectively, as well as all the extracts of *P. pavonica* (24 to 35 mm). In 2023, Afrin et al. studied six types of seaweed from Saint Martin's Island, Bangladesh: *Hydroclathrus clathratus, Padina tetrastromatica, Botryocladia wrightii*, *Sargassum muticum, Gracilaria* *parvispora*, and *Porphyra* sp. All methanolic seaweed extracts were significantly more effective at killing all four types of bacteria (*E. coli, Enterococcus faecalis, S. aureus,* and *Pseudomonas aeruginosa*) than those that were ethanolic or acetone-based. Different parts of *S. muticum, P. tetrastromatica,* and *H. clathratus* were extracted using methanol, effectively inhibiting *S. aureus* and *E. faecalis* more efficiently.

The antibacterial efficacy of seaweeds may be affected by various factors, including harvest time, drying method, growth stage, species, the extraction method's capacity to recover active substances, and the extracting solvent (Virnala & Poonghuzhali 2017; Madkour et al 2019; Amirsharifi et al 2020; Cabial et al 2021; Nofal et al 2022; Hejna et al 2024).

Conclusion. The study revealed that *G. rugosa* extracts demonstrated the most potent antibacterial activity, effectively inhibiting Gram-positive and Gram-negative bacteria. This underscores the exceptional potential of *G. rugosa* as a natural antibacterial agent. *U. intestinalis* and *H. opuntia* followed as the second and third most effective species, further emphasizing the diverse antibacterial capabilities present in seaweeds.

The findings are significant not only for their immediate applications but also for their broader implications. The demonstrated efficacy of these seaweed extracts offers a promising alternative to conventional antibiotics, addressing the growing global concern over antibiotic resistance. In addition, the bioactive compounds in seaweed can be used to develop new medicines for more than just antibacterial use, but also their potential as antifungal, antiviral, or even anti-inflammatory.

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