Antibacterial Activity Of Seaweed (*Gymnogongrus* sp) Extract Against *Salmonella typhimurium, Escherichia coli* and *Bacillus subtilis*

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Abstract : Seaweed *Gymnogongrus* sp is endemic to the Maluku region, with the local name "vegetable coral" which usually begins to grow early in the east (April) season along the south coast of Ambon island (Hutumuri - Mahia) and is harvested from July to September. People usually consume them as side dishes. Seaweed can be explored as a natural preservative. In order to preserve fresh food business, especially fish in a sustainable manner using natural preservatives containing bioactive components as antibacterial. To obtain the content of organic compounds from dried plants is to extract the continuous connecting powder of the starting material using a hexane solvent series of solvents alternately, ranging from lowest to highest polarity level or from non polar (Hexane) to produce hexane 1, semi polar extract (Ethyl acetate) to polar (Methanol). In this study also tried the opposite of polar, semi polar and non polar yielding hexane 2 extract. In this study hexane 1 and hexane 2 extracts are used as test extracts. The purpose of this research is to know the rendement and power of antibacterial of hexane extract from seaweed vegetable type *Gymnogongrus* sp. The results showed that the yield of hexane 1 extract (0.149%) was higher than hexane 2 extract (0.108%). The antibacterial power to *Escherichia coli, Salmonella thypi* and *Bacillus subtilis* from n-hexane 2 extracts is, 42.33; 37.50 and 20.83 mm better when compared with n-hexane 1 extract and control (DMSO) with the result of drag zone diameter respectively: 26.00; 39.33 and 20.17 mm and 11.00; 19.50 and 13.00 mm.

Keywords: Antibacterial activity, *Gymnogongrus* sp extract, inhibition zone

I. INTRODUCTION

Promotion of the use of natural compounds against foodborne pathogenic microorganisms continues to be done because of consumer perceptions of the most negative chemical preservatives (Reddy et al., 2013;[1] Sant'Ana et al 2014[2]; Mohammed and Omer, 2015)[3]. Seaweeds that have bioactive components as antibacterials should be explored for further study developed for food purposes. Seaweed can preserve food because like other natural ingredients contain phenolics that can preserve food (Abd Elgadir, 2015)[4].

The discovery of antibacterial bioactive compounds from seaweed is a new hope for the community, in response to some people's concerns about antibiotics and preservatives derived from synthetic chemicals. The presence of side effects such as cancer, environmental destruction and disease resistance by these chemicals led to research to explore bioactive compounds in the field of herbs, especially from the sea, especially seaweed as an alternative food preservatives and treatment continues to grow. Extracts of natural ingredients contain phytochemical compounds such as phenol, tannin, steroids, terpenoids, and alkaloids. Brown seaweed as Gram antibacterial (-), Gram (+), pathogens and food decomposition (Kolanjinathan et al, 2009)[5]; Pereira, et al., (2011)[6] reported isolation of epitaondiol monoacetic compounds, stypotriol triacetate and stypodiol from brown seaweed *Stypopodium flabelliforme* can inhibit the activity of *Staphylococcus aureus* bacteria, *Salmonella typhimurium, Proteus mirabilis, Bacilus cereus, Enterococcus faecalis* and *Micrococcus luteus.*

Solvent selection becomes an important factor in the exploration of natural materials, because each type of solvent has different anti-bacterial capabilities. Dubber and Harder (2008)[7] found that methanol extract from *Ceramium rubrum* seaweed had higher inhibitory effect than non polar n-hexane extract, while contradictory was found Hellio et al., (2001)[8] where non-polar solvent had antibacterial power highest on seaweed of the same type but different place. This shows that not only the type of solvent but the geographical location of the growth of seaweed also determines the antibacterial power of the natural ingredients.

Seaweed species (*Gymnogongrus* sp.) Classified as red algae (*Rhodopytha*) are endemic to the Maluku region that usually grows annually in the early east (April) season along the south coast of Ambon Island (Hutumuri - Mahia) and are usually harvested from the moon July - September. During this time people usually consume as a side dish and more sold (Moniharapon, 1984)[9]. Sormin (2012)[10], reported that seaweed extract *Porphyra marcosii* also grouped red algae that grow at the same time and habitat with *Gymnogongrus* sp. able to inhibit the growth of *Escherichia coli bacteria. Staphylococcus aureus,* and *Salmonella typhi*.

The objective of this research was to know the rendement and antibacterial power of n-hexan extract from coral-type seaweed (*Gymnogongrus* sp.) To 3 test bacteria *Escherichia coli* (Gram negative),and *Bacillus subtilis* (Gram positive, destroyer/decomposition).

II. RESEARCH METHODS

Sample Preparation of Seaweed Vegetable Coral *Gymnogongrus* sp.

Seaweeds of coral vegetation (*Gymnogongrus* sp.) Are collected in the village of Hutumuri and Mahia hamlet of Urimesing village of Ambon city. Furthermore, before drying is washed with clean water. Drying is done by drying on a wire screen for 3-4 days. Seaweeds of dried coral species (*Gymnogongrus* sp.) Are weighed and stored for extracts. The chemical composition of the wet and dry vegetable seaweeds (*Gymnogongrus* sp.) Is presented in Table 1.

Table 1. Composition of Seaweed Type Vegetable Coral (*Gymnogongrus* sp.) Wet and Dry

Chemical Composition	Wet	Dry
Water content (%)	87.54	27.26
Protein levels (%)	2.10	5.31
Fat level (%)	0.24	0.42
Ash Content (%)	3.52	10.50
Carbohydrate levels % (by difference)	6.60	56.51

Extract Preparation

Seaweeds of coral vegetation (*Gymnogongrus* sp.) Are collected in the village of Hutumuri and Mahia hamlet of Urimesing village of Ambon city. Furthermore, before drying is washed with clean water. Drying is done by drying on a wire screen for 3-4 days. A total of 750 g of dried seaweed was extracted (maseration method) with 2,500 ml of hexane for 2 days (48 hours). The resulting filtrate was evaporated with a rotary evapotor vacuum resulting in hexane 1 extract (Dubber and Harder (2008)[7] and 750 g were extracted, respectively: with 2,500 ml of methanol for 2 days and the resulting residue was macerated again with 2,500 ml of ethyl acetate for 2 days and then the resulting residue was extracted again with 2,500 ml of hexane for 2 days and the resulting filtrate was evaporated with a vacuum evapotor rotary resulting in a hexane 2 extract (Modified method of Himejima and Kubo, 1991)[11].

Determination of Rendement

Calculation of the value of rendement is the weight ratio of the commodities taken / obtained with the intact weight of the commodity raw materials multiplied by 100% (Nurjanah et al., 2004)[12].

Antibacterial Test

The antibacterial test was carried out by a 2-layer (bilayer) method of the medium according to Balaouri et al., (2016)[13]. The following procedure: The sterilized medium is silenced for several minutes and pour it into 15 ml sterile petri and leave it to medium dry and solid. Prepare media mixed with bacterial media culture bacteria (*Escherichia coli, Salmonella thypi and Bacillus subtilis*) with cell number 107, then poured on the top of the first layer of the media and leave to dry and solid. Make a well (hole) on the surface of the media with a sterile hole with a diameter of 6 mm, then drop 40 µl of each solution extract into the pit and 40 µl DMSO 10% sterile as a negative control. Subsequently the media was incubated in the incubator at 37 ° C for 24 hours. Observe and measure the diameter of the clear zone (mm) formed around the wellbore. The diameter of the inhibit zone is the diameter of the resulting size minus 6 mm.

Data analysis using Completely Randomized Design (RAL) Factorial pattern with 3 (three) (Gasperzs, 1994)[14].

III. RESULT AND DISCUSSION

Rendement *Gymnogongrus* sp. Extract

Result of extraction with maseration method to 750 g seaweed *Gymnogongrus* sp. dried obtained hexane extracts were 1.12 g and 0.81 g, respectively. From the results of this extract then obtained rendement extract of raw materials in the form of dried seaweed as shown in Table 2 below.

Table 2. Rendement extract of seaweed *Gymnogongrus* sp

Extract of seaweed	Rendement (%)
Hexane 1	0.149
Hexane 2	0.108

To obtain the content of organic compounds from dried plants is to extract continuous connections of powdered starting material using a series of solvents alternately, ranging from lowest to highest polarity level or from non polar to polar. This is also done by Dubber and Harder (2008)[7], where they do so by using n-hexane solvent and then methanol on seaweed type *Mastocarpus stellatus*, *Laminaria digitata* and *Ceramium rubrum*.

Rendement results of hexane 1 extract (first extracted) from seaweed type *Gymnogongrus* sp. as shown in Table 1 was larger when compared with extracted hexane 2 extracts after extracting with methanol (extracts obtained 138.58 g or yield of 18.477%) and ethyl acetate (extracts obtained 5.59 g or yield of 0.745 %). The yield of Table 1 shows that Hexan 1 extract 0.149% is slightly higher and the hexane 2 extract is slightly lower than Sormin (2012)[10] reported that the yield of hexane extract from *Porphyra marcosii* seaweed is 0.125%. It is understandable that although these two seaweeds are grouped red algae that grow at the same time and habitat, but the shape of *Gymnogongrus* sp. is a thalus while Porphyra marcosii is leaf-shaped.

Antibacterial Activity Seed Hexan Extracts *Gymnogongrus* sp.

Based on the result of statistical analysis, two way anova test showed that bacterial type factor and extract type factor and interaction between bacterial factor with seaweed extract factor had highly significant effect on antibacterial power (drag zone) with significance value <α (<0,01). Furthermore, the results of the test of Bright Differences (BNJ) type of bacteria, types of extracts and bacteria type interaction treatment and the type of extract as listed in Tables 3, 4 and 5.

Table 3. BNJ Test Results Differences in Bacterial Type

Type of bacteria	Average inhibit zone (mm)
Escherichia coli	26.44b
Salmonella thypi	32.11a
Bacillus subtilis	18.00c

Information:

The numbers in the notation columns followed by different letters (a, b, and c) show a significant difference in (sig <0.01).

Table 4. BNJ Test Results Differences Extract Type

Types of extracts	Average inhibit zone (mm)
n-hexane 1	28.50b
n-hexane 2	33.55a
control	14.50c

Information:

The numbers in the inhibition diameter columns followed by different letters (a, b, and c) show a significant difference in (sig <0.01).

Table 5. BNJ Test Results of Treatment Interaction Type of Bacteria and Type of Extract of Seaweed *Gymnogongrus* sp.

Type of bacteria	Types of extracts (500µg)	Average inhibit zone (mm)
Escherichia coli	n-heksan 1	26.00b
	n-heksan 2	42.33a
	control	11.00d
Salmonella thypi	n-heksan 1	39.33a
	n-heksan 2	37.50a
	control	19.50c
Bacillus subtilis	n-heksan 1	20.17c
	n-heksan 2	20.83bc
	control	13.00d

Information:
The numbers in the inhibition diameter columns followed by different letters (a, b, c, d, e, and f) show significant differences at ($p < 0.01$).

From Table 3 it turns out that the hexane extract of seaweed *Gymnogongrus* sp. has the highest ability to inhibit the growth of *Salmonella thypi* bacteria (Gram negative, pathogen) with inhibit zone 32.11 mm, followed by Escherichia coli (Gram negative, sanitation indicator and also pathogen) 26,44 mm and *Bacillus subtilis* (Gram positive) equal to 18 .00 mm. This is due to the fact that peptidoglycans of Gram positive bacteria are thicker than Gram-negative bacteria (Fardiaz, 1992)[15], so that the active extracts of the *Gymnogongrus* sp. more easily penetrate the cell wall of Gram negative bacteria. Apparently this is also appropriate but much larger than the reported Sormin (2012)[10] that the inhibited bacteria inhibited methanol extract, ethyl acetate and hexane from *Porphyra marcosii* are *Eschericia coli* (9,9817 mm), followed by *Salmonella thypi* (9.5808 mm) and *Staphylococcus aureus* (7.4708 mm). This result is also much higher when compared to the diameter of inhibitory power of acetone seaweed extract *Stocheospermum marginatum* of 11 mm (*E. coli*) of methanol extract to *E. coli* by 10 mm (Kayalvishi et al., 2012)[16]. Previously Kolanjinatan et al., (2009)[5] reported that ethanol extract from *Gracilaria edulis* seaweed was able to inhibit *E. coli, S. aureus, P. aeruginosa* and *Streptococcus faecalis.* Limo-Filho et al. (2002)[17] suggests bacteria that can be inhibited by *Ulva fasciata and Caulerpa cupressoides extracts are Bacillus subtillis, Staphylococcus epidermidis, S. aureus*. Oranday et al., (2004)[18] suggested that extracts of seaweed species *Ulva lactuca, U. fasciata and Sargassum fluitans* are able to inhibit *E. coli and S. aureus bacteria.* Tuney et al., (2006)[19] reported seaweed species of *Ulva rigida, Enteromorpha linza, Padina pavonica, Colpomenkia sinuosa, Dicttyota linearis and Dictyopteris membrannacea* capable of inhibiting *S. aureus, S. epidermis, P. aeruginosa and E. coli bacteria.* Seaweed species of *Chaetomorpha lemonade, Enteromorpha compressa dichotoma and Polysiphonia subtilissima* were able to inhibit *Bacillus brevis, Bacillus subtilis, E. coli, Vibrio cholera and Shigella flexneri.* Pareira et al. (2011)[6] suggests that seaweed *Stypopodium flabelliforme* able to inhibit bacteria *Staphylococcus aureus, Salmonella typhimurium, Proteus mirabilis, Bacilus cereus, Enterococcus faecalis and Micrococcus luteus*. Jeyanthi et al., (2013)[20] reported seaweed *Gracilaria cortica able to inhibit the bacteria Klebsiella sp.*and *E. coli* respectively 1.5 mm and 1.9 mm and seaweed *Enteromorpha flexuosa* each 2.5 and 3.7 mm.

Based on further tests (Table 4), the n-hexane 2 extract was significantly different from the n-hexane 1 extract capable of producing the highest inhibit zone of 33.55 mm which was very significant with n-hexane 1 (28.50 mm) and control (14, 50 mm). These results are much higher as reported Sormin (2012)[10] inhibition zone of n-hexane extract, ethyl acetate and methanol from *Porphyra marcosii* and control respectively are: 6.39; 10.35 and 13.29 mm and 6.01 mm.

The antibacterial power of the two n-heksan extracts of seaweed *Gymnogongrus* sp. ranging from 20.17 to 42.33 mm is still within the range and higher than the antibacterial power of seaweed extract of Malaysian red alga (*Laurencia* sp) which ranges from 7 to 30 mm, but in its ability to inhibit the growth of *Escherichia coli* the seaweed is lower than seaweed *Gymnogongrus* sp.

When compared with the study of Hellio et al., (2001)[8] then seaweed extract *Gymnogongrus* sp. has a higher antibacterial inhibitory power where *Ceramium ruprum* seaweed inhibitory has a resistance range of 5-6 mm.

Antibacterial inhibition of seaweed *Gymnogongrus* sp. is generally in the range slightly lower and higher when compared with the antibacterial power of land plants such as the leaves and seeds of lotus flower that is 19.33 - 29.57 mm (Fitrial, 2009)[21], while the betel leaf ranges from 10-24 mm (Suliantri, 2009)[22].

Based on the advanced test (Table 5), the inhibition zone of n-hexane 2 extract to the highest *Escherichia coli* bacteria was 42.33 mm which was significantly different with all inneractic n-hexane 1 extracts against *E. coli* with 26.00 mm inhibition zone and control of all test bacteria. Also significantly different from

hexane 2 extract inhibition zone to *Bacillus subtilis* of 20.83 mm and hexane 1 extract against *Bacillus subtilis* that is 20.17 mm. This result is much higher as reported by Sormin (2012)[10], ie zone of n-hexane extract from *Porphyra marcosii* to *E. coli, Salmonella thypi* and *Staphylococcus aureus*: 12.23, 11.61 and 7.20 mm.

From the above explanation, the choice of solvent becomes an important factor in the exploration of natural materials, because each type of solvent has different anti-bacterial capabilities. Dubber and Harder (2008)[7] found that methanol extract from *Ceramium rubrum* seaweed had higher inhibitory effect than non polar n-hexane extract, while contradictory was found Hellio et al., (2001)[8] where non-polar solvent had antibacterial power highest on seaweed of the same type but different place. This shows that not only the type of solvent but the geographical location of the growth of seaweed also determines the antibacterial power of the natural ingredients.

Apparently *Gymnogongrus* seaweed extract from hexane solvent has a very large inhibition zone so it is most able to inhibit the three test bacteria compared to previous research from other researchers related to seaweed. This shows the possibility of non-polar compounds of broad-spectrum *Gymnogongrus* phytochemicals.

IV. CONCLUSION

The yield of n-hexane 1 extract was 0.149% higher than the n-hexane 2 extract of 0.108%. The antibacterial power to *Escherichia coli, Salmonella thypimurium* and *Bacillus subtilis* from n-hexane 2 extract is 42,33; 37.50 and 20.83 mm better when compared with n-hexane 1 extract and control (DMSO) with result of drag zone diameter respectively: 26.00; 39.33 and 20.17 mm and 11.00; 19.50 and 13.00 mm. *Gymnogongrus* extract most actively inhibits *Salmonella typhimurium* bacteria.

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