Jurnal Teknologi

Full Paper

ANALYSIS OF BIOACTIVE COMPOUNDS OF *Caulerpa recemosa*, *Sargassum* sp. AND *Gracillaria verrucosa* USING DIFFERENT SOLVENTS

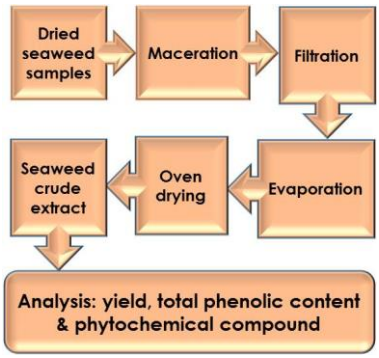
Arham Rusli[a*], Metusalach[b], Mulyati Muhammad Tahir[c], Salengke[c], Syamsuar[a]

[a]Department of Fisheries Products Processing Technology, Pangkep State Polytechnic of Agricultural, Jalan Poros Makassar-Parepare Km 83 Pangkep – 90655, Indonesia
[b]Department of Fisheries, Hasanuddin University, Jalan Perintis Kemerdekaan Km 10 Makassar – 90245, Indonesia
[c]Department of Agricultural Engineering and Food Technology, Hasanuddin University Jalan Perintis Kemerdekaan Km 10 Makassar – 90245, Indonesia

Article history
Received
4 December 2015
Received in revised form
15 February 2016
Accepted
23 February 2016

*Corresponding author
a_rusli06@yahoo.com

Graphical abstract



Abstract

This study aimed to analyze the bioactive compounds of *Caulerpa recemosa, Sargassum* sp., and *Gracillaria verrucosa* from Makssar Strait using three different solvents i.e. acetone, ethanol , and methanol. Samples of the seaweed *C. recemosa* collected from marine waters of Takalar regency, while *Sargassum* sp. and *G. verrucosa* were collected from marine waters of Pangkep regency which is part of the Makassar Strait. Seaweed crude extract qualitatively analyzed using Thin Layer Chromatography (TLC). The treatment applied in this studi is *Sargassum* sp. with acetone solvent, *Sargassum* sp. with ethanol solvent, *Sargassum* sp. with methanol solvent, *C. recemosa* with acetone solvent, *C. recemosa* with ethanol solvent, *C. recemosa* with methanol solvent, *G. verrucosa* with acetone solvent, *G. verrucosa* with ethanol solvent, and *G. verrucosa* with methanol solvent. Parameters measure were the total extract yield, total phenolic content and phytochemical compound of seaweed crude extrac. The result showed that the content of bioactive compounds crude extract of seaweed is affected by the species of seaweed and solvents used in extraction process. The crude extract of *C. recemosa* produces better bioactive compounds than the crude extract of *G. verrucosa* and *Sargassum* sp. as shown by the yield and total phenolic content of crude extract. The best solvent used in extracting bioactive compounds of *C. recemosa* is methanol. Identified phytochemical compounds from the crude extract of *C. recemosa*, are flavonoids, terpenoids, alkaloids, and phenols.

Keywords: Bioactive, seaweed, *Caulerpa recemosa*(Forsskål) J. Agardh, 1873, *Gracillaria verrucosa* (Hudson) Papenfuss 1950, *Sargassum*

1.0 INTRODUCTION

Seaweed is a promising source of bioactive secondary metabolites that can be used as antimicrobial substances [1–3]. These bioactive secondary metabolites include alkaloids, flavonoids, phenols, saponins, tannins, steroids, terpenoids, and glycosides [4, 5]. However, the content of the bioactive compounds of seaweed are largely determined by species of seaweed [6, 7]. The crude

extract of *Sargassum duplicatum* Bory, 1828 contains alkaloids, saponins, tannins, steroids, and glycosides [8] while *Caulerpa scalpelliformis* (R. Brown ex Turner) C. Agardh 1817 contains tannin, flavonoids, glycosides, phenols, saponins, and terpenoids [5]. Similarly, seaweed *Caulerpa recemosa* (Forsskål) J. Agardh, 1873 which is obtained from the northern Sardinia area contains phytochemical compounds alkaloids, sesquiterpenes, diterpenes and sterols [9]. The crude extract of *Gracilaria crassa* Harvey ex J. Agardh, 1876 contains alkaloids, glycosides, saponins, phenols, and flavonoid [10], whereas *Gracilaria corticata* J. Agardh, 1852 contains alkaloids, coumarins, tannins, saponins, flavonoids, quinine, phenols, and steroids [11]. Previous research reported that *G. corticata* and *Kappaphycus alvarezii* (Doty) Doty ex P. C. Silva contain phytochemical compounds include anthraquinones, flavonoids, lignin and saponin [12].

In addition to the species of seaweed, the content of the bioactive compound of seaweed is also influenced by the type of solvent [13, 14]. Elsie *et al.* [15] analyzed the phytochemical constituents of seaweed species *Sargassum wightii* Greville ex J. Agardh 1848 and *Gracillaria edulis* using three solvents, namely ethanol, acetone and methanol. Research results indicated that among the three solvents, ethanolic extract showed maximum number of phytochemical constituents when compared to methanol and acetone extract. Similarly, Anjum *et al*. [11] reported that chloroform was the most effective solvent to extract the bioactive compound from seaweed, followed by ethanol, petroleum ether and water. Extraction of the seaweed species *G. corticata* and *K. alvarezii* by using three types of solvents was reported that tannins were presented only in the ethanol extracts, while alkaloids were presented in *K. alvarezii* using ethyl acetate and hexane. The highest phenol content was observed in the ethyl acetate extracts of *G. corticata* and ethanol extracts of *K. alvarezii* [6]. Similarly, alkaloids and coumarins were detected in the methanol and ethanol extracts of *Sargassum cinereum* J. Agardh 1848, while there was no detection in the acetone extract and distilled water [16].

The chemical composition including bioactive compound of the seaweed is also affected by environmental factors and geographical origin or area of cultivation [12]. Makassar Strait is an area where a wide variety seaweed species grow, either naturally or cultivated. Information on the content of bioactive compounds of seaweed from the Makassar Strait are limited, especially for *C. recemosa*, *Sargassum* sp. and *G. verrucosa* (Hudson) Papenfuss 1950. Whereas the three species of seaweed that contains bioactive compounds has potency as a source of antimicrobial agents. This study aimed to evaluate the bioactive compounds of *Caulerpa recemosa*(Forsskål) J. Agardh, 1873, *Sargassum* sp, and *Gracillaria verrucosa* (Hudson) Papenfuss 1950 from Makassar Strait using three different solvents i.e. acetone, ethanol and methanol. This is an initial stage of research on the incorporation of crude extract of seaweed in the manufacture of antimicrobial edible films.

2.0 EXPERIMENTAL

2.1 Sample Preparation

Samples of the seaweed *C. recemosa* were collected from marine waters of Takalar regency, while *Sargassum* sp. and *G. verrucosa* were collected from marine waters of Pangkep regency which is part of the Makassar Strait. The seaweed were collected regardless of the age of the plant. Specimen were cleaned from the epiphytes and washed thoroughly. Furthermore, samples were dried at room temperature for 2 × 24 h, then dried in a vacuum drying oven at 50 °C for 24 h. The samples were cut into small pieces and then crushed using a blender.

2.2 Design of Experiment and Data Analysis

The experiment was conducted using a completely randomized factorial design with triplicates. The treatment applied was seaweed species (*C. recemosa, Sargassum* sp. and *G. verrucosa*) and type of solvent (acetone, ethanol and methanol). The treatment combination were *Sargassum* sp. with acetone solvent (SA), *Sargassum* sp. with ethanol solvent (SE), *Sargassum* sp. with methanol solvent (SM), *C. recemosa* with acetone solvent (CA), *C. recemosa* with ethanol solvent (CE), *C. recemosa* with methanol solvent (CM), *G. verrucosa* with acetone solvent (GA), *G. verrucosa* with ethanol solvent (GE), and *G. verrucosa* with methanol solvent (GM). Total extract yield, content of total phenolic and phytochemical compound of seaweed crude extract including flavonoids, terpenoids, alkaloids, phenols, and tannins were measured.

The effects of the treatments were analyzed using ANOVA, and the difference between treatment effects was tested using Duncan's multiple range test. Significant difference was considered at 95 % probability. Data analysis was performed using SPSS software (SPSS Inc.). Phytochemical compound of seaweed crude extract by species and type of solvent were analyzed descriptively.

2.3 Extraction Procedure of Secondary Metabolites Compound

Extraction of secondary metabolites refers to methods of extraction that has been done by Iswani [17]. Samples of dried seaweed, which have been grinded (250 g), were macerated separately using different solvent namely acetone, ethanol and methanol (2.0 L) for 3 × 24 h. The seaweed-solvent mixture was then filtered using filter paper to obtain debris-free filtrate. Filtrate was evaporated using a rotary vacuum evaporator at 45 °C. Then the filtrate was dried in an oven for ± 3 h at 50 °C to eliminate solvent that still remain in the bioactive compound. Crude extract of seaweed was stored in sealed bottles before analyzed.

2.4 Determination of Total Phenolic Contents

The amount of total phenolics was determined with the Folin–Ciocalteu reagent [18]. Gallic acid was used as a standard and the total phenolics were expressed as mg g^{-1} gallic acid equivalents (GAE). The Folin-Ciocalteu reagent is sensitive to reducing compounds including polyphenols, thereby, producing a blue colour upon reaction. This blue colour is measured spectrophotometrically at 760 nm. The test was done in triplicates and the results were expressed as mg GAE g^{-1} of the sample.

2.5 Phytochemical Compound Analysis

Phytochemical compound analysis was performed according to the standard protocol [19]. Seaweed crude extract was qualitatively analyzed using Thin Layer Chromatography (TLC). Crude extract of seaweed that has been diluted with a solvent mixture of chloroform and methanol (1:1 v/v) was spotted on silica gel G60 F_{254} plates, and inserted into a chamber containing *n*-hexane and ethyl acetate (2:1 v/v). Then the chromatogram was dried and sprayed using appropriate reagents for phytochemical compounds to be further analyzed. All the prepared seaweed crude extracts were subjected to phytochemical screening for the presence of flavonoids, terpenoids, alkaloids, phenols, and tannins.

3.0 RESULTS AND DISCUSSION

3.1 Yield and Total Phenolic Content

The extraction yield of the seaweed crude extract is presented in Table 1, which indicates that the crude extract of *C. recemosa* had the highest yield for all types of solvents. The yield of crude extract of *C. recemosa* was significantly different from that of *G. verrucosa* and *Sargassum* sp. This indicates that the content of crude extract of seaweed was affected by seaweed species.

The results showed that the yield of total phenolic content of *C. recemosa* crude extract was significantly different than that of *G. verrucosa* and *Sargassum* sp., where crude extract of *C. recemosa* with methanol solvent produced the highest value of total phenolic content (Table 1). The value of total phenolic content also showed that seaweed species affected the total phenolic content of the seaweed crude extract.

Table 1 Yield and total phenolic content of seaweed crude extract

Sampel*)	Yield (%)	Total Phenolic (mg · g^{-1})
SA	0.44 ± 0.15^{a}	158.83 ± 3.75^{c}
SE	2.82 ± 0.52^{a}	242.61 ± 4.48^{d}
SM	3.38 ± 0.79^{a}	254.75 ± 0.82^{e}
CA	14.32 ± 3.70^{b}	494.99 ± 0.44^{f}
CE	14.23 ± 3.41^{b}	693.49 ± 5.96^{g}
CM	12.83 ± 0.85^{b}	711.36 ± 4.72^{h}
GA	0.60 ± 0.01^{a}	94.21 ± 0.40^{a}
GE	2.08 ± 0.11^{a}	96.35 ± 4,87^{a}
GM	2.15 ± 0.32^{a}	150.37 ± 8.07^{b}

Values are expressed as mean ± sandard deviation, n = 3.
Different superscript letters in the same column indicate significant differences between samples at the level of $p < 0.05$.
*)SA = *Sargassum* sp. with acetone , SE = *Sargassum* sp. with ethanol, SM = *Sargassum* sp. with methanol, CA = *C. recemosa* with acetone, CE = *C. recemosa* with ethanol, CM = *C. recemosa* with methanol, GA = *G. verrucosa* with acetone, GE = *G. verrucosa* with ethanol, GM = *G. verrucosa* with methanol

Table 1 also shows that the use of solvent affected the total phenolic content of the crude extract of seaweed. The highest total phenolic content of the crude extract for three species of seaweed was obtained from methanol solvent. This is in agreement with the research conducted on seaweed *Padina australis* where the highest of total phenolic content was obtained from the methanol extract, followed by ethyl acetate and *n*-hexane extract [20].

3.2 Phytochemical Compound

Results of qualitative analysis of the phytochemical compound of seaweed crude extract is presented in Table 2. The content of the phytochemical compound of *C. recemosa* crude extract produced in this study is consistent with previous report [21] in which *C. recemosa* contained phytochemical compounds such as phenols, flavonoids, alkaloids, and steroids.

The results showed that the species of seaweed and solvents affected the phytochemical compounds of the seaweeds crude extract. It is indicated by the phenols compounds detected in the crude extract of *C. recemosa* for all types of solvents while for other crude extracts, phenols compounds were not detected, except in the crude extract of *G. verrucosa* extracted with methanol solvent. The influence of solvent on the type of phytochemical compounds from crude extract of seaweed was consistent with that reported in the extraction of the seaweed species *G. edulis* and *G. acerosa*, where tannins, saponins, and alkaloids were only detected in the ethyl acetate and ethanol solvent, whereas the the petroleum ether and hexane solvents were not detected [22].

Table 2 Phytochemical compound of seaweed crude extract

Sampel*)	Phytochemical compound				
	Fla	Ter	Alk	Phe	Tan
SA	+	+	+	-	-
SE	+	+	+	-	-
SM	+	+	+	-	-
CA	+	+	+	+	-
CE	+	+	+	+	-
CM	+	+	+	+	-
GA	+	+	+	-	-
GE	+	+	+	-	-
GM	+	+	+	+	-

Fla = Flavonoids, Ter = Terpenoids, Alk = Alkaloids, Phe = Phenols, Tan = Tannins, + = presence, - = absence

*)SA = *Sargassum* sp. with acetone , SE = *Sargassum* sp. with ethanol, SM = *Sargassum* sp. with methanol, CA = *C. recemosa* with acetone, CE = *C. recemosa* with ethanol, CM = *C. recemosa* with methanol, GA = *G. verrucosa* with acetone, GE = *G. verrucosa* with ethanol, GM = *G. verrucosa* with methanol

Similarly, tannins and phenols compounds at *Amphiroa fragilissima* extracts were only detected in methanol and ethyl acetate solvent, while in the chloroform, they were not detected. Terpenoids was detected in chloroform extract, while glycosides was not detected in ethyl acetate and methanol extract [23]. The result of qualitative phytochemical analysis of *C. scalpelliformis* in aqueous and five different solvent extracts (acetone, benzene, chloroform, diethyl ether and methanol) showed that phenol was only detected in benzene extract and steroid was in methanol extract [5].

The presence of phytochemical compound such as flavonoids, terpenoids, alkaloids, and phenols in seaweeds crude extracts indicated presence of antimicrobial properties [5, 6, 24].

4.0 CONCLUSION

The content of bioactive compounds from seaweed crude extract was affected by the species of seaweed and solvents used in extraction. The crude extract of *C. recemosa* produced better bioactive compounds than the crude extract of *G. verrucosa* and *Sargassum* sp., indicated by the value of the yield and total phenolic content of crude extract of *C. recemosa*, which were higher than the crude extract of *G. verrucosa* and *Sargassum* sp. The best solvent used in bioactive compound extraction of *C. recemosa* was methanol. Identified phytochemical compounds from the crude extract of *C. recemosa*, were flavonoids, terpenoids, alkaloids, and phenols. The presence of phytochemical compound in seaweeds crude extracts indicated the presence of antimicrobial properties.

Acknowledgement

The authors wish to thank the Director of the Directorate of Research and Community Services Ministry of Research, Technology and Higher Education, Director of the Pangkep State Polytechnic of Agricultural and Head of research and community service units for financial support.

References

[1] Manivannan, K., G. K. Devi, P. Anantharaman, and T. Balasubramanian. 2011 Antimicrobial Potential of Selected Brown Seaweeds from Vedalai Coastal Waters, Gulf of Mannar. *Asian Pacific Journal of Tropical Biomedicine*. 1(2): 114–120.

[2] Choi, J. S., H. J. Bae, S. J. Kim, and I. S. Choi, 2011. *In vitro* Antibacterial and Anti-inflammatory Properties of Seaweed Extracts against Acne Inducing Bacteria, *Propionibacterium acnes*. *Journal of Environmental Biology*. 32(3): 313–318.

[3] Kumar, J. I. N., M. Barot, and R. N. Kumar. 2014. Phytochemical Analysis and Antifungal Activity of Selected Seaweeds from Okha Coast, Gujarat, India. *Life Science Leaflet*. 52: 57–70.

[4] Siregar, A. F., A. Sabdono, and D. Pringgenies. 2012. Potensi Antibakteri Ekstrak Rumput Laut terhadap Bakteri Penyakit Kulit *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, dan *Micrococcus luteus* [Potential of Seaweed Extract Antibacterial Against Bacterial Skin Disease *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, and *Micrococcus luteus*]. *Journal of Marine Research*. 1(2): 152–160.

[5] Karthick, N., M. A. Fathimal, K. Ramesh, H. Sridhar, M. Natrajan, V. V. Divya, *et al*. 2014. Screening of Phytochemicals and Antimicrobial Activity of *Caulerpa scalpelliformis* Collected from Manapad Coast, Tuticorin District, Tamilnadu, South India. *Journal of Coastal Life Medicine*. 2(2): 107–111.

[6] Kumbhar, R. D., S. P. Rode, and A. B. Sabale. 2014. Phycochemical Screening of Seaweeds from Sindhudurg District of Maharashtra. *International Journal of*

Pharmaceutical Sciences Review and Research. 29(1): 77–81.
[7] Seenivasan, R., M. Rekha, H. Indu, and S. Geetha. 2012. Antibacterial Activity and Phytochemical Analysis of Selected Seaweeds from Mandapam Coast, India. *Journal of Applied Pharmaceutical Science*. 2(10): 159–169.
[8] Firdaus, M., S. S. Karyono, and M. Astawan. 2009. Penapisan Fitokimia dan Identifikasi Ekstrak Rumput Laut Coklat (*Sargassum duplicatum*) [Phytochemical Screening and Identification of Brown Alga (*Sargassum duplicatum*) Extract]. *Jurnal Ilmu-Ilmu Hayati*. 21(1): 60–65. [Bahasa Indonesia].
[9] Ornano, L., Y. Donno, C. M. Ballero, M. Serafini, and A. Bianco. 2014. Phytochemical Study of *Caulerpa racemosa* (Forsk.) J. Agardh, an Invading Alga in The Habitat of La Maddalena Archipelago. *Natural Product Research*. 28(20): 1795–1799.
[10] Shanmugam, J., K. D. Raman, S. Viswanathan, and T. Nallamuthu. 2013. Antibacterial and Antioxidant Activity of Red Seaweeds from Kilakarai, Rameswaram, Tamilnadu, India. *Journal of Pharmaceutical and Biomedical Sciences*. 32(32): 1386–1395.
[11] Anjum, A., G. Aruna, and C. M. Noorjahan. 2014. Qualitative Phytochemical Analysis and Evaluation of Total Phenolic Content of Two Selected Red Seaweeds from Sea Coast of Mandapam, Tamilnadu. *Weekly Science Research Journal*. 2(7): 1–5.
[12] Chithra, R. and S. Chandra. 2014. Phytochemical Variation and Genetic Diversity of *G. corticata* and *K. alvarezii* from Different Environment. *International Journal of Research in BioSciences*. 3(2): 1–5.
[13] Rahul, M., N. Suresh, and T. Anil. 2014. Evaluation of Physicocehmical Properties of Seaweed, *Caulerpa recemosa*. *International Journal of Research in Ayurveda and Pharmacy*. 5(4): 540–546.
[14] Cox, S., Abu-Ghannam, N. and Gupta, S. 2010. An Assessment of The Antioxidant and Antimicrobial Activity of Six Species of Edible Irish Seaweeds. *International Food Research Journal*. 17: 205–220.
[15] Elsie, B. H., P. N. Sudha, and M. S. Dhanarajan. 2010. *In Vitro* Studies on Antimicrobial Activity and Phytochemical Analysis of Marine Algae (Seaweeds) *Sargassum witti* and *Gracilaria edulis* Using Three Different Solvents Extracts. *The Ecoscan*. 4(4): 343–345.
[16] Al-Saif, S. S. A., N. Abdel-Raouf, H. A. El-Wazanani, and I. A. Aref. 2014. Antibacterial Substances from Marine Algae Isolated from Jeddah Coast of Red Sea, Saudi Arabia. *Saudi Journal of Biological Sciences*. 21: 57–64.
[17] Iswani, S. 2007. Proses Preparasi Ekstrak Kasar Etanol dari Makroalga untuk Uji Farmakologi [The Process of Preparation of Crude Extract Ethanol from Macroalgae for Pharmacological Assay]. *Buletin Teknologi Akuakultur*. 6(1): 57–60.
[18] Singleton, V. L. and J. A. Rossi. 1965. Colorimetry of Total Phenolics with Phosphomolybdic Phosphotungstic Acid Reagents. *American Journal of Enology and Viticulture*. 16: 144–158.
[19] Harbone, J. B. 1998. *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*. London: Chapman and Hall.
[20] Podungge, F. 2012. *Kandungan Fenol, Senyawa Fitokimia, dan Aktivitas Antioksidan Rumput Laut* Padina australis [The Content of Phenols, Phytochemicals Compound and Antioxidant Activity of *Padina australis* Seaweed]. [Final Project]. Bogor: Institut Pertanian Bogor. [Bahasa Indonesia].
[21] Srivastava, N., K. Saurav, V. Mohanasrinivasan, K. Kannabiran, and M. Singh. 2010. Antibacterial Potential of Macroalgae Collected from the Madappam Coast, India. *British Journal of Pharmacology and Toxicology*. 1(2): 72–76.
[22] Divya, S. R., M. Padmaja, M. S. Dhanarajan, and C. Mohan. 2013. Preliminary Phytochemical Screening, Total Phenolics, Flavonoid and Tannin Contents of *Gracilaria edulis* and *Gelidium acerosa*. *International Journal of Bioscience Research*. 2(3): 33–39.
[23] Viswanathan, S., C. Ebciba, R. Santhiya, and T. Nallamuthu. 2014. Phytochemical Screening and *In Vitro* Antibacterial, Antioxidant and Anticancer Activity of *Amphiroa fragilissima* (Linneaus) J V Lamoroux. *International Journal of Innovative Research in Science Engineering and Technology*. 3(5): 12933–1294.
[24] Widowati, I., D. Lubac, M. Puspita, and N. Bourgougnon. 2014. Antibacterial and Antioxidant Properties of The Red Alga *Gracilaria verrucosa* from The North Coast of Java, Semarang, Indonesia. *International Journal of Latest Research in Science and Technology*. 3(3): 179–185.