Bidang Fokus : Kemaritiman

Laporan Akhir Tahun
PENELITIAN DASAR UNGGULAN PERGURUAN TINGGI

Judul :

Karakterisasi Senyawa Antioksidan Rumput Laut *Eucheuma spinosum* Sebagai Pangan Fungsional dari Perairan Sulawesi Utara

Tahun ke 3 dari Rencana 3 Tahun

Dr. Ir. Lena J. Damongilala, M.Si NIDN: 0021026203
Defny S. Wewengkang, SPIK.,MSc.,PhD. NIDN: 0009127302
Ir. Fitje Losung, M.Si NIDN:0010026104

UNIVERSITAS SAM RATULANGI
Nopember 2019

Dibiaya oleh :
Direktorat Riset dan Pengabdian Masyarakat
Direktorat Jenderal Penguatan Riset dan Pengembangan
Kementrian Riset, Teknologi, dan Pendidikan Tinggi
Sesuai dengan Kontrak Penelitian Tahun Anggaran 2019
Nomor : 165/UN 12.13/LT/2019

LAPORAN AKHIR PENELITIAN MULTI TAHUN

ID Proposal: aea4ac33-0314-4aa4-96c4-a70e89153a8e
Laporan Akhir Penelitian: tahun ke-3 dari 3 tahun

1. IDENTITAS PENELITIAN

A. JUDUL PENELITIAN

Karakterisasi Senyawa Antioksidan Rumput Laut Eucheuma spinosum Sebagai Pangan Fungsional dari Perairan Sulawesi Utara

B. BIDANG, TEMA, TOPIK, DAN RUMPUN BIDANG ILMU

Bidang Fokus RIRN / Bidang Unggulan Perguruan Tinggi	Tema	Topik (jika ada)	Rumpun Bidang Ilmu
Kemaritiman	-	Pemanfaatan Sumber Daya Alam (SDA): Non hayati dan hayati berbasis potensi megadiversitas secara berkelanjutan	Bioteknologi Perikanan

C. KATEGORI, SKEMA, SBK, TARGET TKT DAN LAMA PENELITIAN

Kategori (Kompetitif Nasional/ Desentralisasi/ Penugasan)	Skema Penelitian	Strata (Dasar/ Terapan/ Pengembangan)	SBK (Dasar, Terapan, Pengembangan)	Target Akhir TKT	Lama Penelitian (Tahun)
Penelitian Desentralisasi	Penelitian Dasar Unggulan Perguruan Tinggi	SBK Riset Dasar	SBK Riset Dasar	3	3

2. IDENTITAS PENGUSUL

Nama, Peran	Perguruan Tinggi/ Institusi	Program Studi/ Bagian	Bidang Tugas	ID Sinta	H-Index
LENA JEANE DAMONGILALA Ketua Pengusul	Universitas Sam Ratulangi	Teknologi Hasil Perikanan		6101098	0
DEFNY SILVIA WEWENGKANG S.PIK.,MSc.,PhD Anggota Pengusul 1	Universitas Sam Ratulangi	Farmasi		6110033	8

Ir LOSUNG FITJE M.Si Anggota Pengusul 2	Universitas Sam Ratulangi	Ilmu Kelautan		6104516	9

3. MITRA KERJASAMA PENELITIAN (JIKA ADA)

Pelaksanaan penelitian dapat melibatkan mitra kerjasama, yaitu mitra kerjasama dalam melaksanakan penelitian, mitra sebagai calon pengguna hasil penelitian, atau mitra investor

Mitra	Nama Mitra

4. LUARAN DAN TARGET

CAPAIAN Luaran Wajib

Tahun Luaran	Jenis Luaran	Status target capaian (*accepted, published, terdaftar atau granted, atau status lainnya*)	Keterangan (*url dan nama jurnal, penerbit, url paten, keterangan sejenis lainnya*)
3	Publikasi Ilmiah Jurnal Internasional	accepted/published	International journal of Chem Tech Research CODE (USA) :

Luaran Tambahan

Tahun Luaran	Jenis Luaran	Status target capaian (*accepted, published, terdaftar atau granted, atau status lainnya*)	Keterangan (*url dan nama jurnal, penerbit, url paten, keterangan sejenis lainnya*)

5. ANGGARAN

Rencana anggaran biaya penelitian mengacu pada PMK yang berlaku dengan besaran minimum dan maksimum sebagaimana diatur pada buku Panduan Penelitian dan Pengabdian kepada Masyarakat Edisi 12.

Total RAB 3 Tahun Rp. 96,660,000

Tahun 1 Total Rp. 0

Tahun 2 Total Rp. 0

Tahun 3 Total Rp. 96,660,000

Jenis Pembelanjaan	Item	Satuan	Vol.	Biaya Satuan	Total
Analisis Data	HR Pengolah Data	P (penelitian)	1	3,500,000	3,500,000
Analisis Data	Tiket	OK (kali)	1	5,610,000	5,610,000
Analisis Data	Biaya analisis sampel	Unit	2	6,000,000	12,000,000
Analisis Data	Uang Harian	OH	3	400,000	1,200,000
Analisis Data	Penginapan	OH	3	600,000	1,800,000
Analisis Data	Transport Lokal	OK (kali)	6	100,000	600,000
Analisis Data	Honorarium narasumber	OJ	40	25,000	1,000,000
Bahan	ATK	Paket	1	2,300,000	2,300,000
Bahan	Bahan Penelitian	Unit	1	25,050,000	25,050,000

Jenis Pembelanjaan	Item	Satuan	Vol.	Biaya Satuan	Total
	(Habis Pakai)				
Pelaporan, Luaran Wajib, dan Luaran Tambahan	Biaya seminar nasional	Paket	1	3,000,000	3,000,000
Pelaporan, Luaran Wajib, dan Luaran Tambahan	Biaya seminar internasional	Paket	1	6,000,000	6,000,000
Pelaporan, Luaran Wajib, dan Luaran Tambahan	Publikasi artikel di Jurnal Internasional	Paket	1	8,000,000	8,000,000
Pelaporan, Luaran Wajib, dan Luaran Tambahan	Luaran KI (paten, hak cipta dll)	Paket	1	2,000,000	2,000,000
Pengumpulan Data	Transport	OK (kali)	2	1,000,000	2,000,000
Pengumpulan Data	Uang Harian	OH	3	500,000	1,500,000
Pengumpulan Data	Biaya konsumsi	OH	6	100,000	600,000
Pengumpulan Data	HR Pembantu Peneliti	OJ	300	20,000	6,000,000
Sewa Peralatan	Ruang penunjang penelitian	Unit	1	2,500,000	2,500,000
Sewa Peralatan	Peralatan penelitian	Unit	6	2,000,000	12,000,000

6. HASIL PENELITIAN

A. RINGKASAN: Tuliskan secara ringkas latar belakang penelitian, tujuan dan tahapan metode penelitian, luaran yang ditargetkan, serta uraian TKT penelitian.

Antioksidan merupakan senyawa bioaktif yang sangat diperlukan sebagai bahan baku dalam berbagai sediaan makanan, sediaan farmasi, dan kosmetika. Keberadaan senyawa ini tersimpan dalam jaringan sel tumbuhan. Penelitian terdahulu dari rumput laut Eucheuma spinosum memiliki aktifitas antioksidan yang lebih tinggi dari jenis lain. Tujuan jangka pendek penelitian ini yaitu : 1) Mendapatkan ekstrak antioksidan jenis rumput Eucheuma spinosum yang diekstraksi menggunakan metode maserasi dan sokletasi dengan berbagai konsentrasi pelarut methanol 2) Mendapatkan senyawa yang beraktifitas antioksidan sebagai bahan pangan dan farmasi dari rumput laut. 3) Mendapatkan karakteristik senyawa murni melalui proses isolasi. Urgensi penelitian ini ialah mendapatkan struktur senyawa dan kemampuan dari isolat murni Eucheuma spinosum sebagai antiosidan yang berpotensi sebagai pangan fungsional. Metode yang digunakan yaitu ekstraksi dengan pelarut metanol diikuti tahap pemurnian dengan metode fraksinasi kromatografi kolom dan KLT dengan pengujian kualitatif antioksidan dengan metode kromatografi lapis tipis bioautografi. Tahapan penelitian yang direncanakan yaitu : Tahun I, ekstraksi rumput laut Eucheuma spinosum dan menguji aktifitas senyawa aktif antioksidan dengan metode DPPH IC50. Tahun kedua (II), isolasi senyawa aktif antioksidan dengan metode kromatografi kolom (KK) dan kromatografi lapis tipis (KLT) uji DPPH penghambatan radikal bebas hayati yang kaya bahan bioaktif dan uji fitokimia. Tahun ketiga (III), Penentuan struktur senyawa murni dengan metode spektoskopi NMR, IR, dan UV, dan mengukur aktivitas antioksidan DPPH dari senyawa murni antioksidan dengan SOD, serta Menguji sifat antibakteri senyawa murni dengan metode difusi agar dengan cara sumur Kirby-Baurer.Luaran penelitian yaitu : Internasional journal dan jurnal nasional terakreditasi Jurnal Pengolahan Hasil Perikanan Indonesia (JPHPI) ISSN 2303-2111, serta bahan ajar. Dari penelitian tahap ke-2 pada sampel rumput laut Eucheuma spinosum yang menggunakan dua jenis pelarut masing-masing metanol dan etanol dan dua macam ekstraksi yaitu maserasi dan soksletasi, ternyata mengandung cukup banyak senyawa antioksidan yang berpotensi sebagai bahan pangan dan bahan baku farmasi. Hal

ini dibuktikan dengan hasil rendemen masing-masing jenis sampel dihasilkan tertinggi pada ekstrak sampel etanol 50% yang dilakukan maserasi sebesar 39,26%. Selanjutnya dilakukan uji aktifitas antioksidan dengan nilai IC50 yang menunjukkan besarnya aktifitas antioksidan dalam konsentrasi larutan sampel untuk menghambat 50% radikal bebas DPPH. Hasil pengujian aktifitas antioksidan dengan DPPH yang memiliki IC50 (Nilai Inhibition Concentration : nilai penghambatan aktifias antioksidan terkecil, berarti memiliki aktifitas antioksidan besar) mendapatkan aktifitas antioksidan pada ekstrak etanol 50% dengan maserasi menghasilkan nilai IC50 terkecil yaitu 97, 522 ppm. Hal ini berarti dengan penggunaan pelarut etanol pada konsentrasi 50%, aktifitas penghambatan sangat besar dan baik bagi sampel. Demikian halnya dengan etanol merupakan pelarut yang aman bagi bahan pangan. Untuk penelitian tahap ke-3 (Tahun terakhir), sudah dan sementara dilaksanakan sesuai tujuan yang direncanakan, yaitu : Mendapatkan karakteristik senyawa murni melalui proses isolasi dan berbagai pengujian sebagai konfirmasi hasilnya. Penelitian pada tahap ke-3, telah dan sementara dilaksanakan sesuai tujuan yang direncanakan. Tahap penyiapan sampel rumput laut merah E. spinosum, dikumpulkan dari petani rumput laut di kecamatan Tatapaan Kabupaten Minahasa Selatan-Sulawesi Utara. Sampel segar dicuci dan dipotong menjadi ukuran kecil. Diekstraksi dengan etanol 50%, kemudian dipartisi air-n-heksana dan air-etil asetat, masing-masing ekstrak yang diperoleh disaring,dipekatkan dalam evaporator vacuum dan dibuat dalam serangkaian konsentrasi untuk pengujian Proses isolasi dari sampel target telah dilakukan berbagai pengujian : fitokimia, aktifitas antioksidan DPPH dan SOD. Data yang telah diperoleh adalah sebagai berikut. Senyawa triterpenoid dan tannin ditemukan pada semua Ekstrak rumput laut E. spinosum. Ekstrak n-heksana mengandung alkaloid,triterpenoid dan steroid. Ekstrak etil asetat mengandung flavonoid, triterpenoid, steroid, dan saponin. Data keberadaan senyawa metabolit sekunder dalam sampel dianalisis fitokimia yang ditunjukkan pada Tabel 1.

Tabel 1. Data Analisis Fitokimia dari ekstrak E. spinosum

No.	Secondary metabolites	Reagent	Samples MeOH	n-Hexane	EtOAc	H2O
1	Flavonoid	a. HCl + Mg	-	-	-	-
		b. H2SO4 (2N)	-	-	-	-
		c. NaOH (10%)	-	-	+	-
2	Alkaloid	Pereaksi Dragendorf	-	+	-	-
3	Steroid	Lieberman-Burchard	+	-	+	-
4	Triterpenoid		+	-	+	-
5	Saponin	HCl + H2O	-	-	+	-
6	Tanin	FeCl3 (1%)	+	+	+	+

Hasil pengujian aktifitas antioksidan dengan radikal DPPH menunjukkan peningkatan konsentrasi,terutama pada ekstrak etil asetat yang dilakukan berulang-ulang. Namun ekstrak air, etil asetat, dan ekstrak n-heksana dari E. Spinosum aktif dengan zona inhibisi masing-masing 2,478; 402,8; dan 1, 537 ppm seeperti terlihat pada Tabel 2.

Tabel 2. Aktifitas Antioksidan (DPPH) dari ekstrak E. spinosum

No.	Samples	Inhibition Activity (IC50 /ppm) n-Hexane	EtOAc	H2O	MeOH
1	Extracts	1,537.1	402.8	2,478.1	-
2	3-(3-methoxyphenyl)propanal	-	87,97	-	-
3	Quercetin	-	-	-	20.98
4	Catechin	-	91.82	-	-

Hasil pengujian aktifitas antioksidan dengan SOD pada ekstrak rumput laut E. spinosum mengalami peningkatan konsentrasi dalam menangkap radikal bebas, terutama dalam ekstrak etil asetat.

B. KATA KUNCI: Tuliskan maksimal 5 kata kunci.

Eucheuma spinosum; isolasi; antioksidan; pangan fungsional;

Pengisian poin C sampai dengan poin H mengikuti template berikut dan tidak dibatasi jumlah kata atau halaman namun disarankan seringkas mungkin. Dilarang menghapus/memodifikasi template ataupun menghapus penjelasan di setiap poin.

C. HASIL PELAKSANAAN PENELITIAN: Tuliskan secara ringkas hasil pelaksanaan penelitian yang telah dicapai sesuai tahun pelaksanaan penelitian. Penyajian dapat berupa data, hasil analisis, dan capaian luaran (wajib dan atau tambahan). Seluruh hasil atau capaian yang dilaporkan harus berkaitan dengan tahapan pelaksanaan penelitian sebagaimana direncanakan pada proposal. Penyajian data dapat berupa gambar, tabel, grafik, dan sejenisnya, serta analisis didukung dengan sumber pustaka primer yang relevan dan terkini.

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Penelitian pada tahap ke-3, telah dilaksanakan sesuai tujuan dan target yang direncanakan. Tahap penyiapan sampel rumput laut merah E. spinosum, dikumpulkan dari petani rumput laut di kecamatan Tatapaan Kabupaten Minahasa Selatan-Sulawesi Utara. Sampel segar dicuci dan dipotong menjadi ukuran kecil agar senyawa bioaktifnya dapat diikat oleh pelarut. Selanjutnya diekstraksi dengan etanol 50%, kemudian dipartisi air-n-heksana dan air-etil asetat, masing-masing ekstrak yang diperoleh disaring,dipekatkan dalam evaporator vacuum dan dibuat dalam serangkaian konsentrasi untuk pengujian Proses isolasi dari sampel target telah dilakukan berbagai pengujian : fitokimia, aktifitas antioksidan DPPH dan SOD, Analisis dgn NMR, dan konfirmasi dengan UV,IR,MS. Data yang telah diperoleh adalah sebagai berikut. Pada pengujian fitokimia, Senyawa triterpenoid dan tannin ditemukan pada semua Ekstrak rumput laut E. spinosum. Ekstrak n-heksana mengandung alkaloid,triterpenoid dan steroid. Ekstrak etil asetat mengandung flavonoid, triterpenoid, steroid, dan saponin. Data keberadaan senyawa metabolit sekunder dalam sampel dianalisis fitokimia yang ditunjukkan pada Tabel 1.

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		H2SO4 (2N)	-	-	-	-
		NaOH (10%)	-	-	+	-
2	Alkaloid	Pereaksi Dragendorf	-	+	-	-
3	Steroid	Lieberman-Burchard	+	-	+	-
4	Triterpenoid		+	-	+	-
5	Saponin	HCl + H2O	-	-	+	-
6	Tanin	FeCl3 (1%)	+	+	+	+

Hasil pengujian aktifitas antioksidan dengan radikal DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) menunjukkan peningkatan konsentrasi, terutama pada ekstrak etil asetat yang dilakukan berulang-ulang. Namun ekstrak air, etil asetat, dan ekstrak n-heksana dari E. Spinosum aktif dengan zona inhibisi masing-masing 2,478; 402,8; dan 1, 537 ppm seeperti terlihat pada Tabel 2.

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2	3-(3-methoxyphenyl)propanal	-	87,97	-	-

3	Quercetin	-	-	-	20.98
4	Catechin	-	91.82	-	-

Hasil pengujian aktifitas antioksidan dengan uji SOD(superoxide dismutase) pada ekstrak rumput laut E. spinosum mengalami peningkatan konsentrasi dalam menangkap radikal bebas, terutama dalam ekstrak etil asetat. Uji ini penting karena berkaitan erat denganperannya sebagai suatu enzim yang melindungi sel-sel tubuh dari kerusakan dan mencegah proses peradangan akibat radikal bebas (Jun et al, 2003; Gargouri et al, 2011; Kalender et al, 2005)

Peningkatan konsentrasi dalam ekstrak E. spinosum dapat menangkap radikal bebas, terutama dalam ekstrak etil asetat. Peningkatan aktivitas antioksidan dari ekstrak E. spinosum air, etil asetat, dan n-heksana tergantung pada konsentrasi masing-masing (Wardani et al., 2017). Nilai zona hambat ekstrak E. spinosum masing-masing adalah 155, 25, dan 870 ppm, seperti yang ditunjukkan pada Tabel 4.

Tabel 4. Aktifitas Antioxidan (SOD) dari ekstrak E. spinosum

No.	Samples	Inhibition Activity (IC50 / ppm)			
		n-Hexane	EtOAc	H2O	MeOH
1	E. spinosum extract	870	25	155	160
2	Quercetin	-	-	-	5
3	Catechin	-	-	-	13

Uupaya untuk mendapatkan aktivitas antioksidan aktif dari rumput laut yang dapat dimakan Eucheuma spinosum, maka penelitian ini telah mengkonfirmasi bahwa ekstrak n-heksana, etil asetat dan H2O aktif terhadap uji antioksidan masing-masing DPPH dan SOD. Berdasarkan data uji terhadap uji DPPH menunjukkan bahwa ekstrak etil asetat adalah yang paling aktif untuk mencari radikal bebas dengan nilai IC50 402,8 ppm, sedangkan jika dibandingkan dengan referensi senyawa quercetin dan chatechin dengan nilai IC50 masing-masing 20,98 dan 91,82 ppm, masing-masing, aktivitas ekstrak etil asetat kurang aktif.32,33 Dalam makalah yang diterbitkan sebelumnya, Damongilala et al., (2013) telah melaporkan bahwa ekstrak metanol dari E. spinosum dan E. cottonii dapat mengais radikal DPPH dengan nilai IC50 75,27 ppm dan 64,73 ppm.20 Menurut analisis fitokimia, metabolit sekunder flavonoid, steroid, terpenoid, saponin dan tanin yang terkandung dalam ekstrak etil asetat memiliki korelasi linier dengan aktivitas antioksidannya. Jadi, data awal ini sama pentingnya dengan temuan untuk panduan lebih lanjut untuk mengisolasi konstituen aktivanya. 34,35,36

Dengan demikian penelitian ini mendapatkan senyawa antioksidan alami yang diharapkan dapat direkomendasikan dan dimanfaatkan sebagai bahan pangan fungsional dari rumput laut E. spinosum, karena senyawa antioksidan penting bagi kesehatan dan untuk mempertahankan mutu produk pangan. Hal mana juga memberi dampak positif bagi petani rumput laut untuk terus berupaya meningkatkan produksi rumput laut. .....

D. **STATUS LUARAN**: Tuliskan jenis, identitas dan status ketercapaian setiap luaran wajib dan luaran tambahan (jika ada) yang dijanjikan pada tahun pelaksanaan penelitian. Jenis luaran dapat berupa publikasi, perolehan kekayaan intelektual, hasil pengujian atau luaran lainnya yang telah dijanjikan pada proposal. Uraian status luaran harus didukung dengan bukti kemajuan ketercapaian luaran sesuai dengan luaran yang dijanjikan. Lengkapi isian jenis luaran yang dijanjikan serta mengunggah bukti dokumen ketercapaian luaran wajib dan luaran tambahan melalui Simlitabmas mengikuti format sebagaimana terlihat pada bagian isian luaran

Dalam upaya untuk mengejar aktivitas antioksidan aktif dari alga yang dapat dimakan Eucheuma spinosum, penelitian ini telah mengkonfirmasi bahwa ekstrak n-heksana, etil asetat dan H2O aktif terhadap uji antioksidan masing-masing DPPH dan SOD. Berdasarkan data uji terhadap uji DPPH menunjukkan bahwa ekstrak etil asetat adalah yang paling aktif untuk mencari radikal bebas dengan nilai IC50 402,8 ppm, sedangkan jika dibandingkan dengan referensi senyawa quercetin dan chatechin dengan nilai IC50 masing-masing 20,98 dan 91,82 ppm, masing-masing, aktivitas ekstrak etil asetat kurang aktif.32,33 Dalam makalah yang diterbitkan sebelumnya, Damongilala et al., (2013) telah melaporkan bahwa ekstrak metanol dari E. spinosum dan E. cottonii dapat mengais radikal DPPH dengan nilai IC50 75,27 ppm dan 64,73 ppm.20 Menurut analisis fitokimia, metabolit sekunder flavonoid, steroid, terpenoid, saponin dan tanin yang terkandung dalam ekstrak etil asetat memiliki korelasi linier dengan aktivitas antioksidannya. Jadi, data awal ini sama pentingnya dengan temuan untuk panduan lebih lanjut untuk mengisolasi konstituen aktivanya. 34,35,36

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E. **PERAN MITRA:** Tuliskan realisasi kerjasama dan kontribusi Mitra baik *in-kind* maupun *in-cash* (jika ada). Bukti pendukung realisasi kerjasama dan realisasi kontribusi mitra dilaporkan sesuai dengan kondisi yang sebenarnya. Bukti dokumen realisasi kerjasama dengan Mitra diunggah melalui Simlitabmas mengikuti format sebagaimana terlihat pada bagian isian mitra

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F. **KENDALA PELAKSANAAN PENELITIAN**: Tuliskan kesulitan atau hambatan yang dihadapi selama melakukan penelitian dan mencapai luaran yang dijanjikan, termasuk penjelasan jika pelaksanaan penelitian dan luaran penelitian tidak sesuai dengan yang direncanakan atau dijanjikan.

Kendala atau kesulitan yang dihadapi selama melaksanakan penelitian adalah sebagai berikut. Tahap penelitian Tahun ke-3, penyediaan bahan/sampel penelitian mengalami kendala akibat terjadinya cuaca ekstrim pada lokasi tempat pengambilan sampel rumput laut, mengakibatkan keterlambatan penyediaan sampel rumput laut untuk dianalisis. Karena itu, pengusulan artikeluntuk jurnal internasionalsebagailuaran wajib mengalami juga keterlambatan. Namun demikian, artikeljurnal sudah dalam proses revisi lanjut.....

G. **RENCANA TINDAK LANJUT PENELITIAN:** Tuliskan dan uraikan rencana tindaklanjut penelitian selanjutnya dengan melihat hasil penelitian yang telah diperoleh. Jika ada target yang belum diselesaikan pada akhir tahun pelaksanaan penelitian, pada bagian ini dapat dituliskan rencana penyelesaian target yang belum tercapai tersebut.

Rencana tindak lanjut penelitian selanjutnya adalah melanjutkan proses uji yang sementara dikerjakan diantaranya untuk uji konfirmasi dari hasil NMR dengan UV, MS, IR, sehingga didapatkan hasil sesuai target penelitian tahap-3 untuk penentuan struktur senyawa. Hasil yang sudah diuji pada proses isolasi dan karakterisasi senyawa menunjukkan hasil positif atau terbukti sebagai senyawa antioksidan alami.
.....
.....

H. DAFTAR PUSTAKA: Penyusunan Daftar Pustaka berdasarkan sistem nomor sesuai dengan urutan pengutipan. Hanya pustaka yang disitasi pada laporan akhir yang dicantumkan dalam Daftar Pustaka.

1. Damongilala, L.J., Widjanarko, S.B., Zubaidah, E., Runtuwene, M.R.J., 2013. Antioxidant activity against methanol extraction of Eucheuma cottoni and E. spinosum collected from North Sulawesi waters, Indonesia. Food Science and Quality Management 17, 7-13.....

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Judul artikel: Tropical Red Algae of Eucheuma spinosum as Natural Food from North Sulawesi Waters, Indonesia : Evaluation of Phytochemical and Antioxidant activities

Tropical Red Algae of *Eucheuma spinosum* as Natural Functional Food from North Sulawesi Waters, Indonesia: Evaluation of phytochemical and Antioxidant activities

[1]Lena J. Damongilala, [2]Defny S. Wewengkang, [1]Fitje Losung

[1]Faculty of fisheries and marine science, Sam Ratulangi University, Indonesia

[2]Faculty of Mathematics and Natural Sciences, Sam Ratulangi University, Indonesia

Corresponding e-mail: lenajeane@unsrat.com
Phone/Fax: 62-0431-868027

ABSTRACT

Recently, research to discovery new marine natural sources as functional foods have pay attention by many researchers to characterize their phytochemicals and biological activities. One of the potential sources of red algae *Eucheuma spinosum* are mainly used as natural daily food sources and contained bioactive components of flavonoids, alkaloids, saponins, tannins and derivatives. This research purposes is to identify and characterize antioxidant constituents and activity of tropical algae *Eucheuma spinosum* as new prospective natural antioxidant sources for functional food source. The sample was extracted by organic solvents and purified by combination technique of normal- and reverse phase chromatography methods. The biological activity evaluations as antioxidant of extract and pure compounds to determine of the free radical scavenging values were assayed against DPPH and SOD methods, respectively. The result showed that ethyl acetate extract from *E. spinosum* can scavenging against DPPH and SOD radicals with IC_{50} values of 402 and 25 ppm, while the active compound of 3-methoxycinamic (**1**) showed IC_{50} of 87.97 ppm against DPPH. This finding give valuable information of *Eucheuma spinosum* has antioxidant activity, and suggested the algae can be consumed as a functional food.

Keywords: functional food, *Eucheuma spinosum*, antioxidant, DPPH, SOD

1. Introduction

In past few decades, the extensively research on functional foods and their bioactive constituents have been conducted to provide health benefits though bioactive compounds, as these compounds target mechanisms that manage, prevent, and/or treat infectious and metabolic diseases. The formulation of bioactive constituents of foods is powerful active molecules naturally present in small quantities those have contributes to regulating biological mechanisms. This fact showed the potential application of plant-based bioactive compounds, including marine algae as a novel source of functional food ingredients and food preservative.

The development of the functional foods has been done a lot because natural foods contain many bioactive compounds (Yeung et al, 2018). Functional foods can be defined as natural or processed foods that contain biological active compounds, food in a specified amount, effective and non-toxic, providing clinically proven health benefits for the prevention, management or treatment of chronic diseases (Sarkar, 2018; Fernandes et al, 2019). In general, functional food is strictly regulated but not

recognized by law in most countries, so there is no definition according to the law (Ye et al, 2018). The concept of functional food refers to food products that are a source of nutritional compounds and provide other benefits to consumers (Leidi et al, 2018).

In the last few years, marine resource as a source of new drug development and healthy food is very interesting (Kania et al, 2013). Seaweed or algae are primitive plants that do not flower and do not have stems, leaves and roots (Moubayed et al, 2016). Marine algae and their constituents have a key position in the progression of modern studies and knowledge on biological activity or active substances. The marine environment, which contains a vast array of organisms with unique biological properties, is one of the most underutilized biological resources.

In order to adapt to these extreme conditions, most algae produce a high variety of secondary metabolites that often have potent biological activities. To date, algae and microalgae are referenced in the literature as sources of bioactive compounds for use as functional food ingredients (Plaza et al, 2009; Plaza et al, 2008). Algae contain several compounds including acids, amines, antibacterial substances, antifungal and antiviral agents, lipids, sterols, steroids and fatty acids, phenolic compounds, phytochromes, pigments, sugars, and alcohol. This algae content is a useful source of products and medicines Satya et al, 2013; Matanjun et al, 2008; O'Sullivan et al, 2011). Seaweed can be used as a source of natural antioxidant compounds because its crude extract shows antioxidant activity (Devi et al, 2011). Algae are rich in bioactive ingredients and can be applied to biotechnology, some of the findings from these algae have been explored but there are still many that need to be explored more deeply. Of the three algal classifications, red algae (*Eucheuma spinosum*) have unique and therapeutic properties that must be studied further for the benefit of the world, and mostly found in tropical, coastal, continental, temperate and cold water. Red algae have around 6,000 species that are including of 670 marine genera (Rajasulochana and Preethy, 2015a; 2015b). Red and brown algae are mainly used as human food sources. Fresh and dried seaweed is consumed by many people; especially those who live in coastal areas (Rajasulochana et al, 2009). Red algae have brilliant colors because of the pigment phycoerythrin and phycocyanine. These algae can live at a greater depth than brown and green algae because they absorb blue light (Dayuti, 2018). *E. spinosum* are considered to be the most important source of many biologically active metabolites compared to other classes of algae (Ali and Gamal, 2010). *E. spinosum* can contain bioactive compounds such as flavonoids, alkaloids, saponins, tannins and derivatives that have antibacterial (O'Sullivan et al, 2011) and antioxidant properties (Safitri et al, 2018).

Antioxidants are compounds that can transmit one electron to free radicals to be neutral (Damongilala et al, 2013). Antioxidants are usually added to food to slow oxidative decline and prevent chronic diseases in the body (Alencar et al, 2016). Scavenger free radicals (antioxidants) have an important role in protecting cells against oxidative stress and maintaining the balance of toxic oxygen species (Wresdiati et al, 2008). These active species may include superoxide anions ($O_2^{\bullet -}$), hydroxyl radicals ($OH^{\bullet}$), hydrogen peroxide (H_2O_2), hypochlorite anions (ClO^-), and singlet oxygen ($^1O_2$) (Rossa et al, 2002).

To search for new prospective natural antioxidant sources, the extract of inhibition activity extract of tropical algae *E. spinosum* against 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) have been suggested, while activity against superoxide dismutase (SOD) assay have not been researched. This paper describes extracts preparation, phytochemical analysis and evaluation of antioxidant capacity against superoxide dismutase (SOD) assay.

2. Materials and Methods

2.1 Plant material collection and determination

The red algae of *Eucheuma spinosum* was cultivated and collected on January 2012 from Manado, North Sulawesi, Indonesia. The specimen was deposit and identified at Laboratory of Plant Taxonomy, Department of Biology, Faculty of Mathematic and natural Sciences, Padjadjaran University, Bandung - Indonesia. Kiesel G 60 silica gel resins and the ODS of LiChroprep RP-18 (Merck, Darmstadt, Germany), were used for column chromatography. TLC analysis was carried out using Kiesel gel 60 F_{254} and RP-18 F_{254S} (Merck). Deuterated solvents were purchased from Merck Co. Ltd. and Sigma Aldrich Co. Ltd. (St. Louis, MO, USA).

2.2 Chemicals and reagents:

The distillated organic solvents of *n*-hexane, ethyl acetate and methanol were used for extraction of sample. For antioxidant assay, DPPH was purchased from Wako - Japan, while for SOD assay reagent of NBT, riboflavin, TEMED, PBS purchased from Amresco. The TLC spraying regents of 10% sulfuric acid in ethanol used in this study were purchased from Merck and Sigma Aldrich.

2.3 Instruments:

NMR spectra recorded on a 500 MHz FT-NMR spectrometer (Varian ECA 500 JOEL, Japan). IR spectra were obtained from a Perkin Elmer Spectrum One FT-IR spectrometer (Buckinghamshire, England). Mass Spectra were obtained from an ES-MS Spectrometer (UPLC MS/MS TQD type, Waters). DPPH and SOD assay were measured on Biochrom Ez Read 400 Elisa Reader.

2.4 Preparation of Extract:

The collected fresh fruits of *Eucheuma spinosum* were washed well and cut into small sizes. The samples was extracted with methanol and then subsequently partitioned with water-*n*-hexane and water-ethyl acetate, respectively. Extracts obtained was filtered, concentrated in vacuo and was made in a series concentrations for phytochemical screening and antioxidant of DPPH and SOD activity test.

2.5 Preliminary phytochemical screening:

Screening for alkaloids, terpenoids, and flavonoids secondary metabolites were performed to methanol, *n*-hexane, ethyl acetate, and water extracts of previously mention vegetables and fruits (Harbone, 1973; Kokate, 2005).

2.6 Antioxidant Activity of DPPH Assay:

The percentage of antioxidant activity (%) of *E. spinosum* extract was assessed by DPPH free radical assay. The measurement of the DPPH radical scavenging activity was performed according to methodology described by Brand-Williams *et al* (1995). (Williams et al, 1995). The samples were reacted with a stable DPPH radical in an ethanol solution. The reaction mixture consisted of 0.5 mL sample, 3 mL of absolute ethanol and 0.3 mL of DPPH radical solution 0.5 µM in ethanol. When DPPH reacts with an antioxidant, which donates hydrogen, it is reduced. The changes in color (from deep violet to light yellow) were observed (absorbance (abs)) at 517 nm after 100 min of reaction using a UV-Vis spectrophotometer (Shimadzu UV-Vis 1800 - Japan). The mixture of ethanol (3.3 mL) and sample (0.5 mL) served as blank. The control solution was prepared by mixing ethanol (3.5 mL) and DPPH radical solution

(0.3 mL). Quercetin and catechin were used as positive control. The scavenging activity percentage (%) is determined based on Mensor *et al* (2001) (Mensor et al, 2001).

2.7 Antioxidant of superoxide dismutase (SOD) activity assay:

The SOD-mimic activities of samples were evaluated using an indirect method of riboflavin photoreduction, which was described previously. The method involves the competitive reaction between the complex and reduced NBT (NBT = nitroblue tetrazolium) for O_2 %-generated by riboflavin under illumination at room temperature (25°C). The sample mixture (240 µL) contained the complex (11 different concentrations), 6 µM riboflavin, 0.8 µM of *N*,*N*,*N*',*N*'-tetramethylethylene-diamine (TMEDA) in 0.016 M phosphate buffer (pH 7.4) and 85 µM NBT. The reaction was stopped by switching off the light after 15 min (4 fluorescence tubes, Philips TLD/20 W, 20 cm distance) and the absorbance of reduced NBT was measured at λ 560 nm with a Multiscan Go Thermo Fischer Scientific UV/Vis double beam spectrophotometer (Deawati et al, 2018).

2.8 Structure determination of active compound 1

Compound **1** was isolated from ethyl acetate extract and the structure was characterized by spectroscopic methods of ^{1}H-NMR (JEOL 500 MHz), ^{13}C-NMR (JEOL 125 MHz), DEPT 135 , HMQC, and HMBC.

3. Results and Discussion

3.1 Preparation of Extract:

Marine natural product is one of the prospectus natural sources to discover new functional food contained some active components. Recently, the needed for research and development to discover new potential natural sources of functional food was very fast & high. The information of their bioactivity and actives constituents are the important aspect to be identified as scientific based of that new functional food can be consumed those have health benefits for human body.

The sample of red algae of *Eucheuma spinosum* was cultivated from waters of Nain Island, Manado – North Sulawesi. Fresh samples were extracted with methanol for 3x24 hours and continued with subsequent partitioning between *n*-hexane-water and ethyl acetic-water to yield concentrated extracts as shown in Table 1.

3.2 Phytochemicals screening of *E. spinosum* extracts:

In order to know composition of their secondary metabolite, the preliminary phytochemical was screening and data as described in Table 2. Data of the presence secondary metabolite constituents of samples by phytochemical analysis showed that the ethyl acetate extract containing major secondary metabolites including flavonoid, steroid, terpenoids, saponin and tannin, while the alkaloid was not identified. On the other hand, methanol and *n*-hexane extracts contained same compounds of tannin, but different content of terpenoid, steroid and steroid, respectively. This data is a strong base of edible algae *Eucheuma spinosum* in this study can suggested as potential natural source bioactive compounds. The finding data was supported by previous study those showed that *Eucheuma spinosum* indicated as an important antioxidant

compound (Muawanah et al, 2016).

3.3 Antioxidant (DPPH) activity of extract:

In an attempt to pursue active antioxidant activities of edible algae *Eucheuma spinosum,* this study had confirmed that *n*-hexane, ethyl acetate and H2O extracts were active against both antioxidant assay of DPPH and SOD, respectively. The test shows that *E. spinosum* extract can scavenging DPPH free radicals with increased concentration, especially on ethyl acetate extracts with repeated repetitions. However, water, ethyl acetate, and *n*-hexane extract of *E. spinosum* were active with inhibition zone values of 2,478.1, 402.8, and 1,537.1 ppm, respectively as shown in Table 3.

Based on the assay data against DPPH assay showed that the ethyl acetate extract is the most active to scavenger free radicals with IC50 value of 402.8 ppm, while if compared to references compounds of quercetin and chatechin with IC50 values of 20.98 and 91.82 ppm, respectively, the activity of ethyl acetate extract was less active (Li et al, 2019; Orhan et al, 2019). In the previous published paper, Damongilala *et al*., (2013) have reported that methanol extract from *E. spinosum* and *E. cottonii* can scavenge DPPH radicals with IC50 values of 75.27 and 64.73 ppm (Damongilala et al, 2013). According to phytochemical analysis, the secondary metabolites of flavonoid, steroid, terpenoids, saponin and tannin those contained in ethyl acetate extract to have linier correlation with its antioxidant activity. So, this initial data is as important finding for further guide to isolate their actives constituents (Garza et al, 2018; Siddiqui et al, 2018; Herbeoui et al, 2018).

3.4 Antioxidant (SOD) activity of extract:

Increased concentration in *E. spinosum* extract can capture free radicals, especially in ethyl acetic extracts. Increase in antioxidant activity from *E. spinosum* extracts of H2O, ethyl acetate, and *n*-hexane depending on the concentration of each. Inhibition zone values of *E. spinosum* extract are 155, 25, and 870 ppm, respectively as shown in Table 4.

Further antioxidant analysis against SOD assay give interesting data as shown Table 3 indicated that ethyl acetate extract also the most active compared to other extracts with IC50 value of 25 ppm, while the SOD activities references compounds of quercetin and catechin were 3.33 and 26.51 ppm, respectively (Jun et al, 2003; Gargouri et al, 2011; Kalender et al, 2005). In published paper, Wardani *et al*.,(2017) reported that methanol extract from algae of *E. cottonni* can scavenge SOD radicals with IC50 of 42.52 ppm, which indicated that ethyl acetate extract of *E. spinosum* was more active than methanol extract of *E. cottonni* against SOD assay (Wardani et al, 2017). The other DPPH and SOD assay data was reported that *Pinus maritima* extract, commercial pine, *Quercus robur*, *Cinnamomum zeylanicum*, and *Ilex paraguariensis* can scaveng radical DPPH with values of 94.51, 92.79, 88.60, 84.43, and 71.75%, respectively. The extracts can also scavenger SOD radicals with values of 60.32, 53.48, 81.20, 51.79, and 52.44% (Dudonne et al, 2009). The data indicate that the extract is active in scavenging DPPH radicals, will also be active to scavenger SOD radicals.

Ethyl acetate extract has a lot of phenol because there are tannins, flavonoids, triterpenoids and steroids. Tannin group with relatively high molecular weight, has been shown to be effective in having greater antioxidant activity than simple phenol (Guzman et al, 2007). The hydroxyl phenol group is reported to play a major role in antioxidant activity especially flavonoids (Zhao et al, 2019; Masaek et al, 2017; Nenadis et al, 2005). The combination of phenolic compounds and saponins (crude

extract) has antioxidant activity that is higher than saponins isolated from the same source which shows that the presence of other molecules increases the antioxidant activity of the extract (Garza et al, 2018). Steroid compounds show the highest rinsing activity in the generation of intracellular ROS (Siddiqui et al, 2018), triterpenoid content detected in Merlot and Syrah has antioxidant activity (Herbeoui et al, 2018).

DPPH is a stable free radical that is widely used to evaluate natural antioxidants, algae or algal products because of its stability, simplicity and reproducibility. Some antioxidants can react slowly or not react to DPPH (Balboa et al, 2013). DPPH as a stable free radical with reserve electron delocalisation contributes to intense violet color which is converted to pale yellow after reduction (Lim et al, 2015). SOD catalyzes the conversion of superoxide (SO) to hydrogen peroxide and oxygen, an important reaction in removing SO in cells. SO is very reactive and can cause cell damage, with excessive amounts trigger reactions that cause damage to important biological macromolecules such as DNA, lipids and proteins (Retroningrum et al, 2016; Adzamanesh and Borgstahl, 2018).

Antioxidants based on their mechanism of action are classified, above: primary and secondary antioxidants. Primary antioxidant is as the main antioxidant giving hydrogen atom (AH), because this compound gives hydrogen atoms rapidly to radical compounds, wherein the radicals formed produce lipid derivatives and antioxidant radicals (A*). It is role as a hydrogen atom donor to fat free radicals to reshape fat molecules. Thus if the antioxidant is given to prevent new radical formation, it will inhibit the process of autoxidation.

3.5 Isolation and structure determination of antioxidant compound 1 from the EtOAc of *Eucheuma spinosum*

Active antioxidant extract of ethyl acetate fraction was selected for further separation active compound. Ethyl acetate extract (1.1 g) was chromatographed on Silica G 60 eluted with *n*-hexane-ethyl acetate (5% step wise) to give two active fractions of **I** or compound **1** (165 mg) and **II** (178.8 mg). Purification of fraction I by chromatographed on ODS RP-18 eluted with H_2O-MeOH (5% step wise) give active compound **2** (5.7 mg).

Compound **1** was isolated as brown solid those soluble in methanol. The ^{13}C-NMR spectrum showed ten carbon signals including for one carbonyl carbon at δ_C 192.7 ppm and six olefinic carbons at 156.7, 133.5, 133.2, 130.3, 117.1 and 116.2 ppm, respectively. Other signals were identified for two methylene carbon at δ_C 30.1 and 46.3, together with one methoxy carbon at δ_C 56.4 ppm. Correlated to carbons data was found from ^{1}H-NMR spectrum those indicated four aromatics and one aldehyde protons (δ_H 9.69), respectively. From the *J* value of 8.4 ppm, two protons at δ_H 7.76 (H-5') and 7.70 (H-6') were determined in *orto* position, while two other protons at δ_H 6.68 (H-4') and 6.90 (H-2') ppm were in *meta* positions (*J* = 9.1 ppm). From the HMBC spectrum, the methoxyl group was attached at C -3' by correlations peaks between methoxyl protons to carbon C-3', while one of two methylene groups H_2-2 and H_2-3 were continuously attached to olefin carbon C-1' side and another was attached to olefinic carbon C-1 of aldehyde functional group. Based on the analysis of 1D and 2D-NMR data, the structure of compound **1** was suggested as 3-(3-methoxyphenyl) propanal, as shown in figure 1. Confirmation the structure **1** with reported in the published papers was meet according to synthetic compounds of same compound from 3 -methoxycinamic by Miller M.R (2000) and also by Zhu and Hong at 2013 (Miller et al, 2000; Zu and Hong, 2013). Base on the references in the

reported data, the active compound **1** was isolated from tropical alga *Eucheuma spinosum* is reported for the first time in this report.

C 192.8 (C1), 46.3 (C2), 30.1 (C3), 133.2 (C1'), 117.1 (C2'), 156.7 (C3'), 116.2 (C4'), 133.5 (C5'), 130.3 (C6'), 56.4 (OMe-3'); **H** 9.69 (1H, s, H1), 2.74 (2H, t, *J* = 3.25 & 1.3, H2), 2.10 (2H, s, H3), 6.90 (1H, d, 9.1, H2'), 6.68 (1H, d, *J* = 8.45, H4'), 7.76 (1H, d, 8.4, H5'), 7.00 (1H, d, 8.4, H6'), 3.90 (2H, s, -OMe).

3.6 Antioxidant (DPPH) activity of compound 1:

The antioxidant activity of compound **1** (3-(3-methoxyphenyl) propanal) was assed against DPPH in order to identify bioactive constituents as antioxidant in algae of *Eucheuma spinosum.* The data showed that compound **1** inhibited DPPH free radicals with inhibition value of 87.97 ppm. If compared to the IC_{50} of reference compounds, compound **1** was as active as BHT with IC_{50} of 84.15 ± 3.82 µg/ml, but less active than vitamin C with IC_{50} of 21µg/ml, respectively (Lentario et al, 2008; Tamat et al, 2007).

Natural products are the source of therapeutically viable antibacterial agents. Higher plants synthesize diverse bioactive compounds that act as antifungal and antibacterial agents. This study presents preliminary data of antibacterial activity of some edible vegetables and fruits selected based on the fact that they are consumed in daily diet. Two basic classifications are applied to antioxidants: synthetic and natural. Generally, synthetic antioxidants are phenolic compounds containing various alkyl substitution rate, while natural antioxidants can be phenolic compounds such as quinones and lactones (Cruz et al, 2019).

Various antioxidant food have been classified into different categories based on the structure and chemical function: bioactive soluble in water include citrate, norbixin, betalains, most phenolics, flavonoids and anthocyanins, and components that are soluble in fat such as carotenoids, tocopherols, terpenoids and vitamin E (Ozkan et al, 2019). Foods that are rich in bioactive compounds play an important role in the prevention and treatment of chronic gastrointestinal (GI) diseases (Chen et al, 2019). Functional food has a complex matrix and the composition of its bioactive compounds requires careful assessment because potential risks can arise from the isolated material. It is known that plants produce and accumulate a variety of typical chemical compounds that usually have low concentrations (Passari et al, 2019). *E. spinosum* that are rich in bioactive compounds can be used as functional foods because they are in accordance with the definition of functional foods that can prevent or treat chronic diseases.

4. Conclusion

The red alga of *E. spinosum* suggested as potential natural antioxidant source those active to scavenge DPPH and SOD radicals can be used as an alternatives natural antioxidant from edible marine functional food of natural products for preventing or treating some human metabolic disease, such as diabetes, Alzheimer's disease and stroke (Bashar et al, 2019).

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Table 1.
Data of weight extracts of samples 1

Samples	Extracts Weight (g)			
	MeOH	*n*-Hexane	EtOAc	H_2O
Sample (4.1 kg)	12.9	0.0647	0,4969	8.5265

Table 2
Data of phytochemical analysis the *E. spinosum* extract extracts

No.	Secondary metabolites	Reagent	Samples			
			MeOH	*n*-Hexane	EtOAc	H_2O
1	Flavonoid	a. HCl + Mg	-	-	-	-
		b. H_2SO_4 (2N)	-	-	-	-
		c. NaOH (10%)	-	-	+	-
2	Alkaloid	Pereaksi Dragendorf	-	+	-	-
3	Steroid	Lieberman-	+	-	+	-
4	Triterpenoid	Burchard	+	-	+	-
5	Saponin	HCl + H_2O	-	-	+	-
6	Tanin	$FeCl_3$ (1%)	+	+	+	+

Table3
Antioxidnat (DPPH) activity of the *E. spinosum* extract

No.	Samples	Inhibition Activity (IC_{50} /ppm)			
		n-Hexane	EtOAc	H_2O	MeOH
1	Extracts	1,537.1	402.8	2,478.1	-
2	3-(3-methoxyphenyl)propanal	-	87,97	-	-
3	Quercetin	-	-	-	20.98
4	Catechin	-	91.82	-	-

Table 4
Antioxidnat (SOD) activity of the *E. spinosum* extract

No.	Samples	Inhibition Activity (IC_{50} / ppm)			
		n-Hexane	EtOAc	H_2O	MeOH
1	*E. spinosum* extract	870	25	155	160
2	Quercetin	-	-	-	5
3	Catechin	-	-	-	13

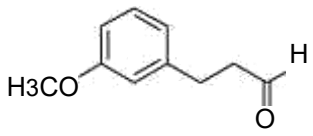

Figure 1 Structure of compound 1 [3-(3-methoxyphenyl) propanal

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