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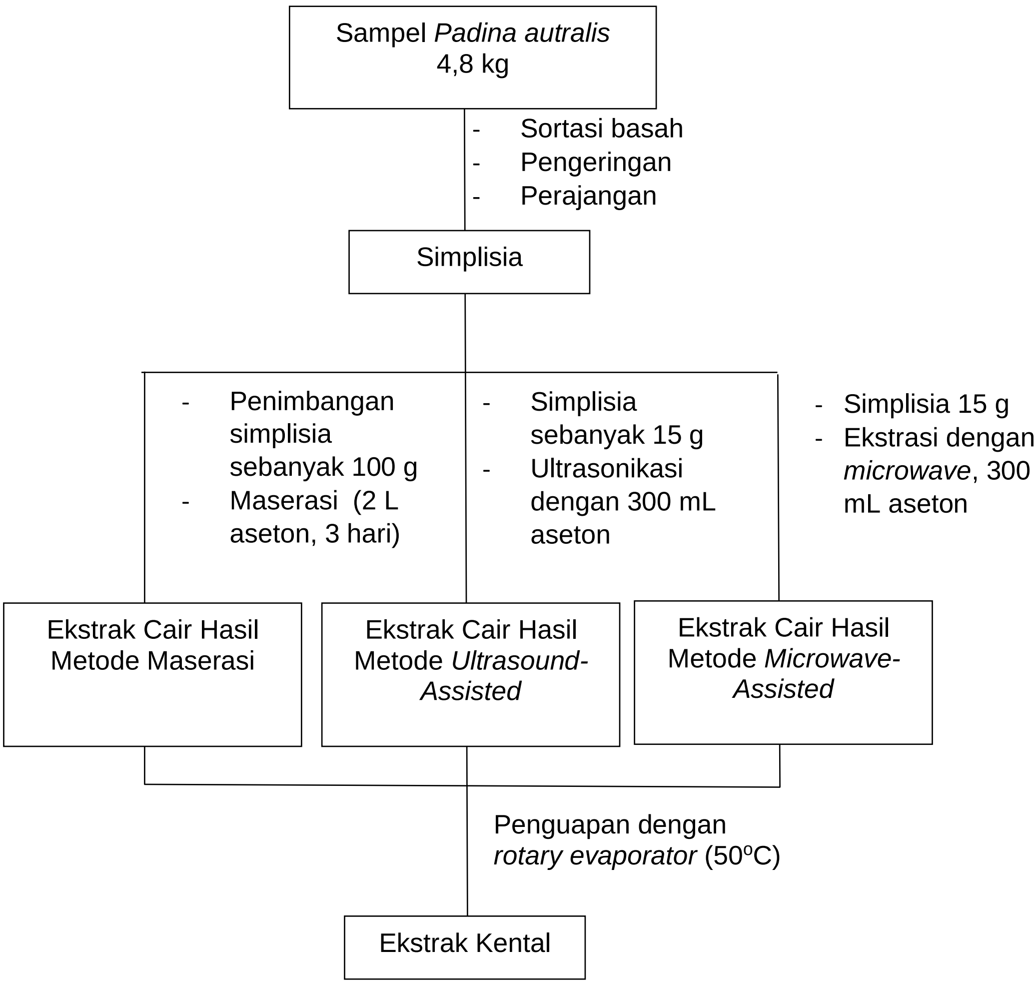
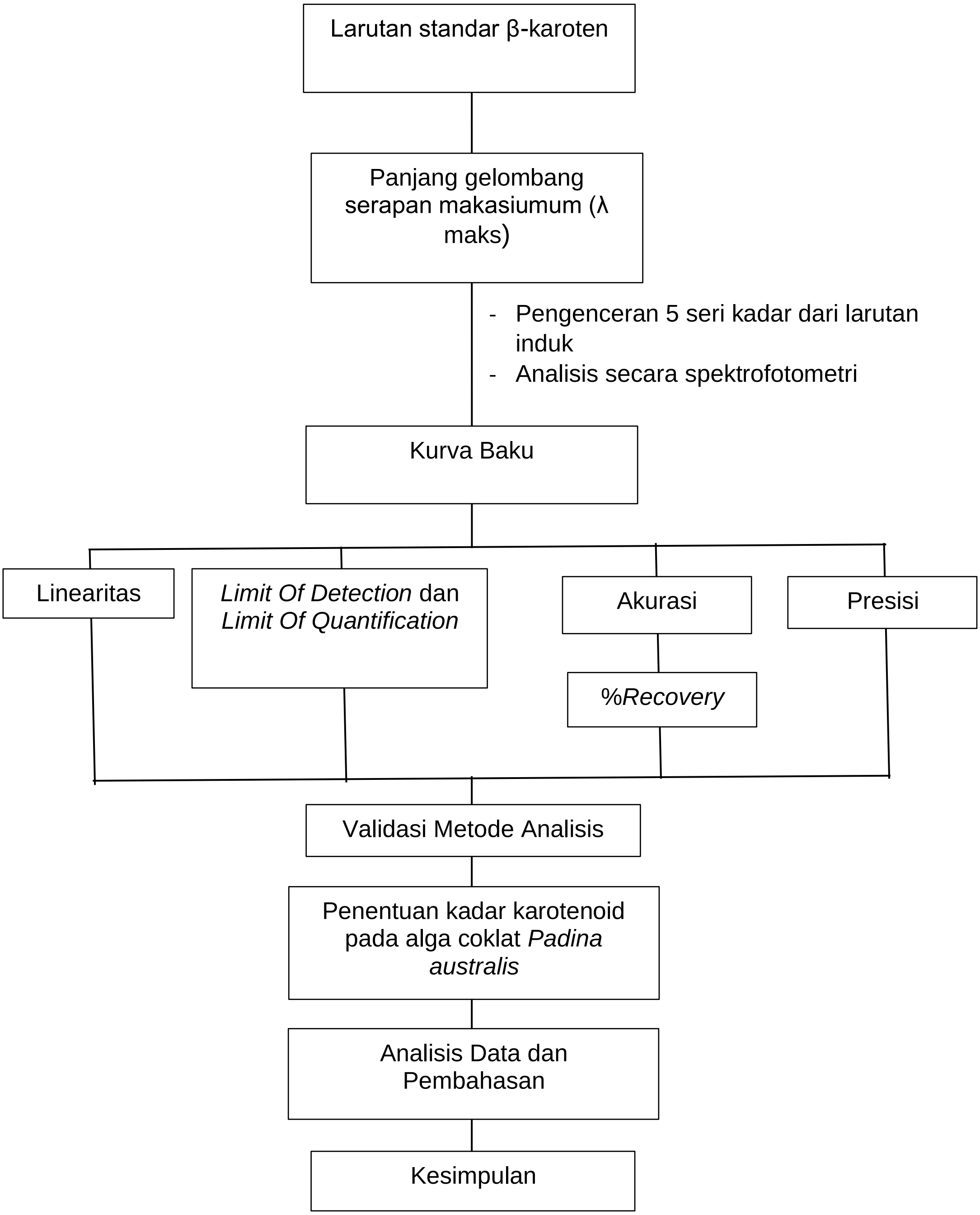
LAMPIRAN

Lampiran 1. Skema Kerja

Lampiran 1.1 Ekstraksi *Padina australis*



Lampiran 1.2 Validasi Metode Analisis *Padina australis* dengan Spektrofotometer UV-Vis



Lampiran 2. Perhitungan

Lampiran 2.1 Perhitungan Validasi Metode Analisis

Lampiran 2.1.1 Linearitas

Larutan baku β-karoten 100 ppm dibuat dengan melarutkan 1 mg baku β-karoten dengan 10 ml metanol p.a dan diencerkan menjadi konsentrasi 1, 2, 3, 4, dan 5 ppm. Lalu dilakukan pengukuran absorbansi sehingga dihasilkan data sebagai berikut:

Konsentrasi Baku (μg/mL)	Absorbansi
1	0,145
2	0,155
3	0,168
4	0,177
5	0,188

Setelah itu dilakukan pemplotan data sehingga diperoleh persamaan sebagai berikut:

$$y = 0{,}0108x + 0{,}1342, \text{ dengan nilai } r^2 = 0{,}997$$

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.998802
R Square	0.997605
Adjusted R	0.996807
Standard E	0.000966
Observatio	5

ANOVA

	df	*SS*	*MS*	*F*	*ignificance F*
Regression	1	0.001166	0.001166	1249.714	4.98E-05
Residual	3	2.8E-06	9.33E-07		
Total	4	0.001169			

	Coefficients	*andard Erro*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*ower 95.0%*	*Jpper 95.0%*
Intercept	0.1342	0.001013	132.4457	9.49E-07	0.130975	0.137425	0.130975	0.137425
X Variable :	0.0108	0.000306	35.3513	4.98E-05	0.009828	0.011772	0.009828	0.011772

Lampiran 2.1.2 Batas Deteksi (LOD) dan Batas Kuantifikasi (LOQ)

Konsentrasi Baku (µg/mL)	Absorbansi	x	y’	y-y’	(y-y’)^2
1	0,145	1,000	0,145	0,0000	0,0000
2	0,155	1,926	0,156	-0,0008	0,0000
3	0,168	3,130	0,167	0,0014	0,0000
4	0,177	3,963	0,177	-0,0004	0,0000
5	0,188	4,981	0,188	-0,0002	0,0000
Jumlah					0,0000

Rumus :

$$LOD = \frac{3{,}3 \times S_{y/x}}{b} \text{ ; } LOQ = \frac{10 \times S_{y/x}}{b}$$

$$S_{y/x} = \sqrt[2]{\frac{\sum(y-y')^2}{n-2}} = \sqrt[2]{\frac{0{,}0000}{5-2}} = \sqrt[2]{\frac{0{,}0000028}{3}} = 0{,}0009$$

$$LOD = \frac{3{,}3 \times 0{,}0009}{0{,}0108} = 0{,}2952 \ \mu g/mL \qquad LOQ = \frac{10 \times 0{,}0009}{0{,}0108} = = 0{,}8945 \ \mu g/mL$$

Lampiran 2.1.3 Akurasi

Konsentrasi (ppm)	Replikasi	Absorbansi	Konsentrasi Terukur (µg/mL)	%Recovery	Rata-Rata
1	1	0,146	1,093	109,26	106,17
	2	0,145	1,000	100,00	
	3	0,146	1,093	109,26	
3	1	0,168	3,130	104,32	107,41
	2	0,169	3,222	107,41	
	3	0,170	3,315	110,49	
5	1	0,186	4,796	95,3	97,78
	2	0,187	4,889	97,8	
	3	0,188	4,981	99,63	

Perhitungan Konsentrasi Terukur

Persamaan regresi linear: y = 0,0108x + 0,1342

$$x = \frac{y-0{,}1342}{0{,}0108}$$

Konsentrasi Baku β-karoten 1 ppm

$$X_1 = \frac{0{,}146-0{,}1342}{0{,}0108} = 1{,}093 \ \mu g/mL$$

$$X_2 = \frac{0{,}145-0{,}1342}{0{,}0108} = 1{,}000 \ \mu g/mL$$

$$X_3 = \frac{0{,}146-0{,}1342}{0{,}0108} = 1{,}093 \ \mu g/mL$$

Konsentrasi Baku β-karoten 3 ppm

$X_1 = \frac{0,168-0,1342}{0,0108} = 3,130$ μg/mL

$X_2 = \frac{0,169-0,1342}{0,0108} = 3,222$ μg/mL

$X_3 = \frac{0,170-0,1342}{0,0108} = 3,315$ μg/mL

Konsentrasi Baku β-karoten 5 ppm

$X_1 = \frac{0,186-0,1342}{0,0108} = 4,796$ μg/mL

$X_2 = \frac{0,187-0,1342}{0,0108} = 4,889$ μg/mL

$X_3 = \frac{0,188-0,1342}{0,0108} = 4,981$ μg/mL

Perhitungan Persen Perolehan Kembali (*%Recovery)*

Rumus: $\frac{\text{Konsentrasi Terukur}}{\text{Konseentrasi Teoritis}} \times 100\%$

%Recovery Baku β-karoten konsentrasi 1 ppm

%Recovery $x_1 = \frac{1,093}{1,00} \times 100\% = 109,26\%$

%Recovery $x_2 = \frac{1,000}{1,00} \times 100\% = 100,00\%$

%Recovery $x_3 = \frac{1,093}{1,00} \times 100\% = 109,26\%$

Rata-Rata %*Recovery* baku 1 ppm = $\frac{109,26+100,00+109,26}{3}$=106,17%

%Recovery Baku β-karoten konsentrasi 3 ppm

%Recovery $x_1 = \frac{3,130}{3,00} \times 100\% = 104,32\%$

%Recovery $x_2 = \frac{3,222}{3,00} \times 100\% = 107,41\%$

%Recovery $x_3 = \frac{3,315}{3,00} \times 100\% = 110,49\%$

Rata-Rata %*Recovery* baku 3 ppm = $\frac{104,32+107,41+110,49}{3}$=107,41%

%Recovery Baku β-karoten konsentrasi 5 ppm

%Recovery $x_1 = \frac{4,796}{5,00} \times 100\% = 95,93\%$

%Recovery $x_2 = \frac{4,889}{5,00} \times 100\% = 97,78\%$

%Recovery $x_3 = \frac{4,981}{5,00} \times 100\% = 99,63\%$

Rata-Rata %*Recovery* baku 5 ppm = $\frac{95,93+97,78+99,63}{3}$ =97,78%

Lampiran 2.1.4 Presisi

Konsentrasi	Replikasi	Absorbansi
5 µg/mL	1	0,152
	2	0,153
	3	0,155
	4	0,154
	5	0,153
	6	0,154
Rata-Rata		0,154
SD		0,001
RSD		0,683

Rata-rata absorbansi = $\frac{0,152+0,153+0,155+0,154+0,153+0,154}{6}$ =0,154

SD = $\sqrt[2]{\frac{\sum(y-y')^2}{n-1}}$ =

$\sqrt[2]{\frac{(0,152-0,154)^2+(0,153-0,154)^2+(0,155-0,154)^2+(0,154-0,154)^2+(0,153-0,154)^2+(0,154-0,154)^2}{6-1}}$

= 0,001

RSD = $\frac{SD}{X}$ ×100% = $\frac{0,001}{0,154}$ ×100% = 0,683%

Lampiran 2.2 Perhitungan Hasil Ekstraksi dan Penetapan Kadar Karotenoid

Perhitungan Persen Rendemen

Metode Ekstraksi	Bobot Simplisia (g)	Bobot Ekstrak (g)	%Rendemen
Maserasi	100	2,74	2,74
UAE	15	0,29	1,93
MAE	15	0,26	1,73

Rumus:

$$\%\text{Rendemen} = \frac{\text{Bobot ekstrak}}{\text{Bobot simplisia}} \times 100\%$$

%Rendemen metode maserasi = $\frac{2{,}74\ \text{g}}{2{,}74\ \text{g}} \times 100\% = 2{,}74\%$

%Rendemen metode UAE = $\frac{0{,}29\ \text{g}}{15\ \text{g}} \times 100\% = 1{,}93\%$

%Rendemen metode MAE = $\frac{0{,}26\ \text{g}}{15\ \text{g}} \times 100\% = 1{,}73\%$

Perhitungan Hasil Penetapan Kadar

Metode Ekstraksi	Absorbansi	Konsentrasi (µg/mL)	Kadar Karotenoid (mg/g)	Rata-Rata ± SD
MAE	0,152	1,648	2,060	1,983±0,067
	0,151	1,556	1,944	
	0,151	1,556	1,944	
UAE	0,161	2,481	12,407	12,562±0,267
	0,162	2,574	12,870	
	0,161	2,481	12,407	
Maserasi	0,183	4,519	1,807	1,844±0,037
	0,184	4,611	1,844	
	0,185	4,704	1,881	

Persamaan regresi linear: y = 0,0108x + 0,1342

$x = \frac{y-0,1342}{0,0108}$

Konsentrasi Sampel Hasil Maserasi

$X_1 = \frac{0,183-0,1342}{0,0108} = 4,519\ \mu g/mL$

$X_2 = \frac{0,184-0,1342}{0,0108} = 4,611\ \mu g/mL$

$X_3 = \frac{0,185-0,1342}{0,0108} = 4,704\ \mu g/mL$

Rata-Rata Konsentrasi = $\frac{4,519+4,611+4,704}{3} = 4,611\ \mu g/mL$

Konsentrasi Sampel Hasil UAE

$X_1 = \frac{0,161-0,1342}{0,0108} = 2,481\ \mu g/mL$

$X_2 = \frac{0,162-0,1342}{0,0108} = 2,574\ \mu g/mL$

$X_3 = \frac{0,161-0,1342}{0,0108} = 2,481\ \mu g/mL$

Rata-Rata Konsentrasi = $\frac{2,481+2,574+2,481}{3} = 2,512\ \mu g/mL$

Konsentrasi Sampel Hasil MAE

$X_1 = \frac{0,152-0,1342}{0,0108} = 1,648\ \mu g/mL$

$X_2 = \frac{0,151-0,1342}{0,0108} = 1,556\ \mu g/mL$

$X_3 = \frac{0,151-0,1342}{0,0108} = 1,556\ \mu g/mL$

Rata-Rata Konsentrasi = $\frac{1,648+1,556+1,556}{3} = 1,586\ \mu g/mL$

Rumus perhitungan kadar:

$$\%\ \text{Kadar} = \frac{[\text{Sampel}] \times V_{(l)} \times Fp}{m_{sampel}}$$

Keterangan:

[Sampel] : Konsentrasi sampel (mg/L)

V : Volume sampel (L)

F_p : Faktor pengenceran

m_{sampel} : Bobot sampel (g)

Kadar Karotenoid Ekstrak Hasil Maserasi 1 = $\frac{4{,}519 \times 0{,}002 \times 1}{0{,}005}$ = 1,807 %

Kadar Karotenoid Ekstrak Hasil Maserasi 2 = $\frac{4{,}611 \times 0{,}002 \times 1}{0{,}005}$ = 1,844 %

Kadar Karotenoid Ekstrak Hasil Maserasi 3 = $\frac{4{,}714 \times 0{,}002 \times 1}{0{,}005}$ = 1,881 %

Rata-Rata Kadar Karotenoid Hasil Maserasi = 1,844 %

Kadar Karotenoid Ekstrak Hasil UAE 1 = $\frac{2{,}481 \times 0{,}002 \times 1}{0{,}0004}$ = 12,407 %

Kadar Karotenoid Ekstrak Hasil UAE 2 = $\frac{2{,}574 \times 0{,}002 \times 1}{0{,}0004}$ = 12,870 %

Kadar Karotenoid Ekstrak Hasil UAE 3 = $\frac{2{,}481 \times 0{,}002 \times 1}{0{,}0004}$ = 12,407 %

Rata-Rata Kadar Karotenoid Hasil UAE = 12,562 %

Kadar Karotenoid Ekstrak Hasil MAE 1 = $\frac{1{,}648 \times 0{,}01 \times 1}{0{,}008}$ = 2,060 %

Kadar Karotenoid Ekstrak Hasil MAE 2 = $\frac{1{,}556 \times 0{,}01 \times 1}{0{,}008}$ = 1,944 %

Kadar Karotenoid Ekstrak Hasil MAE 3 = $\frac{1{,}556 \times 0{,}01 \times 1}{0{,}008}$ = 1,944 %

Rata-Rata Kadar Karotenoid Hasil MAE = 1,983 %

Perhitungan nilai SD

$$SD = \sqrt[2]{\frac{\sum(y-y')^2}{n-1}}$$

$$SD\ Maserasi = \sqrt[2]{\frac{(1{,}807-1{,}844)^2+(1{,}844-1{,}844)^2+(1{,}881-1{,}844)^2}{2-1}} = 0{,}037$$

$$SD\ UAE = \sqrt[2]{\frac{(12{,}407-12{,}562)^2+(12{,}870-12{,}562)^2+(12{,}407-12{,}562)^2}{2-1}} = 0{,}267$$

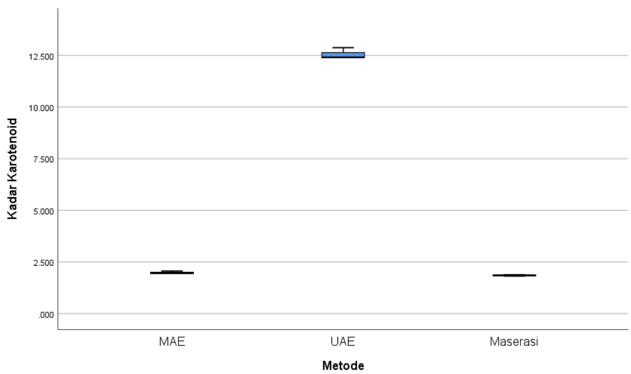
$$SD\ MAE = \sqrt[2]{\frac{(2{,}060-1{,}983)^2+(1{,}944-1{,}983)^2+(1{,}944-1{,}983)^2}{2-1}} = 0{,}067$$

Hasil Perbandingan Kadar Karotenoid Menggunakan Kruskal-Wallis Test

Tests of Normality

	Metode	Kolmogorov-Smirnov[a] Statistic	df	Sig.	Shapiro-Wilk Statistic	df	Sig.
Kadar Karotenoid	MAE	.385	3	.	.750	3	.000
	UAE	.385	3	.	.750	3	.000
	Maserasi	.175	3	.	1.000	3	1.000

a. Lilliefors Significance Correction



Kruskal-Wallis Test

Ranks

	Metode	N	Mean Rank
Kadar Karotenoid	MAE	3	5.00
	UAE	3	8.00
	Maserasi	3	2.00
	Total	9	

Test Statistics[a,b]

	Kadar Karotenoid
Kruskal-Wallis H	7.322
df	2
Asymp. Sig.	.026

a. Kruskal Wallis Test

b. Grouping Variable: Metode

Lampiran 3. Dokumentasi Penelitian

Gambar 9. alga Padina australis

Gambar 10. Proses pencucian

Gambar 11. Proses pengeringan

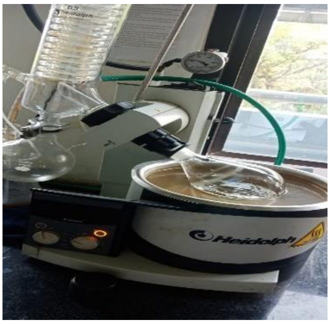
Gambar 12. Penyimpanan simplisia

Gambar 13. Penggerusan simplisia

Gambar 14. Proses maserasi

Gambar 15. Proses ekstraksi UAE

Gambar 16. Proses ekstraksi MAE



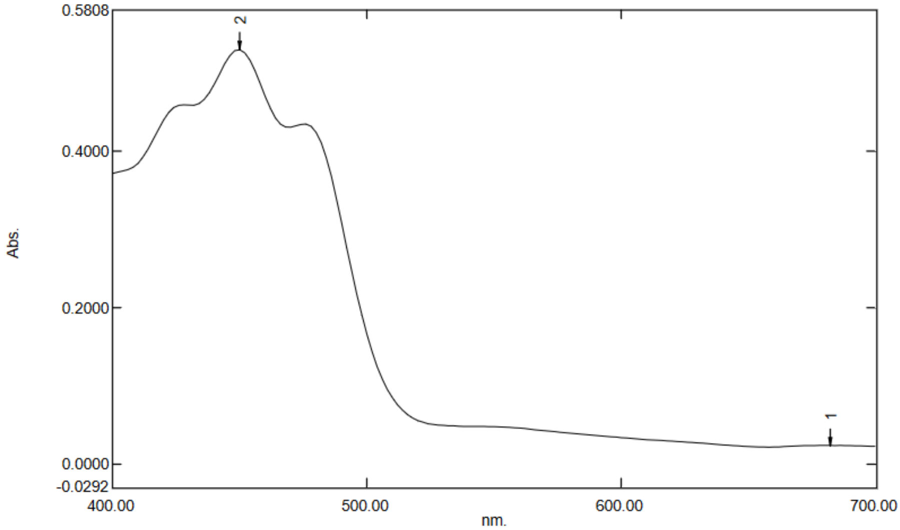
Gambar 17. Proses penguapan pelarut

Gambar 18. Proses penguapan pelarut di *waterbath*

Gambar 19. Proses penimbangan ekstrak kental

Lampiran 4. Spektrum Hasil Validasi Metode Analisis Karotenoid

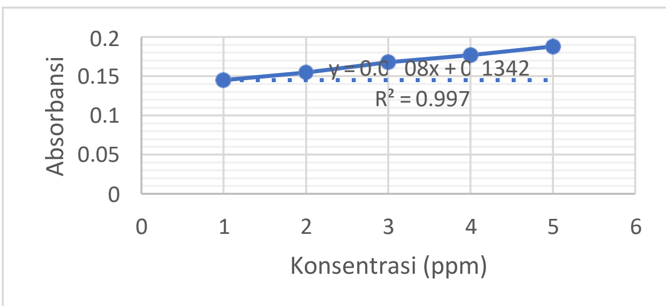
a. Panjang gelombang maksimum



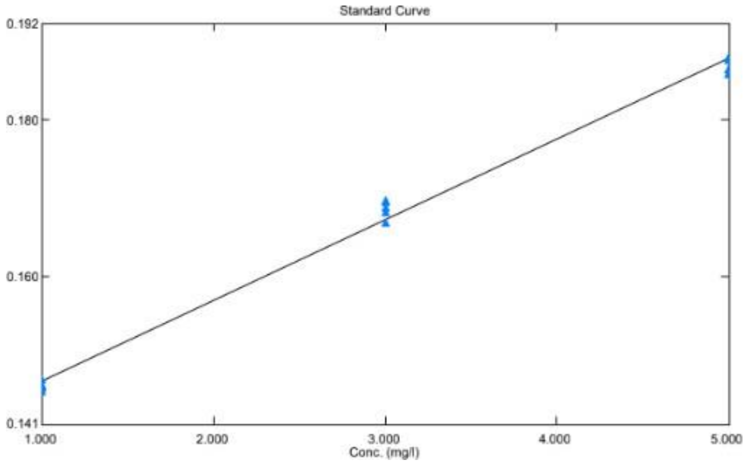
No.	P/V	Wavelength	Abs.	Description
1		682.00	0.0239	
2		450.00	0.5300	
3		658.00	0.0217	

b. Linearitas, LOD, dan LOQ

Sample ID	Type	Ex	Conc	WL556.0	Wgt.Factor
baku11	Standard		1.000	0.145	1.000
beta213	Standard		2.000	0.155	1.000
beta31	Standard		3.000	0.168	1.000
beta45	Standard		4.000	0.177	1.000
beta51	Standard		5.000	0.188	1.000



a. Akurasi



Standard Table

	Sample ID	Type	Ex	Conc	WL450.0	Wgt.Factor	Comments
1	1ppm	Standard		1.000	0.146	1.000	
2	1ppm1	Standard		1.000	0.146	1.000	
3	1ppm2	Standard		1.000	0.147	1.000	
4	1ppm3	Standard		1.000	0.146	1.000	
5	1ppm4	Standard		1.000	0.146	1.000	
6	1ppm5	Standard		1.000	0.145	1.000	
7	2ppm1	Standard		3.000	0.168	1.000	
8	2ppm2	Standard		3.000	0.169	1.000	
9	2ppm3	Standard		3.000	0.170	1.000	
10	2ppm4	Standard		3.000	0.170	1.000	
11	2ppm5	Standard		3.000	0.167	1.000	
12	5ppm1	Standard		5.000	0.186	1.000	
13	5ppm2	Standard		5.000	0.187	1.000	
14	5ppm3	Standard		5.000	0.188	1.000	
15	5ppm4	Standard		5.000	0.188	1.000	
16							

b. Presisi

Sample ID	Type	Ex	Conc	WL450.0	Wgt.Factor
5ppm3	Standard		5.000	0.153	1.000
5ppm4	Standard		5.000	0.153	1.000
5ppm5	Standard		5.000	0.154	1.000
5ppm6	Standard		5.000	0.153	1.000
5ppm7	Standard		5.000	0.154	1.000
5ppm8	Standard		5.000	0.155	1.000

Lampiran 5. Surat Determinasi Sampel

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DEPARTEMEN BIOLOGI
FAKULTAS MATEMATIKA DAN ILMU PENGETAHUAN ALAM
UNIVERSITAS HASANUDDIN, KAMPUS TAMALANREA
JL. PERINTIS KEMERDEKAAN KM.10, MAKASSAR

No	: 094/ILK.BIO/PP.13/12/2023
Hal	: Identifikasi Algae
Lamp	: 1 Lembar

SURAT KETERANGAN

Yang bertanda tangan dibawah ini, menerangkan bahwa setelah mengkaji karakter sampel ganggang algae dan identifikasi maka terdapat spesies yakni *Padina australis* Hauck, 1887.

Sampel	: Terima tanggal 29 November 2023
Kondisi sampel	: Segar, tidak ada holdfast

Genus	: *Padina*
Jenis	: *Padina australis* Hauck, 1887
Diskripsio	: Thallus terdiri dari beberapa helaian bentuk kipas/filament dengan diameter 3-4 cm dengan lingkaran-lingkaran konsentris, thallus berupa lembaran tipis bersegmen-segmen dengan garis-garis yang cenderung melingkar berwarna coklat. Pinggiran talus cenderung melengkung ke dalam. Thallus berwarna cokelat muda kehijauan, cokelat kekuning-kuningan atau kadang keputih-putihan akibat pengapuran. Ukuran filament ini sedikit lebih besar dibandingkan jenis lain dari *Padina*. Tepi luar filament menebal dan permukaan atas filament mempunyai garis konsentris warna putih.

Makassar, 01 Desember 2023
Kepala Laboratorium ILK,

Dr. Ir. Slamet Santosa, M.Si.
NIP. 19620726 198702 1 001

Tembusan :
1. Arsip

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Lampiran

Gambar 1. *Padina australis* Hauck, 1887