Gracilaria

Algae uses

introduction

الجامعة الإسلامية العالمية ماليزيا
INTERNATIONAL ISLAMIC UNIVERSITY MALAYSIA
يونيورسيتي إسلام انتارابغسا ملايسيا
Garden of Knowledge and Virtue

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UNIVERSITAS ISLAM INDONESIA

Marine Algae: Chemistry and Potential

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Introduction

- There are three main macroalgae taxa according to their morphological pigmentations: *Rhodophyta* (red algae), *Chlorophyta* (green algae), and *Phaeophyceae* (brown algae)

- Macroalgae are valuable marine plants that have garnered much attention from the public due to their high bioactive, nutrients and minerals content.

- Environmental factors, such as temperature, salinity, sunlight, pH, physiological status and CO_2 supply could influence the chemical composition of marine algae

Metabolites in macroalgae:

Bioactive components/metabolites	Example	Remarks
Polysaccharides	agar, alginates, galactans, carrageenans, laminarans, fucoidan and ulvans	32% to 50% of dry matter 51-56% of total fibers in green (ulvan) and red algae (agars, carrageenans and xylans), 67-87% in brown algae (laminaria, fucus, etc)
Lipids, fatty acids and sterols	phosphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, phoshatidic acid, fatty acids, oxylipins, sterols	1-5% of dry matter Green algae have much higher oleic and alpha-linoleic acid content Red algae have a high EPA content
Protein, amino acids		Brown algae: 5-11% Red algae: 30-40% of dry matter Green algae: 20% of dry matter
Phenolic compounds	Phloroglucinols, phlorptannins	
Vitamins and minerals		Red and brown algae (provitamin A): 20-170 ppm Red and brown algae (vitamin C): 500-3000 ppm. Vitamin B12

Macroalgae uses:

Food/Nutraceutical	Medicinal	Cosmetic
Hydrocolloids, ashydrocolloids or phycocolloids, as food additive (gelling, water retention, emulsifying agents)	Alginates are used in wound dressings, and production of dental moulds	Photo-protective: sargachromenol, fucoxanthin, tetraprenyltoluquinol chromane meroterpenoid, scytonemin, sargaquinoic acid
Agar is used in foods such as confectionery, meat and poultry products, desserts and beverages and moulded foods	Hormones: Melatonin and thyroid	Whitening effect: eckol, dieckol, diphlorethohydroxycarmalol, dioxynodehydroeckol, fucoxanthin, phloroglucinol
Carrageenan is used in salad dressings and sauces, dietetic foods, and as a preservative in meat and fish products, dairy items and baked goods	Red algae containing carrageenan have been used for millennia as treatments for respiratory ailments, especially intractable sinus infections and lingering pneumonias	
 	Antiobesity: fucoxanthin, alginates, fucoidans and phlorotannins Antiinflammatory: terpenoids, protein, peptides, amino acids, fatty acids etc. Anti-herpes simplex virus: carrageenan, fucan, sulphated polysaccharide Neuroprotective: fucoxanthin, fucosterol, fucoidan, laminarin, porphyran, saringasterol, phlorotannins Immune boosting: fucoidan	

Gracilaria changii

(Port Dickson, Malaysia)

Sampling & Drying method

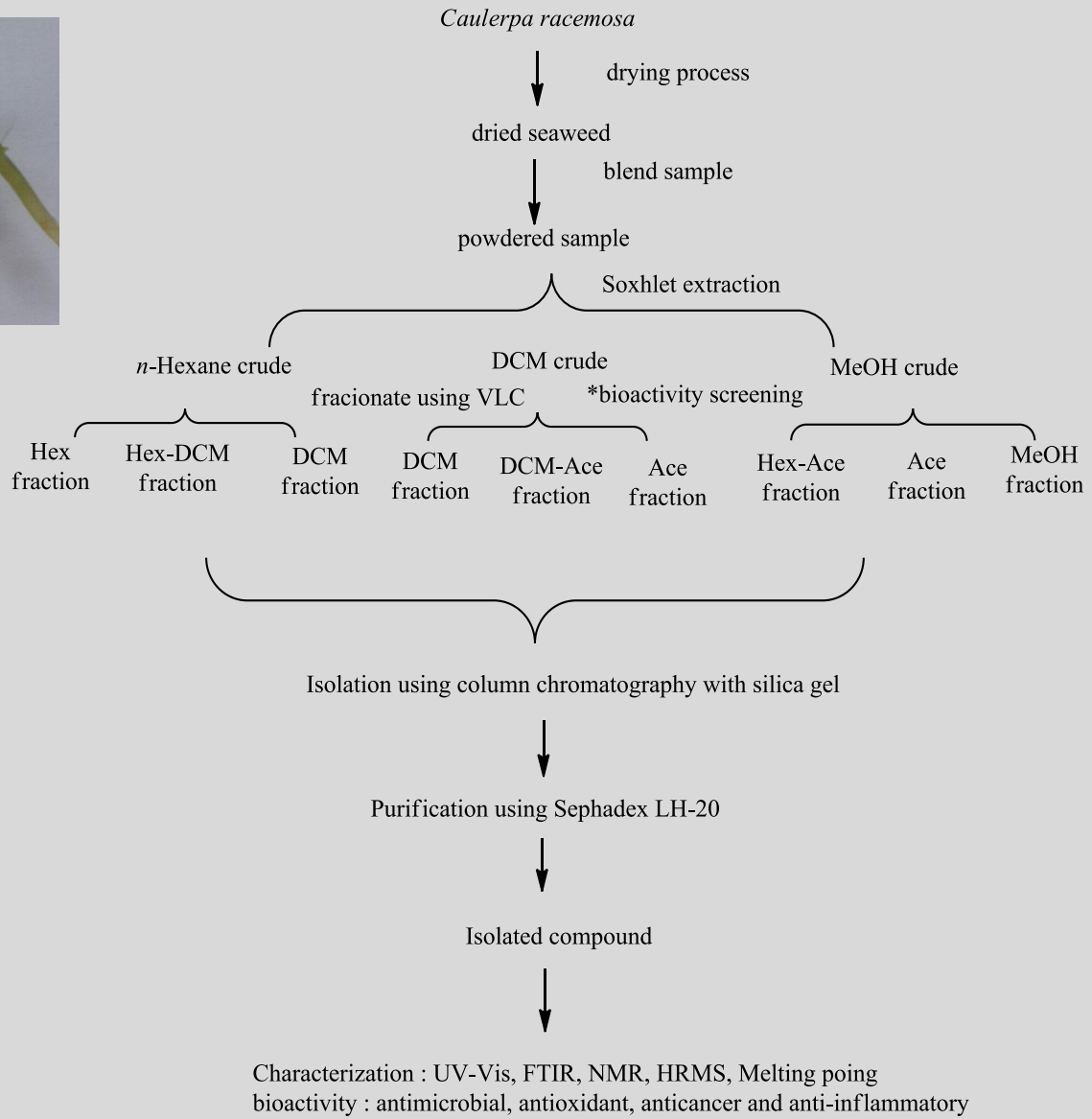
- Sun-drying
- Oven-drying
- Freeze-drying
- Air-drying

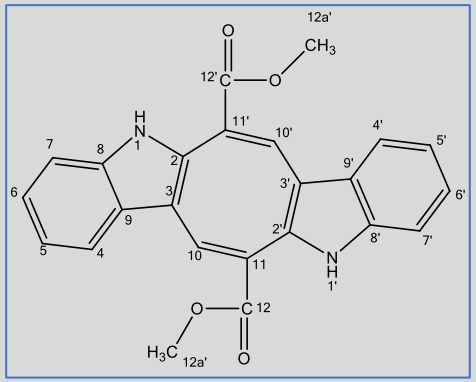
Agar extraction method

- Determination of agar yield
- Determination of gelling temperature
- Determination of melting temperature

Drying Methods	Agar yield (%)	Gelling Temperature (℃)	Melting Temperature (℃)
Oven-drying	10.09±1.44	36.67±0.58	66±1.00
Freeze-drying	10.03±1.13	38.33±0.58	78.67±0.58
Air-drying	13.04±1.36	38.00±1.00	63.00±5.20
Sun-drying	12.11±0.59	39.67±1.15	71.00±1.00

Caulerpa racemosa





Caulerpin

Cytotoxicity

Cell lines	IC_{50}
H1299 (lung cancer)	61.43%
A549 (lung cancer)	50%
MCF-7 (breast cancer)	63.43%

Anti-inflammatory

Nitric oxide production induced by LPS on the RAW 264.7 RAW 264.7 macrophage cells : moderately reduced from 32.71 µM to 23.28

C. lentilifera

Different drying methods will affect the nutrition content in *C. lentillifera*

Drying methods:

- Air-drying
- Sun-drying

Nutrient content:

- Moisture content
- Ash content
- Crude fats content
- Crude fibre content
- Crude protein content
-Crude carbohydrate content

Proximate composition	Sun-dried (%)±SD	Air-dried (%)±SD
Moisture	96.23	95.72
Crude ash	51.57 ± 1.19[a]	57.76 ± 1.26[b]
Crude fat	0.42 ± 0.09[a]	1.25 ± 0.22[b]
Crude protein	6.61 ± 0.21[a]	7.14 ± 0.09[b]
Crude fibre	10.86 ± 1.53[a]	8.88 ± 0.81[a]
Carbohydrate	30.54	24.97

Sargassum olygocystum

S. oligocystum

↓

Dried

↓

Grounded to desired size (125, 500, 1000 µm)

↓

Subjected to different extraction method (either maceration or Soxhlet extraction)

With 3 solvents (methanol, acetone, ethanol)

↓

Vacuum filtered

↓

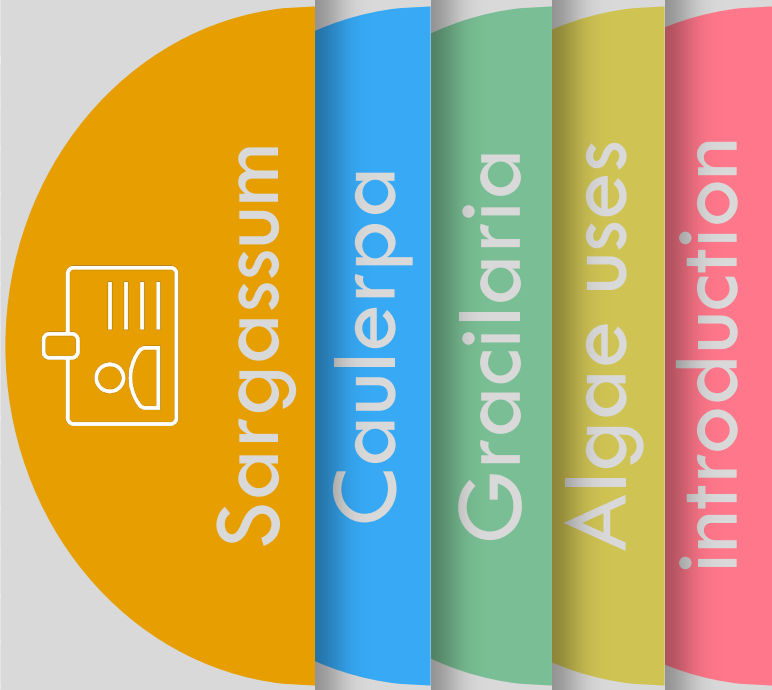
Evaporated, T = 35- 40 °C

↓

HPLC analysis

Sargassum olygocystum

Experiment condition	Solvent	Seaweed passed through sieve size/um	Extraction Method	Yield of extract (mg/g)	Fx content in the extract (mg/ml)	Fx content (mg/g dry weight)
1	Methanol	125	Maceration	9.975 ± 16.6	2.389 ± 1.73	23.84
2	Methanol	125	Soxhlet extraction	26.79 ± 29.1	8.979 ± 7.00	240.5
3	Methanol	500	Maceration	20.73 ± 10.0	4.525 ± 6.59	93.92
4	Methanol	500	Soxhlet extraction	22.13 ± 7.02	4.896 ± 4.08	108.4
5	Methanol	1000	Maceration	24.33 ± 21.9	7.844 ± 0.65	190.8
6	Methanol	1000	Soxhlet extraction	28.73 ± 37.5	12.56 ± 6.27	359.2
7	Acetone	125	Maceration	22.03 ± 26.7	3.120 ± 2.67	68.28
8	Acetone	125	Soxhlet extraction	25.07 ± 15.5	7.084 ± 0.67	177.5
9	Acetone	500	Maceration	14.65 ± 20.0	6.829 ± 11.6	100.0
10	Acetone	500	Soxhlet extraction	34.00 ± 43.4	22.13 ± 11.9	754.8
11	Acetone	1000	Maceration	17.21 ± 19.2	4.625 ± 7.58	79.70
12	Acetone	1000	Soxhlet extraction	34.80 ± 3.61	7.095 ± 6.40	247.1
13	Ethanol	125	Maceration	24.60 ± 24.9	6.599 ± 1.07	162.4
14	Ethanol	125	Soxhlet extraction	41.13 ± 10.6	0.259 ± 0.22	10.70
15	Ethanol	500	Maceration	7.239 ± 4.55	12.52 ± 5.21	90.49
16	Ethanol	500	Soxhlet extraction	26.47 ± 27.6	2.047 ± 2.89	54.26
17	Ethanol	1000	Maceration	14.40 ± 15.4	11.77 ± 2.96	170.0
18	Ethanol	1000	Soxhlet extraction	30.53 ± 37.2	3.298 ± 4.06	100.8



Ulva lactuca - Ulvan

9 to 36%: sulphated rhamnose, uronic acids and xylose by α,β-(1,4) bonds

Two types: aldobiuronic acids and ulvanobioses

Ability to form gel: stabilisers, emulsifiers, tickeners

Moisturizing, protective, antioxidative and antitumour properties

Sample Collection → **Cleaning** → **Drying**
- Air-Drying
- Sun-drying

↓

Extraction
- Acid Extraction with different pH

→ **Sample Preparation for Analysis** → **Characterisation**
- FTIR Analysis
- ^{1}H NMR Analysis

Ulva

Sargassum

Caulerpa

Gracilaria

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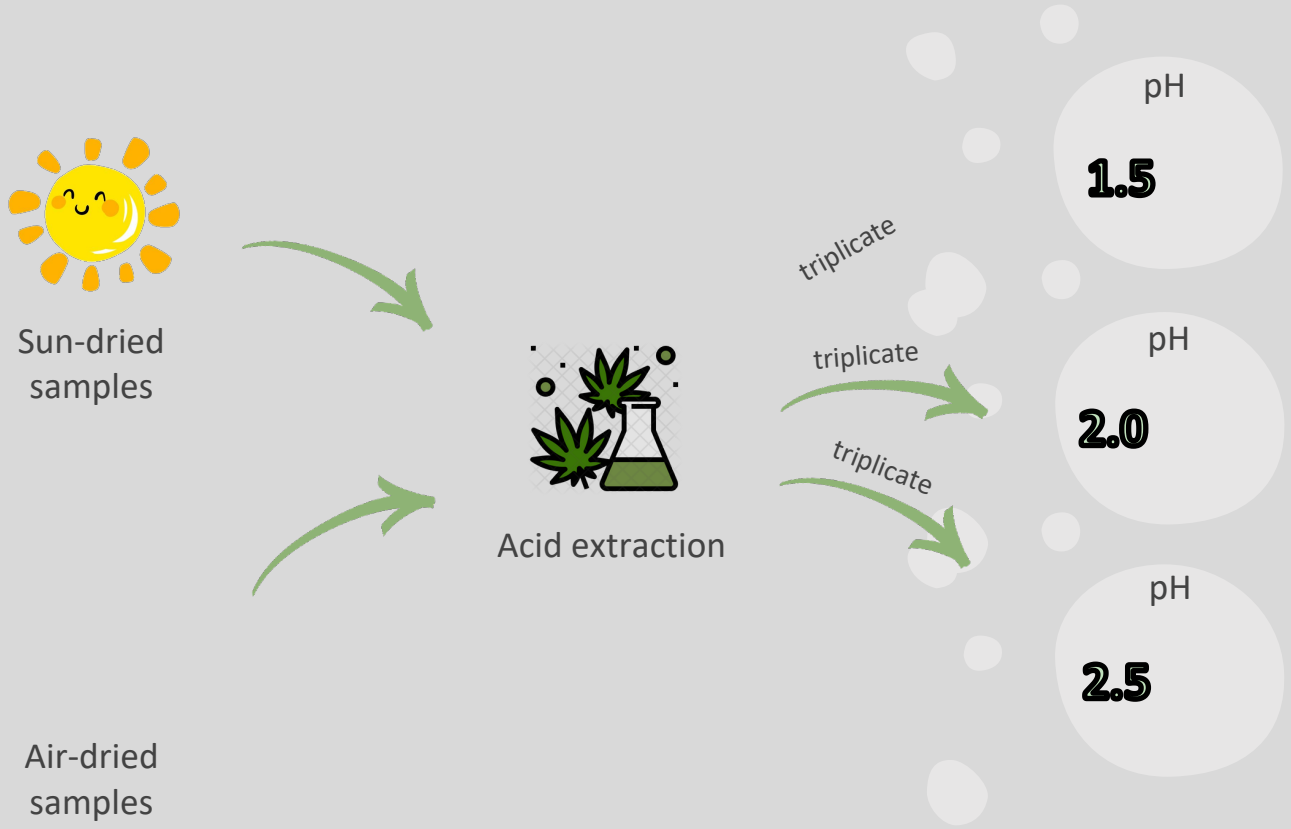
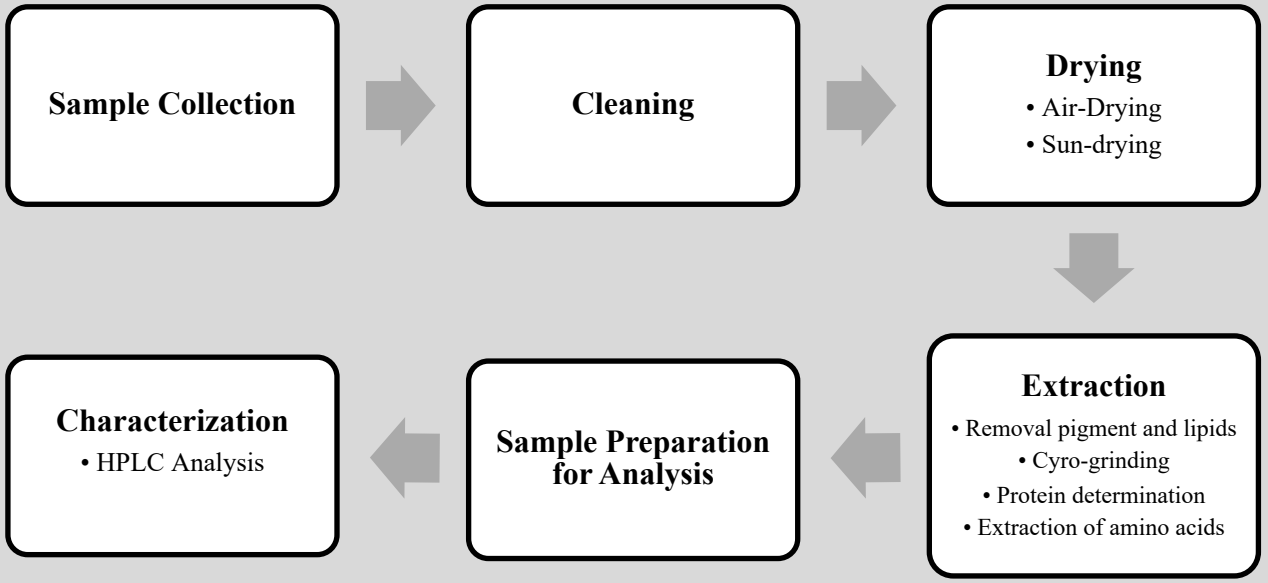


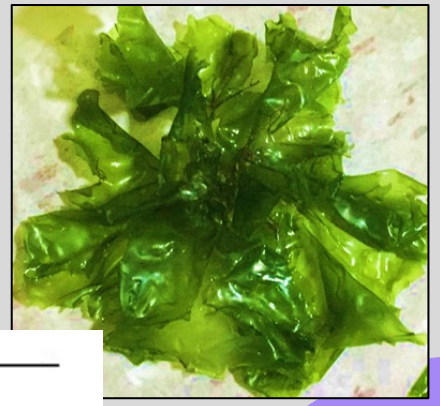
Drying process	pH	Weight, g	Extraction Yield, %
Sun-dry 1	1.5	0.3533 ±0.017^{bcd}	11.73±0.477^{bc}
Sun-dry 2	2.0	0.3821 ±0.008^{bc}	12.5±0.260^{bc}
Sun-dry 3	2.5	0.2135 ±0.005^{e}	7.02±0.164^{e}
Air-dry 1	1.5	0.4102 ±0.067^{ab}	13.50±2.194^{ab}
Air-dry 2	2.0	0.4560 ±0.069^{a}	15.01±2.277^{a}
Air-dry 3	2.5	0.3035 ±0.050^{d}	10.00±1.659^{d}

Ulva lactuca – Lysine, Leucine, Glycine



Amino Acids	Average (%)			
	Air-Dry		Sun-Dry	
	w/w	TAAs	w/w	TAAs
Hyp	0.107 ± 0.023^{mo}	1.410^{kl}	0.107 ± 0.006^{mo}	1.311^{mn}
Asp^{N}	0.902 ± 0.144^{a}	11.889^{a}	0.965 ± 0.039^{a}	11.827^{a}
Ser^{N}	0.447 ± 0.076^{cg}	5.892^{de}	0.471 ± 0.014^{cg}	5.773^{fg}
Glu^{N}	0.881 ± 0.145^{a}	11.612^{a}	0.975 ± 0.032^{a}	11.950^{a}
Gly^{N}	0.550 ± 0.096^{bd}	7.249^{c}	0.582 ± 0.011^{bd}	7.133^{cd}
His^{E}	0.118 ± 0.020^{mo}	1.555^{kl}	0.097 ± 0.005^{mo}	1.189^{mn}
Arg^{N}	0.504 ± 0.064^{bf}	6.643^{d}	0.483 ± 0.025^{cf}	5.920^{ef}
Thr^{E}	0.436 ± 0.069^{ch}	5.747^{df}	0.453 ± 0.101^{ci}	5.552^{fi}
Ala^{N}	0.678 ± 0.100^{b}	8.936^{b}	0.745 ± 0.012^{b}	9.131^{b}
Pro^{N}	0.392 ± 0.063^{ck}	5.167^{ei}	0.438 ± 0.013^{dk}	5.368^{gk}
Tyr^{N}	0.169 ± 0.048^{lm}	2.227^{j}	0.221 ± 0.013^{lm}	2.709^{l}
Val^{E}	0.533 ± 0.078^{be}	7.025^{c}	0.576 ± 0.014^{be}	7.060^{ce}
Met^{E}	0.129 ± 0.057^{mn}	1.700^{jk}	0.150 ± 0.018^{mn}	1.838^{lm}
Lys^{E}	0.403 ± 0.078^{cj}	5.312^{eh}	0.441 ± 0.024^{cj}	5.405^{gj}
Ile^{E}	0.346 ± 0.050^{fl}	4.560^{gi}	0.378 ± 0.012^{fl}	4.633^{jk}
Leu^{E}	0.561 ± 0.082^{bc}	7.394^{c}	0.619 ± 0.020^{bc}	7.587^{c}
Phe^{E}	0.427 ± 0.062^{ci}	5.628^{dg}	0.458 ± 0.014^{ch}	5.613^{fh}

Ulva lactuca – Vitamin B12



Seaweeds	Vitamin (mg per 8 g dry portion)								
	B1	B2	B3	B6	B8	B9	B12	C	E
Ascophyllum nodosum	0.216	0.058	0.000	0.001	0.001	3.648	0.131	0.654	0.029
Laminaria digitata	0.011	0.011	4.896	0.513	0.513	0.000	0.495	2.842	0.275
Undaria pinnatifida	0.403	0.936	7.198	0.259	0.015	0.528	0.345	14.779	1.392
Porphyra umbilicalis	0.077	0.274	0.761	0.119	NA	1.003	0.769	12.885	0.114
Palmaria palmata	0.024	0.080	0.800	0.002	0.002	0.021	1.840	5.520	1.296
Ulva spp.	0.060	0.030	8.000	NA	NA	0.012	6.300	10.000	NA

Component	Value (% db)
Moisture content	9.89 ± 3.37
Ash	31.40 ± 0.62
Protein	9.24 ± 0.31
Fat	0.38 ± 0.24
Crude fiber	3.68 ± 0.16
Carbohydrate	49.09 ± 3.99

Sample collection, drying (Sun-, oven-, air-, freeze-drying) and preparation

Designing screening experimental design by 2-Level Factorial

Sample extraction (ultrasonic water bath, orbital shaker, boiling) and parameters

Purification of vitamin B12 crude extract

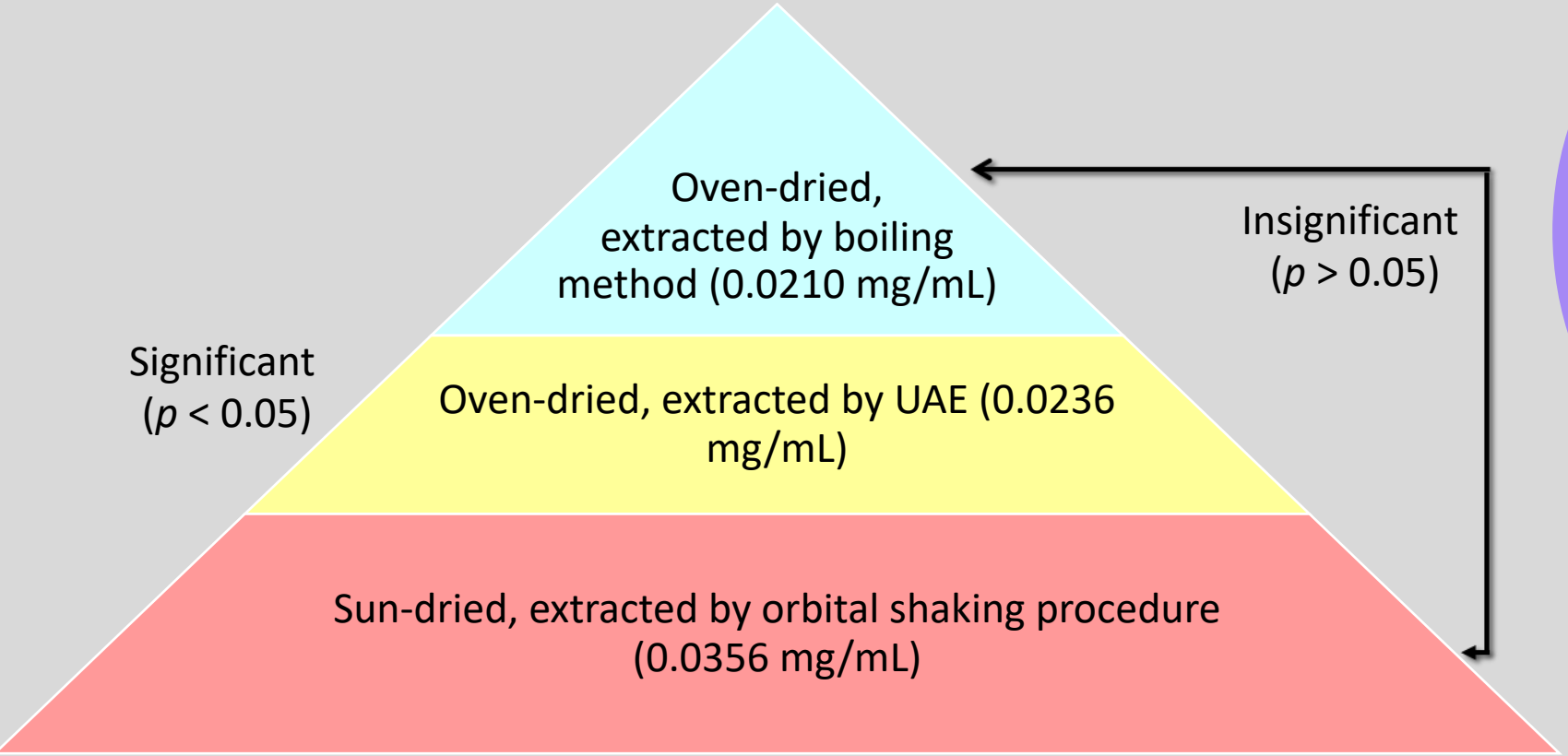
Qualitative and quantitative analysis by HPLC

Statistical analysis

Optimisation by Central Composite Design (CCD)

Run	Solvent:solvent ratio (MeOH:H2O)	pH	Solute:solvent ratio (g/mL)	Conc. of vitamin B12 (mg/mL) *
1	75:25	3	3:60	
2	50:50	4	3:75	
3	25:75	3	3:90	
4	75:25	5	3:60	
5	25:75	5	3:60	
6	25:75	3	3:90	
7	50:50	4	3:75	
8	75:25	3	3:90	
9	50:50	4	3:75	
10	25:75	5	3:90	
11	75:25	5	3:90	
12	75:25	5	3:90	
13	25:75	3	3:60	
14	75:25	3	3:90	
15	75:25	3	3:60	
16	25:75	5	3:90	
17	75:25	5	3:60	
18	50:50	4	3:75	
19	50:50	4	3:75	
20	25:75	3	3:60	
21	25:75	5	3:60	

Standards	Retention time (min)
Cn-Cbl	1.9
OH-Cbl	2.3
Adl-Cbl	2.7
Me-Cbl	3.1

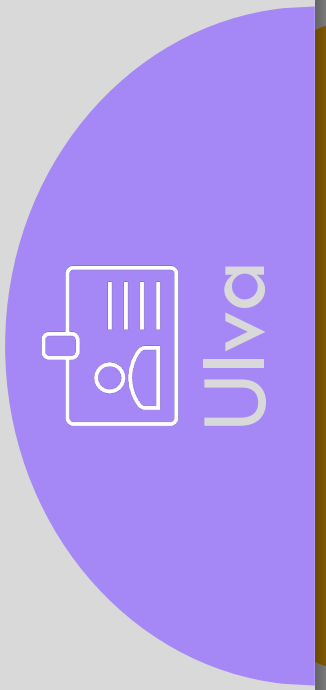


ANOVA analysis for the concentration of CN-Cbl from oven-dried *U. lactuca* using UAE method

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.0008	7	0.0001	76.53	< 0.0001	significant
A-Solvent:solvent ratio (MeOH:H2O)	0.0000	1	0.0000	10.53	0.0070	
B-pH	0.0001	1	0.0001	49.69	< 0.0001	
C-Solute:solvent ratio	2.448E-06	1	2.448E-06	1.69	0.2180	
AB	0.0004	1	0.0004	261.72	< 0.0001	
AC	0.0003	1	0.0003	184.21	< 0.0001	
BC	0.0000	1	0.0000	20.66	0.0007	
ABC	0.0000	1	0.0000	7.22	0.0198	
Curvature	0.0001	1	0.0001	60.40	< 0.0001	

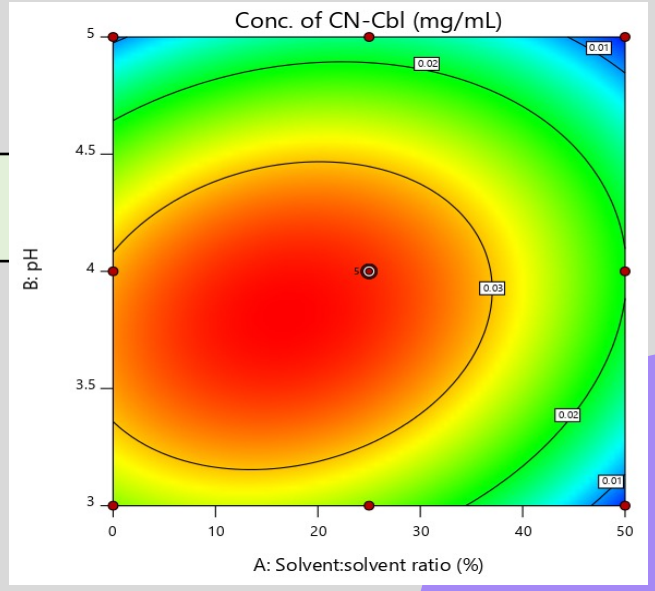
Optimised design layout of Central composite design for UAE of oven-dried sample

Run	Solvent:solvent ratio (MeOH:H_2O)	pH	Conc. of vitamin B12 (mg/mL)
1	0:100	3	0.0250
2	50:50	3	0.0084
3	0:100	5	0.0072
4	50:50	5	0.0055
5	0:100	4	0.0323
6	50:50	4	0.0189
7	25:75	3	0.0225
8	25:75	5	0.0192
9	25:75	4	0.0357
10	25:75	4	0.0355
11	25:75	4	0.0351
12	25:75	4	0.0358
13	25:75	4	0.0305

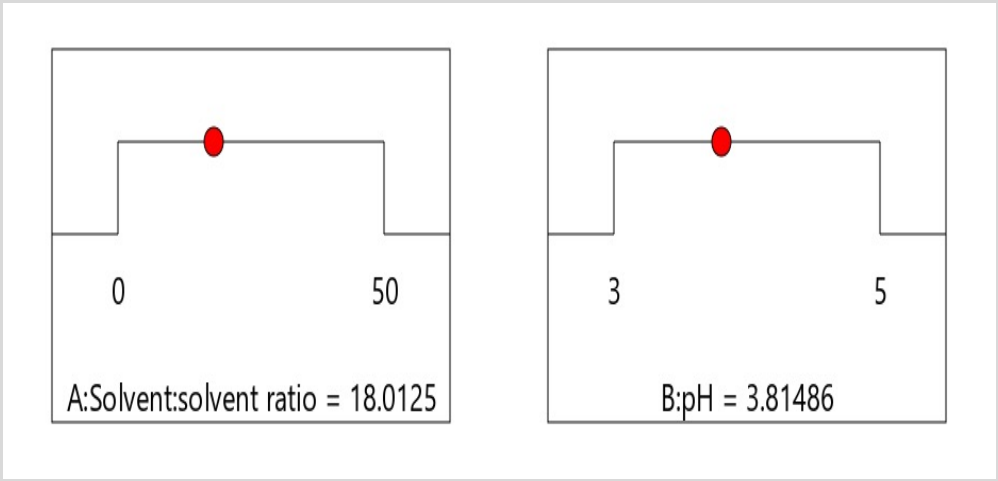


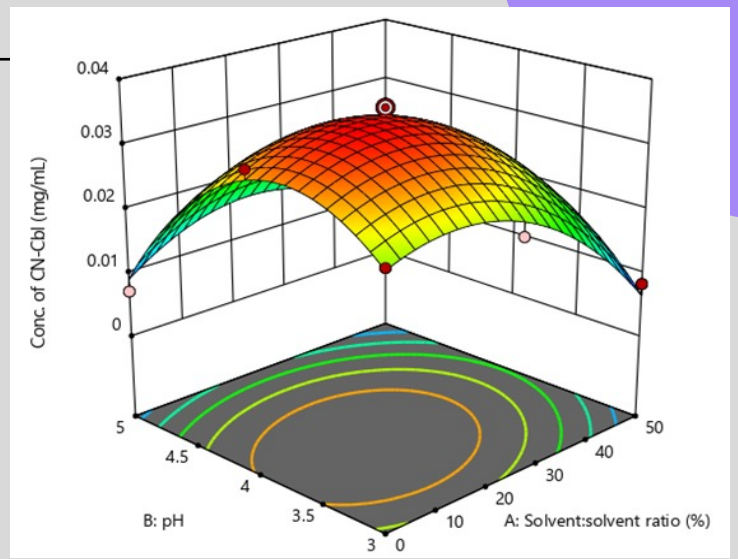
ANOVA analysis for the concentration of CN-Cbl from oven-dried *U. lactuca* using UAE method

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.0003	5	0.0003	49.26	< 0.0001	significant
A-Solvent:solvent ratio	0.0002	1	0.0002	27.14	0.0012	
B-pH	0.0001	1	0.0001	15.64	0.0055	
AB	0.0001	1	0.0001	9.06	0.0196	
A^2	0.0002	1	0.0002	37.27	0.0005	
B^2	0.0005	1	0.0005	85.86	< 0.0001	
Residual	0.0000	7	6.179E-07			
Lack of Fit	0.0000	3	7.511E-06	1.45	0.3538	not significant
Pure Error	0.0000	4	5.180E-06			
Cor Total	0.0016	12				



(i) AB interaction in contour plots
(ii) AB interaction in 3D surface





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