Pharmacogn J. 2020; 12(3): 624-629
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogj.com

Research Article

Characteristics of *Turbinaria conoides* and *Padina Minor* As Raw Materials For Healthy Seaweed Salt

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ABSTRACT

Background: Seaweed is one of the abundant biological resources in Indonesia and contains secondary metabolites. This study was aimed to determine the characteristics and antioxidant activity of brown seaweed salts that fits the standard hence it can be applied as a functional salt preparation for hypertensive patients. **Objective:** The study consisted of identification of raw materials, yield analysis, the levels of Na and K, heavy metals, NaCl and antioxidant activities using the *Ferric Reducing Antioxidant Power* (FRAP) and the *Cupric Reducing Antioxidant Capacity* (CUPRAC) methods. **Materials and Methods:** The experimental design used was a Completely Randomized Design (CRD) with different types of seaweed as a parameter (*Turbinaria conoides* and *Padina minor*), temperature (40⁰C and 55⁰C), and time (10 and 30 minutes) with 3 replications. **Results:** The results demonstrated the interaction between different types of seaweed, temperature and heating time had a significant effect on the level of 5% ($P < 0.05$) on yield, the ratio of Na:K and functional salt NaCl. A functional salt FRAP antioxidant activity of *T. conoides* ranged from 39.12 to 55.31 μM trolox/g and CUPRAC ranged from 98.50 to 113.95 μM trolox/g, while the functional salt from *P. minor* has FRAP antioxidant activity ranging from 18.19 to 24.67 μM trolox/g and CUPRAC 40.05-53.05 μM trolox/g. **Conclusion:** Seaweed *T. conoides* and *P. minor* can be used as raw materials for functional salt preparations for hypertensive patients.

Key words: CUPRAC, DPPH, FRAP, Functional salt, Hypertension.

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History

- Submission Date: 18-02-2020;
- Review completed: 01-03-2020;
- Accepted Date: 16-03-2020.

DOI : 10.5530/pj.2020.12.93

Article Available online

http://www.phcogj.com/v12/i3

INTRODUCTION

Seaweed is one of the abundant biological resources in Indonesian water. Based on data from the Ministry of Marine Affairs and Fisheries (MMAF) in 2016 seaweed production reached 11.69 million tons.[1] The use of seaweed in the industry today prefers using red seaweed (*Eucheuma cottonii, E. spinosum*, and *Gracilaria* sp.) and green seaweed (*Caulerpa racemosa, C. lentilifera* and *Ulva lactuca*), while brown seaweed is limited to extract of alginate content. The most abundant brown seaweed in Indonesian sea is *Turbinaria* sp., *Sargassum* sp. and *Padina* sp.

Brown seaweed produces varieties secondary metabolites such as alkaloids, terpenoids, steroids, tannins, saponins, and glycosides that have a potency in the pharmaceutical industry.[2] It also has the inhibitory activity againts LDL oxidation, *Angiotensin Converting Enzyme* (ACE), α-amylase, α-glucosidase[3] and therapeutic effects and protection against several degenerative diseases, especially cancer.[4] The active ingredients of seaweed can be utilized in a variety of fields, especially in the fields of the pharmaceutical industry, biomedicine, and nutraceuticals, as well as a source of antioxidants. Seaweed as a source of antioxidants has been studied includes seaweed as a cosmetic raw material.[5-17] Seaweed is also studied as seaweed salt dosage for patients with hypertension[18-20] while the study as a source of nutraceutical from *Sargasum aquifolium* extract is conducted by Paradise (2013). Hypertension has reported in the deaths of 8 million people each year; 1.5 million deaths occur in Southeast Asia. The prevalence of hypertension will continue to increase every year and it is predicted in 2025 29% of the population in the world will be affected by hypertension. The diet of salt intake is one of the treatments applied to hypertensive patients, but the weakness is the lack of potassium (K) intake into the body. For a hypertensive condition, potassium (K) has a role in reducing blood pressure. Based on this, hypertensive patients need salts alternatives for a diet of salt intake without lack of potassium (K).

Low-sodium salt is an alternatives product that can be used in hypertensive patients, as a study of salt made from seaweed has been conducted using *U. lactuca* seaweed[21] and Green seaweeds (*C. lentillifera* and *H. opuntia.)*[12,22] Brown seaweed is an alternative in material for low-sodium salt. Minerals and active components in brown seaweed can produce low-sodium salt that had antioxidant activity, but the process of making low-sodium salt from *Turbinaria conoides* and *Padina minor* is still very limited, so the aim of this research was to get the characteristics and antioxidant activity of low-sodium salt that can be applied as a salt for hypertensive patients.

MATERIALS AND METHODS

Materials

The materials used in this research were *T.conoides* and *P. minor* brown *seaweed* obtained from the Scout Island in Thousand Island Regions, purified

Cite this article: Nurjanah, Abdullah A, Diachanty S. Characteristics of *Turbinaria conoides* and *Padina Minor* As Raw Materials For Healthy Seaweed Salt. Pharmacogn J. 2020;12(3):624-9.

water, FeCl $_3$ (*Merck*), ethanol PA 99.9% (*Merck*), ascorbic acid (*Merck*), TPTz ((2,4,6-Tris (2- *pyridyl*)*s-triazine*) (*Sigma-Aldrich*), Neocuproine (*Sigma-Aldrich*), Mg (*Merck*) powder , amyl alcohol (*Merck*), Folin-Ciocalteu (*Merck*), Na_2CO_3 (*Merck*), gallic acid, ascorbic acid (*Merck*), Trolox (*Merck*), CuCl $_{2\,2}$ H_2O (*Merck*) , ammonium acetate (*Merck*), CH_3COOH (*Merck*), CH_3COONa, K_2CrO_4 (*Merck*). The analytical devices were *water bath* (SWBR17), *vortex* (VM-300), glassware, pH meter, micropipette (Gilson ®), AAS (*Atomic* Absorption *Spectrophotometer*) (Shimatzu AA -700), *UV-Vis RS Spectrophotometer* (UV-2500), oven (Memmert, Germany), analytic type 210-LC (Adam, United States).

Methods

This research was conducted in two stages. The first phase includes the sampling, preparation, and identification of the raw materials. The second stage includes the production and characterization of seaweed salts. Characterization of salt consisting of yield analysis, levels of Na and K, heavy metals, NaCl and antioxidant activity using *Ferric Reducing Antioxidant Power* (FRAP) and *Cupric Reducing Antioxidant Capacity* (CUPRAC) methods.

Sample collection and preparation

T.conoides and *P.minor* were obtained from Sulawesi waters and identified in the Marine Bioprospection, Marine Science and Technology Laboratory of Fisheries and Marine Scince Faculty, Bogor Agricultural University. The samples were cleaned from sand and foreign matter and washed the rest of the sample was cut and crushed using *a blender* until smooth and sieved with a size of 30 mesh using sea water, then placed in a container, while part of the samples was separated for identification purposes soaked in ethanol 70% and and dried for 3-5 days.

Seaweed salt production

Seaweed salt was carried out based on the method with modification.[23] The modifications made are the stirring stage during heating. The process starts with smoothing seaweed using a *blender*, followed by sifting. It is carried out by mixing seaweed and purified water (1:10) and heated using *water bath* at temperatures (40 and 55°C) during 10 and 30 minutes, stirred. The results were stirred using 500*mesh nylon* cloth and filter paper, then dried with an oven at 60 °C for 48 hours.

Levels of the heavy metal and Na and K of the functional salt

Analysis of the heavy metal and Na and K levels from functional salts was carried out which is an AAS method (Atomic Absorption Spectrophotometer).[24] This test uses *flame type* air-C_2H_2 with a length of Na waves 589.6 nm and 766.5 nm and Na 0 detection limit 7143 mg/kg and K 1.0083 mg/kg, while the analysis of heavy metals consists of lead (Pb), copper (Cu), and mercury (Hg) with Pb wavelength 283.3 nm, Cu 228.8 nm, and Hg 253.6 nm with the limit detection of Pb at 0.23 mg/kg, Cu 1.2 mg/kg and Hg 0.004 mg/kg.

The seaweed salt NaCl levels

Testing the NaCl level of functional salts was carried out by the Mohr method (modified) based on the Day and Underwood method (1989). 5 g of the sample was turned into ash, then it was transferred to 250 mL Erlenmeyer. 1 mL of 5% potassium chromate solution was added, followed by titration using silver nitrite solution 0.1 M until orange or orange changes occur. The level of NaCl can be calculated by the formula:

$$\text{salt (NaCl) (\%)} = \frac{(T \times N \times 58.4)}{W\ (mg)} \times 100\%$$

Information: T = mL titration

N = Normality of silver nitrate

W = sample weight

The seaweed salt iodine levels

Testing of iodine levels in functional salts was carried out method using ICP-MS.[25] The sample was weighed in a liner, then concentrated HNO_3 added and then *pre-digest* for 10-15 minutes and continued with the stage of sample destruction. It was cooled and diluted by purified water before analyzed using ICP-MS.

Antioxidant activity using FRAP method

The functional antioxidant salt activity with the FRAP method was based on modified methods.[26] FRAP reagent preparation was formed using 300 mM acetate buffer with pH 3.6; 10 mM TPTZ (2,4,6-*tripyridyl-s-triazine)* in 40 mM HCl and 20 mM $F_3Cl_3.6H_2O$ with a ratio of 10:1:1. The absorbance measurement used by mixing 0.1 mL of sample, 0.6 mL of distilled water and 3 mL of FRAP reagent. A mixture of FRAP samples and reagents was mixed in *a vortex*, then incubated using a *water bath* at 37 °C for 30 minutes. Absorbance measurements were carried out at a wavelength of 593 nm. The calibration curve uses the Trolox solution (standard for validation) with various concentrations. Antioxidant capacity is stated in µM trolox/g.

Antioxidant activity using the CUPRAC method

Samples were made by dissolved 0.25 m seaweed salt in 1 mL of distilled water and added with $CuCl_2.2H_2O$ 0.01 M; 1 mL ethanolic neocuproine 0.0075 M; 1 mL of ammonium acetate buffer pH 7 1 M and 0.85 mL distilled water. A mixture of samples and reagents in the *vortex was* then incubated within a dark room temperature for 30 minutes. The absorbance measurement is carried out at a wavelength of 450 nm. The calibration curve was made using Trolox solution with various concentrations. Antioxidant capacity is expressed in µM trolox/g.[27]

Data analysis

The experimental design used was a Completely Randomized Design (CRD). The data obtained were tested with three replication, normality and homogeneity before ANOVA analysis was carried out. Data analysis was performed by *Analysis of Variant* (ANOVA) at 95% confidence interval ($\alpha = 0.05$). The significant results then further tested using the *Duncan* test.

RESULTS

Characteristics of raw materials

Identification of the morphology of the raw material is carried out by observing morphological characteristics macroscopically at the Marine Bioprospection, Marine Sciences and Technology Laboratory of IPB. The results of morphological identification were in the class of *Phaeophyceae*, two family which is *Sargassaceae* and *Dictyotaceae* and the species were *Turbinaria conoides* and *Padina minor.*

The yield, Na and K, and NaCl of the seaweed salt

The yield analysis of seaweed salts was carried out to see the final weight obtained from the treatment, while the analysis of Na and K was carried out to obtain the ratio of Na: K from the seaweed salts. The parameter ratio of Na: K is the main parameter in seaweed salts for the application of a salt diet for hypertensive patients. NaCl levels in seaweed salts are one of the important elements for its application in hypertensive patients. The results of the analysis of yield, Na and K, and NaCl of the seaweed salt can be seen in Table 1.

Beside the NaCl levels, the iodine and heavy metal levels (Hg, Pb, and Cu) should fit the standard in the table salt. The levels of iodine and heavy metal of functional salts from *T. conoides* and *P. minor* salts with a heating temperature of 40 ° C for 10 minutes can be seen in Table 2.

Antioxidant activity

Antioxidant activity was determined using two methods with different mechanisms; *Ferric Reducing Antioxidant Power* (FRAP) and *Cupric Reducing Antioxidant Capacity* (CUPRAC). The measurement of functional antioxidant salt activity was carried out to determine the antioxidant activity in seaweed salts. Antioxidant activity contained in seaweed salts is one indicator that there is an active component in functional salts. Moreover, antioxidant activity is one of the advantages of seaweed salt compared to table salt and low sodium salts on the market. The antioxidant activity of seaweed salts can be seen in Table 3.

DISCUSSION/CONCLUSION

T.conoides habitat is in coral areas with low tides and reef flat areas and the morphology is erect *thallus*, triangular-small *turbinate* leaves with a length of 1 cm, attached to stiffed *holdfast*, forming large and thick colonies and brown.[28] *P. minor* seaweed has a flabellate *thallus*, thin and grows to form colonies with *holdfast rhizoid*, has a 7-12 lobeline that is yellowish brown in color and has a living habitat in areas with sandy substrates.[29]

Duncan's further test results showed that the interaction of different types of seaweed (*T. conoides* and *P. minor*), time (10 and 30 minutes) and temperature (40 °C and 55 °C) affected the yield of seaweed salts. The average yield of seaweed salts ranges from 20%-26%. Generally longer heating times and warmer temperatures can produce higher salt yields, but in industrial applications, shorter heating times and lower temperatures are expected because it can reduce the production costs. Also, the difference in the yield of seaweed salts produced is due to the morphological differences between seaweed species.[23]

Duncan's further test results showed that the interaction of different types of seaweed (*T. conoides* and *P. minor*), time (10 and 30 minutes) and temperature (40 °C and 55 °C) affected the ratio of seaweed salt Na:K. The average value of the ratio Na:K of the functional salt ranges from 0.81-2.54 mg/g. The lowest Na:K ratio was obtained from *T. conoides* seaweed salt with a heating temperature of 40° C for 10 minutes. The Ratio of Na:K from *T. conoides* seaweed salt (0.81-0. 89 mg/g) salt fits the category of functional salt for hypertensive diet, whereas *P. minor* (2.26-2.54 mg/g) only fits the category of table salt. The recommended range of dietary intake Na:K for humans has a ratio between 0.3-1.[30,31] Salt with a low Na:K ratio and high K components provide health benefits for consumers. Seaweed salt with "kombu" raw material has a Na:K ratio of 57.7 mg/g, so it belongs to the table salt category. Salt with a low Na:K ratio and higher K content can provide benefits to consumers because they can replace NaCl and have an effect on improving health. A health perspective, the low ratio of Na:K is related to the application of a salt diet for hypertensive patients.[32]

Duncan's further test results show that the interaction of different types of seaweed (*T. conoides* and *P. minor*), time (10 and 30 minutes) and temperature (40 °C and 55 °C) affect NaCl levels of the functional salt. Functional salt content ranges 27.74-49. 94%. NaCl levels in functional salts compared to table salt is usually classified as having low levels of NaCl. There is two type salt; the first is table salt while the second

Table 1: Yield, Na, K and NaCl of the functional salt.

Functional salt	Temperature (°C)	Time (minute)	Yield(%)	Na (mg/g)	K (mg/g)	Na:K	NaCl (%)
T. conoides	40	10	24 ± 0.69[bdc]	61.64 ± 0.22	76.50 ± 0.31	0.81[d]	46.21 ± 0.20[c]
		30	23 ± 1.39[dc]	63.58 ± 0.40	71.51 ± 0.27	0.89[c]	48.24 ± 0.42[b]
	55	10	24 ± 0.77[d]	62.75 ± 0.08	75.28 ± 0.52	0.83[d]	49.94 ± 0.89[a]
		30	26 ± 0.38[bac]	62.46 ± 0.21	74.58 ± 0.50	0.84[d]	46.21 ± 1.53[c]
P. minor	40	10	24 ± 0.58[dc]	66.77 ± 1.95	26.33 ± 0.03	2.54[a]	28.34 ± 0.10[d]
		30	26 ± 0.37[e]	74.88 ± 0.54	33.10 ± 0.14	2.26[b]	27.91 ± 0.84[d]
	55	10	20 ± 1.68[a]	67.09 ± 0.19	26.59 ± 0.04	2.52[a]	28.77 ± 0.22[d]
		30	26 ± 1.33[ba]	61.93 ± 0.22	27.08 ± 0.16	2.29[b]	27.74 ± 1.27[d]

Notes: The numbers in the same column are followed by the same letters do not differ significantly at the 5% confidence level (Duncan's multiple tests).

Table 2: Iodine and heavy metals content of *T. conoides* and *P. minor* seaweed salt.

Parameter (mg/kg)	Functional salt		Standard
	T. conoides	P. minor	
Lead (Pb)	< 0.04	< 0.04	max. 10 mg/kg**
Mercury (Hg)	< 0.002	< 0.002	max. 0.1 mg/kg**
Copper (Cu)	0.96	0.99	max. 10 mg/kg*
Iodine (I)	240.00	64.10	min.30 mg/kg**

Notes: * (SNI 2000), ** (SNI 2010).

Table 3: Antioxidant activity of seaweed salt.

Seaweed	Temperature (°C)	Time (minutes)	FRAP antioxidant activity (μM trolox/g)	CUPRAC antioxidant activity (μM trolox/g)
T. conoides	40	10	40.00 ± 0.96	98.50 ± 0.07
		30	39.12 ± 1.00	99.02 ± 0.37
	55	10	54.33 ± 1.68	113.62 ± 0.27
		30	55.31 ± 1.54	113.95 ± 0.27
P. minor	40	10	18.19 ± 0.23	40.05 ± 0.04
		30	19.40 ± 0.04	48.31 ± 0.11
	55	10	05.05 ± 0.41	51.69 ± 0.18
		30	24.67 ± 0.15	53.05 ± 0.29

is diet salt.[32] Table salts have a minimum requirement of 94% NaCl concentration, while for a diet salt the maximum value is 60%.

T. conoides functional salt can be used as an alternative for hypertensive patients because it contains a Na:K ratio that meets the standard as diet salt, while *P. minor* functional salt can be used as a table salt. *T. conoides* functional salt and *P. minor* can be used as an alternative due to its standard and benefit for health from the lower Na: K ratio, low NaCl (<60%), more iodine (> 30 mg/kg), and with only a trace of heavy metal.

The phenol compounds antioxidants will be oxidized in the presence of light, heat, and oxygen. Besides that, the phenol in functional salts has acidic, volatile, and it is sensitive to the light and oxygen. Phenol levels in a material will decrease with the treatment of washing, boiling, and further processing.[33]

A seaweed salt FRAP antioxidant activity was 18.19 - 55.31 μM trolox/g belongs to moderate antioxidant capacity. The compound belongs to very strong antioxidant activity if the antioxidant capacity value is more than 500 μmol Fe^2/g, while strong if the antioxidant capacity is 100-500 μmol Fe^2/g, moderate if the antioxidant capacity is 10-100 μmol Fe^2/g, and weak if the antioxidant capacity is <10 μmol Fe^2/g.[34] The result from the heating temperature of 55 °C for 30 minutes has the highest antioxidant activity (55,31 μM trolox/*g).* FRAP (*Ferric Reducing Antioxidant Power*) is a method of determining an antioxidant activity to measure the ability of antioxidants to reduce Fe^{3+} in complexes Fe^{3+}-TPTZ becomes Fe^{2+}-TPTZ by donating electrons.

The CUPRAC seaweed salt antioxidant activity was 40.05 -113.95 μM trolox / g. The result from a heating temperature of 55 ° C for 30 minutes has the highest antioxidant activity (113. 95 μM trolox/g). The CUPRAC (*Cupric Reducing Antioxidant Capacity*) method is based on electron transfer and is considered a good indicator of total antioxidant ability.[35] Antioxidant activity in seaweed such as *Turbinaria* sp. and *P. polysiphonia* has an important role in the aging process, anti-inflammatory, anti-bacterial, anti-fungal, cytotoxic, anti-malarial, antiproliferative, and anti-cancer.[36]

Seaweed *T. conoides* and *P. minor* could be used as raw material for the preparation of low sodium seaweed salts with antioxidant activity. A heat treatment temperature of 40 °C for 10 minutes of *T. conoides* produced seaweed salts that were low in Na:K ratio and heavy metals but high in iodine, NaCl, and antioxidant activity so it could be used as raw material for dietary salt. While *P. minor* could be used as raw material for table salt preparations because it produces a Na:K ratio that exceeds the standard of diet salt.

ACKNOWLEDGEMENTS

Authors gratefully acknowledge the Research Grant from Indonesian Ministry of Higher Education and Technology PTUPT 2019 No. 3/E1/KP.PTNBH/2019 to Prof. Dr. Nurjanah.

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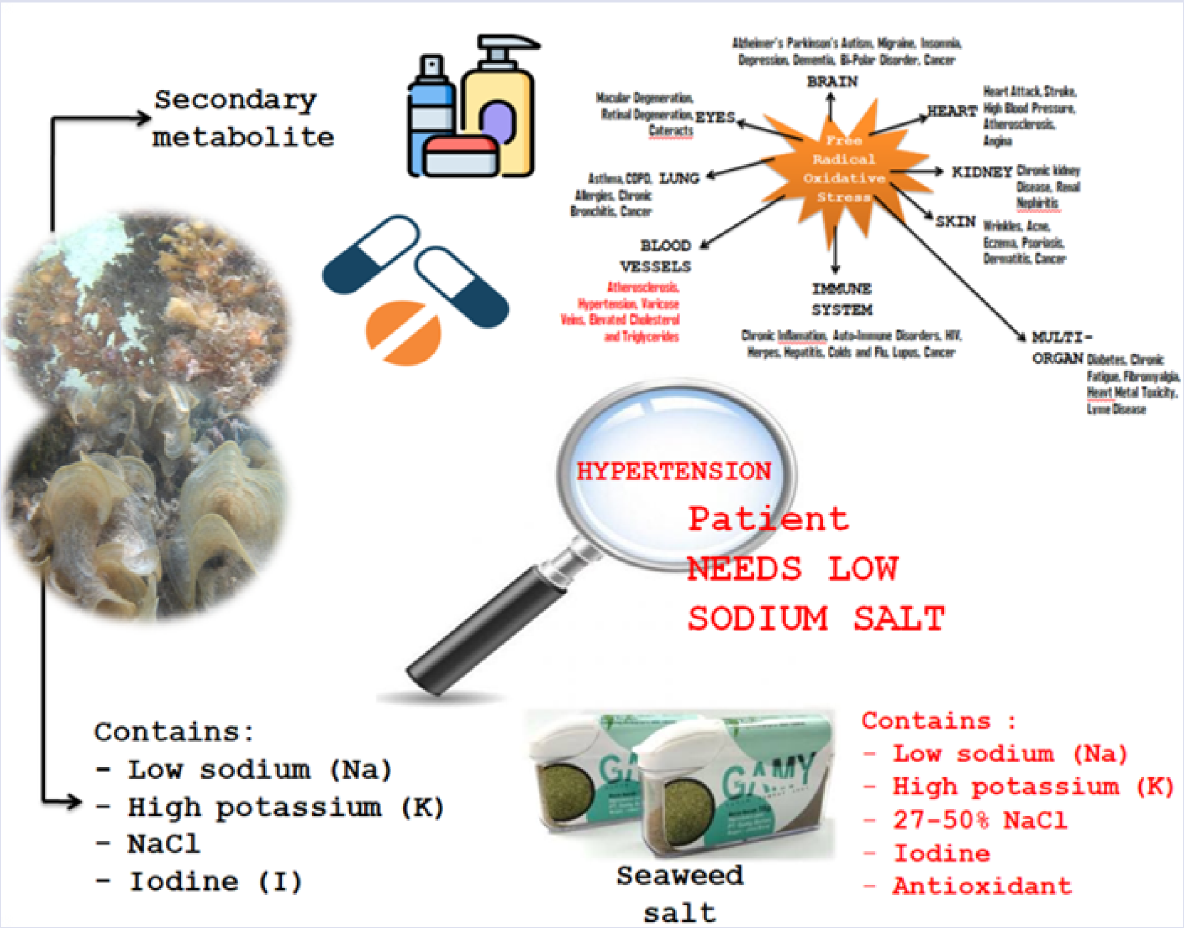
GRAPHICAL ABSTRACT



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Cite this article: Nurjanah, Abdullah A, Diachanty S. Characteristics of *Turbinaria conoides* and *Padina Minor* As Raw Materials For Healthy Seaweed Salt. Pharmacogn J. 2020;12(3):624-9.