# Effects of Various Drying Processes on Malaysian Brown Seaweed, *Sargassum polycystum* Pertaining to Antioxidants Content and Activity

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**ABSTRACT** The objective of this study was to evaluate the effects of different drying methods on phytochemicals and *in vitro* antioxidant activity of brown seaweed, *Sargassum polycystum*. Six different drying methods employed in this study were freeze drying, oven drying at 40 °C, oven drying at 60 °C, sun drying, vacuum drying at 40 °C and vacuum drying at 60 °C. Five different polarity solvents, methanol, ethanol, acetone, ethyl acetate, cold water and hot water were used as extraction solvents to determinephytochemicals content for total phenolic content, total flavonoid content and total carotenoid content while that antioxidant activitywas assessed by employing ferric reducing antioxidant power (FRAP), 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity (DPPH) and beta carotene bleaching (BCB) assay. Ethyl acetate extracts vacuum dried at 40 °C showed highest total phenolic content (58.54 ± 1.30 mg PGE $g^{-1}$ dry extract), total flavonoid content (35.91 ± 1.54 mg RE $g^{-1}$ dry extract), FRAP value 379.41 ± 1.17 µmol TE $g^{-1}$ dry extract and DPPH EC50 values, 3.83 ± 0.18 mg $mL^{-1}$ among all drying methods. Sun dried extracts possessed lowest retention of phytochemicals content and antioxidant activity among other drying methods. As a conclusion, *S. polycystum* was best dried by vacuum drying methodat 40 °C which has the higher retention of phytochemicals and antioxidant content.

**KEYWORDS:***Sargassum polycystum*, brown seaweed, drying, antioxidant, phytochemical
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## INTRODUCTION

In Malaysia, Sabah is a maritime state naturally suited for edible seaweed plantations with more than a quarter of its borders neighbouring the sea (Bono *et al.,* 2011). Tropical seaweed productions include *Eucheuma, Kappaphycus, Caulerpa* and *Gracilaria* species. The abundant supply of seaweeds in Malaysia offers great opportunities for producing and extracting several functional ingredients, such as fucoidan, alginate, agar and carrageenan. Apart from significant strong antioxidants activity as reported by Matanjun *et al.* (2008), Sabah seaweeds, brown seaweed, *Sargassum polycystum* shown to have anti-obesity effect (Awang *et al.,* 2014) and reduces hyperglycaemia (Motshakeri *et al.,* 2013) and dyslipidaemia (Matanjun *et al.,* 2010).

Seaweeds are high in moisture content when consumed fresh. They were reported to contain 75 to 85% water and 15 to 25% organic components and minerals (Matanjun *et al.,* 2010; Gupta *et al.,* 2011). Processing methods are required to reduce moisture content for long-term storage ability. However, the physical and chemical, biological, nutritional, safety and sensory consistency of the seaweed is influenced by such processing methods (Chan *et al.,* 1997). The cell membrane could affect by food processing, such as heating or freezing, triggering the release of membrane-bound phytochemicals, which greatly increases bio-accessibilityduring intake (Wani *et al.,* 2020). According to Gupta *et al.* (2011), seaweed drying at a lower temperature (less than 40 °C) contributed in a 49 and 51% reduction in the total phenol and total flavonoid content, however the reduction tended to decrease as the drying temperature rose to more than 41 °C. The respective disintegration of this matrix led to an increase or minimal exposure to oxidation reactions of antioxidant compounds

(Martínez-Las Heras *et al.*, 2014). The most thoroughly common drying method being studied are air drying, freeze drying, vacuum drying and sun drying (Kamiloglu *et al.*, 2016). Freeze drying is a method of removing moisture from a substance after it is frozen and put under a vacuum, facilitating the ice to change immediately from solid to vapour without going through a liquid phase (Ratti, 2001). Oven drying subjected the surface of the substances to hot air and the heat transfers from the surface to the interior of the materials. Due to rapid drying of the product surface and uneven heat transfer into the products, the rate of water evaporation is quicker than the mass transfer of water to the product surface (Mujumdar & Devahastin, 2000). Sun drying is a simple, inexpensive technique that takes both capital input and operating costs in view. This is because there is no need for energy inputs and skilled labour plus very huge volumes of commodities can be dried at low cost through sun drying. To achieve the drying process, sun drying employs direct solar radiation, ambient air temperature, relative humidity and wind speed (Mujumdar & Devahastin, 2000).

However, to date there is lack of reports on the changes of drying effects on phytochemical and antioxidant activity of seaweed except *Kappaphycus alvarezii* (crocodile morphotype) (Ling *et al.*, 2014). In addition, literature shows that the effects of the drying process on the activity of antioxidants and phenolic compounds are inconsistent and conflicting. Such contradictory outcomes may be attributable to plant samples of various organisms with different phytochemicals, structure and physicochemical properties in nature (Harbourne *et al.*, 2009). It is clearly crucial to investigate the drying effects on nutrients, phytochemicals and antioxidant activity of seaweed in order to promote the survival of the desired chemical constituents and antioxidant properties.

The aim of this study is to determine the influence of drying methods on phytochemical contents (total phenolic content and total flavonoid content) of *S. polycystum*. Six extraction solvents (aqueous methanol, aqueous ethanol, aqueous acetone, hot water, cold water and ethyl acetate) were used to determine phytochemicals content and antioxidant activity.

## METHODOLOGY

*Sample collection*

The fresh seaweed, *S. polycystum* used in this study were obtained from Pulau Sebangkat, Semporna, Sabah, Malaysia between October and November 2015. Only seaweeds without any physical damage were selected. The seaweed identification was based on morphological character as described by Wong *et al.* (2004) and voucher specimen was labelled as SP 001 and stored. The fresh seaweeds were cleaned with tap water and their epiphytes and holdfasts were removed.

*Preparation of Dried Samples*

(1) Freeze Drying (FD): A freeze dryer (Labconco, USA) was used for freeze drying of seaweeds samples. Fresh samples were placed in freezer for 24 hours followed by drying in a freeze dryer at -86 °C for 48 hours.
(2) Oven Drying at 40 °C (OD 40) and 60 °C (OD 60): A heating oven with mechanical convection (Binder, Germany) with exhaust fan was used. Fresh samples were spread evenly in a single layer on trays which were then placed in the dryer and maintained at 40 ± 5 °C for 60 hours and 60 ± 5 °C for 29 hours.
(3) Sun Drying (SD): Fresh samples were spread evenly in a single layer on netted drying rack. The drying rack was then placed under direct sunshine (mean temperature, 32 °C) for 4 days.

(4) Vacuum Drying at 40 °C (VD 40) and 60 °C (VD 60): Fresh samples were placed in a single layer in a vacuum oven with two drying racks (Binder, Germany) at 40 ± 5 °C for 50 hours and 60 ± 5 °C for 24 hours.

The drying duration was based on a pre-test where samples were dried until constant weight was achieved. Each drying treatment was conducted in triplicate. The dried samples were grounded to fine powder and passed through 850 μm sieve to get a uniform powder. The powdered seaweeds were stored in an airtight plastic bag and stored under -20 °C until further analysis.

*Extraction*

Dried seaweed powders were extracted with 80% v/v methanol, 80% v/v ethanol, 80% v/v acetone and ethyl acetate as extraction solvent with the ratio of sample: solvents (1:20, w/v at 24 °C). In the case of water extracts, hot water extract was obtained by extracting 5 g of dried seaweed sample powder with 250 mL of distilled water at 80 °C); cold water extract was extracted with same ratio of sample: water (1:50) with distilled water at room temperature. The mixture was sonicated at room temperature in a sonicator bath (Branson 2510, USA) for 30 mins at 42 kHz, 130 W before shaking in a shaking incubator for 4 hours at room temperature. The extract was then filtered through Whatman No.1., the residual sample was re-extracted and dried extract was obtained using a rotary evaporator (Rotary R-200, Buchi, Switzerland) under vacuum at 40 °C. The extracts were freeze-dried to acquire dried extracts for water extracts. The dried extracts were stored at -21 °C and were further analysed by dissolving them in methanol.

*Total Phenolic Content (TPC)*

Total phenolic content (TPC) was determined using Folin-Ciocalteu method by Matanjun *et al.* (2008) with modification. Thoroughly, about 0.2 mL of seaweed extract was applied (10 times dilution) to 1.0 mL of Folin-Ciocalteu reagent (Sigma). About 0.8 mL of sodium carbonate (7.5 percent, w / v) was applied to the mixture after 4 minutes and was allowed to stand in the dark for 30 minutes. Using a double beam spectrophotometer (Perkin Elmer) against the blank solution, the absorbance was read at 765 nm. Using phloroglucionol as standard, the standard calibration curve was developed while ascorbic acid was used as positive control. The findings were expressed in the equivalent of mg phloroglucinol equivalent per gram dried extract (mg PGE $g^{-1}$). All measurements were performed in triplicate.

*Total Flavonoid Content (TFC)*

The total flavonoid content (TFC) was determined using aluminium chloride calorimetric assay with modification (Zhishen *et al.*, 1999). For a short time, 0.2 mL of seaweed extract was added to 1.0 mL of deionized water and 0.075 mL of 5% $NaNO_2$. After 6 minutes, about 0.15 mL of aluminium chloride (10%, w/v) was added to the mixture. About 0.5 mL of 1 M NaOH was added to the mixture after 5 minutes and the volume was made up to 2.5 mL with deionized water. The solution was well blended and sustained for 15 minutes in the dark. Using a double beam spectrophotometer against the blank solution, the absorbance was read at 510 nm. Rutin was used as a reference to define the standard calibration curve. The results were expressed as mg rutin equivalent per gram dried extract (mg RE $g^{-1}$). All measurements were performed in triplicate.

*Total Carotenoid Content*

Total carotenoid was determined according to the methodology of Chan *et al.* (2014). The seaweed powder (3.0 g) were extracted with 75 mL of a mixture of hexane:acetone:ethanol (2:1:1, v/v) for 1 hour at room temperature. The homogenate was purified and up to 100 mL of extraction solvent was produced for the collected supernatant. Then, to differentiate the stages, 25 mL of water

was added and shaken vigorously. Two layers were detected, an organic upper layer and an aqueous lower layer, after 30 minutes of being shielded from light. The organic layer absorbance was measured at 470 nm.

*Ferric Reducing Antioxidant Potential (FRAP)*

FRAP assay was conducted as described by Benzie & Strain (1996). Three mL the freshly prepared and warmed at 37 °C FRAP solution was added to 0.3 mL of seaweed extracts and kept in the dark for 30 minutes. The FRAP solution was made up of 300 mM acetate buffer at pH 3.6, 10 mM 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) solution in 40 mM HCl and 20mM ferric chloride in the ration of 10:1:1 (v/v). Using a double beam UV-vis spectrophotometer against blank distilled water, readings were taken at an absorption maximum of 593 nm. For calibration, a Trolox concentration of 10 - 1000µM was used. BHT (0.5 mg $mL^{-1}$) was used as positive control. The results were expressed as µM trolox equivalent per gram dried extract (µM TE $mg^{-1}$). All measurements were performed in triplicate.

*DPPH radical scavenging activity*

Free radical scavenging activity was measured based on the scavenging ability of 2-2-diphenyl-1-pircylhydrazyl (DPPH) radicals as described by Brands-William *et al.* (1995). For 0.5 mL of extracts with different concentrations, an aliquot of 0.5 mL of freshly prepared 0.06 mM DPPH in methanol was added. In order to check the linearity of response and to determine the antioxidant activity values in an adequate linear range, five different concentrations of the extracts studied were assayed. The mixture was permitted to stand in the dark for 30 minutes and read at 517 nm using a methanol double beam spectrophotometer as a blank to zero absorption. The BHT was used as positive control solution. The reaction kinetics were plotted for each antioxidant concentration examined. The results were expressed in term of $EC_{50}$ and defined as an amount of extract concentration (mg $mL^{-1}$) required to decrease the initial DPPH concentration by 50%. All measurements were performed in triplicate. Radical Scavenging Abilities (RSA) were stated as $EC_{50}$ and calculated by using Equation (1).

$$RSA\ (\%) = [A_0-(AB-A_s)]/A_0 \times 100 \quad (1)$$

$A_0$ is Absorbance of the control solution, AB is absorbance of the DPPH solution in the presence of extracts and $A_s$ is absorbance of the sample extract without DPPH.

*Beta carotene bleaching assay*

Antioxidant activity was performed spectrophotometrically according to Chan *et al.* (2014). Firstly, β-carotene solution was prepared by dissolving 0.2 mg of β-carotene into 1 mL of chloroform. Then, about 1 mL of the freshly prepared β-carotene solution was added to a round bottom flask containing 0.02 mL of linoleic acid and 0.2 mL of Tween 20. About 50 mL of deionized water was applied after chloroform evaporation at 40 °C (Rotary R-200, Buchi, Switzerland) and the mixture was shaken vigorously to form an emulsion (β-carotene-linoleate emulsion).

Aliquots of the β-carotene linoleic acid emulsion (2.0 mL) were mixed with 1.0 mL of seaweed extract at various concentrations (0.10 – 20 mg $mL^{-1}$). The mixture was then incubated at 50°C for 2 hours in a water bath. Using a UV-vis double-beam spectrophotometer, emulsion oxidation was controlled at 470 nm. As control, an equal amount of methanol and emulsion were used and BHT as the positive control was used. The $IC_{50}$ of the extracts was determined by plotting a graph of percentage inhibition versus extract concentrations. The percentage inhibition (%) of extracts was calculated using Equation (2).

$$\text{Inhibition (\%)} = 1 - [A_{S(0)} - A_{S(120)} / A_{C(0)} - A_{C(120)}] \times 100 \quad (2)$$

$A_{S(0)}$ is absorbance of sample solution at time t=0, $A_{S(120)}$ is absorbance of sample solution at time t=120, $A_{C(0)}$ is Absorbance of control solution at time t=0 and $A_{C(120)}$ is absorbance of control solution at time t=120.

*Statistical Analysis*

All tests have been carried out in triplicate. The data were analysed using version 26.0 of Statistical Package for the Social Sciences software and the mean ± standard deviation (n=3) data was expressed. Using the Duncan post hoc test, statistical comparisons were made using one-way variance analysis (ANOVA) and statistical differences of p-values below 0.05. For correlation determination, the Pearson correlation coefficient was used. At p-value levels below 0.05, a meaningful difference was considered.

## RESULT AND DISCUSSION

*Extraction yield*

The extraction yield of *S. polycystum*ranged from 1.73-7.70% (Table 1). The orders of yield by extracting solvents among drying methods in descending order are as follows: ethanol (5.53 - 7.70%) > methanol (4.56 - 5.96%), hot water (4.28 - 6.44%), cold water (4.47 - 5.49%)>acetone (2.68 - 4.07%)>ethyl acetate (1.73 - 3.15%). Although there are many reports showing the superiority of methanol over other solvent in seaweeds (Matanjun *et al.,* 2008; Ling *et al.,* 2014)) but ethanol extracts of *S. polycystum* showed significantly higher yield ($p < 0.05$) over methanol extracts. Water has the highest solvent strength polarity (10.2) followed by methanol (5.1), acetone (5.1), ethanol (5.2) and ethyl acetate (4.4) (Nyiredy, 2000). This might be due to the selectivity of ethanol (2.74) is slightly higher than methanol (2.18).

**Table 1.** Extraction yield of *Sargassum polycystum* under different drying treatments by various solvents

| Solvent | Percentage of Extraction Yield (%) | | | | | |
|---|---|---|---|---|---|---|
| | Freeze Dried (FD) | Oven Dried 40°C (OD40) | Oven Dried 60°C (OD60) | Sun Dried (SD) | Vacuum Dried 40°C (VD40) | Vacuum Dried 60°C (VD60) |
| **Ethanol** | $7.70\pm0.62^{aA}$ | $7.13\pm1.33^{abA}$ | $6.84\pm1.55^{abA}$ | $5.53\pm0.50^{bA}$ | $7.21\pm0.91^{abA}$ | $6.79\pm0.43^{abA}$ |
| **Methanol** | $5.96\pm0.86^{aB}$ | $5.44\pm0.49^{abB}$ | $4.81\pm0.44^{abBC}$ | $4.56\pm0.87^{bA}$ | $5.48\pm0.30^{abB}$ | $4.86\pm0.68^{abB}$ |
| **Acetone** | $4.07\pm0.89^{aC}$ | $3.95\pm0.25^{aCD}$ | $3.80\pm0.57^{abC}$ | $2.68\pm0.60^{bB}$ | $4.02\pm0.47^{aC}$ | $3.35\pm0.56^{abC}$ |
| **Ethyl Acetate** | $3.15\pm0.72^{aC}$ | $2.89\pm0.33^{abD}$ | $2.08\pm0.24^{cD}$ | $1.73\pm0.25^{cB}$ | $2.86\pm0.31^{abD}$ | $2.18\pm0.29^{bcD}$ |
| **Cold Water** | $5.49\pm0.46^{aB}$ | $4.91\pm0.50^{aBC}$ | $5.27\pm0.76^{aABC}$ | $4.47\pm0.46^{aA}$ | $5.48\pm0.98^{aB}$ | $4.60\pm0.47^{aB}$ |
| **Hot Water** | $6.44\pm0.83^{aAB}$ | $5.97\pm0.61^{aAB}$ | $6.22\pm1.11^{aAB}$ | $4.28\pm0.98^{bA}$ | $5.79\pm0.39^{abB}$ | $5.48\pm0.94^{abB}$ |

**Note:**

1. Values within the same row with different superscripts letters (a-c) are significantly different at $p < 0.05$.
2. Values within the same column with different superscripts letters (A-D) are significantly different at $p < 0.05$.

The combinationof water and organic solvent facilitates the extraction of chemicals that are soluble in water and/or organic solvent (Chan *et al.,* 2014). Highest extraction yield was reflected in freeze dried (FD) ethanol extracts (7.70 ± 0.36%) while lowest yield was exhibited in the sun dried (SD) ethyl acetate extracts (1.73 ± 0.15%). Ethyl acetate showed significantly lower extraction yield among different dried extracts while that ethanol, methanol and hot water extracts were almost 3 times higher that ethyl acetate extracts. The method of drying on *S. polycystum*prior to extraction seemed not much influenced ($p > 0.05$) to the extraction yield, except for SD extracts which showed lower yield as compared to other solvent. The difference found in SD might be due to different mechanism of drying where direct sun drying involved heat transfer through radiation compared to convective drying. Sun drying prolonged exposure to air may leads to leach of potential bioactive compounds such as phenolics and flavonoids in seaweedsprocessing (Ling *et al.,* 2014).

*Total Phenolic Content*

Total phenolic content (TPC) of *S. polycyctum* ranged from 22.76 - 58.54 mg phloroglucinol equivalent (PGE) $g^{-1}$ (Table 2). The order of TPC by various solvent in descending order are as follows: ethyl acetate (39.94 - 58.54 mg PGE $g^{-1}$) > ethanol (26.16 - 32.96 mg PGE $g^{-1}$) > methanol (25.12 - 31.70 mg PGE $g^{-1}$) > acetone (24.05 - 30.05 mg PGE $g^{-1}$) > hot water (26.56 - 38.40 mg PGE $g^{-1}$) > cold water (22.76 - 31.15 mg PGE $g^{-1}$). From this study, highest TPC was found in VD 40 ethyl acetate extracts (58.54 ± 1.30 mg PGE $g^{-1}$) and OD 40 ethyl acetate extracts (56.30 ± 1.38 mg PGE $g^{-1}$), whereas lowest TPC was in SD cold water extracts (22.76 ± 0.77 mg PGE $g^{-1}$) and SD acetone extracts (24.05 ± 0.98 mg PGE $g^{-1}$).

**Table 2.** Total Phenolic Content of *Sargassum polycystum* under different drying treatments by various solvents

| Solvent | TPC Content (mg PGE $g^{-1}$ DE) | | | | | |
|---|---|---|---|---|---|---|
| | Freeze Dried (FD) | Oven Dried 40°C (OD40) | Oven Dried 60°C (OD60) | Sun Dried (SD) | Vacuum Dried 40°C (VD40) | Vacuum Dried 60°C (VD60) |
| **Ethanol** | $29.54\pm0.68^{bB}$ | $32.30\pm0.49^{aBC}$ | $30.47\pm1.18^{bBC}$ | $26.16\pm0.47^{cB}$ | $32.96\pm0.64^{aC}$ | $31.96\pm1.00^{aC}$ |
| **Methanol** | $28.69\pm1.08^{cB}$ | $31.26\pm0.58^{aCD}$ | $29.71\pm0.95^{bcC}$ | $25.12\pm0.78^{dBC}$ | $31.70\pm0.43^{aCD}$ | $30.75\pm0.63^{abCD}$ |
| **Acetone** | $27.42\pm0.96^{cBC}$ | $30.29\pm0.51^{aD}$ | $28.45\pm0.86^{bcC}$ | $24.05\pm0.98^{dCD}$ | $30.65\pm0.46^{aDE}$ | $29.68\pm0.42^{abE}$ |
| **Ethyl Acetate** | $48.60\pm1.50^{dA}$ | $56.30\pm1.38^{abA}$ | $52.44\pm1.53^{cA}$ | $39.94\pm1.33^{eA}$ | $58.54\pm1.30^{aA}$ | $54.01\pm0.70^{bcA}$ |
| **Cold Water** | $25.33\pm1.06^{cC}$ | $28.34\pm1.15^{bE}$ | $25.91\pm1.02^{cD}$ | $22.76\pm0.77^{dD}$ | $29.56\pm0.81^{abE}$ | $31.15\pm1.37^{aCD}$ |
| **Hot Water** | $29.62\pm1.60^{bB}$ | $33.36\pm0.90^{bB}$ | $32.10\pm1.33^{bB}$ | $26.56\pm0.74^{dB}$ | $38.40\pm1.15^{aB}$ | $37.09\pm1.27^{aB}$ |

**Note:**

1. Values within the same row with different superscripts letters (a-e) are significantly different at $p < 0.05$.
2. Values within the same column with different superscripts letters (A-E) are significantly different at $p < 0.05$.

High TPC content in ethyl acetate extracts were in accordance with previous finding by Duan *et al.* (2006). Hot water extraction has also been suggested to be better than cold water extraction, as there would be a release of phenolic cell wall in the case of hot water extraction (Toor & Savage, 2006). The high temperature from hot water also increases the solubility of phenols by increasing the number of compounds with free hydroxyl groups and caused possible breakdown of tannin to simple phenols (Oboh, 2005; Amin *et al.,* 2006). During high temperature drying, reductions in TPC can be due to the binding of polyphenols to other compounds (proteins) or to changes in the

chemical structure of polyphenols that cannot be isolated and calculated by the current methods of extraction (Martín-Cabrejas*et al.*, 2009; Qu *et al.*, 2010). Sun-dried extracts displayed the significantly lower TPC content as compared to other drying methods. Degradation of the total phenolic contents increases prominently due to the fact that processing takes place in an open air and the presence of heat and oxygen favored enzymatic activity of polyphenol oxidase (Ling *et al.*, 2014).

*Total Flavonoid Content*

Total flavonoid content (TFC) of *S. polycystum*extracts ranged from 7.30 - 35.91 mg rutin equivalent (RE) $g^{-1}$ (Table 3). It can be observed that TFC varied with different solvent extractions and different drying methods. The order of extractability of flavonoids in descending order are as follows: ethyl acetate (28.26 - 35.91 mg RE $g^{-1}$) > ethanol (16.20 - 26.96 mg RE $g^{-1}$) > methanol (13.62 - 24.16 mg RE $g^{-1}$) > hot water (11.50 - 23.54 mg RE $g^{-1}$) > acetone (2.13 - 4.51 mg RE $g^{-1}$) > cold water (7.30 - 19.58 mg RE $g^{-1}$). The VD40 ethyl acetate extract exhibited the highest TFC, 35.91 ± 1.54 mg RE $g^{-1}$ while lowest retention inSD cold water extract, 7.30 ± 1.47 mg RE $g^{-1}$ and SD acetone extract, 9.28 ± 1.28 mg RE $g^{-1}$.

**Table 3.** Total Flavonoid Content of *Sargassum polycystum* under different drying treatments by various solvents

| Solvent | TFC Content (mg RE $g^{-1}$ DE) | | | | | |
|---|---|---|---|---|---|---|
| | Freeze Dried (FD) | Oven Dried 40°C (OD40) | Oven Dried 60°C (OD60) | Sun Dried (SD) | Vacuum Dried 40°C (VD40) | Vacuum Dried 60°C (VD60) |
| **Ethanol** | $17.89\pm0.95^{cB}$ | $25.04\pm1.36^{abB}$ | $23.38\pm0.71^{bB}$ | $16.20\pm0.85^{cB}$ | $26.96\pm0.84^{aB}$ | $26.08\pm1.55^{aB}$ |
| **Methanol** | $16.67\pm0.48^{cBC}$ | $23.34\pm0.40^{aBC}$ | $21.49\pm1.26^{bB}$ | $13.62\pm1.29^{dBC}$ | $24.06\pm1.08^{aC}$ | $24.16\pm0.17^{aBC}$ |
| **Acetone** | $15.19\pm0.61^{cC}$ | $18.03\pm1.45^{bD}$ | $18.54\pm0.78^{bC}$ | $9.28\pm1.28^{dDE}$ | $22.58\pm1.67^{aC}$ | $17.34\pm1.24^{bcD}$ |
| **Ethyl Acetate** | $30.93\pm1.55^{bcA}$ | $32.53\pm0.98^{bA}$ | $30.64\pm1.73^{bcA}$ | $28.26\pm1.85^{cA}$ | $35.91\pm1.54^{aA}$ | $32.69\pm0.55^{bA}$ |
| **Cold Water** | $12.12\pm0.92^{cD}$ | $16.33\pm1.08^{bD}$ | $17.51\pm1.06^{abC}$ | $7.30\pm1.47^{dE}$ | $19.58\pm0.86^{aD}$ | $17.60\pm1.52^{abD}$ |
| **Hot Water** | $15.94\pm0.53^{cC}$ | $22.88\pm0.78^{abC}$ | $21.26\pm1.33^{bB}$ | $11.50\pm1.74^{dCD}$ | $22.64\pm0.35^{abC}$ | $23.54\pm0.76^{aC}$ |

**Note:**

1. Values within the same row with different superscripts letters (a-d) are significantly different at $p < 0.05$.
2. Values within the same column with different superscripts letters (A-E) are significantly different at $p < 0.05$.

Vacuum drying (VD) at 40 °C showed significantly higher ($p < 0.05$) TFC retention than oven dryingat 40 °C in acetone, ethyl acetate and cold water. Vacuum drying is an ideal drying method to heat sensitive samples to dry under sub-atmospheric pressures (Ratti, 2001). The effect of temperature affects the release of flavonoids and increased chemical extraction of flavonoid compounds (Olivera *et al.*, 2008). According to Korus (2011) flavonoid losses could be explained by their oxidation, which might be due to the harsh drying conditions, the temperature and duration used.

*Total Carotenoid Content*

Total carotenoid content (TCC) of *S. polycystum*extracts ranged from 47.21 - 301.38 mg beta carotene equivalent (BE) $g^{-1}$ (Table 4). The TCC varied with different solvent extractions across different drying methods. The order of extractability of carotenoids in descending order are as follows: ethyl acetate (200.43 - 301.38 mg BE $g^{-1}$) > ethanol (99.58 - 149.53 mg BE $g^{-1}$) > hot water (96.48 - 147.03 mg BE $g^{-1}$) > methanol (90.41 - 139.51 mg BE $g^{-1}$) > acetone (50.45 - 99.50 mg BE $g^{-1}$) > cold water (47.21 - 95.50m g BE $g^{-1}$).

**Table 4.** Total Carotenoid Content of *Sargassum polycystum* under different drying treatments by various solvents

| Solvent | TCC Content (mg BE $g^{-1}$ DE) | | | | | |
|---|---|---|---|---|---|---|
| | Freeze Dried (FD) | Oven Dried 40°C (OD40) | Oven Dried 60°C (OD60) | Sun Dried (SD) | Vacuum Dried 40°C (VD40) | Vacuum Dried 60°C (VD60) |
| **Ethanol** | $149.53\pm0.89^{aB}$ | $120.35\pm0.81^{dB}$ | $117.27\pm0.68^{eB}$ | $99.58\pm0.81^{fB}$ | $143.90\pm0.78^{bB}$ | $125.17\pm0.43^{cB}$ |
| **Methanol** | $139.51\pm0.91^{aD}$ | $110.61\pm0.89^{dD}$ | $107.10\pm0.85^{eD}$ | $90.41\pm0.88^{fD}$ | $134.39\pm0.58^{bD}$ | $116.38\pm0.61^{cD}$ |
| **Acetone** | $99.50\pm0.76^{aE}$ | $67.72\pm0.82^{dE}$ | $60.42\pm0.87^{eE}$ | $50.45\pm0.71^{fE}$ | $93.60\pm0.66^{bE}$ | $72.25\pm0.73^{cE}$ |
| **Ethyl Acetate** | $301.38\pm0.82^{aA}$ | $234.70\pm0.84^{dA}$ | $220.47\pm0.83^{eA}$ | $200.43\pm0.65^{fA}$ | $294.37\pm0.85^{bA}$ | $241.39\pm0.69^{cA}$ |
| **Cold Water** | $95.50\pm0.93^{aF}$ | $64.41\pm0.65^{dF}$ | $53.61\pm0.64^{eF}$ | $47.21\pm0.80^{fF}$ | $89.47\pm0.68^{bF}$ | $69.36\pm0.93^{cF}$ |
| **Hot Water** | $147.03\pm0.86^{aC}$ | $116.33\pm0.81^{dC}$ | $115.44\pm0.82^{dC}$ | $96.48\pm0.78^{eC}$ | $140.39\pm0.84^{bC}$ | $121.43\pm0.76^{cC}$ |

**Note:**

1. Values within the same row with different superscripts letters (a-f) are significantly different at $p < 0.05$.
2. Values within the same column with different superscripts letters (A-F) are significantly different at $p < 0.05$.

In all solvent systems, the TCC of freeze-dried extract has a substantially higher ($p < 0.05$) content that may be associated with the development of ice crystals within the seaweed matrix during pre-freezing, that may also cause cell destruction leading to increased release of cellular components (Chan *et al.*, 2009). In the freeze dryer, the low temperatures and oxygen-free atmosphere reduce carotene degradation. Carotenes can be either converted into other carotenoids in other thermal drying processes or fully degraded during drying (Ghafoor *et al.*, 2020). Vacuum dried showed significantly ($p < 0.05$) higher retention of TCC as compared to oven drying treatment. Beta-carotene is a very reactive compound due to its highly unsaturated structure, which renders it electronically rich by delocalization of $\pi$-electrons. Consequently, it is also prone to degradation and more precisely to isomerization, especially at high temperature, and oxidation, due to the occurrence of oxygen in food (Wani *et al.*, 2020).

*Ferric Reducing Ability Power (FRAP)*

The ferric reducing ability of *S. polycystum*extracts ranged from 162.50-379.41 μM TE $mg^{-1}$ (Table 5). The order of reducing ability in descending order are as follows: ethyl acetate (318.733 - 379.41 μM TE $mg^{-1}$) > ethanol (261.07 - 303.92 μM TE $mg^{-1}$) > methanol (245.37 - 296.70 μM TE $mg^{-1}$) > acetone (246.34 - 287.95 μM TE $mg^{-1}$) > hot water (246.33 - 295.63 μM TE $mg^{-1}$) > cold water (221.43 - 271.96 μM TE $mg^{-1}$). In the FRAP assay, the highest antioxidant activities were detected in VD 40 and OD 40 ethyl acetate extractwhile lowest value was found in SD cold water extract, 221.43 ± 4.55 μM TE$mg^{-1}$.

Generally, drying at lower temperature under vacuum condition VD 40, *S. polycystum* extract showed significant ($p < 0.05$) higher reducing ability than OD 60 and VD 60 extracts except in hot water extracts and VD 60 ethyl acetate extract. This discovery may be because oven drying was done at atmospheric pressure instead of under vacuum, so the use of vacuum enabled water to evaporate at lower temperatures and thereby minimized potential oxidation without air from the drying setting, contributing to the preservation of antioxidant components. For VD 40 and VD 60 ethyl acetate extract there were no significant difference, this might indicate that the lipophilic compound responsible for antioxidant activity was not affected by drying temperature but more prone to

oxidation at high temperature as oven drying ethyl acetate extract at 60 °C exhibited lower antioxidant activity. Yen *et al.* (1993) and Siddhuraju *et al.* (2002) documented that the reduction power could be widely ascribed to the antioxidant activity-associated bioactive compounds. These indicate that phenolics, flavonoids, carotenoids and other bioactive compounds, present in VD 40 *S. polycystum* extract, are good electron donors and could terminate the radical chain reactions by converting free radicals to more stable products. SD extracts revealed lowest reducing ability among all extracts. Slow drying rate possibly increased the leaching effect and extended the duration of seaweed exposure to air.

**Table 5.** Ferric Reducing Ability Power (FRAP) of *Sargassum polycystum* under different drying treatments by various solvents

| Solvent | FRAP values (μM TE $g^{-1}$ DE) | | | | | |
|---|---|---|---|---|---|---|
| | Freeze Dried (FD) | Oven Dried 40°C (OD40) | Oven Dried 60°C (OD60) | Sun Dried (SD) | Vacuum Dried 40°C (VD40) | Vacuum Dried 60°C (VD60) |
| **Ethanol** | 278.24±3.77 cB | 288.76±6.63 bB | 274.05±4.34 cB | 261.07±3.58 dB | 303.92±4.50 aB | 290.51±4.26 bB |
| **Methanol** | 274.89±6.54 bcB | 279.29±10.31 bC | 264.05±6.79 cC | 245.37±6.93 dC | 296.70±5.10 aBC | 272.06±5.47 bcC |
| **Acetone** | 257.25±2.62 bC | 276.15±2.54 abC | 263.49±4.85 bC | 246.34±5.26 cC | 287.95±6.81 aC | 263.05±4.66 bcCD |
| **Ethyl Acetate** | 356.43±5.00 bcA | 369.55±7.52 aA | 349.13±3.26 cA | 318.73±3.58 dA | 379.41±1.17 aA | 367.48±9.96 abA |
| **Cold Water** | 255.02±2.85 cC | 275.53±2.49 cD | 242.37±3.82 cD | 221.43±4.55 dD | 271.96±7.06 aD | 256.67±2.79 bD |
| **Hot Water** | 255.02±2.85 cdD | 275.53±2.49 bC | 261.97±4.86 cC | 246.33±9.25 dC | 295.63±7.52 aBC | 290.51±8.04 bB |

Note:
1. Values within the same row with different superscripts letters (a-d) are significantly different at $p < 0.05$.
2. Values within the same column with different superscripts letters (A-D) are significantly different at $p < 0.05$.

*DPPH Radical Scavenging Activity*

The $EC_{50}$ of *S. polycystum* extracts ranged from 3.83 - 6.69 mg $mL^{-1}$ (Table 6). A lower value of $EC_{50}$ indicates a higher antioxidant activity. The order of $EC_{50}$ in ascending order are as follows: ethyl acetate (3.83 - 4.70 mg $mL^{-1}$) < hot water (5.02 - 5.99 mg $mL^{-1}$) < ethanol (5.07 - 5.91 mg $mL^{-1}$) < methanol (5.23 - 5.98 mg $mL^{-1}$) < acetone (5.42 - 6.15 mg $mL^{-1}$) < cold water (5.72 - 6.69 mg $mL^{-1}$). The highest radical scavenging activity (RSA) was demonstrated by VD 40 ethyl acetate extracts with $EC_{50}$ value, 3.83 ± 0.18 mg $mL^{-1}$ while poorest RSA was found in FD cold water extracts with $EC_{50}$ value, 6.69 mg $mL^{-1}$.

The highest radical scavenging activity (RSA) was illustrated by VD 40 ethyl acetate extracts with $EC_{50}$ value, 3.83 ± 0.18 mg $mL^{-1}$. Vacuum drying has some distinct features, such as reduced drying temperature and inadequate processing of the oxygen environment FD extracts exhibited poor RSA in acetone, cold water and hot water extracts. In hot water extracts, VD 40 extracts was found to be significantly ($p < 0.05$) higher RSA than OD 60 extracts. Deduction could be made that during drying process at high temperature with the occurrence of oxygen leaching happened and caused the oxidation of compounds.

**Table 6.** DPPH Radical Scavenging Activity of *Sargassum polycystum* under different drying treatments by various solvents

| Solvent | EC $_{50}$ (mg mL$^{-1}$ Dried Extract) | | | | | |
|---|---|---|---|---|---|---|
| | Freeze Dried (FD) | Oven Dried 40°C (OD40) | Oven Dried 60°C (OD60) | Sun Dried (SD) | Vacuum Dried 40°C (VD40) | Vacuum Dried 60°C (VD60) |
| **Ethanol** | 5.64±0.24$^{cB}$ | 5.21±0.30$^{abB}$ | 5.52±0.17$^{bcB}$ | 5.91±0.19$^{dB}$ | 5.07±0.13$^{aB}$ | 5.24±0.12$^{abB}$ |
| **Methanol** | 5.73±0.12$^{bcB}$ | 5.52±0.19$^{abAB}$ | 5.54±0.10$^{abB}$ | 5.98±0.23$^{cAB}$ | 5.23±0.15$^{aB}$ | 5.35±0.24$^{aB}$ |
| **Acetone** | 6.28±0.35$^{cC}$ | 5.95±0.24$^{bcC}$ | 5.65±0.07$^{abB}$ | 6.19±0.16$^{cAB}$ | 5.42±0.36$^{aBC}$ | 5.67±0.16$^{abC}$ |
| **Ethyl Acetate** | 4.25±0.22$^{bcA}$ | 4.00±0.13$^{abA}$ | 4.44±0.21$^{bcA}$ | 4.70±0.21$^{cA}$ | 3.83±0.18$^{aA}$ | 4.25±0.20$^{bcA}$ |
| **Cold Water** | 6.68±0.29$^{aC}$ | 5.90±0.29$^{aC}$ | 5.94±0.12$^{aC}$ | 6.39±0.37$^{aC}$ | 5.72±0.25$^{aB}$ | 6.41±1.18$^{aC}$ |
| **Hot Water** | 6.24±0.34$^{cC}$ | 5.23±0.25$^{abB}$ | 5.47±0.16$^{bB}$ | 5.99±0.11$^{cAB}$ | 5.02±0.12$^{aC}$ | 5.15±0.16$^{abB}$ |

Note:
1. Values within the same row with different superscripts letters (a-d) are significantly different at $p < 0.05$.
2. Values within the same column with different superscripts letters (A-C) are significantly different at $p < 0.05$.

*Beta Carotene Bleaching Assay*

The $EC_{50}$ of *S. polycystum* extracts ranged from 5.49 - 14.20 mg mL$^{-1}$ (Table 7). The lower the $EC_{50}$ indicated, the better the seaweed extract ability to prevent the discoloration of the beta-carotene. The following hierarchy for inhibition, $EC_{50}$ in ascending order are as follows: ethanol (3.83 - 5.22 mg mL$^{-1}$) < methanol (3.97 - 5.52 mg mL$^{-1}$) < ethyl acetate (4.64 - 5.75 mg mL$^{-1}$) < acetone (5.04 - 6.33 mg mL$^{-1}$) < hot water (8.32 - 9.24 mg mL$^{-1}$) < cold water (9.48 - 10.57 mg mL$^{-1}$).

**Table 7.** Beta Carotene Bleaching Assayof *Sargassum polycystum* under different drying treatments by various solvents

| Solvent | EC $_{50}$ (mg mL$^{-1}$ DE) | | | | | |
|---|---|---|---|---|---|---|
| | Freeze Dried (FD) | Oven Dried 40°C (OD40) | Oven Dried 60°C (OD60) | Sun Dried (SD) | Vacuum Dried 40°C (VD40) | Vacuum Dried 60°C (VD60) |
| **Ethanol** | 4.45±0.34$^{bcA}$ | 4.50±0.33$^{bcA}$ | 4.84±0.19$^{cdA}$ | 5.22±0.27$^{dA}$ | 3.83±0.21$^{aA}$ | 4.20±0.22$^{abA}$ |
| **Methanol** | 4.70±0.44$^{bAB}$ | 4.68±0.19$^{bA}$ | 4.95±0.12$^{bcA}$ | 5.52±0.39$^{cAB}$ | 3.97±0.43$^{aA}$ | 4.40±0.27$^{abA}$ |
| **Acetone** | 5.54±0.36$^{aC}$ | 5.41±0.29$^{aB}$ | 5.52±0.35$^{aB}$ | 6.33±0.27$^{bC}$ | 5.04±0.25$^{aB}$ | 5.57±0.31$^{aC}$ |
| **Ethyl Acetate** | 5.21±0.25$^{bcBC}$ | 5.07±0.07$^{bB}$ | 5.45±0.13$^{cdB}$ | 5.75±0.22$^{dB}$ | 4.64±0.13$^{aB}$ | 5.03±0.15$^{bB}$ |
| **Cold Water** | 9.84±0.19$^{aE}$ | 9.74±0.22$^{aD}$ | 9.86±0.14$^{aD}$ | 10.57±0.16$^{bE}$ | 9.48±0.38$^{aD}$ | 9.75±0.21$^{aE}$ |
| **Hot Water** | 8.61±0.10$^{abD}$ | 8.49±0.05$^{abC}$ | 8.76±0.22$^{bC}$ | 9.24±0.13$^{cD}$ | 8.32±0.27$^{aC}$ | 8.69±0.20$^{bD}$ |

**Note:**
1. Values within the same row with different superscripts letters (a-d) are significantly different at $p < 0.05$.
2. Values within the same column with different superscripts letters (A-E) are significantly different at $p < 0.05$.

The BCB displayed a distinct pattern of antioxidant activity in comparison to the findings obtained from the DPPH and FRAP assays, with the ethanol extract showing greater inhibition of β-carotene bleaching. This could indicate that there were more antioxidant compounds in the ethanol extract, which could work better in a water-oil emulsion method compared to other extracts (Chan *et al.*, 2014). The highest inhibition was found in VD 40 ethanol extracts $EC_{50}$, 3.83 ± 0.21 mg mL$^{-1}$. This property may be due to the antioxidant components present in the *S. polycystum* extract that can reduce the extent of beta-carotene destruction by neutralizing the linoleate-free radical and other

free radicals formed in the system. VD 40 and VD 60 extracts showed significant ($p<0.05$) higher inhibition capacity than OD 60 extracts using ethanol as solvent.

All drying treatments of *S. polycystum* extracts showed FRAP and DPPH radical scavenging activity with phytochemical content exhibited good correlation with the regression coefficient of R2 > 0.80. The weak relationship of *S. polycystum* extracts with the beta-carotene activity (BCB) implied a relatively weak relationship, $R^2 < 0.30$ was found between TPC, TFC, TCC with BCB assay.The weak relationship of *S. polycystum* extracts with the beta-carotene activity (BCB) implied a relatively weak relationship, $R^2 < 0.30$ was found between TPC, TFC with BCB assay. This could demonstrate that the extracts' antioxidant pathway was preferable to electron transfer rather than hydrogen transfer. In addition, the TPC provides an indicator of both lipophilic and hydrophilic compound levels. On the other hand, the BCB only offers an indicator of the levels of lipophilic compounds.

## CONCLUSIONS

Overall, different drying methods showed effects onTPC, TFC, TCC and antioxidant activity indicating that theyvary according to drying conditions. Some reportssuggested that freeze drying is the most appropriatedrying technique in retaining chemical composition ofseaweeds. However, in this study freeze dried contained significantly higher total carotenoid content. Generally, vacuum dried 40 °C retained higher phytochemicals (TPC and TFC) and antioxidant activity as compared to other drying methods. The effects of solvents shared a general trend across different drying treatments with ethyl acetate exhibited highest phytochemical contents and antioxidant activities in FRAP and DPPH assay; ethanol portrayed highest extraction yield and second-best solvent after ethyl acetate; hot water revealed considerable results in extraction yield, phytochemicals and antioxidant with organic solvents.

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