

## ***In vitro* Antioxidant and Anti-Tyrosinase Activities along with Phytochemical Constituents of Brittle Wort (*Chara corallina*) Extracts**

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### **ABSTRACT**

Brittle wort (*Chara corallina*), a widespread freshwater green macroalga found in southern Thailand, is one of the most widely consumed edible species. Recently, many studies revealed potential of freshwater algae for use as a dietary supplement. This study aimed to screen the phytochemicals and assess the biological activity of *C. corallina* as an anti-tyrosinase and antioxidant, as well as the total phenolic and flavonoid contents. Samples of *C. corallina* was extracted using ethanol and distilled water. Crude extracts were screened for the phytochemicals as well as evaluated for total phenolic content, total flavonoid content, antioxidant and anti-tyrosinase activity. The yields of the aqueous and ethanolic crude extracts were 10.84% and 12.27%, respectively. The phytochemical screening of the ethanolic extract revealed the presence of terpenoids, flavonoids, saponins, and alkaloids, whereas anthraquinones, flavonoids, saponins, and alkaloids were identified in the aqueous extract. Aqueous extract had higher total phenolic content ( $9.3 \pm 0.2$  mg gallic acid equivalent  $\cdot$  mg<sup>-1</sup> crude extract) and total flavonoid content ( $1.9 \pm 0.2$  mg quercetin equivalent  $\cdot$  mg<sup>-1</sup> crude extract) compared to ethanolic extract. Through DPPH and ABTS scavenging activities, the biological activities of *C. corallina* aqueous extract demonstrated its potential as an antioxidant whereby tyrosinase inhibition activities showed an IC<sub>50</sub> of  $231.6 \pm 3.8$   $\mu$ g  $\cdot$  mL<sup>-1</sup> and  $555.8 \pm 12.1$  and  $611.5 \pm 8.7$   $\mu$ g  $\cdot$  mL<sup>-1</sup>, respectively. The present study suggests that *C. corallina* is the potential source of antioxidants and anti-tyrosinase substances and is vital in the development of new skin-lightening substance for the cosmetics industry.

**Keywords:** Bioactive compound, *Chara corallina*, Cosmetic application, Freshwater algae

### **INTRODUCTION**

Bioactive chemicals are natural or synthetic components that have recently received a lot of attention due to their many health-promoting properties (Ratana-Arporn and Chirapart, 2006; Ahn *et al.*, 2007; Choudhary *et al.*, 2021). The phytochemical components present in the plant, such as anthraquinones, terpenoids, flavonoids, saponins, tannins, and alkaloids, can unveil the plant's therapeutic physiological potential, showcasing notable antioxidative, antibacterial, anti-inflammatory, anti-tyrosinase, antiviral, and anti-aging properties (Khongsai and Vittaya, 2020; Irawan *et al.*, 2022).

Concerning biological activity, antioxidant characteristics assist organisms in dealing with oxidative stress generated by free radical damage, including protecting the skin from oxidative stresses, while anti-tyrosinase capabilities aid in skin whitening. All antioxidant and anti-tyrosinase compounds can protect the skin from being induced by UV radiation (Sari *et al.*, 2019). Antioxidants are widely incorporated into a variety of anti-aging skin care systems, accounting for the majority of the skin health substances in cosmeceutical products. The primary function of anti-tyrosinase is to decrease skin pigmentation by preventing the enzyme catalysis to pigmentation relating to the formation of melanin

in the melanogenesis process. Excessive melanin or hyperpigmentation cause darker or uneven skin tone, age spots, and melisma (Dej-adisai *et al.*, 2016). Therefore, numerous tyrosinase inhibitors are involved in removal of pigment treatments and whitening cosmetics, whereas substances that encourage pigmentation may protect the skin of humans from the effects of ultraviolet irradiation (Miyazawa and Tamura, 2007).

Nowadays, macroalgae are widely used as a natural source of bioactive substances that have a significant impact on the development of nutraceuticals. Macroalgae differ substantially in relation to species, age, habitat, harvesting period and environmental conditions, providing the difference in nutritional profile and functionality. The species, temperature and UV radiation exposure are all important elements in the development of biological compounds in macroalgae. The biological substances found in macroalgae are also increasingly being utilized in biochemical and medicinal research (Setthamongkol *et al.*, 2015; Choudhary *et al.*, 2021; Klomjit *et al.*, 2021). Several studies have been reported on seaweed-derived compounds with beneficial properties for reducing skin aging, indicating that they should be included in cosmetic formulations (Ahn *et al.*, 2007; Choi *et al.*, 2013; Tomas and Kim, 2013; Chang and Teo, 2016; Kim *et al.*, 2019; Sari *et al.*, 2019; Arguelles and Sapin, 2020; Arguelles, 2021; Choosuwan *et al.*, 2023).

Both freshwater and marine algae are abundant in pharmacologically active metabolites (Messyasz *et al.*, 2018; Choudhary *et al.*, 2021). Natural compounds also contain antioxidant and antiprotease activity, which is beneficial in preventing skin aging (Arguelles and Sapin, 2020; Arguelles, 2021). The existence of bioactive secondary metabolites with biological activities has been identified in many macroalgae because they include active biological phytochemicals. Terpenoids, saponins, steroids, alkaloids, flavonoids, and tannins are some of the biologically active substances found in the extracts, which could be useful in cosmetics. For examples, the methanolic extracts of red marine algae (*Eucheuma cottonii*) and brown marine algae (*Sargassum plagyophyllum*) have strong tyrosinase inhibitory activity, the

acetone extract of brown marine algae (*Ascophyllum nodosum*) exhibits antioxidant, tyrosinase inhibition, and antibacterial properties and the aqueous extract of the brown seaweed (*Hydroclathrus clathratus*) has strong antiviral activity (Kantachumpoo and Chirapart, 2010; Taş *et al.*, 2015; Arguelles and Sapin, 2020).

Despite the lack of data on the phytochemical and bioactivity of freshwater algae (Shah *et al.*, 2022), there has been an increase in research on the algae, particularly with its characterization as natural antioxidants for nutrients or therapeutic materials to replace synthetic antioxidants in cosmetic product formulation (Messyasz *et al.*, 2018). The cosmetic business is now working on producing novel skincare items that satisfy the requirements and preferences of customers by using affordable, sustainable, and natural raw materials that include a variety of bioactive compounds as a source of active chemicals (Ghazala and Shameel, 2005). Green freshwater algae such as *Chara vulgaris*, *Cladophora glomerata*, and *Spirogyra crassa* contained some different valuable compounds with cosmeceutical, agricultural importance, nutraceutical and medicinal (Shah *et al.*, 2022) while *C. glomerata* contains amino acids, fatty acids, and other bioactive compounds due to its high concentration of chlorophyll *a* and carotenoids (Messyasz *et al.*, 2018). *Chara globularis* was found to possess insecticidal chemicals, methanolic and ethanolic extract of *C. wallichii*, as well as isolated sterols, which demonstrated considerable phytotoxic action against *Lemna aequinoctialis*, and *Chara contraria* had strong anticancer activity including methanolic extract and ethyl acetate-soluble portions of *C. corallina*, which shown antimicrobial activity against gram-positive and gram-negative bacteria while four sterols extracted of *C. corallina* demonstrated high antifungal activity against a variety of fungal species (Ghazala and Shameel, 2005).

The current study was designed to screen phytochemical constituents from ethanolic and aqueous extracts of green algae *C. corallina* and determine their total phenolic and flavonoid contents. The *in vitro* anti-tyrosinase of the extracts were further evaluated as it possesses anti-oxidative

activity. To improve their benefits for cosmetic applications, the correlation of the potential anti-tyrosinase and antioxidant activities of extracts to their phytochemical constituents was also validated using qualitative analysis.

## MATERIALS AND METHODS

### *Collection of macroalgae*

The freshwater macroalga, *Chara corallina*, (Figure 1) was obtained from Lamtab district, Krabi Province, Thailand. The collected macroalgae were rinsed many times with freshwater to remove any extraneous matter such as epiphytes and other contaminants. Before extraction, the cleaned macroalgae were air-dried for 4 days, chopped into small portions, and crushed into a fine-grained powder.

### *Macroalgae extraction*

Two kg of the powdered macroalgae were soaked three times with 5 L of 95% ethanol and extracted for 3 days, shaking occasionally, to obtain the ethanolic extract. To obtain aqueous extract, another 2 kg of powdered macroalgae was boiled in distilled water for 1 h. The mixture was filtered after

extraction to separate the residue and supernatant. A rotary evaporator (Laborota 4000 efficient rotary evaporator; Heidolph instruments GmbH & Co. KG, Germany) was then used to evaporate the supernatant under vacuum at 40 °C. Both extracts were stored in tight and light-resistant containers at 4 °C in order to preserve effectiveness before being used in an alternative experiment.

### *Phytochemical screening tests*

Phytochemical screening tests of anthraquinones, terpenoids, flavonoids, saponins, tannins, and alkaloids from *C. corallina* extracts were performed according to recent standardized methods as described by Khongsai and Vittaya (2020).

### *Total phenolic content (TPC)*

TPC determination of *C. corallina* extracts was performed by the Folin-Ciocalteu method based on a protocol modified by Messyasz *et al.* (2018). The absorbance of the mixture was compared to the gallic acid calibration curve with a concentrations range from 0 to 80  $\mu\text{g}\cdot\text{mL}^{-1}$  ( $y = 0.1004x + 0.0444$ ;  $r^2 = 0.9982$ ). Each sample was prepared and measured in triplicate. The results of TPC were presented as mg of gallic acid equivalent (GAE) per mg of crude extract.



Figure 1. *Chara corallina*.

### Total flavonoid content (TFC)

TFC determination of *C. corallina* extracts was performed by the aluminum chloride method based on a protocol modified by Messyas *et al.* (2018). The absorbance of the mixture was compared to a quercetin calibration curve with a concentrations range from 0 to 1 mg·mL<sup>-1</sup> ( $y = 0.0598x + 0.0073$ ;  $r^2 = 0.9983$ ). Each sample was prepared and measured in triplicate. The results of TFC were presented as mg of quercetin equivalent (QE) per mg of crude extract.

### Antioxidant activities

#### 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity

The scavenging activity of *C. corallina* extracts was determined using DPPH (Sigma Aldrich, USA) assay according to a methodology modified from Ling *et al.* (2015). The 500 µL of sample solution with variable concentrations (0–10 mg·mL<sup>-1</sup>) were mixed with 500 µL of 0.01 mM DPPH in absolute ethanol. The mixture was vigorously shaken and kept in the dark for 30 min at room temperature. The absorbance of the mixture was measured using a UV-Vis Spectrophotometer at 517 nm. Ascorbic acid (0–10 µg·mL<sup>-1</sup>) was used as the positive control. The absorbance of blank sample containing the same amount of ethanol and DPPH was measured as a control. Each sample was prepared and measured in triplicate. The free-radical scavenging activity was calculated as follows:

$$\text{DPPH inhibition (\%)} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

where  $A_{\text{control}}$  is the absorbance of control;  $A_{\text{sample}}$  is the absorbance of the sample.

DPPH inhibition percentage of *C. corallina* extracts was plotted and the results were expressed as IC<sub>50</sub> value which is described as the concentration (mg·mL<sup>-1</sup>) of extract that can exhibit 50% scavenging of the DPPH radical.

#### 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) free radical scavenging activity

The scavenging activity of *C. corallina* extracts was determined using ABTS assay according to a methodology modified from Thring *et al.* (2009). The 7 mM of ABTS<sup>+</sup> was mixed with 2.45 mM of K<sub>2</sub>SO<sub>4</sub> in a ratio of 1:0.5 and then left to stand in the dark for 12–16 hrs. This stock solution was then diluted in ethanol to give an absorbance of 0.70±0.02 at 734 nm. The 100 µL of sample solution with variable concentrations (0–10 mg·mL<sup>-1</sup>) was mixed with 10 mL of stock solution. The mixture was vigorously shaken allowed to stand at room temperature for 6 min. The absorbance of the mixture was measured using a UV-Vis Spectrophotometer at 734 nm. Ascorbic acid (0–10 µg·mL<sup>-1</sup>) was used as the positive control. The absorbance of the blank sample containing the same amount of ethanol and stock solution was measured as a control. Each sample was prepared and measured in triplicate. The free-radical scavenging activity was calculated as follows:

$$\text{ABTS inhibition (\%)} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

where  $A_{\text{control}}$  is the absorbance of control;  $A_{\text{sample}}$  is the absorbance of the sample.

ABTS inhibition percentage of *C. corallina* extracts was plotted and the results were expressed as IC<sub>50</sub> value which is described as the concentration (mg·mL<sup>-1</sup>) of extract that can exhibit 50% scavenging of the ABTS radical.

#### Anti-tyrosinase activity

The tyrosinase inhibitory activity of *C. corallina* extracts were determined by tyrosinase inhibitory assays according to a methodology modified from Laosirisathian *et al.* (2020) and Rangkadilok *et al.* (2007). The 100 µL of the test sample (0–1 mg·mL<sup>-1</sup>), 50 µL of 200 unit·mL<sup>-1</sup> tyrosinase enzyme, 100 µL of methanol with a pH of 6.8, and 50 µL of 1 mg·mL<sup>-1</sup> of tyrosine were

mixed and incubated for 60 min at room temperature. The absorbance of the mixture was measured using a microplate reader at 450 nm. Kojic acid (0–10  $\mu\text{g}\cdot\text{mL}^{-1}$ ) was used as the positive control. Each sample was prepared and measured in triplicate. The tyrosinase inhibitory activity was calculated as follows:

$$\text{Tyrosinase inhibition (\%)} \\ = [(A-B)-(C-D)/(A-B)] \times 100$$

where A is the absorbance of methanol, 200  $\text{unit}\cdot\text{mL}^{-1}$  of the tyrosinase enzyme and tyrosine; B is the absorbance of methanol, 200  $\text{unit}\cdot\text{mL}^{-1}$  of the tyrosinase enzyme and tyrosine and methanol with pH of 6.8; C is the absorbance of sample, 200  $\text{unit}\cdot\text{mL}^{-1}$  of the tyrosinase enzyme and tyrosine; D is the absorbance of sample, 200  $\text{unit}\cdot\text{mL}^{-1}$  of the tyrosinase enzyme and methanol with pH of 6.8.

The tyrosinase inhibition percentage of *C. corallina* extracts was plotted and the results were expressed as  $\text{IC}_{50}$  value which is described as the concentration ( $\text{mg}\cdot\text{mL}^{-1}$ ) of extract that can exhibit 50% inhibition of tyrosinase enzyme.

#### Statistical analysis

The results were presented as mean  $\pm$  standard deviation (SD) based on triplicate observations. Statistical analysis was performed by t-test for TPC and TFC. The antioxidant activities and anti-tyrosinase activities were evaluated by one-way analysis of variance (ANOVA), followed by Duncan's new multiple range test with a statistically

significant level set at  $p < 0.05$  employing SPSS 22 for Windows. The correlation between TPC, TFC, antioxidant, and anti-tyrosinase activities were analyzed using Pearson's method.

## RESULTS

### Phytochemical screening

The yields of ethanolic and aqueous crude extracts of *Chara corallina* were 12.27% and 10.84%, respectively. The presence of terpenoids, flavonoids, saponins, and alkaloids were identified in the preliminary phytochemical screening of the ethanolic extract, whereas anthraquinones, flavonoids, saponins, and alkaloids were also identified in the screening of the aqueous extract (Table 1).

### TPC and TFC

TPC and TFC were significantly affected by the extraction solvent. The aqueous extract showed a higher TPC and TFC than the ethanolic extract,  $p < 0.05$  (Table 2).

### DPPH and ABTS free radical scavenging activities

The antioxidant activities of *C. corallina* extracts are expressed in terms of  $\text{IC}_{50}$  (Table 3). Aqueous extract presented higher DPPH ( $\text{IC}_{50} = 555.8 \pm 12.1 \mu\text{g}\cdot\text{mL}^{-1}$ ) and ABTS inhibitions ( $\text{IC}_{50} = 611.5 \pm 8.7 \mu\text{g}\cdot\text{mL}^{-1}$ ) than ethanolic acid ( $p < 0.05$ ). However, the antioxidant activities of the two extracts were weaker than those of ascorbic acid (positive control).

Table 1. The preliminary phytochemical screening of *Chara corallina* extracts.

Phytochemical	<i>Chara corallina</i>	
	Ethanolic extract	Aqueous extract
Anthraquinones	-	+
Terpenoids	+	-
Flavonoids	+	+
Saponins	+	+
Tannins	-	-
Alkaloids	+	+

**Note:** Symbols + and – indicate presence and absence of the compounds.



Table 2. The total phenolic and flavonoid contents of *Chara corallina* extracts.

Sample	Total phenolic content (mg GAE·mg <sup>-1</sup> crude extract)	Total flavonoid content (mg QE·mg <sup>-1</sup> crude extract)
Ethanol extract	6.9±0.5 <sup>b</sup>	0.2±0.1 <sup>b</sup>
Aqueous extract	9.3±0.2 <sup>a</sup>	1.9±0.2 <sup>a</sup>

**Note:** GAE and QE are gallic acid equivalent and quercetin equivalent, respectively; Means±SD in the same column superscripted with different lowercase letters are significantly ( $p<0.05$ ) different.

Table 3. The antioxidant activities of *Chara corallina* extracts.

Sample	IC <sub>50</sub> (µg·mL <sup>-1</sup> )	
	DPPH free radical scavenging	ABTS free radical scavenging
Ethanol extract	8,846.5±52.5 <sup>a</sup>	7,527.9±62.5 <sup>a</sup>
Aqueous extract	555.8±12.1 <sup>b</sup>	611.5±8.7 <sup>b</sup>
Ascorbic acid	6.6±0.1 <sup>c</sup>	8.5±0.3 <sup>c</sup>

**Note:** IC<sub>50</sub> = inhibitory concentration at 50%; DPPH = 2,2-diphenyl-1-picrylhydrazyl; ABTS = 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt; Means±SD in the same column superscripted with different lowercase letters are significantly ( $p<0.05$ ) different.

#### Anti-tyrosinase activity

The anti-tyrosinase activity of the extracts is expressed in terms of IC<sub>50</sub> (Table 4). The aqueous extract presented significant higher tyrosinase inhibition than ethanol extract ( $p<0.05$ ). However, the anti-tyrosinase activity of the two extracts were weaker than those of kojic acid (positive control).

#### Relationships among the observed parameters

Highly significant relationships were observed in all pairs of observed variables (Table 5). Positive relationships were found between TFC and TPC, tyrosinase inhibition and DPPH inhibition, ABTS inhibition and DPPH inhibition, and tyrosinase inhibition and ABTS inhibition, providing correlation coefficients ( $r$ ) ranging from 0.961 to 1. The other remaining pairs showed highly negative relationships ( $r = -0.967$  to  $-0.992$ ).

Table 4. The anti-tyrosinase activity of *Chara corallina* extracts.

Sample	IC <sub>50</sub> (µg·mL <sup>-1</sup> )
Ethanol extract	435.0±12.7 <sup>a</sup>
Aqueous extract	231.6±3.8 <sup>b</sup>
Kojic acid	5.9±0.8 <sup>c</sup>

**Note:** Means±SD in the same column superscripted with different lowercase letters are significantly ( $p<0.05$ ) different.

Table 5. Correlation coefficient (r) between TPC, TFC, antioxidant activities, and anti-tyrosinase activity ( $p < 0.01$ ).

Variable	TPC	TFC	DPPH inhibition (IC <sub>50</sub> )	ABTS inhibition (IC <sub>50</sub> )	Tyrosinase inhibition (IC <sub>50</sub> )
TPC	1	-	-	-	-
TFC	0.961	1	-	-	-
DPPH inhibition (IC <sub>50</sub> )	-0.967	-0.985	1	-	-
ABTS inhibition (IC <sub>50</sub> )	-0.970	-0.984	1	1	-
Tyrosinase inhibition (IC <sub>50</sub> )	-0.992	-0.977	0.991	0.993	1

**Note:** TPC = Total phenolic content; TFC = Total flavonoid content; DPPH inhibition (IC<sub>50</sub>) = 50% inhibition concentration of DPPH radical free radical; ABTS inhibition (IC<sub>50</sub>) = 50% inhibition concentration of ABTS radical free radical; Tyrosinase inhibition (IC<sub>50</sub>) = 50% inhibition concentration of tyrosinase enzyme

## DISCUSSION

The results revealed the existence of common groups of secondary metabolites such as phenol and flavonoid compounds in *Chara corallina* extracts and the aqueous extract with a high TPC and TFC has higher antioxidant and anti-tyrosinase activity than ethanolic extract. TPC and TFC of the extract are primarily reliant on the polarity of the extractant used during the extraction process, particularly polyphenols that are highly soluble in polar solvents (Arguelles and Sapin, 2020). One possible explanation is that the chemical constituents of *C. corallina* extracts dissolve better in water than in ethanol. It should be emphasized, however, that the observed free radical scavenging activity could also be attributable to the presence of antioxidant capacity (Messyas et al., 2018).

TPC and TFC are a significant group in connection to biological activity, particularly antioxidant capabilities. Although flavonoids are classified as polyphenolic substances, their chemical structure is principally composed of two phenyl rings and a heterocyclic ring that can be substituted with hydroxyl and methyl groups at various positions (Ghazala and Shameel, 2005). The suggested approach is explained by the ability to provide hydroxyl hydrogen and resonance stabilization from the resultant antioxidant radicals (Laosirisathian et al., 2020). Among their many biological attributes, polyphenols have a potent capacity to scavenge free radicals; as a result, they have notable antioxidant

activity (Shah et al., 2022). It might happen as a result of reducing ability, free radical binding, metal ion chelation, free radical stabilization, and oxidase inhibition (Ling et al., 2015). This is because the extract not only has antioxidant activity but probably contain a variety of biologically active components such as anticancer, antitumor and antiviral activity (Choudhary et al., 2021). In the present study, the extracts exhibited lower antioxidant activity compared to standard antioxidants, such as ascorbic acid, tocopherol, quercetin, butylated hydroxyanisole and butylated hydroxytoluene. The conventional antioxidants proved to be more effective in radical scavenging capabilities than freshwater algal extracts. This difference may be attributed to the likelihood that the algal extracts contain various biologically active substances along with antioxidant compounds (Taş et al., 2015).

The study of antioxidant activities revealed that both extracts inhibited DPPH and ABTS free radicals. The extract with the lowest IC<sub>50</sub> value has the most potential to scavenge free radicals. The aqueous extract was more effective than the ethanolic extract in scavenging DPPH and ABTS free radicals, but less effective than ascorbic acid. The inhibitory activities of both free radicals of aqueous extract were much higher than that of ethanolic extract. Because of the similarities of mechanisms, the ABTS assay showed the same ranking of scavenging activity as the DPPH assay (Pientaweeratch et al., 2016). In this case, IC<sub>50</sub> of the two extracts inhibited ABTS was lower than that of DPPH.

In addition, *C. corallina* extract had significant anti-tyrosinase activity, although its activity was lower than kojic acid. Additionally, the hydroxyl groups found in the phenolic compounds in the *C. corallina* extract can result in the formation of hydrogen bonds to the tyrosinase enzyme active site, resulting in steric hindrances and changes in enzyme conformation that result in decreased activity, which is the reason of the extract's inhibitory effect in this experiment (Arguelles and Sapin, 2020). Furthermore, the inclusion of other recognized tyrosinase inhibitors, such as polyphenols, may play an important role in tyrosinase suppression utilizing *C. corallina* extract. The mechanism that results of antioxidants is skin whitening by inhibiting tyrosine oxidation to quinone dihydroxyphenylalanine and minimizing free radicals in keratinocytes generated by UV exposure. According to the findings, the aqueous extract of *C. corallina* was more powerful as a tyrosinase inhibitor than the ethanolic extract. Furthermore, phenol and flavonoids are secondary metabolites with the ability to inhibit excess melanin synthesis processes. Flavonoids have the ability to directly reduce tyrosinase activity in melanogenic pathways, which contributes to depigmentation. Based on the similarities of the dihydroxyphenyl group in dihydroxyphenyl alanine and the  $\alpha$ -keto containing flavonoids, functional prior research in flavonoids revealed that flavonoids with  $\alpha$ -keto groups possess tyrosinase inhibition activity (Sari *et al.*, 2019).

Based on the results, it is possible to confirm a relationship between the antioxidant activity of the extracts and the abundance of phenolic compounds. The extract with high TPC and TFC exhibited strong radical scavenging activity in DPPH and ABTS. This phenomenon can be attributed to the electron-transfer process, as explained by Pientaweeratch *et al.* (2016). These results align with previous studies, suggesting that the content of phenolic component plays a significant role in the antioxidant activity of natural products (Laosirisathian *et al.*, 2020). It is noteworthy that phenolic compounds are known for their ability to reduce organic matter oxidation by transferring the H atom from the OH group. Therefore, the TPC of the extract directly influences its antioxidant activity (Khongsai and Vittaya, 2020).

The instability of this particular compound group, the age of the sample, the different extraction methods and the different materials are related to different chemical properties. These categories of compounds also possess antioxidant activity, therefore samples enriched in flavonoids and polyphenols may have significant radical scavenging activity in the DPPH assay or any other antioxidant experiment (Ghazala and Shameel, 2005; Choudhary *et al.*, 2021). The results of this study suggest that *C. corallina* extract has a potential to be further developed as a natural source of free radical scavenging phytochemicals. This suggests a promising avenue for mitigating the impact of UV irradiation, a known cause of skin disorders, and reducing skin hyperpigmentation by inhibiting tyrosinase. This metabolite would be beneficially incorporated as a lightening additive in pharmaceutical cosmetics with natural skin-whitening products and used for deeming it as an active ingredient in anti-aging products for its sun protection capabilities (Roy *et al.*, 2013). However, the toxicity of these *C. corallina* extracts should be thoroughly studied before continued use in formulated goods or consumption.

## CONCLUSION

Our recent findings show that the *Chara corallina* extract exhibited both antioxidant and anti-tyrosinase actions, depending on the total phenolic and total flavonoid contents. The aqueous extract of *C. corallina* could be utilized in skin-lightening ingredients for the pharmaceutical and cosmetic industries. Furthermore, the identification and purification of phenolic compounds should be of interest for a better understanding of the mechanisms underlying the active components found in the *C. corallina* extract. The present study also shed more light on the effectiveness of *C. corallina* extract as a phenolic component source.

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