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Original Article

Antioxidant and Tyrosinase Inhibitor Activities of Ethanol Extracts of Brown Seaweed (*Turbinaria conoides*) as Lightening Ingredient

Diani Mega Sari[1], Effionora Anwar[2*], Nurjanah[3], Ayun Erwina Arifianti[4]

ABSTRACT

Background: *Turbinaria conoides* is one of abundant brown macroalgae in Indonesian oceans contains phenolic derivatives. Phloroglucinol is a multifunctional phenolic derivative in brown algae. Phloroglucinol has been known to have activity inhibitor tyrosinase, because phloroglucinol is copper chelating agent. The antioxidant and the tyrosinase inhibitor activities are parameters of the skin lightening active ingredient. The mechanism of tyrosinase inhibitors is to decrease skin pigmentation by inhibit the catalytic steps of the enzyme to the pigmentation associated with melanin production in the pathway of melanogenesis. Antioxidant activity protects skin against oxidative stress mediated by UVR. Numerous report have determine phenolic compound of *T. conoides* as antioxidant but none is reported as tyrosinase inhibitor**. Objective:** The objective of this study is to determine the activities of *T. conoides* as antioxidant and tyrosinase inhibitor. **Methods:** *Turbinaria conoides* were extracted with ethanol with different concentration (30%, 50% and 70%) by maceration method. Crude ethanolic extracts of *T. conoides* were Determined its total phenolic content, antioxidant activity (DPPH) and tyrosinase inhibitor. **Results:** E50 showed the highest total phenolic content (27.63±1.05 mg PGE/g extract). Antioxidant and tyrosinase inhibitor activities of E50 showed IC_{50} value of 215.96 µg/mL and 188.85 µg/mL, respectively. **Conclusion:** Ethanol extract of *Turbinaria conoides* is potential as antioxidant agent and tyrosinase inhibitor.
Key words: *Turbinaria conoides*, Phloroglucinol, Total phenolic content, Antioxidant, Lightening agent.

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INTRODUCTION

Skin lightening is a basic requirement for people who live in tropical countries such as Asia. Asian cultures consider bright skin as a beautiful standard. The growth of skin lightening products in Asia is increasing rapidly.[1] Most skin lightening products contained drug that have side effects on long-term use. Side effects that arise are dermatological reactions such as burning, itching, irritation and causing the effects of dependence. These side effects are related to the safety of the product.[2] Lightening active ingredients that are considered unsafe such as mercury and hydroquinone because mercury can cause nephrotoxicity, due to the accumulation of heavy metals contained in the material.[2]

Turbinaria conoides is one of brown macroalgae which can be found in the oceans of Indonesia.[3] *T. conoides* has bioactive compound such as carotenoid, laminarin, alginate, fucoidan and mannitol.[3] Fraction of *T. conoides* has reported by Karthik (2016), contained phloroglucinol (0,49 mg TAE/g).[4] Phloroglucinol is a multifunctional phenolic derivative that only can be found in brown algae. Phloroglucinol showed various activities such as anti inflammatory, antioxidant, antiphotoaging, antitumor, antivirus and matrix-metalloproteinase.[4,5] In the study of Babitha and Kim (2011), phloroglucinol has been known to have activity inhibitor tyrosinase, because of its copper chelating agent.[6,7]

The activity of tyrosinase inhibitors is one of the parameters on skin lightening agent. The mechanism of tyrosinase inhibitors is to decrease skin pigmentation by inhibition the catalytic of enzyme to the pigmentation associated with melanin production in the pathway of melanogenesis.[1] Skin lightening agent are associated with antioxidant activity that can protect the skin against oxidative pressures mediated by UVR.[8] Skin exposed to UVR exposure in both acute and chronic can cause various cellular and biological changes such as DNA damage, loss of homeostasis and cell function, abnormal pigmentation due to changes in melanin synthesis, immunosuppression and inflammation that can cause skin problems, such as photoaging and non-invasive skin cancers such as non melanoma and melanoma.[9]

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MATERIAL AND METHODS

Material

The brown seaweed, *T. conoides*, obtained from Pasauran Beach, Banten, West Java. *T. conoides* has been identified in LIPI Oceanography. Phloroglucinol (Sigma-Aldrich), Folin-Ciocalteu reagent (Merck), kojic acid (Sigma-Aldrich), ascorbic acid (Sigma-Aldrich), mushroom tyrosinase EC 1.14.18.1 (Sigma-Aldrich), L-tyrosine (Sigma-Aldrich), 2,2-Diphenyl-1-picrillhydrazyl (DPPH) (Sigma-Aldrich).

Method

Extraction of *T. conoides*

T. conoides was washed immediately using sea water after collected from ocean. *T. conoides* was packaged with ice box during the expedition. Then *T. conoides* rinsed and stored in the freezer at -20^{0}C before use.[10] The extraction method of *T. conoides* was a modification from Li *et al.* (2017). Maceration of *T. conoides* using 3 concentrations of ethanol such as 30%, 50% and 70% during 3 days. Before maceration, they were shaken using a homogenizer 15000 rpm for 5 min. After extraction, each mixture was filtered to separate residue and supernatant. The supernatant was then evaporated using a rotary vacuum evaporator until crude extract was obtained.[10]

Total Phenolic Content of *T. conoides* Extract

Determination total phenolic content of *T. conoides* extract was measured by *Folin-Ciocalteu* method with slight modification from Bobo-garcia G. *et al.* (2015) method. Extract solution (25 µL) were mixed with 100 µL of reagent Folin-Ciocalteu 25% and shaken for 1 min in 96-well microplate. The mixture was incubated at room temperature until 4 min. Then the mixture added 75 µL of sodium carbonate solvent (10%) in 96-well microplate and shaken for 1 min. The mixture was incubated at room temperature until 2 h. The absorbance of mixture was measured using the microplate reader at 760 nm and compared to phloroglucinol calibration curve (10-320 µg/mL).[11,12]

Antioxidant Activity of *T. conoides* Extract

DPPH methods

Determination of antioxidant activity was based on Bobo-garcia G. *et al.* (2015) with some modification. Activity of *T. conoides* was measured using microplate reader with DPPH method. 20 µL of diluted sample was added to 180 µL DPPH solvent (150 µM) in 96-well microplate and shaken for 60 s. Then, the mixture was incubated at room temperature for 30 m and absorbance of the mixture measured at 517 nm.[11] The result was compared with ascorbic acid. Antioxidant activity of *T. conoides* extract was calculated using the following equation:

$$\text{Scavenging effect (\%)} = [(\text{Asample}-\text{Asample blank})/(\text{Acontrol}-\text{Acontrol blank})] \times 100$$

Where Asample is the absorbance of the test sample, Asample blank is the absorbance of the sample only, Acontrol is the absorbance of the control and Acontrol blank is the absorbance of the methanol only. The result was described as IC_{50} value. The IC_{50} value was calculated as the concentration of sample (µg/ml) requiring scavenging 50% of the DPPH in the reaction mixture.[10,11]

Tyrosinase Inhibitor Activity of *T. conoides* Extract

The best of antioxidant activity of *T. conoides* extract was determined tyrosinase inhibitor activity using microplate reader by Kang *et al.* (2004) method with slight modification.[13] 40 µL enzim (240 unit/mL) and 40 µL sample (*T. conoides* extract) was added into 96-well microplate containing 40 µL L-tyrosin and 80 µL buffer solution (50 mM, pH 6.5). The mixture shaken 60 s and incubated for 30 m at room temperature. Then, the absorbance was measured using microplate reader at 475 nm. The result was compared with kojic acid. The percent inhibition of *T. conoides* extract was expressed as activity tyrosinase inhibitor and calculated by the following equation:

$$\text{\% Inhibition: } [(A-B) - (C-D)] / (A-B) \times 100\%$$

Where A is the absorbance at 475 nm blanko (enzyme without sample), B is the absorbance at 475 nm blanko control (without enzyme and sample), C is the absorbance at 475 nm sample (enzyme and sample) and D is the absorbance at 475 nm sample control (sample without enzyme). The result was descirbed as IC_{50} value. IC_{50} value is the concentration of inhibitors required to inhibit half the activity of the enzyme under test conditions.[13]

RESULTS

Extraction of *T. conoides*

The yield of rendemen of E30, E50 and E70 were obtained 6%, 4.4% and 4.8%, respectively.

Total Phenolic Content of *T. conoides* Extract

The total phenolic content of all *T. conoides* extracts were expressed as mg Phloroglucinol Equivalent (PGE) per gram of the extract. The total phenolic content of all *T. conoides* extracts were calculated using curve calibration of Phloroglucinol (ranging from 1.25 to 40 µg/mL at 96-well microplate). E50 showed the highest of total phenolic content (27.64± 1.05 mg PGE/g extract), while E30 and E70 were 20.69± 1.69 mg PGE/g extract and 21.04±0.35 mg PGE/g extract, respectively (Figure 1).

Antioxidant Activity of *T. conoides* Extract

Inhibition activity of E50 showed higher than E30 and E70. IC_{50} value of E50 was 215.96 µg/mL while E30 and E70 were 1062.093 µg/mL and 493.76 µg/mL, respectively (Figure 2). Antioxidant activity of *T. conoides* extract was compared with positive control of ascorbic acid that showed IC_{50} value 5.29 µg/mL.

Tyrosinase Inhibitor Activity of *T. conoides* Extract

Tyrosinase inhibitor activity was measured by microplate reader at a wavelength of 475 nm. E50 showed the presence of tyrosinase inhibitor activity with IC_{50} value 188.85 µg/mL (Figure 3). The results was compared with IC_{50} value of kojic acid (6.97 µg/mL), which is a positive control in this study (Figure 4).

DISCUSSION

The polarity and type of solvent for extraction affects the results of the total phenolic content in the extract so that those need to be considered.

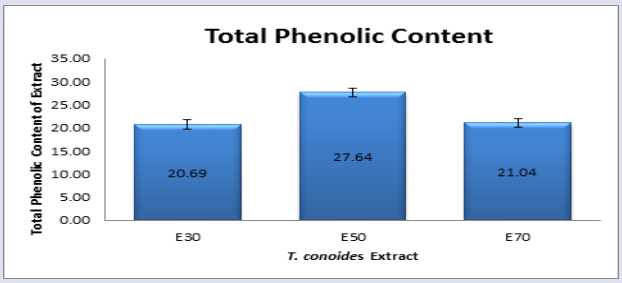

Figure 1: Total Phenolic Content of *T. conoides* Extract.

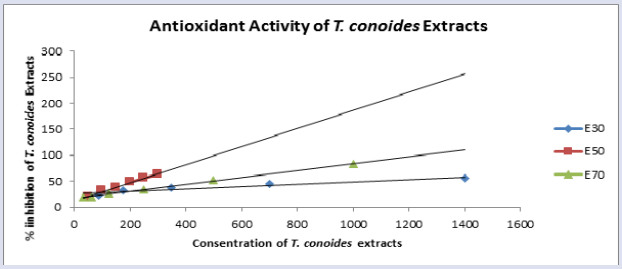

Figure 2: Antioxidant Activity of *T. conoides* Extract.

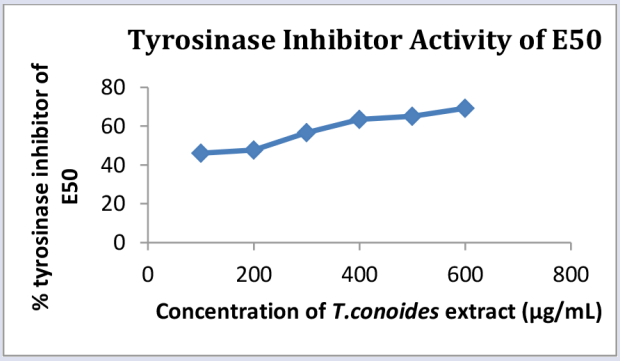

Figure 3: Tyrosinase Inhibitor Activity of E50.

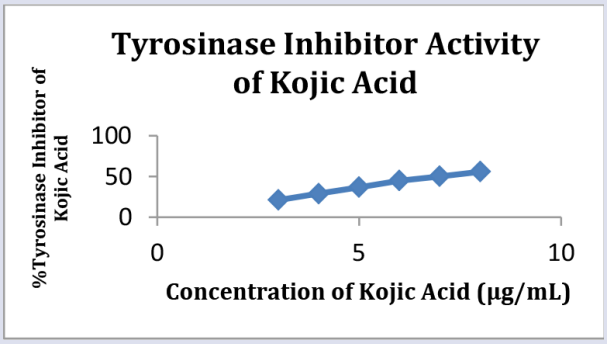

Figure 4: Tyrosinase Inhibitor Activity of Kojic Acid.

Phloroglucinol including other polyphenol derivatives contained in brown alga have solubility in methanol, ethanol, acetone, tetrahydrofuran, acetonitrile, 1,4-dioxane, chloroform and dichloromethane.[14]

Based on the result of this study, E50 showed the highest total phenolic content (27.63±1.05 mg PGE/g extract) than E30 and E70. In the study from Chakraborty (2013) showed total phenolic content of methanol extract of *T. conoides* was 16.64±0.10 mg GAE/g extract.[15] The total phenolic content in extract affects antioxidant activity, because phenolic compounds are known to reduce the oxidation levels of an organic matter by transferring a H atom from the OH group.[16] This is evidenced by the results obtained that the extract has a high total phenolic content has higher antioxidant activity than others. E50 exhibited higher antioxidant activity with IC_{50} value 215.96 µg/mL than E30 and E70. However, potential of antioxidant activity of *T. conoides* extracts still not able as good as ascorbic acid (5.29 µg/mL). *T. conoides* extract showed more potential as antioxidant than *S. Plagyophyllum*, that is brown alga with same family (Sargassaceae). Antioxidant activity of 50% ethanol extract of *S. Plagyophyllum* extract showed 50 mg/mL can inhibition 39.16±0.01%, it was reported by Lifie, Mansauda, Anwar, Nurhayati and Anwar (2018).[17] In the study from Ponnan *et al.* (2017), Ethyl acetat fraction of ethanol extract of *T. conoides* (EAF) has reported potential as antioxidant activity with superoxide radical scavenging activity method, that is EAF showed 57%-77% (40-80 µg/mL) of inhibition activity.[18]

Antioxidants are molecules that inhibit free radical reactions and can delay or inhibit cell damage.[19] Skin exposed to UV exposure, requiring exogenous antioxidants acting as ROS scavenge, may improve antioxidant or pro-oxidant balance.[20] ROS can activate melanocytes to increase the production of melanin pigment causing pigmentary disorders such as melisma.[20] ROS that is formed will induce oxidative stress on the skin that will lead to the depletion of both intra and intercellular endothelial antioxidants, increased intracellular lipid peroxidation and induce a specific pathway that modulates inflammatory, immunosuppressive, or skin apoptotic processes.[20]

The mechanism of action of antioxidants as skin lightening by inhibition from tyrosine oxidation to quinone dihydroxyphenilalanine and decrease free radical in keratinocytes caused by ultraviolet exposure.[21] Based on the result, tyrosinase inhibitor activity of E50 showed IC_{50} value 188.85 µg/mL. E50 showed potential as tyrosinase inhibitor activity than methanol extract of *Ecklonia stolonifera* OKAMURA (Laminariaceae) which was 345 µg/mL.[13] *T. conoides* showed more potential as tyrosinase inhibitor, if it compared to previous study with another genus from the same family such as *Sargassum plagyophyllum* and Sargassum *polycystum*. In the study of Arifianti *et al.* (2017) reported fresh powder of *S. plagyophyllum* have tyrosinase inhibitor activity with IC_{50} value 4.97 mg/mL, while based the result Chan *et al.* 2011, extract ethanol *S. polycystum* showed did not have any effect at the highest dose of tyrosinase concentration (500µg/mL).[22,23]

Phenol, flavonoids and coumarin are secondary metabolites that have activity inhibit excess melanin production processes. This metabolite was developed in pharmaceutical cosmetic as lightening ingredient.[24] Flavonoids are one of the polyphenols that have activity as anti-inflammatory, antiviral, antioxidant, anti-cancer and scavenger ROS. In depigmentation activity, flavonoids can directly inhibit tyrosinase activity in melanogenic pathways. Functional structure analysis in flavonoids showed that flavonoids with α-keto groups had activity in inhibiting tyrosinase, due to the similarity between the dihydroxyphenyl group in DOPA and α-keto containing flavonoids.[25]

CONCLUSION

Ethanol extract of *T. conoides* showed activity as antioxidant and tyrosinase inhibitor, hence, it will be potential to be developed in pharmaceutical cosmetics as lightening ingredient.

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CONFLICT OF INTEREST

The authors declares no conflict of interest.

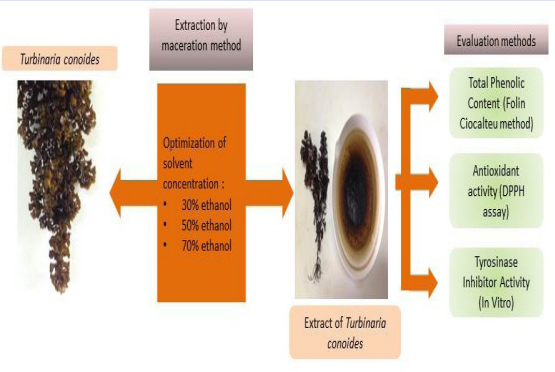
ABBREVIATIONS

DPPH: 2,2-Diphenyl-1-picrylhydrazyl; **E30:** 30% Ethanol Extract of *T. conoides*; **E50:** 50% Ethanol Extract of *T. conoides*; **E30:** 70% Ethanol Extract of *T. conoides*; **GAE:** Galic Acid Equivalent **TAE:** Tannic Acid Equivalent; ***T. conoides*:** *Turbinaria conoides*; **PGE:** Phloroglucinol Equivalent.

REFERENCES

1. Pillaiyar T, Manickam M, Jung S. Downregulation of melanogenesis : drug discovery and therapeutic options. Drug Discover Today. 2017;22(2):282-98. Cited in ScienceDirect: ELSEVIER Ltd.
2. Burger P, Landreau A, Azoulay S, Michel T, Fernandez X. Skin Whitening Cosmetics: Feedback and Challenges in The Development of Natural Skin Lighteners. Cosmetics. 2016;3(4):36.
3. Nursid M, Wikanta T, Susilowati R. Antioxidant Activity, Cytotoxicity and Fukosantin Content of Brown Seaweed Extract from Binuangeun Beach, Banten (Aktivitas Antioksidan, Sitotoksisitas dan Kandungan Fukosantin Ekstrak Rumput Laut Coklat dari Pantai Binuangeun, Banten). Postharvest and Marine and Fisheries Biotechnology Journal (Jurnal Pascapanen dan Bioteknologi Kelautan dan Perikanan, IND). 2013;8(1):73-84.
4. Karthik R, Manigandan V, Sheeba R, Saravanan R, Rajesh PR. Structural characterization and comparative biomedical properties of phloroglucinol from Indian brown seaweeds. Journal of Applied Phycology. 2016;28(6):3561-73.
5. Thomas NV, Kim SK. Beneficial effects of marine algal compounds in cosmeceuticals. Marine Drugs. 2013;11(1):146-64.
6. Babitha S, Kim EK. Effect of marine cosmeceuticals on the pigmentation of skin. Marine cosmeceuticals: Trends and Prospect. CRC Press; Boca Raton, FL, USA. 2011;63-6.
7. Wang HMD, Chen CC, Huynh P, Chang JS. Exploring the potential of using algae in cosmetics. Bioresource Technology. 2015;184:355-62.
8. Jadoon S, Karim S, Hassan MH, Akram MR, Khan AK, Malik A, *et al.* Anti-aging potential of phytoextract loaded-pharmaceutical creams for human skin cell longetivity. Oxidative Medicine and Cellular Longevity. 2015;1-17.
9. Panich U. Antioxidant Defense and UV-Induced Melanogenesis: Implications for Melanoma Prevention. Current Management of Malignant Melanoma 2010. InTech. 2011.
10. Li Y, Fu X, Duan D, Liu X, Xu J, Gao X. Extraction and Identification of Phlorotannins from the Brown Alga, Sargassum fusiforme (Harvey) Setchell. Marine Drugs. 2017;15(2):49.
11. Bobo-garcia G, Davidov-pardo G, Arroqui C, Marin-arroyo MR, Navarro M. Intra-laboratory validation of microplate methods for total phenolic content and antioxidant activity on polyphenolic extracts and comparison with conventional spectrophotometric methods. J Sci Food Agric. 2015;95(1):204-9.
12. Montero L, Sanchez-camargo AP, Garcia-canas V, Tanniou A, Stiger-pouvreau V, Russo M, *et al.* Anti-proliferative activity and chemical characterization by comprehensive two-dimensional liquid chromatography coupled to mass spectrometry of phlorotannins from the brown macroalga *Sargassum muticum* collected on North-Atlantic coasts. Journal of Chromatography A. 2016;1428:115-25.
13. Kang HS, Kim HR, Byun DS, Son BW, Nam TJ, Choi JS. Tyrosinase inhibitors isolated from the edible brown alga Ecklonia stolonifera. Archives of Pharmacal Research. 2004;27(12):1226-32.
14. Shao X, Tian G, Ren C, Li Z. Solubility of Phloroglucinol Tris (cyclic 2,2-dimethyl-1,3-propanediol phosphate) in Different Solvents. J solution Chem. 2015;44(6):1310-9.
15. Chakraborty K, Praveen NK, Vijayan KK. Evaluation of phenolic contents and antioxidant activities of brown seaweeds belonging to Turbinaria spp. (*Phaeophyta*, *Sargassaceae*) collected from Gulf of Mannar. Asian Pacific Journal of Tropical Biomedicine. 2013;3(1):8-16.
16. Foti MC. Antioxidant properties of phenols. Journal of Pharmacy and Pharmacology. 2007;59(12):1673-85.
17. Mansauda KLR, Anwar E, Nurhayati T. Antioxidant and Anti-Collagenase Activity of Sargassum plagyophyllum Extract as an Anti-Wrinkle Cosmetic Ingredient. Pharmacogn J. 2018;10(5):932-6.
18. Ponnan A, Ramu K, Marudhamuthu M, Marimuthu R, Siva K, Kadarkarai M. Antibacterial, antioxidant and anticancer properties of *Turbinaria conoides* (J. Agardh) Kuetz. Clinical Phytoscience. 2017;3(1):5.
19. Nimse SB, Pal D. Free radicals, natural antioxidant and their reaction mechanisms. Royal Socety of Chemistry. 2015;5(35):27986-8006.
20. Srisayam M, Weerapreeyakul N, Barusrux S, Kanokmedhakul K. Antioxidant, antimelanogenic and skin-protective effect of sesamol. J cosmet Sci. 2014;65(2):69-79.
21. Naidoo L, Khoza, Dlova NC. A Fairer Face, a Fairer Tomorrow?. A Review of Skin Lighteners Cosmetics. 2016;3(3):33.
22. Arifianti AE, Anwar E, Nurjanah N. Tyrosinase Inhibitor and Antioxidant Activity of Fresh and Dry Powder of Seaweed of *Sargassum plagyphyllum* (Aktivitas Penghambatan Tirosinase dan Antiokisdan Serbuk Rumput Laut dari *Sargassum plagyphyllum* Segar dan Kering). Indonesian Fishery Product Processing Journal. 2017;20(3):488-93.
23. Chan YY, Kim KH, Heah SH. Inhibitory effects of *Sargassum polycystum* on tyrosinase activity and melanin formation in B16F10 murine melanoma cells. Journal of Ethnopharmacology. 2011;137(3):1183-824.
24. Lin JW, Chiang HM, Lin YC, Wen KC. Natural Products with Skin– Whitening Effects. Journal of Food and Drug Analysis. 2008;16(2):1-10.
25. Ebanks JP, Wickett RR, Boissy RE. Mechanisms regulating skin pigmentation: The rise and fall of complexion coloration. International Journal of Molecular Sciences. 2009;10(9):4066-87.

GRAPHICAL ABSTRACT



SUMMARY

- 50% ethanol extract of *T. conoides* showed the highest of total phenol content and antioxidant activity. The extract has the ability as a tyrosinase inhibitor and can be used as a skin lightening ingredient.

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