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Original Article

In-vitro Evaluation of Antioxidant Activity and Anti-collagenase Activity of *Thalassia hempricii* as a Potent Ingredients for Anti-Wrinkle Cosmetics

Kiki Zakiah[1], Effionora Anwar[2*], Tati Nurhayati[3]

ABSTRACT

Background: *Thalassia hemprichii* have reported containing antioxidant effects. However, information on other biological activities relating to the anti-wrinkle properties is limited. The free radical scavenging activity and enzyme inhibitory activity of the plant extracts investigated that they can help restore skin elasticity and thereby slow the wrinkling process. **Objective:** To evaluate *in-vitro* antioxidant and anti-collagenase activity of *T. hemprichii* as a potent ingredient for anti-wrinkle cosmetic. **Methods:** *T. hemprichii* was extracted with ethanol 100% (E1) 75% (E2) and ethanol 50% (E3) using maceration extraction method for 24 h, thrice. The extract was examined for total phenolic content, antioxidant activity compared with vitamin C, and the best extract was tested for the inhibitory activity of collagenase. **Results:** The total phenolic content of *T. hempricii* extract was 0,525 ± 0,016 mg GAE/100 g extract (E1) 0,538 ± 0,006 mg GAE/100 g extract (E2) and 0,558 ± 0,090 mg GAE/100 g extract (E3) respectively. The antioxidant activity (% inhibition) of the extract was 38,035 ± 0,252 % (E1), 52,502 ± 6,225 % (E2) and 57,261 ± 0,505 % (E3). Ascorbic acid as a positive control of antioxidant activity showed much higher % inhibition than the sample with 78,055 ± 0,756 %. As the best extract from antioxidant activity, E2 and E3 have inhibited collagenase activity with 51,809 ± 0,164 % and 52,212 ± 0,735 % at 20µg/mL respectively. **Conclusion:** In general the (*T. hempricii*) extract can be used as a potential active ingradient for anti-wrinkles cosmetic.
Key words: *Thalassia hempricii*, Seagrass, Antioxidant, Collagenase, Anti-wrinkle.

Kiki Zakiah[1], Effionora Anwar[2*], Tati Nurhayati[3]

[1]Master student at Faculty of Pharmacy, Universitas Indonesia, Depok, INDONESIA.
[2]Professor at Department of Pharmacy Technology, Faculty of Pharmacy, Universitas Indonesia, Depok, INDONESIA.
[3]Doctor, Faculty of Fisheries and Marine Sciences, Bogor Agricultural University, Bogor, INDONESIA.

Correspondence

Prof. Effionora Anwar

Faculty of Pharmacy, Universitas Indonesia, Kampus UI Depok, 16424, West Java, INDONESIA.

Phone no : +62-21-7270031

E-mail: effionora.anwar@farmasi.ui.ac.id

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INTRODUCTION

Wrinkle, as a manifestation of skin aging, is a natural process that will be experienced by every living creature. But the aging process of each person is not the same, because it is influenced by several factors, both extrinsic and intrinsic. Extrinsic factor such as environmental exposure like sunlight recurring to the skin causing sunburn and dry skin or wrinkle. The intrinsic such as genetics, hormonal changes, metabolic processes factor.[1] Skin repeatedly exposed to ultraviolet light is made up of two type of harmfull ray that penetrate deep into the dermis, so cause physical changes and finally generated Reactive Oxigen Scavenger (ROS). ROS cause damage lipid, protein and DNA of the skin.[2] ROS indirectly induce production of MMPs via MAP- kinase pathway.[3] Like all other organs, undergoes chronological aging. In addition, unlike other organs, skin is in direct contact with the environment and therefore undergoes aging as a consequence of environmental damage. The primary environmental factor that causes human skin aging is UV irradiation from the sun. This sun-induced skin aging (photoaging MMPs are a group of zinc-dependent extracellular proteinases divided into five sub-groups based on their substrate such as collagenase. Collagenase is responsible for extracellular matrix (ECM) cause of collagen breakdown. Elastase is serine proteinase that responsible for the breakdown of elastin in ECM. When collagen and elastin always maintain the skin structural integrity and elasticity, collagen and elastin contribute to undesired wrinkles. Plant extracts usually content phenol, polyphenol, flavonoid they possess antioxidant activity, that used as anti-collagenase and anti-elastase activities.[4] several studies have reported investigations on plant extracts as inhibitors of proteinases and as anti-oxidants. The anti-ageing and anti-oxidant properties of 23 plant extracts (from 21 plant species

The activity of the potential antioxidants to prevent skin damage cause by ROS. Previous study have revealed that seagrass polyphenols has antioxidant activity.[5] Seagrass also reported has a richer sources of natural antioxidant than seaweeds.[6] Other study reported that *Thalassia hempricii*, one of Indonesian seagrass species, has IC_{50} values 123.72 ± 9.99.[5]

Nowadays, research on marine plants has been done a lot, to get more information and evidence. However, research on the inhibition of enzymes by sea-

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grass is limited. Therefore, this study aims to test the phenolic content, the capacity of antioxidant and inhibitory activity of collagenase enzyme from *Thalassia hemprichii* extract. Inhibition of collagenase activities by natural plant compounds might be a promising approach to prevent extrinsic skin aging like a wrinkle.

MATERIALS AND METHODS

Material

Thalassia hemprichii obtained from Pasauran Beach, Serang, Banten, West Java-Indonesia, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), Gallic acid, Ascorbic acid, *C. histolyticum* collagenase (ChC) (type IA), N-[3-(2-furyl) acryloyl]-Leu-Gly-Pro-Ala (FALGPA) and Epigallocatechingallate (≥ 95%) were purchased from Sigma-Aldrich. $FeCl_3$, Folin-Ciocalteu reagent, and Sodium carbonate were purchased from Merck.

Methods

Preparation of seagrass extract

After collected from Binuangeun Beach, Banten, Indonesia. *T. hemprichii* leaves and rhizome were washed immediately using sea water to remove sand particles, and maintained with ice box throughout the expedition. Then *T. hempricii* rinsed several times, dried and stored. Next, *T. hempricii* was conducted determination at Biology Center of Indonesian Institute of Sciences (LIPI) to ensure the species.

To get the extract, dried leaves and root were extracted using maceration method for 24 h in 100%, 75% and 50 % with ethanol at room temperature under dark condition. Then the mixture was centrifuged (5000 rpm for 10 min) to separate the residue and filtrate. The filtrate was evaporated using a rotary vacuum evaporator (Buchi, Switzerland).

Phytochemical Screening of Thalassia hempricii

Qualitative analysis of the *T. hempricii* extract determined by Meyer and Dragendorff reagents for alkaloid, ferric chloride test for phenol, magnesium turning test for flavonoids and $FeCl_3$ for tannins according to previous study.[7]

Total Phenolic Content (TPC) of T. hempricii extract

Total phenols was measured by the modified Folin-Ciocalteu method.[8]such as treatment of fever, skin diseases, muscle pains, wounds and stomach problems. Hence it is essential to study their bioactive metabolites and medicinal properties when considering their food applications. In the present study, the leaves of six seagrasses Enhalus acoroides, Thalassia hemprichii, Halodule pinifolia, Syringodium isoetifolium, Cymodocea serrulata and Cymodocea rotundata were extracted with aqueous methanol and tested for their antioxidant capacity. Among them, H. pinifolia recorded high phenolic (21.64 mg g-1 using a Shimadzu UV-VIS spectrophotometer (UV-2450). 0, 5 ml extract was mixed with 1 mL of 10% Folin-ciocalteu reagent (1:10 v/v) and 2 mL (75 g/L) of sodium carbonate. The tubes were homogenized by vortex for 15 sec and allowed to stand for 30 min for the reaction. Absorbance was then measured at 720 nm using UV-VIS Spectrophotometer. The calibration curve was prepared by putting the value of absorbance vs. concentration.[9] The total phenolic content was expressed as Gallic Acid Equivalent (GAE) in milligram per 100 g dried extract.[5]

Antioxidant activity of *T. hempricii extract*

Free radical scavenging activity was determined by Molyneux method.[10] with slight modifications. As much as 1.0 mL of sample was added to 3.0 mL of 40 ppm DPPH solution. In the dark condition, the mixture was homogenized by vortex for about 20 sec, then kept at room temperature for 30 min, and the absorbance was measured at 517 nm. Ascorbic acid was used as positive control. Antioxidant activity of *T. hempricii* was expressed as percentage of DPPH scavenging effect (%). The scavenging effect of DPPH radicals was calculated using the equation:

$$\text{DPPH Scavenging effect}(\%) = 1 - \frac{A_0 - A_1}{A_0} \times 100$$

Note: A_0 is the absorbance of the control, A_1 is the absorbance of the sample.

Anti-Collagenase Activity of T. hempricii extract

Inhibitory activity of *Thalassia hempricii* extract against *C. histolyticum* collagenase (ChC) was measured using spectrophotometry microplate reader by Wittanauer methods.[11] with slight modification. 50 µltricine buffer solution (pH 7.5), 50 µl of sample and 50 µl of enzyme (125 Unit/ml ChC, type IA) were added into 96-well microplate. Then 50 µl FALGPA (0.5mM) was added to start the reaction. The solution was then incubated for 15 min. The ChC inhibitory activities of the individual samples were measured by continuously monitoring the decrease in absorbance of FALGPA and expressed as percentage inhibition (%). The relative inhibition was calculated according to Equation below:

$$\text{Percentage inhibition}(\%) = 1 - \frac{Os}{Oc} \times 100$$

Note: O_s is the corrected absorbance enzyme in the presence of samples, O_c is the corrected absorbance of enzyme without samples.

RESULTS

Phytochemical Screening

Sample that we used in this study is true *Thalassia hempricii*, base on determination at The Biology Center of Indonesian Institute of Sciences (LIPI). We found that phytochemical qualitative analysis chemical substances of 100 % (E1), 75 % (E2) and 50 % (E3) ethanolic extract of all parts of the plants (roots and leaves), showed that *T. hempricii* contained alkaloids, flavonoids, phenols and tannins (Table 1).

Total Phenolic Content (TPC) of T. hempricii extract

A calibration curve of Gallic acid (ranging from 100 to 700 µg) was prepared and the total phenolic content was standardized against gallic acid and is expressed as mg Gallic acid equivalents per 100 g of sample on a dry weight basis. R^2 = 0, 9903, y = 0,0007x - 0,0853. Total phenol in the extract of *T. hempricii* was 0,525 ± 0,016 mg GAE/100 g extract (E1) 0,538 ± 0,006 mg GAE/100 g extract (E2) and 0,558 ± 0,090 mg GAE/100 g extract (E3) respectively (Figure 1). As we can see in Figure 1, Compared to E1 and E2, E3 contains higher amount of phenol with 0,558 ± 0,090 mg GAE/100 g extract. We can say that E1 < E2 < E3.

Table 1: Qualitative test of phytochemical constituent.

Phytochemical constituents	*T. hempricii* ethanolic extract		
	50%	75%	100%
Alkaloids	++	++	++
Flavanoids	++	++	+
Phenols	++	++	++
Tannins	++	++	+

Note : (++++): very high content; (+++) : high content; (++) : moderate content; (+) : low content; (-) : not detected

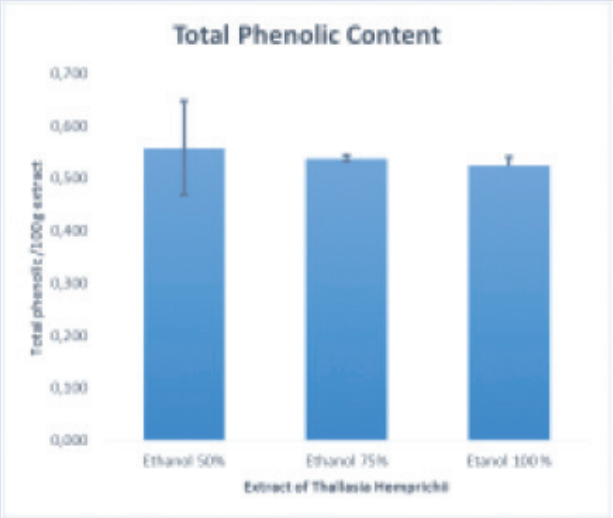

Figure 1: Total Phenolic Content of *Thalassia hempricii*.

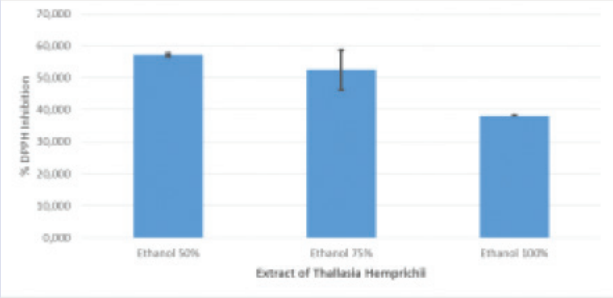

Figure 2: % DPPH Inhibition of *Thalassia hempricii*.

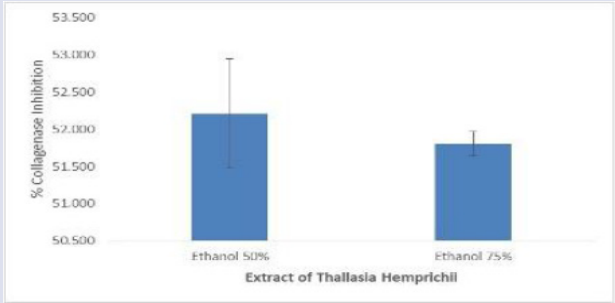

Figure 3: % Inhibition of Collagenase of *Thalassia hempricii*.

Antioxidant activity of *T. hempricii extract*

In this study, the % inhibition of *T. hempricii* extract using DPPH assay was 38,035 ± 0,252 % (E1), 52,502 ± 6,225 % (E2) and 57,261 ± 0,505 % (E3). This result are presented in Figure 2. Positive control of ascorbic acid showed much higher % inhibition than the sample with 78,055 ± 0,756 %. As in the Figure 2, E2 and E3 gives a greater % inhibitory value, compared to E1.

Anti-Collagenase Activity of T. hempricii extract

We used microplate reader with spectrophotometry method at wavelength 340 nm to determine the inhibitory activity of E2 and E3 that showed highest % antioxidant activity. E3 gives a greater % inhibitory value, with 52,212 ± 0,735 at 20µg/mL, as well as a higher % inhibition DPPH value compared to E2 which gives 51,809 ± 0,164 % at 20 µg/mL (Figure 3).

DISCUSSION

Based on phytochemical screening, data show that the extract contains alkaloid, flavonoid, phenol, and tannin (polyphenol). Alkaloids compounds are discovered can stop free radical chain by donating H atoms in free radicals.[12] Ferric reducing ability of plasma and metal chelating assays. Results: Malaria infection caused the formation of free radicals which subsequently led to oxidative stress and apoptosis. The antioxidant properties of the alkaloids under investigation revealed that in addition to the antiplasmodial activity, the alkaloids could also prevent oxidative stress. (+ Other study reported that Marine Alkaloids can inhibit MMP-1 (human fibroblast collagenase).[13] Flavanoids claimed have impact on the antioxidant capacity because OH group in flavanoid can be used to donate H atoms in free radicals. The antioxidant activity of flavonoids and their metabolites *in vitro* depends upon the arrangement of functional groups about the nuclear structure.[14] Phenolic and flavonoid content are associated with antioxidant activity especially in terms of DPPH and nitric oxide free radical scavenging.[15] Tannins or polyphenols scavenge free radicals with a complex kinetic that involves quick and slow steps of scavenging.[16]

Seagrasses are rich in proanthocyanidin (condensed tannins) as one of the phenolic compounds.[17] Phenolic compounds has been reported that against oxidative stress-mediated disorders and have received intense attention as potential therapeutic agents against a wide range of diseases including neurodegenerative diseases, cancer, diabetes, cardiovascular dysfunction, inflammatory diseases as well as aging.[18] In many cases, phenolic compounds are claimed to be the main active constituent explaining the seagrass antioxidant activity.[8,18]such as treatment of fever, skin diseases, muscle pains, wounds and stomach problems. Hence it is essential to study their bioactive metabolites and medicinal properties when considering their food applications. In the present study, the leaves of six seagrasses Enhalus acoroides, Thalassia hemprichii, Halodule pinifolia, Syringodium isoetifolium, Cymodocea serrulata and Cymodocea rotundata were extracted with aqueous methanol and tested for their antioxidant capacity. Among them, H. pinifolia recorded high phenolic (21.64 mg g-1).

The pharmacological actions of phenolic antioxidants against free radical scavenging is very important to inhibit wrinkle, because skin which repeatedly exposed to ultraviolet light every day, can occurred wrinkles.[19] The presence of some phytoconstituents, such as phenols, flavonoids and tannins in seagrasses may be the cause of antioxidant activity in preventing occurred wrinkles or inhibit collagenase activity.[20]

The selection of solvent types is essential for efficiency of the extraction process, because the compound of solvent with different polarity provides different antioxidant potential.[21] The most widely solvents applied for extraction so far is methanol, ethanol and acetone or mixtures with water can bring up various polyphenols from various phenolic structures.[22] mainly for quality control purposes. The effects of experimental variables, such as solvent composition and volume and time and temperature on extraction, have been studied. A unique gradient program for the separation of several phenolic classes (hydroquinones, hydroxybenzoic acids, flavan-3-oles, hydroxycinnamic acids, coumarins, flavanones, flavones,

dihydrochalcones and flavonols The use of water with other organic solvents as a combination contributes to the formation of fairly small polar media ensuring the extraction of polyphenols.[23] This research choose ethanol-water solvent for making the extract. However, difference state of the seagrass growth site can result difference total content of phenol.[24] For example, TPC for *T. hempricii* that was collected from Chinnapallam, Gulf of Mannar Biosphere Reserve, Tamilnadu, India has 2.76 ± 0.170 mg GAE/g sample [8]such as treatment of fever, skin diseases, muscle pains, wounds and stomach problems. Hence it is essential to study their bioactive metabolites and medicinal properties when considering their food applications. In the present study, the leaves of six seagrasses *Enhalus acoroides, Thalassia hemprichii, Halodule pinifolia, Syringodium isoetifolium, Cymodocea serrulata* and *Cymodocea rotundata* were extracted with aqueous methanol and tested for their antioxidant capacity. Among them, *H. pinifolia* recorded high phenolic (21.64 mg g-1. Meanwhile, *T. hempricii* that was collected from Palk bay, India has 6 mg GAE/g sample.[25] Environmental conditions have great influences in the antioxidant property and the accumulation of the phytochemicals.[26]

The DPPH assay has been commonly used to measure the free radical-scavenging ability of plants.[27] The antioxidant effect on DPPH radical scavenging may be due to those ability to donate hydrogen. When the DPPH solution is mixed with a substrate acting as a hydrogen atom donor, a stable non-radical DPPH formed characterized by a simultaneous change of violet color to pale yellow.[10] DPPH assay is most suitable for polar compounds because the DPPH crystals can dissolve easily in a commonly solvent and give maximum absorbance.[28] Based on Figure 2, E2 and E3 shows greater % scavenging effect, compared to E1. TPC of *T. hempricii* related with the antioxidant activity. Other research reported 38.62 ± 0.62 % for inhibitory activity of DPPH assay of *T. hempricii*.[8] such as treatment of fever, skin diseases, muscle pains, wounds and stomach problems. Hence it is essential to study their bioactive metabolites and medicinal properties when considering their food applications. In the present study, the leaves of six seagrasses *Enhalus acoroides, Thalassia hemprichii, Halodule pinifolia, Syringodium isoetifolium, Cymodocea serrulata and Cymodocea rotundata* were extracted with aqueous methanol and tested for their antioxidant capacity. Among them, *H. pinifolia* recorded high phenolic (21.64 mg g-1 The varying antioxidant capacity of various plant extracts can be attributed to differences in chemical composition of plants such as phenolic acids, and flavonoids.[8,26]such as treatment of fever, skin diseases, muscle pains, wounds and stomach problems. Hence it is essential to study their bioactive metabolites and medicinal properties when considering their food applications. In the present study, the leaves of six seagrasses *Enhalus acoroides, Thalassia hemprichii, Halodule pinifolia, Syringodium isoetifolium, Cymodocea serrulata* and *Cymodocea rotundata* were extracted with aqueous methanol and tested for their antioxidant capacity. Among them, *H. pinifolia* recorded high phenolic (21.64 mg g-1

The collagenase inhibition by E3 related with the total phenolic content and antioxidant activity of DPPH. Additionally, Antioxidant activities of plants extracts were correlated with their total phenolic content.[29] The characteristics of polyphenols may differ, depend on the solvent used during the extraction of the two solvents.[23] There's can be different mechanism of anti-aging or anti-wrinkle properties on plants extract.[27] So, E3 might be used as an active ingredient for anti-aging or anti-wrinkle products.

CONCLUSION

In general, the *T. hempricii* extract can be used as a potential active ingradient for anti-wrinkles cosmetic.

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ABBREVIATIONS

T. hempricii: Thalassia hempricii; **ECM:** extracellular matrix; **DPPH:** 2,2-Diphenyl-1-picrylhydrazyl; **ChC:** C. histolyticum collagenase; **FALGPA**: N-[3-(2-furyl) acryloyl]-Leu-Gly-Pro-Ala; **GAE**: Gallic Acid Equivalent.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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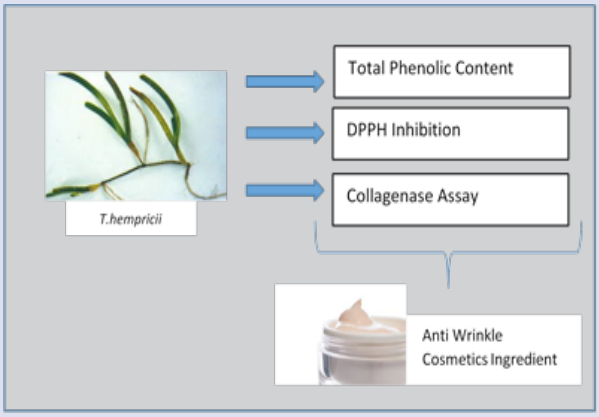
GRAPHICAL ABSTRACT



SUMMARY

- Previous study have revealed that seagrass polyphenols has antioxidant activity. Seagrass also reported has a richer sources of natural antioxidant than seaweeds. Other study reported that *Thalassia hempricii*, one of Indonesian seagrass species, has potential IC_{50} value.
- Nowadays, research on marine plants has been done a lot, to get more information and evidence. However, research on the inhibition of enzymes by seagrass is limited. Therefore, this study aims to test the inhibitory activity of collagenase enzyme from *Thalassia hemprichii* extract. Inhibition of collagenase activities by natural plant compounds might be a promising approach to prevent extrinsic skin aging like a wrinkle.
- The present study describe anti-wrinkle activity of *T. hempricii* assessed by collagenase inhibitory activities compared with the positive control.
- This scientific study support and suggest *T. hempricii* has the capability as an anti-wrinkle agent that is suitable for use in cosmetic products.

ABOUT AUTHORS

Kiki Zakiah., Kiki Zakiah Is a Pharmacy Technology student at Faculty of Pharmacy, Universitas Indonesia.

Effionora Anwar is a Professor at Department of Pharmacy Technology, Faculty of Pharmacy, Universitas Indonesia. She has many experienced in semisolid formulation from medicinal plants.

Tati Nurhayat Just got her Professorship on May, 2018 from The Faculty of Fisheries and Marine Sciences, Bogor Agricultural University - Indonesia. She has many experienced in enzymatic assay

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