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Original Article

Antioxidant and Anti-Collagenase Activity of *Sargassum plagyophyllum* Extract as an Anti-Wrinkle Cosmetic Ingredient

Karlah Lifie Riani Mansauda[1], Effionora Anwar[2*], Tati Nurhayati[3*]

ABSTRACT

Background: Sea algae are widely used as food and cosmetics in the world. There are several algae including brown algae which are us for human used to maintain health and skin care. Brown algae have various potential biological activities because contain substantial phytochemical constituent. Numerous report has identified phytochemical compound of *Sargassum* sp. extract but the activity as anti-collagenase almost none. **Objective:** To study the antioxidant, and anti-collagenase activity of *Sargassum plagyophyllum* extract as active pharmaceutical ingredient for anti-wrinkle cosmetics. **Methods:** *Sargassum plagyophyllum* obtained from Pasauran Beach, Banten, West Java, Indonesia. The extract *Sargassum plagyophyllum* extracted with three concentration ethanol-water: (E1) ethanol 25%, (E2) ethanol 50% and (E3) ethanol 75%, by using maceration extraction method for 24 h, thrice. The extract was evaluated include total phenolic content, antioxidant activity, and the best extract was tested for the anti-collagenase activity. **Results:** Total phenol in the extract were 0.588 ± 0.01 (E1), 0.272 ± 0.01 (E2), and 0.220± 0.03 (E3) mg PGE/ 100 g extract, respectively. Antioxidant activity of the extract (50 mg/mL) was 41,61±0,02% (E1), 39,16±0,01% (E2), 37,58±0,03% (E3) and ascorbic acid 78.03±0,65 % (22.44 µg/mL) as a standard. The best extract (E1) had inhibited the activity of collagenase by 54.46 ± 0.37%. **Conclusion:** The brown seaweed (S*argassum plagyophyllum*) extract can be used as an active pharmaceutical ingredient for anti-wrinkles cosmetic

Key words: Anti-collagenase, *Sargassum plagyophyllum*, Antioxidant, Anti-wrinkle.

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INTRODUCTION

Wrinkle can be caused by intrinsic and extrinsic factors. Intrinsic factors include metabolic processes, genetic factors, unbalanced antioxidant components in the skin and free radicals and hormonal factors.[1] Extrinsic aging like wrinkle is caused by exposure UV radiation, stress, cigarettes, pollution, drugs and food.[2] There are various ways to inhibit the skin aging process, one of them by inhibiting free radical activity. Materials that can be used to inhibit free radicals activity are called antioxidants.[3] In general plants and marine algae containing polyphenols, flavonoids, phenolic acids were also known to have the ability as anti-aging like anti-wrinkle and antioxidant activity.[4] One of natural antioxidants source was the brown seaweed (*Sargassum sp.*),[5] and antioxidant activity mostly demonstrated by its polyphenols compound.[6] Brown seaweed commonly content phlorotannin or phloroglucinol compounds that have antioxidant activity as radical scavenging activity (RSA) .[7] Another study reported that phenol compounds in brown seaweed were more effective than α-tocopherol and almost comparable to synthetic antioxidants, BHA and BHT.[8] *Sargassum sp*. has ability to inhibit and reactive oxygen species (ROS) by donating protons so as to speed up the process of free radical termination.[9] ROS can derived by uv-ray from sun light and can damage the skin and cause wrinkle.

In this study, S*argassum plagyophyllum* extracts might be one of natural ingredients that could be used for anti-wrinkle cosmetics. Therefore, determinate the total content of phenolic compound, antioxidant activity and the ability to inhibit collagenase enzyme is the importance factors to study as anti-wrinkle.

MATERIALS AND METHODS

MATERIAL

Sargassum plagyophyllum obtained from Pasauran Beach, Serang, Banten, West Java Indonesia. *Folin-Ciocalteau* Reagents (Sigma-Aldrich), Sodium Carbonate (Merck), Standard Phloroglucinol (Sigma-Aldrich), 2,2-Diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich), Ascorbic acid standard (Sigma-Aldrich), *N*-[3-(2-furyl)acryloyl]-Leu-Gly-Pro-Ala (FALGPA) (Sigma-Aldrich), Epigallocatechingallate (EGCG) (Sigma-Aldrich), collagenase derived from *Clostridium histolyticum* (Chc) type IA (Sigma-Aldrich).

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METHODS

To ensure species of *Sargassum sp.* that used in this research as *Sargassum plagyophyllum* was conducted determination at Oceanographic Research Centre, Indonesian Institute of Sciences (LIPI), Ancol, North Jakarta.

Extraction of Sargassum sp.

The method of extraction was done by maceration for 24 hr[10] using ethanol in different concentrations of 25%, 50% and 75% as a solvent. After filtered, the filtrate separated from residue, then evaporated by *rotary evaporator* at 40°C until produced viscous extract. To produce the dry extract evaporation process was continued by using a water bath.

Phytochemical Screening of Extract Sargassum sp.

Qualitative analysis of the *S. plagyophyllum* extract determined by ferric chloride test for phenol, magnesium turning test for flavonoids, and Meyer and Dragendorff reagents for alkaloids, according to previous study.[11]

Total Phenolic Content (TPC) of S. plagyophyllum extract

Total Phenolic Content (TPC) of the extract was determined using Folin-Ciocalteu method.[5] To 0.5 ml of *Sargassum* sp. extract was added one ml of 10% folin-ciocalteu solution. The solution was mixed by vortex and incubated in dark place with room temperature for 5 min. After that, two ml of 7.5% sodium carbonate solution was added and shaken until homogeneous. The solution was stored for 70 min and the absorbance were measured at 707 nm using a UV-Vis spectrometer. Total phenolic content was calculated based on a standard curve of phloroglucinol. The curve was made by plotting concentration (ppm) versus absorbance. Total phloroglucinol was expressed in mg Phloroglucinol Equivalents (PGE) per 100 g of dried extract.

Antioxidant activity of S. plagyophyllum extract

Antioxidant activity was tested using DPPH radical-scavenging activity method [12] with modification. 0.5 ml of sample (with different concentrations) was added three ml of DPPH-ethanol solution. The mixture of DPPH-sample was homogenized and kept at room temperature also protected from light for 30 min, then measured using UV-Vis spectrophotometer at 517 nm. The same treatment was used to determined ascorbic acid as control. The antioxidant activity is calculated by following equation:

$$\%\ \text{inhibition} = \frac{As - Ac}{As} \times 100$$

Information: A_s is absorbance and A_c absorbance of sample solution without DPPH.

Anti-Collagenase activity of S. plagyophyllum extract

Anti-collagenase activity of *S. plagyophyllum* extract was evaluated following the Wittenauer method[13] with modification. 50 µL trisine buffer solution (pH 7.5), 50 µL of sample and 50 µL of enzyme (125 U/ml ChC, type IA) were added into 96-well microplate. To start the reaction was added 50 µL N- (3- [2-Furyl] -acryloyl)-Leu-Gly-Pro-Ala (FALGPA) 0.5 mM. The solution was then incubated for 15 min after the reaction started. The decrease of FALGPA absorbance was monitored at 340 nm in the microplate reader at a constant temperature of 25°C after the addition of FALGPA. Positive controls were used epigallocatechin gallate (EGCG), percentage inhibition was calculating by:

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

Information: OS is the corrected absorbance enzyme in the presence of samples, and OC is the corrected absorbance of enzyme without samples.

RESULTS

Sample that used in this study is *Sargassum plagyophyllum* based on determination at The Oceanographic Research Centre (P_2O) Indonesian Institute of Sciences (LIPI) (Figure 1). Qualitative analysis for *S. plagyophyllum* showed that brown seaweed extracted with ethanol 25%, 50%, and 75% showed also positive contained phenol, flavonoids, and alkaloids.

Total Phenolic Content (TPC) of S. plagyophyllum extracts

The yield of total phenol in the extract was 0.588 ± 0.01 mg PGE/ 100 g for extract ethanol 25% (E1), 0.272 ± 0.01 mg PGE/ 100 g for extract ethanol 50% (E2), and 0.220± 0.03 mg PGE/ 100 g for extract ethanol 75% (E3) (Figure 2). Ethanol 25% was proved more effected to extract phloroglucinol compounds in the brown seaweed *S. plagyophyllum* than extract with different solvent.

Antioxidant activity of S. plagyophyllum extracts

Antioxidant activity was tested using DPPH radical-scavenging activity method. The antioxidant activity of *S. plagyophyllum* extracts (50 mg/mL) was 41,61±0,02% for extract ethanol 25% (E1), 39,16±0,01% for extract ethanol 50% (E2), 37,58±0,03% for extract ethanol 75% (E3) and ascorbic acid 78.03±0,65 % (at 22.44 µg/mL) as antioxidant activity comparison (Figure 3). It showed that ethanol 25% extract of *S. plagyophyllum* had the highest antioxidant activity than other extracts. Based on these the data, concentration of phenol content in the extracts consistent with their antioxidant activity but were no higher than ascorbic acid as the standard.

Anti-Collagenase activity of S. plagyophyllum extract

Result showed that 50 µg/mL *S. plagyophyllum* extract inhibited 54.46 ± 0.37% activity of collagenase enzyme. Relationship between anti-collagenase activity extracts and concentration of the extracts can be seen in Figure 4 with y = 0.2386x + 42.464 and coefficient of determination R^2 = 0.9136. Where Y is the yields of anti-collagenase, and X is the concentration of extract.

DISCUSSION

Sample that used in this study is true *Sargassum plagyophyllum* based on determination at The Oceanographic Research Centre (P_2O) Indonesian Institute of Sciences (LIPI), and have length of herb 44.5 cm, leaves length 6.1 cm and a width 1.2 cm (Figure 1). Phytochemical qualitative analysis of chemical substances all parts of plants indicated that they have contained alkaloids, flavonoids, and phenols. Qualitative tests were performed to identify the pharmacological compounds present in the extract using a simple method.[14] Phenol is one of the compounds that can prevent aging, cardiovascular disease, and protect from free radicals.[15] Qualitative analysis for *S. plagyophyllum* showed that brown seaweed extracted with ethanol 25%, 50%, and 75% showed also positive contained phenol, flavonoids, and alkaloids.[1] Total phenol in the extract was 0.59 ± 0.01 (E1), 0.27 ± 0.01 (E2), and 0.22± 0.03 mg PGE/ 100 g (E3), respectively (Figure 2). The measurement of phenol content was conducted by Folin-Ciocalteu method because it was a quick and simple method to determine the phenolic content of a sample.[16] The most important phenolic compounds contained in the extract of seaweed is phlorotannin. Phenolic compounds had been reported to have antioxidant properties. Earlier reports revealed that polyphenols in brown seaweed extracts had antioxidant activity.[17] To obtain extracts with maximum phenolic content, the selection of suitable solvents is an important factor in the extraction process. In this study, we used ethanol. Ethanol widely used in the extraction of polyphenol compounds because of its non-toxic nature. The content of phloroglucinol in the *S. plagyophyllum* extracts in various concentration of ethanol can be found in Figure 1. It showed that

S. plagyophyllum immersed in 25% ethanol (E1) had the most phenolic compounds of 0.59± 0.009 mg PGE/ 100 g extract compared to other ethanol concentration and higher the water content in solvent the more phloroglucinol was drawn.

Water as a solvent can attract phlorotannin with strong polarity. It can have affected algae cell to swell and caused the substance out of the cell, indicated that polarity of the solvent was very important in choosing solvent for the extraction process for polar substances.[18] According to previous study *Sargassum serratum* was extracted with ethanol 100% could extracted phenolic compound (4.102 ± 0.005 mg phloroglucinol equivalent/g extract).[18] On the other hand, ethanol 25% was proved more effected to extract phloroglucinol compounds in the brown seaweed *S. plagyophyllum*. There were several factors that affected such a difference, it is not only polarity factor but also difference kind of the structure of phlorotannin in algae, also depending on the geographical area of collection and the genetic variation of the alga itself[19] for example, TPC for *F. serratus* that was collected from Finnavarra, Clare, Ireland has 18.055 mg PGE/ 100 g sample[20] meanwhile, TPC for F. *serratus* was collected from Hvassahraun coastal area, Iceland has 24.0 g PGE/ 100 g sample.[21] Plants with different species are also shown to have different antioxidant activity although included in the same genus.[22]

Antioxidant activity was tested using DPPH radical-scavenging activity method, this method was measured the occurrence of color changes due to the neutralization reaction between free radical molecules by donating hydrogen (H) atom.[23,24] One of the most common method for testing preliminary antioxidant activity of plant extracts is DPPH radical-scavenging.[25] In this test, the antioxidant activity of *S. plagyophyllum* extract (50 mg/mL) was 41,61±0,02% (E1), 39,16±0,01% (E2), 37,58±0,03% (E3) and ascorbic acid 78.03±0,65 % (22.44 µg/mL) as antioxidant activity comparison (Figure 3.). Extract ethanol 25% of *S. plagyophyllum* had the highest antioxidant activity than other extract. Based on these the data, indicated the higher TPC value of *S. plagyophyllum* the higher its antioxidant activity (E1). This result supported by previous studies who found that there are high correlations between concentrations of TPC and antioxidant activity.[17,26]

Figure 1: Brown seaweed (*Sargassum plagyophyllum*) obtained from Pasauran Beach, Serang, Banten.

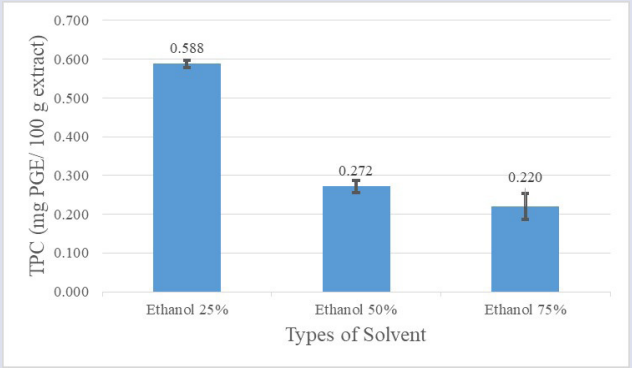

Figure 2: Total Phenolic Content (TPC) of *Sargassum plagyophyllum* extracts

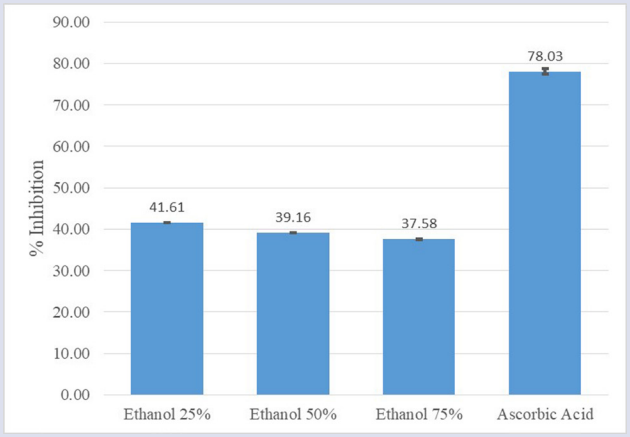

Figure 3: Antioxidant activity of *Sargassum plagyophyllum* extracts (at 50 mg/mL) and ascorbic acid (at 22.44 µg/mL)

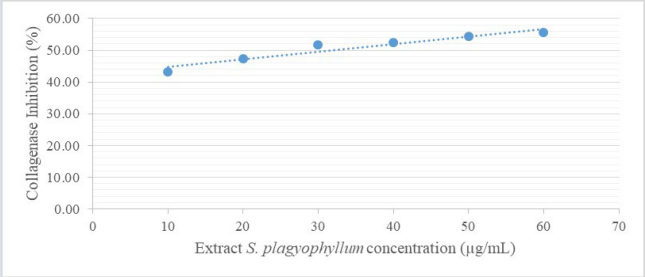

Figure 4: Relationship between anti-collagenase activity extracts and concentration of the extracts.

Anti-Collagenase activity of S. plagyophyllum extract

Activity of anti-collagenase was determined by with microplate readers at 340 nm. Collagenase assay only conducted to ethanol 25% *S. plagyophyllum* extract. Result showed that 50 µg/mL *S. plagyophyllum* extract inhibited 54.46 ± 0.37% activity of collagenase enzyme. Relationship between anti-collagenase activity extracts and concentration of the extracts can be seen in Figure 4. Pientaweeratch[27] revealed that extracts offered anti-aging or anti-wrinkle properties in different mechanisms. Amla showed moderate anti-collagenase activity with the highest phenolic content and antioxidant property. Sapota showed the higher anti-collagenase activity and anti-elastase activity with moderate antioxidant effect.[27] Thus, extracts might be added as a mixture to gain the anti-wrinkle effects. Study on phenolic content might be related to the antioxidants of an extract but has no correlation with anti-proteinase activity[27,28] for the example pomegranate showed no phenolic content but observed inhibit

50% collagenase at 0.5 µl of pomegranate peel extract.[29] Therefore, although the extract has a low TPC value, it is necessary to screen anti-proteinase activity such as anti-collagenase to determine the anti-aging properties of the extracts.[27] It was indicated that structure and physico-chemical properties of the substance can influence activity pharmaceutical ingredient in pharmaceutical product like drug or cosmetics.

CONCLUSION

The present study revealed *Sargassum plagyophyllum* extracts as a new alternative potent anti-collagenase activity for active pharmaceutical ingredient cosmetic anti-wrinkle. We presume that the collagen enzymes inhibitory effects of *Sargassum plagyophyllum* extracts are independent on their total phenolic compound and antioxidant activity. Further research on anti-proteinase activity and determination of active compounds related to anti-wrinkle are necessary to treat wrinkle.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

BHA: Butylated hydroxyanisole; **BHT:** Butylated hydroxytoluene; **Chc:** *Clostridium histolyticum*; **DPPH:** 2,2-Diphenyl-1-picrylhydrazyl; **EGCG:** Epigallocatechingallate; FALGPA : N-[3-(2-furyl)acryloyl]-Leu-Gly-Pro-Ala; ***F. serratus:*** *Fucus serratus*; **LIPI :** Indonesian Institute of Sciences; **PGE :** Phloro Glucinol Equivalents; **P2O :** The Oceanographic Research Centre; **ROS :** Reactive Oxygen Species; **RSA :** Radical Scavenging Activity; ***S. plagyophyllum*:** *Sargassum plagyophyllum*; TPC : Total Phenolic Content; **UV-Vis spectrophotometer:** Ultraviolet-Visible spectrophotometer.

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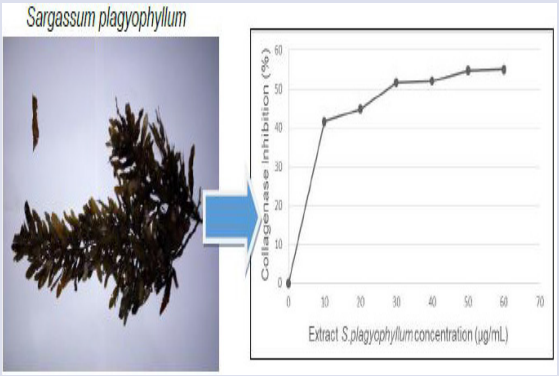
GRAPHICAL ABSTRACT



SUMMARY

- This study revealed that *Sargassum plagyophyllum* have shown anti-collagenase activity against collagenase from *Clostridium histolyticum.*
- Collagen enzymes inhibitory effects of *Sargassum plagyophyllum* extracts are independent on their total phenolic compound and antioxidant activity.
- *Sargassum plagyophyllum* reported being a new promising alternative that can be used as anti-wrinkle ingredient.

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