

ABSTRAK

AKTIVITAS INHIBISI ALPHA-AMILASE HIDROLISAT PROTEIN ALGA MERAH *Gracilaria* sp. SECARA *IN VITRO*

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Pengobatan diabetes mellitus (DM) yang umum diadministrasikan adalah senyawa insulin dan non-insulin masih memiliki risiko efek samping tinggi. Ekstrak alga merah *Gracilaria* sp. telah diteliti memiliki efek inhibisi alpha-amilase (α -A) yang dilaporkan menjadi alternatif pengobatan DM yang lebih aman. Namun, penggunaan *Gracilaria* sp. hanya terbatas pada metabolit sekundernya dan belum dimanfaatkan hidrolisat proteinnya, padahal *Gracilaria* sp. dilaporkan memiliki kandungan total protein yang lebih tinggi dibandingkan *Ulva* sp. dan *Sargassum* sp. Penelitian ini dilakukan untuk mengamati kandungan protein total serta profil protein pada *Gracilaria* sp. dan menganalisis aktivitas inhibisi α -A yang diperoleh dari hidrolisat protein *Gracilaria* sp. Metode yang dilakukan adalah koleksi sampel *Gracilaria* sp. di Pantai Krakal, DI Yogyakarta, preparasi sampel dan uji proksimat, teknik isolasi protein dengan ammonium sulfat (AS) dengan kejenuhan 75%, 80%, 85%, dan 90% yang dikombinasikan dengan etanol 100%, analisis profil protein SDS-PAGE dengan pewarnaan CBB dan *silver-stain*, pengamatan aktivitas inhibisi enzim α -A dengan hidrolisat protein oleh hidrolisis tripsin pada rasio enzim:substrat (b/b): 1:40, 1:50, dan 1:60, serta analisis statistik dengan *one-way* ANOVA dan uji DMRT dengan tingkat kepercayaan 95%, di mana $p < 0,05$ adalah signifikan. Hasil menunjukkan isolasi terbaik pada presipitasi AS 85% dengan rendemen protein 43,67%, kemudian profil protein dengan pita tertebal pada pewarnaan CBB berada pada ukuran <17 kDa dan 25 kDa, sedangkan pada pewarnaan *silver-stain* didapatkan pita yang tebal pada ukuran ~9, ~11, ~20, dan ~48 kDa. Sedangkan aktivitas inhibisi α -A terbaik ada pada hidrolisat protein 1:60, walaupun secara signifikan lebih rendah dari akarbosa pada 1000 ppm. Penelitian dapat dilanjutkan dengan perbedaan teknik presipitasi protein, purifikasi protein lebih lanjut, dan pengukuran inhibisi α -A dengan enzim protease yang berbeda.

Kata kunci: *alpha-amilase, ammonium sulfat, diabetes mellitus, Gracilaria* sp., *hidrolisat protein*

ABSTRACT

***IN VITRO* ANALYSIS OF ALPHA-AMILASE INHIBITORY ACTIVITY FROM PROTEIN HYDROLYSATE OF RED ALGAE *Gracilaria* sp.**

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Treatment of Diabetes mellitus (DM) that is commonly applied is insulin and non-insulin compounds which still have a high risk of side effects. *Gracilaria* sp. extract has been studied to inhibit the enzyme alpha-amylase (α -A), as considered safer drug target of DM. Even though it has a high protein content, the use of *Gracilaria* sp. is only limited to its secondary metabolites and its protein hydrolysate has not been utilized. Therefore, this research was conducted to analyze the best protein extraction method, the protein profile on SDS-PAGE, as well as to investigate α -A inhibitory activity on protein hydrolysates. The method used is the collection of samples of *Gracilaria* sp. at Krakal Beach, D I Yogyakarta, sample preparation and proximate analysis, ammonium sulfate protein precipitation with saturation of 75%, 80%, 85%, and 90% along with ethanol precipitation, protein profiling on CBB-stained and silver -stained SDS-PAGE, proceeded to analysis of α -A inhibitory activity from tryptic protein hydrolysis with enzyme:substrate: 1:40, 1:50, and 1:60 and last, statistical analysis using one-way ANOVA and DMRT test with 95% confidence level, where $p < 0.05$ is significant. The results showed that the best protein isolate resulted from protein isolation with 85% saturated AS with a protein yield of 43,67%. Then, protein profiles can be observed on the thickest band appeared at <17 and ~ 25 KDa on CBB staining while sizes of ~ 9 , ~ 11 , ~ 20 , and ~ 48 kDa on silver-staining. The highest alpha-amylase inhibition activity was observed in the E:S treatment, namely 1:60, but significantly lower than the positive control of acarbose at 1000 ppm. Research can be continued with different protein precipitation techniques, further protein purification, and measurement of α -A inhibition with different protease enzymes.

**Keyword(s): *alpha-amylase, ammonium sulfate, diabetes mellitus, Gracilaria* sp.,
protein hydrolysate**