ISOLASI DAN KARAKTERISASI PROBIOTIK, PENENTUAN KADAR KATEKIN SERTA EKSPRESI GEN PPAR-α DARI TEH HIJAU (*Camellia sinensis*) FERMENTASI PADA MENCIT (*Mus musculus*) DIABETES

DISERTASI

ALIYAH FAHMI
2030412004

Ketua Pembimbing: Prof. Dr. Sumaryati Syukur, M.Sc
Anggota Pembimbing 1: Prof. Dr. Adlis Santoni, M.S
Anggota Pembimbing 2: Dr. Sri Melia, STP., MP

PROGRAM STUDI S3 ILMU KIMIA
FAKULTAS MATEMATIKA DAN ILMU PENGETAHUAN ALAM
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ABSTRAK

Penelitian ini dilakukan dengan membuat teh hijau fermentasi (THF) secara alami, yaitu THF-madu dan THF-gula aren, serta mempelajari probiotik yang terkandung di dalam THF tersebut melalui pendekatan mikrobiologi. Produk fermentasi ini diuji secara in vivo pada model mencit diabetes (*Mus musculus*), dengan fokus pada analisis jaringan, biokimia, ekspresi gen PPAR-α, dan kadar katekin pada teh hijau non-fermentasi, THF-madu hutan, dan THF-gula aren. Sampel teh diambil dari Perkebunan Teh Sidamanik di Sumatera Utara, yang dikenal dengan kandungan katekin, tinggi, namun sifatnya kurang stabil dan bioaksesibilitas rendah di saluran pencernaan. Fermentasi teh hijau dilakukan untuk meningkatkan bioaksesibilitas katekin.

Penelitian ini bertujuan untuk melakukan fermentasi teh hijau dengan penambahan sumber karbohidrat berbeda (madu dan gula aren), mengisolasi dan mengkarakterisasi bakteri asam laktat (BAL) probiotik dari THF tersebut secara mikrobiologi, serta mengevaluasi efeknya pada model mencit diabetes (*Mus musculus*), melalui uji in vivo (histopatologi, biokimia, ekspresi gen PPAR-α) dan penentuan kadar katekin dari TH non-fermentasi, THF-madu dan THF-gula aren menggunakan HPLC.

Probiotik diidentifikasi secara mikrobiologi meliputi morfologi, karakterisasi biokimia, dan identifikasi molekuler dengan 16S rRNA. Uji in vivo dilakukan pada mencit diabetes yang dibagi menjadi lima kelompok: K1 (kontrol negatif), K2 (kontrol positif dengan glibenklamid), K3 (mencit diberi THF-gula aren), K4 (mencit diberi THF-madu hutan), K5 (mencit diberi teh hijau non-fermentasi), dan K0 (mencit normal). Analisis dilakukan dengan histopatologi (pewarnaan hematoksilin dan eosin untuk jaringan pankreas dan usus), imunohistokimia (jaringan usus), dan biokimia (penentuan kadar glukosa, profil lipid, MDA, dan CAT menggunakan spektrofotometer UV-Vis), serta ekspresi gen PPAR-α menggunakan rt-PCR. Kadar katekin pada TH-non fermentasi, THF-madu hutan, dan THF-gula aren diukur dengan teknik HPLC.

Hasil penelitian menunjukkan bahwa THF-madu mengandung probiotik *Lactobacillus plantarum* Y-1, sementara THF-gula aren mengandung

Lacticaseibacillus paracasei HBUAS62903. Analisis histopatologi menunjukkan hasil terbaik pada kelompok K0, dengan rerata area 0,57 dan perimeter 3,12, serta hasil imunohistokimia yang paling baik pada K4. Uji biokimia menunjukkan bahwa K4 memiliki kadar glukosa terendah (184,67±85,38 mg/dL), profil lipid terbaik (kolesterol 44,79±8,35 mg/dL; trigliserida 122,02±57,61 mg/dL; HDL 101,77±5,3 mg/dL), kadar MDA terendah (3,04±0,071 nmol/mg), dan kadar CAT tertinggi (5,31±0,43 nmol/mg). Ekspresi gen PPAR-α tertinggi juga ditemukan pada kelompok K4 (21,88±1,27). Kadar katekin pada TH-non fermentasi adalah 16,9%, THF-madu hutan 4,08%, dan THF-gula aren 0%.

Berdasarkan hasil penelitian ini, dapat disimpulkan bahwa THF-madu hutan lebih efektif dibandingkan THF-gula aren dan teh hijau non-fermentasi dalam uji jaringan, biokimia, dan ekspresi gen PPAR-α, sehingga berpotensi sebagai kandidat suplemen untuk mengatasi diabetes mellitus.

Kata Kunci: teh hijau fermentasi, probiotik, katekin, *Mus musculus* diabetes, gen PPAR-α

ABSTRACT

This study was conducted by making naturally fermented green tea (FGT), namely FGT-honey and FGT-palm sugar, and studying the probiotics contained in the FGT through a microbiological approach. These fermented products were tested in vivo on a diabetic mouse model (*Mus musculus*), focusing on tissue analysis, biochemistry, PPAR-α gene expression, and catechin levels in non-fermented green tea, FGT-forest honey, and FGT-palm sugar. Tea samples were taken from the Sidamanik Tea Plantation in North Sumatra, known for its high catechin content. Still, its nature is less stable and has low bio-accessibility in the digestive tract. Green tea fermentation is carried out to increase the bio-accessibility of catechins.

This study aims to ferment green tea with the addition of different carbohydrate sources (honey and palm sugar), isolate and characterize probiotic lactic acid bacteria (LAB) from FGT microbiologically, and evaluate its effects on diabetic mice (*Mus musculus*) models, through in vivo tests (histopathology, biochemistry, PPAR-α gene expression) and determination of catechin levels from non-fermented GT, FGT-honey and FGT-palm sugar using HPLC.

Probiotics were identified microbiologically including morphology, biochemical characterization, and molecular identification with 16S rRNA. In vivo tests were conducted on diabetic mice divided into five groups: K1 (negative control), K2 (positive control with glibenclamide), K3 (mice given FGT-palm sugar), K4 (mice given FGT-forest honey), K5 (mice given non-fermented green tea), and K0 (normal mice). Analysis was performed using histopathology (hematoxylin and eosin staining for pancreatic and intestinal tissue), immunohistochemistry (intestinal tissue), and biochemistry (determination of glucose levels, lipid profiles, MDA, and CAT using a UV-Vis spectrophotometer), as well as PPAR-α gene expression using rt-PCR. Catechin levels in GT-non-fermentation, FGT-forest honey, and FGT-palm sugar were measured using the HPLC technique. The results showed that FGT-honey contained the probiotic *Lactobacillus plantarum* Y-1, while FGT-palm sugar contained *Lacticaseibacillus paracasei* HBUAS62903. Histopathology analysis showed the best results in group K0, with an average area of 0.57 and a perimeter of 3.12, and the best immunohistochemistry results in K4. Biochemical tests showed that K4 had the

lowest glucose levels (184.67±85.38 mg/dL), the best lipid profile (cholesterol 44.79±8.35 mg/dL; triglycerides 122.02±57.61 mg/dL; HDL 101.77±5.3 mg/dL), the lowest MDA levels (3.04±0.071 nmol/mg), and the highest CAT levels (5.31±0.43 nmol/mg). The highest PPAR-α gene expression was also found in the K4 group (21.88±1.27). The catechin levels in TH-non-fermentation were 16.9%, FGT-forest honey 4.08%, and FGT-palm sugar 0%.

Based on the results of this study, it can be concluded that FGT-forest honey is more effective than FGT-palm sugar and non-fermented green tea in tissue, biochemical, and PPAR-α gene expression tests, so it has the potential as a supplement candidate to overcome diabetes mellitus.

Keywords: fermented green tea, probiotics, catechins, *Mus musculus* diabetes, PPAR-α gene