

**IDENTIFICATION OF ANTIBACTERIAL PEPTIDE FRACTIONATED
WITH CATION EXCHANGE SPE COLUMN OF JATROPHA SEED
(*Ricinus communis* L.) TRYPSIN HYDROLYZED PROTEIN
EXTRACTED WITH TRIFLUOROACETIC ACID**

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17/414622/PA/18122

ABSTRACT

A research to identify antibacterial peptides from jatropha seed containing RIP (ribosome inactivating protein) which is one of the antibacterial mechanisms has been carried out. This study aims to determine the amino acid sequence of peptides with antibacterial activity resulting from the enzymatic hydrolysis of jatropha seed protein (*Ricinus communis* L.) which was extracted using acid in order to obtain a high amount of protein.

The jatropha seed powder was defatted by extraction using petroleum ether before extracting the protein using trifluoroacetic acid. Protein was reduced and alkylated using dithiothreitol and iodoacetamide then hydrolyzed with trypsin enzymes. The hydrolyzate was fractionated with a cation exchange SPE column using various pH of elution solvent. The antibacterial activity test was carried out on *Escherichia coli* and *Staphylococcus aureus*, qualitatively through the disc diffusion method and determination of the MIC and IC₅₀ values which was performed using the microdilution method. Peptides which have antibacterial activity will be analyzed by High Resolution Mass Spectroscopy.

Protein obtained from jatropha seed extraction using trifluoroacetic acid is 60.4%. The pH 3 and pH 4 peptide fractions show inhibitory zones against *E. coli* with inhibitory zone diameters of 22 and 20 mm, respectively. While inhibitory zone diameters against *S. aureus* were 16 and 10 mm, respectively. The values of MIC and IC₅₀ of pH 3 fraction against *E. coli* were 6.60 and 17.95 µg/mL, while those against *S. aureus* were 6.60 and 17.03 µg/mL, respectively. Fraction obtained at pH 4 fraction show antibacterial activity against *E. coli* with MIC and IC₅₀ values of 18.30 and 37.30 µg/mL, respectively and those against *S. aureus* were 36.70 and 95.31 µg/mL, respectively. Both fractions at pH 3 and pH 4 were found to contain 3 peptides. Peptides found in pH 3 fraction were AAGASG; GAGLVPR; and MGACCSKEPSFAEGR, while those in pH 4 fraction were VALASLLSQPLPQISDK; AAGASG; and GAGLVPR.

Keywords: antimicrobial peptide, protein hydrolysis, *Ricinus communis* L. seed, trifluoroacetic acid, trypsin enzyme