Antibacterial mechanism of Kratom (Mitragyna speciosa) methanol extract on Streptococcus pneumoniae and Eschericia coli bacteria

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\textbf{ABSTRACT}

\textbf{Introduction:} Kratom (Mitragyna speciosa) is a tropical herbal plant native to the North Malay Peninsula, Malaysia and Thailand. In Indonesia, kratom is a typical plant of West Kalimantan, especially in Putussibau. Kratom leaf has many pharmacological effects, one of them is antioxidant. However, the antioxidant and antimicrobial activities of Mitragyna speciosa leaf extracts are lacking. This study was to examine the effect of the methanol extract of Mitragyna speciosa (Rubiaceae Family) leaves on microorganisms Escherichia coli and Streptococcus pneumoniae.

\textbf{Methods:} Kratom leaves were extracted with methanol as solvent. In this study, S. pneumoniae and E. coli bacteria have been used. Antibacterial activity tests were carried out at concentrations of 0.78%, 1.56%, 3.125%, 6.25%, 12.5%, 25%, 50% and 100%. Insilico was used to search for secondary metabolites and metabolite interactions in Mitragyna speciosa.

\textbf{Results:} Mitragyna speciosa leaf methanol extract was effective in inhibiting the growth of E. coli and S. pneumoniae significantly (P<0.001). The growth of E. coli was not identified from a concentration of 25% to 100%. However, there was no bacterial growth in S. pneumoniae from a concentration of 6.25% to 100%. In the insilico study it was found that the secondary metabolite of Mitragyna speciosa is quercetin, which interacts with the ATPI protein as a predictor in the mechanism of Mitragyna speciosa as an antibacterial.

\textbf{Conclusion:} In conclusion, there is no correlation between nutritional status and remission outcome of patients with ALL in the induction phase of therapy. However, high percentage of underweight patients shows nutrition needs special attention to improve therapy outcomes.
treatment for diabetes and reduce sugar levels. Previous studies on kratom leaves show pharmacological effect activity, among those; analgesic and stimulants, antidepressant, anti-inflammatory and antinociceptive, antioxidant and antibacterial. The effectiveness of kratom as an antibacterial has been carried out on several types of bacterial, including the bacterial that cause acne namely Propionibacterium acnes, in *Salmonella typhi* and *Bacillus subtilis*.

The activity of kratom as an antimicrobial cannot be separated from the composition contained in the kratom leaves, including the high content of phenolics and flavonoids in the methanol extract of kratom leaves.

Phytochemical studies have shown that there are several chemical constituents of *Mitragyna speciosa* plants including indole alkaloids, including mitragynine, 7-hydroxymitraginine, 5-desmethylmitragynine, 17-desmethyldihydro-mitragynine, speciogynine, speciocilliaine, and paynantheine, and also contain secondary metabolites such as flavonoids, saponin, monoterpenes, triterpenoids, secoiridoids and polyphenolic compounds.

The antimicrobial potential of kratom is also expected to have an effect on the bacteria that cause respiratory and digestive tract infections. Therefore, this study aimed to examine the effect of kratom leaf as an antimicrobial on Staphylococcal pneumonia and *E. coli* bacteria.

### Methods

#### Plant material

The plant of leaves of *Mitragyna speciosa* were collected from Putussibau state, West Kalimnatan, Indonesia. *Mitragyna speciosa* leaves are washed with water to remove dirt before the drying process. Then the leaves are cut and dried in direct sunlight for 2-3 days. After cutting the leaves and grounded into powder.

#### Methanolic Extract

The dried leaves of M. speciosa were ground in powder form. A total of 2.08 kg of *Mitragyna speciosa* powder was extracted using 96% methanol as solvent. The solvent was changed every 1x24 hours and macerated for 7x24 hours. After maceration, it was concentrated using a rotary evaporator and a water bath to obtain a thick extract of 20 g of crude methanol extract and stored at -20°C.

#### Test microorganisms and growth media

We collected *S. pneumoniae* and *E. coli* provided by the Surabaya Health Laboratory Center (BBLK). Bacterial colonies were taken by means of inoculum suspension for 24 hours of culture, then suspended in sterile 0.9% NaCl solution.

#### Antibacterial testing

The antibacterial activity of *Mitragyna speciosa* leaf extract was tested by agar diffusion method using NA media. Bacterial suspension of 0.02 ml 1:40 dilution was mixed with 10 ml of NA medium in a diluent bottle, then shaken until homogeneous. Take the inoculant in the tube and scratch it on a petri dish, and incubate at 37°C for 18-24 hours, then observe the growth of bacteria. Testing the antibacterial activity of kratom leaf extract against *S. pneumoniae* and *E. coli* as carried out with 8 (eight) variations of concentration namely 0.78%, 1.56%, 3.72%, 6.25%, 12.5%, 25%, 50% and 100%. The whole test tube incubated in an incubator at 37°C for 18-24 hours. Then make observations of the whole tube against tube clarity by viewing control.

#### Statistical analysis

This research was repeated three times. All results are expressed as mean ± SEM. Differences between groups were analyzed by one-way analysis of variance (ANOVA), followed by Tukey’s post hoc analysis. Dose-response curve comparisons were made with a two-factor repeated-measurement ANOVA, followed by Tukey’s post hoc test for comparison between groups. P<0.05 was considered significant. analysis using SPSS version 21 program.

### Results

#### Minimum Inhibitory Concentration of Methanol Extract of Mytragina Speciosa of *E. coli*

Minimum Inhibitory Concentration of extract ethanol Mytragina Speciosa of *E. coli* showed that the activity decreased at a concentration of 25%, 50% and 100% (figure.1A), where there was no visible bacterial growth compared to the control group. The bacterial count also showed that the lower the extract concentration, the more bacterial growth (figure.1B). Figure.1 Antimicroba activity (MIC) of Mytragyna speciosa extract in *E. coli*

#### Minimum Inhibitory Concentration of Methanol Extract of Mytragina Speciosa in Strepococcus pneumoniae

Minimum Inhibitory Concentration of Extract Methanol Mitragyna Speciosa of *S. pneumoniae* showed that the activity decreased at a concentration of 6.25%, 12.5%, 25%, 50% and 100% (figure.2A), where there was no visible bacterial growth compared to the control group. The bacterial count also showed that the lower the extract concentration, the more bacterial growth (figure.2B). Figure.2 Antimicroba activity (MIC) of Mytragyna speciosa extract in Strep.pneumonia

#### Interaction of kratom secondary metabolite compounds

Based on previous research, the composition of the methanol extract of *Mitragyna speciosa* has been obtained. In this study we examine the interaction of the active compound from the methanolic extract of mitragyna speciosa, namely quercetin which is the equivalent of the flavonoid content. (Figure.3)
Figure 1. Minimum Inhibitor Concentration of Methanol Extract of *Mytragina Speciosa* in *E.Coli*. Ctrl; Control, MS; *Mitragyna Speciosa*. **; P<0.01, ***; P<0.001 vs control group.

Figure 2. Minimum Inhibitory Concentration of Methanol Extract of *Mytragina speciosa* in *Streptococcus pneumoniae*. Ctrl; Control, MS; *Mitragyna Speciosa*. **; P<0.01, ***; P<0.001 vs control group.

Figure 3. Prediction of interactions between chemicals (quercetin) and proteins in the body insilico.
Quercetin is a flavonoid that is widely found in plants. In this insilico quercetin has an interaction bond with atp1. ATP synthase (F(1)F(0) ATP synthase or Complex V) generates ATP from ADP in the presence of a proton gradient across the membrane generated by the electron transport complex of the respiratory chain. This quercetin interaction is predictive of its antimicrobial activity by inhibiting ATP1 synthesis at the cellular level. While osl and xd1 are proteins that do not have special characteristics.

Discussion
In this study, it was found that the methanolic extract of Mitragyna speciosa (Kratom) had an antimicrobial effect on bacteria that cause respiratory tract infection and bacteria in the digestive tract. The results of this study showed that Mytragina speciosa methanol extract was effective as an antimicrobial at higher concentrations. Where in this study found at a concentration of more than 12.5% Escherichia coli bacteria were not found, and at concentrations > 3.72% Streptococcus pneumoniae bacteria. In line with this study, previous studies have shown the effectiveness of Mytragina speciosa as an antimicrobial of several types of extract solvents. Antimicrobial activity was found to be effective in MeoH and Alkaloid extracts, which have the ability to inhibit growth and kill larger bacteria Salmonella typhi and Bacillus subtilis. Other studies have also found that kratom has an antimicrobial effect that has an inhibitory effect on Aeromonas hydrophylia.

Antibacterial activity test shows that increasing the concentration of extract given can increase the killing power of the extract against bacteria. However, if the concentration of the extract continues to increase, it can reduce the killing power. This finding suggested that the increasing concentration of Kratom can lead to an increase in viscosity of the extract, thus affecting the rate of diffusion of extract in agar media. In addition to the concentration factor, the type of antimicrobial material can also determine the ability to inhibit bacterial growth.

The effectiveness of kratom as an antimicrobial has been known for a long time, based on the results of phytochemicals in kratom leaves, it has been found that kratom leaves contain alkaloid, flavonoids, steroid, saponin and tannins and have antioxidant effect. It has been found in previous studies that compounds derived from plants have antibacterial activity based on phytochemical data. The components of the active substances in mitragyna speciosa extract have been proven, including alkaloids, flavonoids, saponin, tannins, phenols and other active compounds.

In nature, flavonoid compounds are products extracted from plants and they are found in several parts of the plant. Flavonoids play a variety of biological activities in plants, animals and bacteria. Flavonoids are associated with a wide spectrum of health-promoting effects and are indispensable components in a wide range of nutraceutical, pharmaceutical, medicinal and cosmetic applications. This is due to its antioxidant, anti-inflammatory, antimutagenic and anti-carcinogenic properties coupled with its capacity to modulate the function of key cellular enzymes. In previous studies, it was found that the active substance of the flavonoid class is quercetin in methanol extract of Mitragyna speciosa. The quercetin content in the MeOH Kratom extract is quite high. The presence of quercetin as a sub-flavonoid gives hope that the content of kratom has a good effect for use as herbal medicine. In this study we found that quercetin has a high pharmacokinetic effect on absorption in the gastrointestinal tract. In addition, to find out of mechanism target of quercetin, we examine in insilico studies. this study showed predictions of quercetine's target of action on enzymes, oxidoreductase, G-protein receptors, enzymes, proteases, lyase and cytochrome p450. Based on the results of this study, it is possible that the quercetin content in the Methanol kratom extract has the ability as an antibacterial against Streptococcus pneumoniae and Eschericia coli. This study is supported by the results of previous studies which found that quercetin inhibits the formation of streptococcus pneumoniae biofilms.

In addition, other studies also found that quercetine in surgacane bagasse extract showed bacteriostatic activity against the growth of S. aureus, L. monocytogenes, E. coli, and S. typhimurium, and could change ATP activity, thus effecting the growth of E.coli. In an in vivo study, it was found that quercetin can protect mice from s.aureus infection by inhibiting thrombin activity. To confirm the interaction of quercetine with proteins in the body, we performed an insilico screening. In this study it was found that quercetine can interact with ATP synthase FO subunit 1 (ATP1) in humans. As we know that ATP is the main energy compound in biological synthesis, and most of it is provided by the F1FO-ATP synthase which is located in the membrane and can be found in bacteria, mitochondria and chloroplasts.

The findings in this study can be used as a basis for explaining the mechanism that methanol extract of Mitragina speciosa (kratom) leaves has an antibacterial effect on Streptococcus pneumoniae and Eschericial coli, through the mechanism of inhibition of ATP synthase Fo subunit 1 (ATP1).

Conclusion
Mitragyna speciosa (kratom) methanol extract has an antimicrobial effect on streptococcus pneumonia and e.coli through the effect of quercetine contained in kratom. This antimicrobial mechanism is thought to be by inhibiting ATP synthesis through the ATP synthase Fo subunit 1 (atp1) pathway. The broad spectrum antimicrobial properties of quercetin can be used in the prevention and treatment of various infectious bacterial diseases and may provide a treatment option to reduce the use of antibiotics.

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Conflict of Interest
The author stated there is no conflict of interest
References


