Determination of Minimum Effective Concentration of Cashew Nut Shell (CNS) Pyrolysis Products for Antibacterial Escherichia coli Using Kinetics Approach

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Abstract. Determination of minimum effective concentration of cashew nut shell (CNS) pyrolysis products as an antibacterial Escherichia coli using kinetics approach has been done. The purpose of this study is to determine minimum concentration of CNS pyrolysis products which are effective as antibacterial E. coli using chemical kinetics and determine reaction order (n) and rate constant (k), equipped with the rate of reaction equation. And it also determine the relation of initial concentration [A]₀, concentration in time [A]ₜ and time variable (t). The results showed that the CNS pyrolysis products consist of two groups: phenolic compounds and alkane compounds. GCMS results also showed that main constituent of the compound is m-octyl-phenol (13.86%). Inhibitory zone on variation in concentration of 100%, 75%, 50%, 25% and 12.5% was 1.47; 1.20; 1.19; 0.87; and 0.75 cm respectively. Reaction order (n) = 0.3 and rate constants (k) = 3.3 so reaction rate equations is r = 3.3[A]₀³. Relations of initial concentration [A]₀ and concentration in time [A]ₜ and time variable (t) obtained [A]ₜ = [A]₀-0.05t. Minimum concentration making the CNS pyrolysis products effective as an antibacterial E. coli is 24.06%.

1. Introduction
Cashew (Anacardium occidentale L) is one of the commodities with a significant contribution to the economy of the Indonesian people. As one of the largest producers in Asia [1], Indonesia produces 139,698 tons of cashews with a productivity level of 539 kg/ha [2]. Main products of cashew plants are cashew nuts, while the by-products are cashew apples and cashew nutshells (CNS), the latter of which are considered as waste and may become an environmental problem if not handled properly [3]. Therefore, special handling of CNS waste is needed.

CNS, when extracted, will produce a liquid called Cashew Nut Shell Liquid (CNSL) in the form of oil, which is composed of complex phenolic compounds with long branched and unsaturated carbon chains [4]. It is a viscous liquid, blackish-brown in colour, and highly reactive in oxidation and polymerisation reactions [5]. Its main components are anacardic acid, cardanol, and cardol, which are natural phenolic compounds with many advantages when compared to the synthesized ones [4]. The phenolic compounds in CNSL potentially function as an antibacterial that can inhibit bacterial growth by working to poison the cytoplasm, damage and penetrate walls, and precipitate bacterial cell proteins [6].
One alternative technology to obtain natural phenolic compounds from CNS is the pyrolysis technique. It is chemical decomposition of organic material through a process of heating without oxygen, in which the raw material undergoes chemical structure breakdown into a gas phase [7]. Pyrolysis process produces a useful product in the form of hot steam, which is then condensed into liquid (bio-oil), syngas (biogas), and char (bio-charcoal). Components of the distilled bio-oil comprise phenolic, acidic, and aromatic polycyclic hydrocarbon compounds [4].

Antibacterial activity of CNS pyrolysis results can be determined based on inhibitory power measured by studying the inhibitory pattern using a chemical kinetics approach by calculating reaction orders and activity rate. The rate law means a constant multiplied by some functions of concentrations of A and B that is written as rate = k [A]^x [B]^y, where x and y are exponents of concentration of A and B, respectively. In this law, k is rate constant, and exponents x and y are reaction orders concerning A and B, respectively [8]. Reaction orders can only be calculated experimentally and predicted if all reaction orders are determinable as the numbers of exponents for each reactant are known [9].

An antibacterial agent such as a CNS pyrolysis product obtained through a long process should be used with consideration of efficiency, namely with the lowest possible concentration but high effectiveness. Based on this description, an experiment took place to determine the minimum effective concentration of CNS oil pyrolysis product through kinetics approach to be an agent against *E. coli*.

2. Experimental Method

2.1. Preparation and Treatment
Cashew nuts were obtained from cashew plantations in Kapota Village, Wakatobi Regency, South Wangi-wangi District, Sulawesi Tenggara. They were dried up and then separated from the shells. The CNS sample was weighed as much as 2.5 kg and then pyrolyzed using a simple pyrolysis reactor.

2.2. Sample characterisation
CNS pyrolysis products were determined using GCMS. The results were obtained in the form of chromatogram and mass spectrum, and further interpreted by comparing them with the literature [10].

2.3. Culture of Test Microorganisms
The nutrient agar medium contained 2% peptone, 1.5% yeast extract, 4% agar, and 1% NaCl. It was prepared by dissolving 22.1 g of nutrient agar with 260 mL of aquadest within Erlenmeyer flask, and then sterilized in an autoclave at 121°C for 15 minutes [11]. The microorganism used was *Escherichia coli* ATCC 25923. The bacteria were rejuvenated by transferring 1 or 2 inoculating loops from the stock that has been supplied to the reaction tube containing 10 mL of sterile liquid media (2% peptone, 1.5% yeast extract, and 4%NaCl) and incubated for 24 hours at 37 ± 2°C [12].

2.4. Antibacterial Activity Testing
The NA medium was piped as much as 20 mL and then put into eppendorf tube and added with 10 µL of bacterium *E. coli*, and then shaken until homogenous. Having been homogenous, it was poured in a petri dish with a circular motion until the media docked at a surface of petri dish, and let it stand a few minutes until solid. It was then placed with a disk paper (d: 0.5 cm) that has been soaked in the test solution CNS pyrolysis products (100%, 75%, 50%, 25%, 12.5%), positive control of amoxicillin, negative control (tween oil and aquadest) on the surface of the solidified agar medium. Afterwards, the petri dish is closed tightly and wrapped in plastic wrap. It was then incubated for 1 x 24 hours in room temperature and inhibitory zone formed was measured [13].

2.5. Data Processing and Analysis
For reactions involving a single reactant, the rate of r can generally be written as follows:

$$r = k [A]^n$$

therefore, taking logarithms from both sides of the equation results in:
\[ \ln r = \ln k + n \ln [A] \]

a plot of values that is obtained for \( \ln r \) versus \( \ln [A] \) gives a linear relation with the slope of \( n \), the reaction sequence with respect to \( A \). It is because the intercept is equal to \( \ln k \) [8]. The rate of reaction in inhibiting the antibacterial activity of \( E. coli \) by pyrolysis products can be determined:

\[ r = \frac{iz}{\text{incubation time}}. \]

If negotiations are carried out at the same time then the change in time in regression analysis can be changed as needed:

\[ \ln iz = \ln k + n \ln [A] \]

Note: \( r \) = Rate, \( iz \) = inhibitory zone, \( k \) = reaction rate constant, \( [A] \) = substance concentration, \( n \) = Order reaction. If \( \ln \) plot is made of relation \( [A] \) to \( \ln iz \), we will obtain [9]:

\[ a = \ln k; k = e^a; b = n = \text{order} \]

Determination of minimum concentration of CNS pyrolysis products needed to provide inhibitory power on \( E. coli \) is calculated using regression analysis: \( \ln iz = \ln k + n \ln [A] \), where \( iz \) is the standard inhibitory zone of inhibition by positive control. If \( iz \), \( k \) and \( n \) are determined then the value of \( [A] \) sought can be calculated by:

\[ n \ln [A] = \ln (iz/k) \] and \[ \ln[A] = \left\{ \ln (iz / k) \right\} / n \]

\[ [A]_{\text{min}} = e^{(\ln (iz / k)/n)} \]

3. Results and Discussion

3.1. Analysis of CNS Pyrolysis Products Compound Components with GCMS

CNS pyrolysis products obtained in this study amounted to 770 mL and brownish black. The sample of CNS pyrolysis products was analyzed with its constituent components with GCMS and displayed many components of which 5 main compounds are shown in Table 1 and Fig. 1

| No | Compound               | Retention Time (Minute) | Area (%) |
|----|------------------------|-------------------------|----------|
| 1  | o-methyl phenol        | 16.900                  | 4.59     |
| 2  | m-butyl phenol         | 31.296                  | 3.35     |
| 3  | m-pentadecyl phenol    | 33.823                  | 4.14     |
| 4  | m-octyl phenol         | 50.013                  | 13.86    |
| 5  | m-undecyl phenol       | 50.281                  | 3.48     |
3.2. Antibacterial activity of CNS pyrolysis products against *E. coli*

Activity test results of CNS pyrolysis products in inhibiting the growth of *E. coli* bacteria can be seen in Figures 2 and 3:

**Figure 1.** Chromatogram of CNS pyrolysis products

**Figure 2.** The result of antibacterial activity of CNS pyrolysis products
Figure 3. Antibacterial activity of CNS pyrolysis products against *E. coli*

Figure 3 disputes that each variation of CNS pyrolysis products gives different inhibitory zone (mm) values to *E. coli*. The above data proves that diameter of the inhibitory zone (mm) of CNS pyrolysis products for concentrations of 12.5%, 25%, 50%, 75%, and 100% gives the value of the inhibitory zone 7.50; 8.70; 11.90; 12.00 and 14.70 mm, respectively. Positive controls produce 9.00 mm inhibitory zones, while negative controls do not produce inhibitory zones. Based on these results, it is known that each increase in the sample will enlarge the diameter of inhibitory zone. Measurement results indicate that CNS pyrolysis products provides an insensitive inhibitory response to a concentration of 12.5%; sensitive enough for concentrations of 25%, 50%, and 75%, and sensitive for 100%. According to previously published studies, the diameter of inhibitory zones is valued as follows: Insensitive (diameter ≤ 8.0 mm), moderate sensitive (8.0 < diameter < 14.0 mm), sensitive (14.0 < diameter < 20.0 mm), and extreme sensitive (diameter ≥ 20.0 mm) [14].

The inhibitory zone caused by the results on CNS pyrolysis contains most phenolic-derived compositions. The content of phenolic compounds can inhibit the growth of bacteria that work by poisoning cytoplasm, damaging and penetrating the walls and precipitating bacterial cell proteins [15]. When phenolic group compounds penetrate the bacterial cell membrane then interact with enzymes and proteins in the membrane, it can cause adhesion of bacterial cell membrane so that the osmotic pressure increases. This can cause damage to the cell membrane and inhibit bacterial respiration, which in turn causes interference with transfer of ion in the cell so that the bacteria experience died [16] [17].

3.3. Review of Chemical Kinetics of CNS Pyrolysis Products on Antibacterial Activity of *E. coli*

Chemical kinetics of CNS pyrolysis products on antibacterial activity can be studied through a plot of ln iz (inhibitory zone) with ln A (antibacterial monitoring). The analysis is performed using linear regression result that can be shown in Figure 4, as follows:
Equations in Fig.4 represents $y = 0.3146x + 1.966$. It can be seen that the CNS pyrolysis products reaction as an antibacterial *E. coli* amounted to 0.3146, meanwhile, the rate constant can be obtained with $e^{1.966} = 3.3088$. Reaction order is 0.3146 and it show influences concentration to rate reaction. This reaction occurs because the reaction order is between 0 order and 1 order. Cornish-Bowden [18] states that the analysis results are observed to be zero sequence with a constant speed. Based on the one-order reaction that is directly proportional to the concentration of reagent A. It can be understood that the reaction order produced in this study is 0.3146 and indicates the influential reactant concentration to the formation of antibacterial inhibitory zones research by increasing research results of CNS pyrolysis products' effect but not bigger than 1 order reaction effect. The equation is obtained:

$$iz = 3.3088[A]^{0.3146}$$

It can represent linearity between relations $\ln iz$ vs $\ln [A]$ is $R^2 = 0.9599$, so it can be interpreted as more than variable that has very strong relation. The coefficient changes from 0.90 to 1.00 then can be interpreted to be very strong or very high [19]. Minimum effective CNS pyrolysis products concentration used as an antibacterial *E. coli* can be account:

$$[A] = \left(\frac{9.00}{3.3088}\right)^{1/0.3146} = 24.06\%$$

The account results represent minimum concentration of an effective CNS pyrolysis product as antibacterial *E. coli* so that it obtains same inhibitory power as positive control, it is necessary to use CNS pyrolysis products of 24.06%.

4. Conclusion
The results of antibacterial activity test of the CNS pyrolysis products against *E. coli* showed different inhibitory variations for each concentration of 12.5%, 25%, 50%, 75% and 100% with the inhibit zone 7.50; 8.70; 11.90; 12.00 and 14.70 mm, respectively. Reaction order (n) of the CNS pyrolysis products in antibacterial activity of *E. coli* is $n = 0.3146$, with $k = 3.3088$. Minimum effective concentration in inhibition is 24.06%.

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