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Bioactivity of Sengkubak (*Pycnarrhena cauliflora* Diels.) Leaf Ethanolic Extract as Antioxidant and Antibacterial *Streptococcus Sanguinis*

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Abstract: *Pycnarrhena cauliflora* Diels. known as Sengkubak, is a natural-based potential antioxidant and antibacterial from the Menispermaceae family. *Streptococcus sanguinis* is a primary colonizer bacteria that is consistent in biofilm formation. This study attempted to identify *S. sanguinis* inhibition from antibacterial and antioxidant bioactivity of sengkubak leaf extract. Sengkubak leaves samples were extracted with 70% ethanol through 72 hours (3x24) maceration. Phytochemicals were identified qualitatively and quantitatively through analysis of total quercetin and phenolic content. Antioxidants were tested using the DPPH method. *S. sanguinis* sensitivity was tested through liquid microdilution method. The extract of sengkubak leaves has a yield of 16.30% (w/w). Qualitative screening showed positive results for flavonoids, phenols, alkaloids, saponins, terpenoids, tannins, and steroids. TFC (quercetin) in extract was 5.22 ± 0.969 QE/g and TPC was 4.08 ± 1.005 mg GAE/g. The IC₅₀ antioxidant value of extract was 40.136 µg/mL. The minimum inhibitory concentration value is shown at 5×10^4 µg/mL. This research study proves that ethanol extract of sengkubak leaves holds various phytochemical compounds which are likely utilized as potent antioxidants and have inhibitory effect against *S. sanguinis*.

Keyword: Sengkubak extract, Phytochemicals, *Streptococcus sanguinis*, Minimum Inhibitory Concentration.

INTRODUCTION

Indonesia biodiversity of plants reaches 20,000 species with the presence of 40% of endemic plants. There is a variety of local forest resources in Kalimantan, *Pycnarrhena cauliflora* Diels. which has a local name sengkubak. It is known that sengkubak can live in secondary forest and lowland habitats at an altitude of 80-700 m above sea level according to Global Biodiversity Information Facility Secretariat (2022). Due to its quite varied nature of

adaptation, the number of distribution distributions and the diversity of *Pycnarrhena* species is still not known significantly. This statement is supported by the pattern of plant distribution found in Papua New Guinea (*P.ozantha*), Borneo (*P.borneensis*), Java (*P.macrocarpa*), Sulawesi (*P.calocarpa*), Timor-Leste (*P.longifolia*), Philippines (*P.manillensis*), and Himalayas (*P.longiflora*) (Iriani E. S. et al., 2021).

The use of sengkubak (*Pycnarrhena cauliflora* Diels.) leaves by local people in the food sector is often used as an alternative to natural cooking ingredients. According to the Center for Research on Spices and Medicinal Plants, although it is still minimally cultivated by a wide audience, sengkubak leaves are still used as a traditional medicinal plant that can relieve symptoms of nausea, headaches, and even prolonged fever. This was confirmed in previous research which stated that sengkubak leaves are often used in the health sector as a traditional medicine that can treat ailments such as fever or headaches and in the food sector as a substitute for cooking spices. According to previous research who identified the phytochemicals in sengkubak found that alkaloids, phenols, steroids, flavonoids, terpenoids contain various biological activities such as antifungal, anticancer, antioxidant and antibacterial which have the potential to be used in the health sector (Masriani, 2019).

Biofilm is a growth layer of microorganisms such as bacteria that can adhere to the surface epithelium of the teeth and oral cavity. The formation of biofilm or plaque has been treated through brushing, flossing, or using commercial products such as mouthwash. Even so, this method is not always effective in inhibiting biofilm formation, so it is felt necessary to start exploring the use of low-risk natural ingredients as antibacterial and antioxidant agents, especially for dental and oral health. Based on research data and various dental and oral health prevalence in Indonesia which are still quite high, concern is needed to the content of food consumed and to people who have immunodeficiencies. Eating habits and patterns that are not maintained when the body's condition is vulnerable to infection attacks are often the cause of oral and dental health problems which are commonly caused by various bacterial microorganisms such as *Streptococcus sanguinis*. *S. sanguinis* itself is known as a primer colonizer bacterium that create formation of biofilm or plaque on the tooth surface through attachment to the protein components of its cell wall, that there will be replication of other secondary colonizing bacteria to form microcolonies who cause health problems in the oral (Septiani et al., 2022). From these scenes, it is necessary to make efforts and more studies specifically on the potential use of sengkubak leaves as antibacterial and antioxidant ingredients as well as candidates for sustainable natural medicines in the field of oral and dental health.

METHOD

Materials

The research was conducted from March to May 2023 at Microbiology Laboratory, Faculty of Medicine, Laboratory of Basic and Industrial Biotechnology, Faculty of Biotechnology UKDW. The main material used in this study include dried sengkubak (*Pycnarrhena cauliflora* Diels.) leaves taken from the forest interior of Kalimantan, ethanol 70%, *Streptococcus sanguinis* 1.5×10^8 CFU/ mL suspension, *Brain-Heart Infusion* or BHI broth media and antibiotics (gentamicin).

Tools

The tools include bacterial incubator, rotary evaporator, filter paper, 96-well plate culture, spectrophotometer, and microplate reader that analyzed through One Way ANOVA using SPSS v.25 software.

Method

This research was conducted with a quantitative approach through experimental methods using a completely randomized design with 3 replications. Identification of plant secondary metabolites was carried out qualitatively, while tests for total flavonoid and

phenolic content, antioxidant, and antibacterial activity from the Minimum Inhibitory Concentration (MIC) parameter were carried out quantitatively. Antioxidant bioactivity was observed through the IC_{50} value and the percentage of inhibition parameter, while the susceptibility of the test bacteria to treatments with varying concentrations of the ethanol extract of sengkubak leaves (100,000 - 390.625 $\mu\text{g/ml}$) with a positive control (gentamicin) was observed from the MIC value.

Plant Identification

Identification of sengkubak plants obtained from inland forests in the West Kalimantan region is brought in a dry state (herbarium) and not exposed to direct sunlight to the Laboratory of the Faculty of Biology UGM for a determination process so that valid plant certification results are obtained as research samples recommendations.

Extract Preparation

Sengkubak leaves are dried through an air-dry process for ± 1 week and in an oven at 40°C before being mashed. 100 g of dried leaf samples were weighed for extraction by maceration method for 3x24 hours using 1000 ml of 70% ethanol (1:10). The extract mixture was stored for 24 hours at 40°C temperature. The maceration results were filtered by filter paper beforehand, and the solvent was volatilized using a Rotary Evaporator at 40°C to obtain the ethanol extract of sengkubak leaves with yield formulation $\{(\text{total weight of paste extract (g)} / \text{total dry weight (g)}) \times 100\%\}$. Storage of the extract in the form of a paste is stored at 4°C until used. The leaf extract was prepared in a concentration series of 1×10^5 $\mu\text{g/ml}$ by dissolving it in 1% DMSO as a stock for bacterial susceptibility testing. The stock extract solution was filtered before being diluted with BHI broth in a bacterial culture medium (Hutomo et al., 2021; Purba et al., 2014; Sholikhah et al., 2021; Soelama et al., 2015).

Phytochemical Screening

Phytochemical screening was carried out to detect alkaloid, flavonoid, phenols, steroid, terpenoids, saponin and tannins in sengkubak leaf ethanol extract solution using the color method. These studies also tested the total content of flavonoid and phenolic using the spectrophotometric method (Sari et al., 2021; Suoth et al., 2013; Syafitri et al., 2014).

Antioxidant Tests

Antioxidant activity was tested using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method through a spectrophotometer. The stock solution was prepared in a concentration of 100 ppm from a mixture of 1 mg of extract per 5 mL of methanol PA. Take 2 mL of the solution and add 50 ppm 3 mL of DPPH solution, then vortex and incubate in the dull light for 30 minutes. Three replications or repetitions were made for each concentration series of 5; 25; 50; 75; and 100 $\mu\text{g/mL}$. These results were read at λ 517 nm with methanol blank then compared with the standard ascorbic acid curve in same way to be calculated from percentage (%) inhibition with formula $[(\text{OD control} - \text{OD sample}) / (\text{OD control}) \times 100\%]$. The result is shown in the IC_{50} value which will be grouped into categories of weak, medium, or strong antioxidant activity (Paraswati, 2015).

Preparation of Test Bacterial

Bacterial suspension preparation

Preparation of bacterial suspension stock from *Streptococcus sanguinis* ATCC 10556 colony begins with a centrifugation process at 3000 rpm for 15 minutes to obtain the pellet portion which will be mixed with liquid peptone media. To obtain the same level of turbidity as the standard McFarland solution 0.5 (1.5×10^8 CFU/mL), the mixture was added slowly to the peptone medium until it showed the same ratio of lines or was not curved using line paper. Bacterial suspension can be used as an inoculum for a maximum of 15 minutes (Hutomo et al., 2018; Nurhayati et al., 2020).

Bacterial activity tests

The sensitivity or more likely susceptibility of *S. sanguinis* bacteria to ethanol extract of sengkubak leaves was first determined by the MIC test using the liquid microdilution method. Bacterial culture 1.5×10^8 CFU/ml as much as 10 μ l was inoculated into 90 μ l BHI broth which already contained ethanol extract of sengkubak leaves with concentrations ranging from 100.000 (10^5) to 390,625 μ g/ml in 96-well plates with 3 repetitions. Gentamicin was used as an antibacterial standard at a concentration of 5 μ g/ml. The plate cultures were stored for 24 hours at 37°C to visually observe the presence or absence of turbidity and to measure the bacterial absorbance (OD) value at λ 595 nm using a microplate reader. The results of sample absorbance were calculated by percentage (%) of inhibition using the same formula for antioxidant test. The result is shown in the MIC value which will be grouped into categories of weak, medium, or strong antibacterial activity (Zakki, 2017).

Data Analysis

The results of data are in the form of descriptive observations and comparison of the susceptibility values of bacteria from the MIC test to the treatment of ethanolic sengkubak concentration in the media through the liquid microdilution method which is quantitatively described by the Optical Density or OD value from the ELISA reader results which are then analyzed statistically using ANOVA One Way at a significance level of 0.05 with advanced Tukey Honestly Significant Difference test.

RESULTS AND DISCUSSION

Plant Identification Results

Determination test is one of the initial stages in determining the name or species of plants that will be used specifically. Sengkubak plants were taken from the forest of Kapuas Hulu Putussibau, West Kalimantan in dark condition to minimize exposure from direct UV rays which have the potential to damage research samples for determination tests to be carried out in the laboratory. The result of sengkubak plants that were identified can be seen in Figure 1.

Kingdom : Plantae
Division : Magnoliophyta
Class : Magnoliopsida
Order : Ranunculales
Family : Menispermaceae
Genus : *Pycnarrhena*
Species : *Pycnarrhena cauliflora* (Miers) Diels.
Local name : Sangkubak


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telah melakukan identifikasi tumbuhan dengan hasil sebagai berikut,

Kerajaan : Plantae
Divisi : Magnoliophyta
Kelas : Magnoliopsida
Ordo : Menispermales (Ranunculales)
Famili : Menispermaceae
Genus : *Pycnarrhena*
Spesies : *Pycnarrhena cauliflora* (Miers) Diels
Sinonim : *Antioxis cauliflora* Miers
Nama lokal : Sangkubak

Identifikasi tersebut dibantu oleh Prof. Dr. Purnomo, M.S.
Demikian surat keterangan ini diberikan untuk dapat dipergunakan seperlunya.

Mengetahui,
an. Dekan
Wakil Dekan Bidang Penelitian, Pengabdian
Kepada Masyarakat, Sosial Sama dan Alumni

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Yogyakarta, 9 Maret 2023
Kepala Laboratorium
Sistematiika Tumbuhan
Fakultas Biologi UGM

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Figure 1. Certification of sengkubak (*Pycnarrhena cauliflora* Diels.)

The results of plant determination were obtained from Laboratory of the Faculty Biology, Gadjah Mada University showed that the plant used in this study was indeed sengkubak (*Pycnarrhena cauliflora* Diels.) with a description of the letter number: 0285/S.Tb./III/2023. This is in accordance with the classification of plants according to the GBIF Backbone Taxonomy (2022) with sengkubak which belongs to the Menispermaceae family.

Extraction Sample

Sengkubak leaves which were taken in a simplified form within the forest area of Kapuas Hulu Putussibau, West Kalimantan subjected to an extraction stage using the standard maceration method. The result for ethanolic extract of sengkubak leaves in the form of a viscous liquid or paste is shown in Figure 2.



Figure 2. Sengkubak leaves ethanolic extract

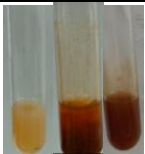
The characteristics of the sengkubak leaves ethanolic extract here are green brown in color with a layer of oil on the surface and had sweet smell that is typical of sengkubak. The plant extraction process resulted in a total yield of 16.30% (w/w). The results showed that the maceration method used was precise and effective because the extract could dissolve properly with ethanol 70% that shown by obtaining a high amount of yield. Compared to previous research that yielded 2.6% yield of 50% sengkubak methanol extract from maceration extraction, and 9.17% yield from 96% maceration of sengkubak leaves taken from the Sanggau region, Kalimantan (Pamuji et al., 2015; Purba et al., 2014).

The amount of yield obtained is supported by many factors such as the plant's habitat related to geographical conditions, the type and concentration of the solvent used related to the polarity of the sengkubak leaf components, temperature, and length of contact time as well as the ratio of the amount of dry sample preparation to the amount of solvent used (Sangadah & Kartawidjaja, 2020). Overall, the yield of the extract shows that the bioactive content detected in sengkubak leaves is high enough so that it can be used as a research sample that has antioxidant and/or antibacterial bioactivity.

Phytochemical Content**Qualitative Result**

One way to identify the potential biological activity contained in a plant is to test its phytochemical content. As it is known that the metabolite content found in each plant varies in terms of type and amount, so the analysis test of compounds in these study was carried out on sengkubak plants to observe those metabolites that have potential as antioxidants and antibacterial against *S. sanguinis* inhibition. The results of tests carried out on alkaloid, flavonoid, steroid, saponin, tannin, phenol and terpenoid are shown in Table 1.

Table 1. Phytochemicals test results for ethanolic extract sengkubak leaves

Phytochemicals test	Description (Colour)	Result	Visual indicator
Alkaloid	Yellow - Mayer Orange - Dragendorff Chocolate – Wagner	Positive	
Flavonoid	Brick red precipitate	Positive	
Steroid	Blue - green ring	Positive	
Saponin	Constant foam	Positive	
Tannin	Bluish black	Positive	
Phenol	Blackish green	Positive	
Terpenoid	Brown ring	Positive	

The results presented that ethanolic extract of sengkubak leaves had alkaloids, flavonoids, steroids, saponins, tannins, phenols and terpenoids. Previous related studies that observed metabolites of sengkubak qualitatively, found that sengkubak leaves extracted with ethanol contained various phytochemical such as alkaloids, flavonoids, steroids, terpenoids, phenols, saponins and tannins. Other studies mentioned it contain alkaloids, steroids, terpenoids, and flavonoids which have the potential to be used as antifungal, antioxidant and antibacterial in the health sector (Masriani et al., 2014; Pamuji et al., 2015). Based on the results of the study, various potential compounds were found to be used as antioxidants and/or antibacterials such as alkaloid, flavonoid, steroid, terpenoid, phenol, saponin and tannin which proved that the extraction and solvent techniques used were effective so these target compounds could be identified. The presence of target compounds in this test could provide an overview of the metabolite content of sengkubak leaves which have a role as an antioxidant and antibacterial against the inhibitory activity of *S. sanguinis*.

Total phenols content

Determination of total flavonoids (quercetin) and phenolic compound which are phenols groups from the ethanol extract of sengkubak leaves in this study was carried out as a form of quantitative phytochemical analysis. According to related literature sources, the presence of these compounds is believed to have antibacterial properties which predominate in medicinal plants (Pamuji et al., 2015; Sarifati et al., 2020). This is supported by similar studies that have examined the phytochemical content of sengkubak leaves qualitatively, where it is known that the phenols content dominates (+++) compared to other existing

compound groups (Masriani et al., 2014; Mohammed et al., 2020). Quercetin and phenolic as part of phenolic compounds are known to have bioactivity as potent antioxidants and antibacterial, so in this study were carried out to test the total content of these compounds. The results of the total quercetin content in Total Flavonoid Content (TFC) units were obtained as shown in Table 2.

Table 2. Total flavonoids content

Sample concentration (g/ml)	Absorbance	Phenol concentration QE (mg/ml)	TFC [mg QE/g sample $\pm$ SD]
0.001	0.055	0.00632	6.3223
0.001	0.037	0.00483	4.8347
0.001	0.033	0.00450	4.5041
Total Content			5.2204 $\pm$ 0.969

Total quercetin value stated in Quercetin Equivalent (QE) with the line equation $y = 0.0121x - 0.0215$. Calculations from this equation will yield the total concentration of flavonoids (TFC) of 5.22 ± 0.969 mg QE/g sample extract. This measurement used a spectrophotometric method with the principle of forming an aluminium complex. Identification of total quercetin in this study were conducted because it belongs to the flavonol class of flavonoids which have strong antioxidant activity by neutralizing oxidative stress by reducing enzyme components or binding to radical molecules while at the same time providing optimal antibacterial effects from the interaction of phenolic hydroxyl groups (Ahmad et al., 2015; Peka, A., & Pyrzynska, 2014). As a comparison, total flavonoid content 7.05 ± 0.26 mg QE/g yellow wood extract (Umayah & Rachmawati, 2005) and the flavonoid total content 9.937 ± 0.009 mg QE/g brotowali stem extract with n-hexane solvent (Roni et al., 2022) both of which come from the Menispermaceae family. In addition, the output of Sengkubak total phenolic in Total Phenolic Content (TPC) units was obtained as shown in Table 3.

Table 3. Total phenolic content result

Sample concentration (g/ml)	Absorbance	Phenol concentration GAE (mg/ml)	TPC [mg GAE/g sample $\pm$ SD]
0.0001	0.130	0.00043	4.3497
0.0001	0.089	0.00030	2.9683
0.0001	0.147	0.00049	4.9225
Total Content			4.0802 $\pm$ 1.005

Total phenolic value stated in Gallic Acid Equivalent (GAE) with the line equation $y = 0.2968x + 0.0009$. Calculations from this equation yielded a total phenolic concentration (TPC) of 4.08 ± 1.005 mg GAE/g sample. The measurement standard for total phenolic is gallic acid, which is a component of hydrolysed tannins. Gallic acid used as standard in this study because it belongs to the phenolic group which is stable and reactive to the Folin-Ciocalteu reagent (Syafitri et al., 2014). Phenolic compounds and quercetin in the leaves of sengkubak are assumed to have a role in reducing cell damage due to oxidative stress or toxicity so that they have the potential to be used as antioxidants and antibacterial agents, which in this study had test against the inhibitory activity of *S. sanguinis* (Arnanda & Nuwarda, 2019; Sarifati et al., 2020).

Antioxidant Activity

Antioxidant activity in this study was conducted by 2,2-diphenyl-1-picrylhydrazyl (DPPH) method because it is considered simpler and more efficient with the help of a UV-Vis spectrophotometer. The principle is to look at the capture of DPPH radicals by compounds that have antioxidant activity through spectrum waves (λ), so it will be known quantitatively the inhibition of radicals expressed in the IC_{50} value (Inhibitory Concentration) or test compound concentration that can suppress free radicals by 50% (Paraswati, 2015). The outcome of antioxidant activity in study point out that ethanolic extract of sengkubak leaves had the ability to scavenge radicals of 40.136 $\mu\text{g/mL}$ in the form IC_{50} value with the highest inhibitory activity at concentration of 100 $\mu\text{g/mL}$ (Ln concentration—4.605 $\mu\text{g/mL}$) of 96.96 % (Figure 3).

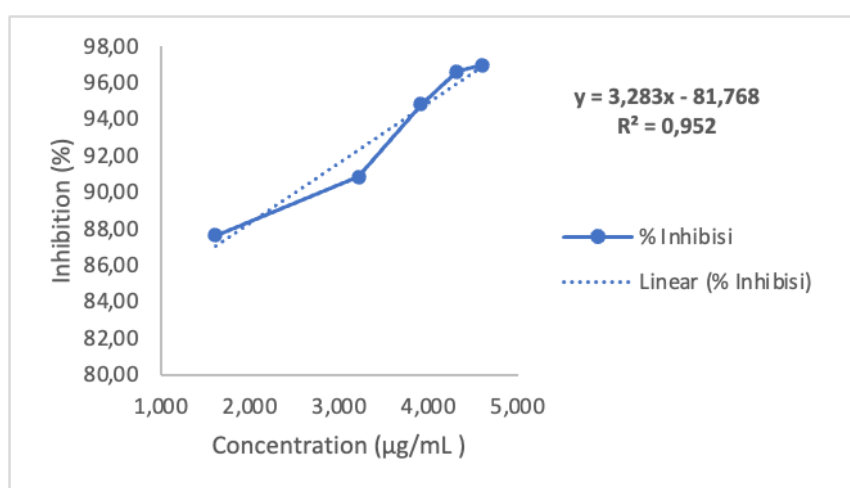


Figure 3. Linear regression curve of sengkubak leaf ethanol extract

The outcome has concluded that ethanolic extract sengkubak leaves has bioactivity as an antioxidant with a strong radical scavenging category from the IC_{50} (<1000 ppm) value and it is proven from the increasing inhibition percentage as a form of extract activity as an antioxidant which is proportional to the high sengkubak leaves ethanol extract concentration.

Previous studies that tested antioxidant activity of sengkubak stated methanol extract of the leaves had strong activity as an antioxidant (Purba et al., 2014) same as that carried out in this study. It said that most of the phenolic compounds such as phenolic acids, tannins, and flavonoids have a significant role in the antioxidant activity of plants, precisely in neutralizing free radicals such as *Reactive Oxygen Species* (ROS) that produced by cell defensive mechanisms when there is an increase in environmental stress. The presence of ROS enzymes indicates mediating activity increased by phenolic compounds such as quercetin and phenolics which serve as cell antioxidants in targeting hydroxyl radicals (Mansoor et al., 2022). Overall, there is strong antioxidant bioactivity in sengkubak from inhibition value that shown in this study, within presence of 0.209 mg QE/g quercetin and 0.163 mg GAE/g phenolic which were obtained from total phenols content with extract inhibition value. Related to structural function of phenol which has an inhibitory effect on bacteria, an antibacterial test was carried out on inhibition of *S. sanguinis*.

Streptococcus sanguinis MIC

The minimum inhibitory concentration or MIC test was carried out using the liquid microdilution standard method from a solution concentration 1×10^5 $\mu\text{g/mL}$ (ppm) ethanolic extract of sengkubak leaves. The growth of *S. sanguinis* in this research was observed visually through the turbidity of the ethanolic extract of sengkubak leaves against the control

and by determining the % bacterial inhibition from the comparative analysis of concentration and absorbance test microplate which can be seen in Figure 4.

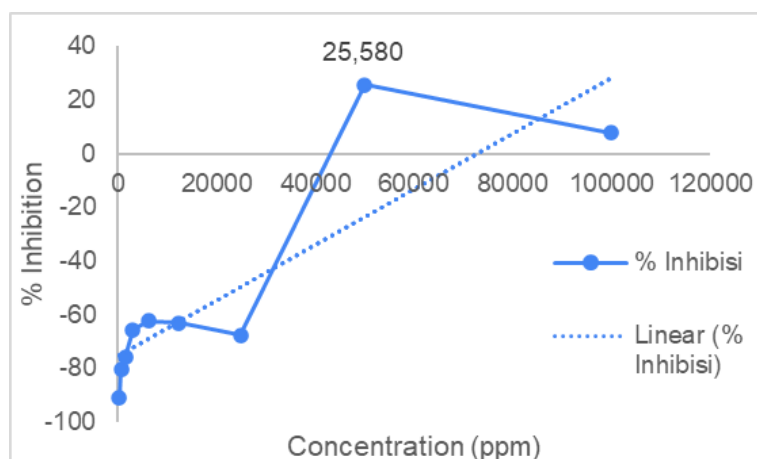


Figure 4. Sample inhibition (%) of MIC test result

From the parameters, it was shown that *S. sanguinis* MIC at concentration 5×10^4 (50.000) $\mu\text{g/mL}$ and there was antibacterial activity from the ethanolic extract sengkubak leaves in the presence of 261 mg QE/g quercetin and 204 mg GAE/g phenolic content. MIC here was defined as the smallest concentration of the sample treatment that were able to inhibit the growth of *S. sanguinis* which could be observed visually from the comparison of the clarity level of test bacteria in the MIC concentration culture media (*well 3*) which was the same as the clarity of control media (*well 1*) after incubation process as shown in Figure 5.

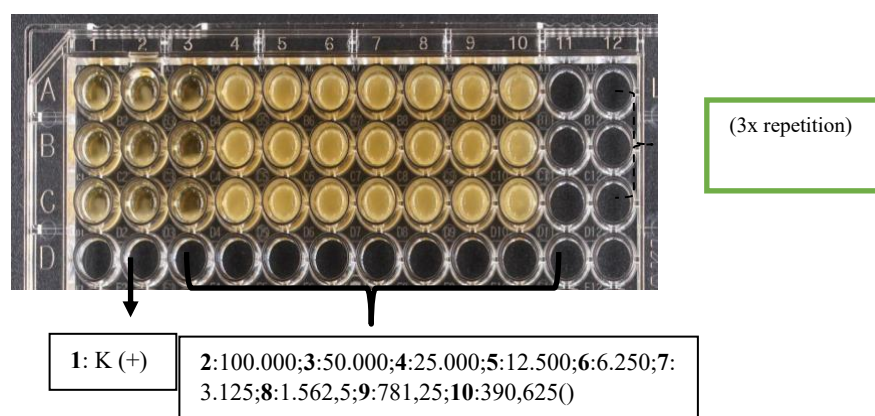


Figure 5. MIC visual of extract against *Streptococcus sanguinis*

These results found that the inhibition ability of extract at MIC concentration of 50.000 $\mu\text{g/mL}$ for each test repetition is 25.58%. The treatment showed extract concentration of 5×10^4 $\mu\text{g/mL}$ (ppm) as the most significant result as the MIC value, where the bacterial density was closest to zero from the ratio of absorbance values which began to show inhibition activity of bacteria through the clarity of the test medium. This is supported by studies that stated the minimum inhibitory concentration of bacteria was determined from the range of clarity levels seen in the MIC test medium (Mardiani et al., 2012).

This study found that there is antibacterial activity of the extract against bacteria test, although it can be said that its nature is not too strong. According to Tsuchiya (1999) in (Zakki, 2017) strong antibacterial activity from natural ingredients has an MIC value in the range of 0.05 – 0.5 mg/mL, moderate between 0.6 – 1.5 mg/mL, and weak above 1.5 mg/mL. In addition, statistical analysis was carried out through the ANOVA test on the OD value of the MIC test results to determine the significance of the effect between the variable extract

concentration and the inhibition percentage of the tested bacteria and obtained a probability value (sig.) of 0.000 (≤ 0.05) based on a 95% confidence level, which means the research hypothesis was accepted or showed that there was a significant effect between treatment groups of sengkubak leaves ethanol extract on the inhibition activity of the *S. sanguinis* test bacteria.

Relevant studies confirm that the level of effectiveness from antibacterial agent including natural ingredients is determined by the nature of the test bacteria, the concentration and the length of contact or incubation time. The inhibition of *S. sanguinis* bacterial growth is thought to be due to interactions between compounds contained in the ethanolic extract of sengkubak leaves such as flavonoids, terpenoids, tannins and saponins. The presence of compounds other than phenol in the flavonoid (quercetin) and phenolic groups is assumed to play a role in the inhibition of *S. sanguinis* which tends to work by destroying membrane structures or proteins resulting in lysis and disrupting bacterial metabolism. It is known that the presence and position of hydroxyl group (-OH) found in the phenol structure provides crucial antibacterial activity against the inhibition of *S. sanguinis*, including the hydroxylation of C3', C4', C5, and C7 as well as prenylation or geranylation of C6 flavonoids (Kemala et al., 2018; Sarifati et al., 2020; Wu et al., 2013).

CONCLUSION

Sengkubak (*Pycnarrhena cauliflora* Diels.) leaves ethanol extract with maceration method can inhibit the growth of *Streptococcus sanguinis* bacteria at concentration 5×10^4 $\mu\text{g/mL}$ based on MIC test parameter. The phytochemical tests of extract revealed positive results for alkaloids, flavonoids, steroids, saponins, tannins, phenols and terpenoids as well for phenolic content tests of total quercetin 5.22 mg QE/g sample and total phenolic 4.08 mg GAE/g sample. It is known that the ethanolic extract of sengkubak leaves has the ability as an antioxidant with IC_{50} value of 40.136 $\mu\text{g/mL}$ and an inhibition percentage of 96.96% which is strong enough to scavenge free radicals with the results of statistical analysis showing that there is a significant effect between the variable concentrations of extract with the percentage of inhibition test bacteria.

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