

Antibacterial Activity Test of Mahkota Dewa Fruit (*Phaleria macrocarpa*) Extract against *Escherichia Coli* Bacteria

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ABSTRACT

Exposure to *Escherichia coli* during pregnancy may cause serious problems such as anemia, premature birth and an increased risk of inflammation of the lining of the brain in the fetus. Mahkota Dewa (*Phaleria macrocarpa*) is a medicinal plant that is widely known and used to treat allergies and chronic diseases. One of the compounds contained in Mahkota Dewa is flavonoids. There is certain mechanism of action of flavonoids in inhibiting bacteria namely by damaging the cytoplasmic cell wall in bacteria. This study aims to analyze the antibacterial properties of various flavonoid concentrations of Mahkota Dewa fruit (*Phaleria macrocarpa*) extract against *Escherichia coli* bacteria through a laboratory experimental study design by testing the antimicrobial potency of the well method, namely 10%, 20%, 40%, 60%, 80% and 100%. Statistical analysis using One-Way Anova Test showed a significant difference between changes in the concentration of flavonoids in Mahkota Dewa fruit extract and the inhibition zones towards the growth of *Escherichia coli* bacteria ($p < 0.05$). Pearson correlation test showed a very strong relationship with a positive direction ($r = 0.874$) which indicated that the higher the concentration of flavonoids in Mahkota dewa fruit extract, the stronger the antimicrobial effect. Based on the study findings, it can be concluded that the flavonoid extract in Mahkota Dewa fruit extract had an antimicrobial potential against *Escherichia Coli* in vitro.

Paparan *Escherichia coli* pada ibu hamil menimbulkan masalah serius seperti anemia, ISK, kelahiran prematur dan meningkatnya resiko radang selaput otak pada janin. Penyakit alergi ataupun penyakit kronis banyak memanfaatkan buah Mahkota dewa (*Phaleria macrocarpa*) sebagai alternatif pengobatannya. Senyawa yang terkandung pada buah mahkota dewa salah satunya yaitu flavonoid. Pada pemberian flavonoid dinding sel sitoplasma akan rusak, hal tersebut yang akan menghambat perkembangan bakteri. Tujuan penelitian ini mendapatkan analisis antibakteri ekstrak buah mahkota dewa atas *Escherichia coli* menggunakan desain penelitian eksperimental laboratorium dengan uji potensi antimikroba metode sumuran. Uji efektivitas flavonoid ekstrak mahkota dewa dari beberapa konsentrasi yaitu 10%, 20 %, 40%, 60%, 80% dan 100%. Perbedaan signifikan ditunjukkan dalam analisa statistik memakai One-Way Anova Test pada perubahan konsentrasi flavonoid ekstrak buah Mahkota Dewa terhadap zona inhibisi perkembangan bakteri *Escherichia coli* ($p < 0,05$). Jalinan yang sangat kuat melalui arah positif di tunjukkan pada uji korelasi Pearson ($r = 0,874$) maka dari itu diartikan semakin bertambahnya konsentrasi flavonoid ekstrak buah Mahkota Dewa, maka semakin kuat efek antibakterinya. Berlandaskan penelitian ini, secara in vitro dapat diartikan bahwa flavonoid ekstrak buah Mahkota Dewa mempunyai potensi sebagai antimikroba atas bakteri *Escherichia coli*.

Introduction

There are several types of diseases that can arise due to bacterial infections, one of which is *Escherichia coli* bacteria. *Escherichia coli* or abbreviated *E. coli* is able to infect the human body and tend to exist around everyday life that we should be aware of (Denamur et al., 2020). *Escherichia coli* belongs to *Enterobacteriaceae* family, which is involved in the group of coliform bacteria. *Enterobacteriaceae* are called enteric bacteria because they are able to survive in the digestive tract (Bruyand et al., 2019) (Vihta et al., 2018). *Escherichia coli* is a natural flora in the human body in the form of rods that are Gram-negative, facultative anaerobes, and do not grow spores (Lindsey et al., 2017). Some of its bacterial strains are pathogenic and some provide benefits to humans, for example preventing the colonization of pathogenic bacteria in the human digestive system (Cassini et al., 2019). Pathogenic *Escherichia coli* was first identified in 1935 as a cause of diarrhea (Downing et al., 2017).

Escherichia coli is divided into 3 major groups based on its interaction with the host (human), namely non-pathogenic (commensal), intestinal pathogenic (in the digestive tract), and extra-intestinal pathogenic (outside the digestive tract). This classification is mainly based on the detection of DNA regions that are often associated with certain pathotypes. Sanitation and hygiene indicator bacteria is another name for bacteria *Escherichia coli*, which means that the low level of sanitation applied indicates the presence of these bacteria in a food product (Touchon et al., 2020). Most of *Escherichia coli* bacteria live in the intestines of humans and animals so that the presence of these bacteria is often associated with a state of contamination originating from feces. Therefore, processes that come into contact with feces indicate the presence of these bacteria. In many developing countries, regarding food safety, many cases of enteric disease in children are caused by *Escherichia coli*. The main etiologic cause of diarrhea is *Escherichia coli* (Adibi et al., 2017). Symptoms of haemolytic uraemic syndrome (HUS) occur in some cases which can lead to kidney failure. The infection can even result in death (Eko and Zahriani, 2016).

Antibiotics are the main treatment in the management of infectious diseases. Antibiotics are microorganisms that produce chemical compounds mainly by fungi or synthetic products that can inhibit or kill the development of bacteria and other organisms (Adelberg, Jawetz, & Melnick., 2017). There is no need to doubt the benefits of antibiotics. However, the use of most antibiotics can result in germ resistance to antibiotics, thereby reducing the benefits of antibiotics (Halden, 2016). Bacterial resistance, especially multi-drug resistance, is a problem that is difficult to overcome. This problem results from the use of antibiotics with inaccurate doses, types, and duration of administration, causing germs to become resistant (Yusuf et al., 2020).

Many people switch to using plants for alternative medicine due to the increase in the incidence of antibiotic resistance. Many researchers are interested to study herbal ingredients as alternative treatment for infections caused by microorganisms (Rahmawati and Retnaningrum, 2022). One of the herbal ingredients that can be used to treat infectious diseases is Mahkota dewa (*Phaleria macrocarpa*) (Okzelia et al., 2017; Cordita, 2019). Mahkota dewa fruit lives in the tropical region. The compounds in Mahkota Dewa fruit are alkaloids, flavonoids, tannins, polyphenols and saponins. Alkaloids,

terpenoids, polyphenols, flavonoids and resin compounds are the group of compounds in plants that are related to anti-cancer and antioxidant activities (Retnaningrum et al., 2021). Saponins, alkaloids, flavonoids and tannins are the active compounds in Mahkota dewa that are effective as antibacterial (Addo et al., 2018; Rahmawati et al., 2020).

A previous study on the effect of young Mahkota dewa extract showed an inhibitory effect on *Klebsiella pneumonia* with a concentration of 1–25% (Adelberg, Jawetz, & Melnick., 2017). In the current study, the authors are intended to know whether the extract of the Mahkota dewa fruit also showed an inhibitory effect on the growth of diarrheal infection-causing bacteria. This study aims to analyze the antibacterial properties of various flavonoid concentrations of Mahkota Dewa fruit (*Phaleria macrocarpa*) extract against *Escherichia coli* bacteria.

Methods

This study applied an in vitro laboratory experimental research design. This study aims to analyze the antibacterial properties of various flavonoid concentrations of Mahkota Dewa fruit (*Phaleria macrocarpa*) extract against *Escherichia coli* bacteria. Extraction of mahkota dewa fruit applied maceration method using 96% ethanol solvent. The result was further partitioned to get flavonoid extract in paste form using n-hexane and ethanol solvents. Then it was put in an oven to get pure flavonoid extract. Effectiveness test towards flavonoid in mahkota dewa extract was performed for several concentrations, namely 10%, 20%, 40%, 60%, 80% and 100%. In addition, the negative control treatment was also given in the form of 0% extract and positive control in the form of BAP media. *Escherichia Coli* bacterial isolates used in this study came from the Microbiology Laboratory of the Faculty of Medicine, Brawijaya University. The anti-microbial test was conducted through the well diffusion method to determine the diameter of the inhibition zones of the flavonoid compounds in Mahkota dewa fruit extract. Data were collected from the measurement of the inhibition zones around the well holes using a caliper in millimeters (mm). Data analysis applied One-Way Anova Test and Pearson correlation.

Results

The diameter of inhibition zones of flavonoids in the Mahkota dewa extract against *Escherichia coli* bacteria were obtained from the results of the well diffusion test using a ruler. The results of the calculation of the effect of each concentration of flavonoids in Mahkota Dewa fruit extract on the inhibition zones created around the well holes can be seen in Table 1. Meanwhile, the graph regarding the effect of the concentration of flavonoids on the diameter of the inhibition zones formed is presented in Figure 1.

Table 1. Diameter of Inhibition Zones against *Escherichia coli*

Concentration	Repetition of diameter of the inhibition zone				Rate (mm)	Description
	I	II	III	IV		
Control -	0	0	0	0	0	Weak
Control +	0	0	0	0	0	Weak
10%	5	5	4	6	5	Medium

Concentration	Repetition of diameter of the inhibition zone				Rate (mm)	Description
	I	II	III	IV		
20%	7	8	7	8	7.5	Strong
40%	14	16	15	16	15.25	Strong
60%	17	18	17	18	17.5	Strong
80%	19	19	18	20	19	Strong
100%	20	20	20.8	20.5	20.325	Strong

Note: Results of observation on inhibition zones on the plates for 18-24 hours at room temperature showed that the largest zone of inhibition was at a concentration of 100%. Treatment effect became better along with the increase in concentration.

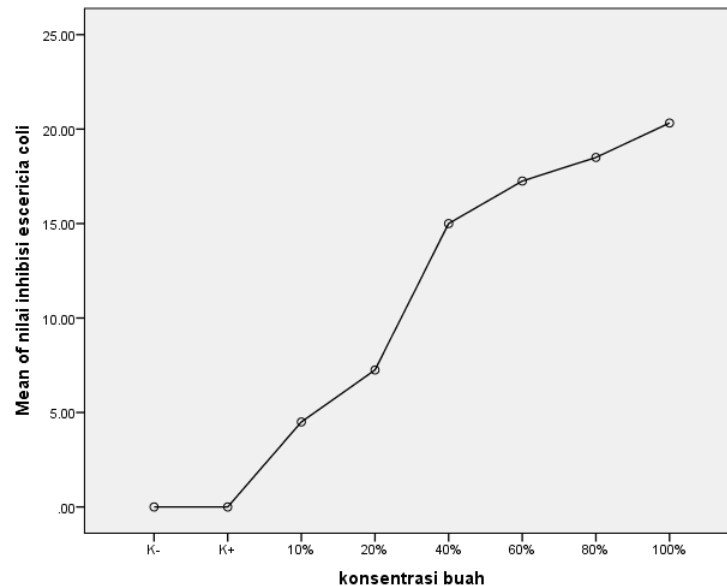


Figure 1. Mean Inhibition Zones Formed Around Well Holes After Treatment using Various Flavonoid Concentrations of Mahkota Dewa Fruit Extract.

The results of normality and homogeneity tests showed that the samples were normally distributed and had homogeneous variations so that one way ANOVA test could be performed with a significance value of 0.000 ($p < 0.5$).

Discussion

This study aims to analyze the antibacterial properties of various flavonoid concentrations of Mahkota Dewa fruit (*Phaleria macrocarpa*) extract against *Escherichia coli* bacteria in vitro. Well diffusion method was applied by using Brain Heart Infusion Agar (BHIA) media. Such method was used to determine the concentration of flavonoids in Mahkota dewa fruit extract which have the potential to inhibit growth of *Escherichia coli* bacteria by measuring and observing the diameter of the inhibition zones formed around the well holes. Mahkota dewa fruit (*Phaleria macrocarpa*) is the test material used in the current study, wherein flavonoid compounds were used as the active substances. The method of extracting flavonoid compounds from the Mahkota dewa was carried out in 2 stages. First, the maceration process was performed using 96% ethanol solvent followed by partitioning with n-hexane solvent. In fact, a previous study found the antifungal and antibacterial effects of flavonoid compounds (Ananda *et al.*, 2017; Clermont *et al.*, 2019).

Well diffusion was applied in this study for several concentrations of 10%, 20%, 40%, 60%, 80% and 100% to determine the effective concentration. After that, there were 4 repetitions for each

concentration. Given the concentration of flavonoids in the Mahkota dewa fruit extract, inhibition zones were created at 10% concentration of 5 mm, at 20% concentration of 7.5, at 40% concentration of 15.25, at 60% concentration of 17.5, 80% concentration of 19 and at 100% concentration of 20.325, as well as in the positive control. In contrast, and inhibition zone was not found in the negative control. It can be concluded that an increase in the concentration of Flavonoids in Mahkota dewa fruit (*Phaleria macrocarpa*) extract led to an increase in the diameter of the inhibition zone created in the well holes. The strength of the antibacterial substance can be seen from the diameter of the inhibition zone that was categorized into 4 groups, namely very strong (> 20mm), strong (11-20 mm), medium (6-10 mm) and weak (<5mm) (Halden, 2016). In this study, the potential of flavonoids in Mahkota dewa fruit extract was categorized as strong. Based on the results of statistical test, it can be concluded that flavonoids in Mahkota dewa fruit extract were effective as antimicrobials against the growth of *Escherichia coli* (Dewi, 2020). Such finding is in line with a similar previous study on the effect of Mahkota dewa fruit extract on the growth of *Escherichia coli*. The difference was the tube test method applied in the current study.

The existence of antibacterial power in mahkota dewa fruit against *Escherichia coli* is due to the content of active substances that act as antibacterial substances. One of the chemical contents contained in mahkota dewa fruit is polyphenols which are thought to have a function as antibacterial substances. Flavonoids are phenolic group compounds that interact with bacterial cells through an absorption mechanism, involving hydrogen bonds with phenol groups. At low levels, a protein complex is formed in the bacterial cell wall with phenol which is weakly bound and immediately undergoes decomposition. It is further followed by penetration of phenol into the cell which causes precipitation and denaturation of plasma proteins. At high levels, phenol affects the permeability of the cell membrane, causing leakage and loss of intracellular compounds (Suryani, 2007).

The statistical analysis applied here was the One-Way Anova Test, which showed a significant difference between changes in the concentration of flavonoids in Mahkota Dewa fruit extract and the inhibition zones towards the growth of *Escherichia coli* bacteria ($p < 0.05$). The Pearson correlation test proved a very strong effect in the positive direction ($r = 0.874$) which indicated that the higher the concentration of flavonoids in the Mahkota dewa fruit extract, the stronger the antimicrobial effect. Based on this study, it can be perceived that the flavonoids in Mahkota Dewa fruit extract in vitro had antimicrobial potential against *Escherichia Coli* bacteria.

Conclusions

Flavonoids content in Mahkota dewa fruit (*Phaleria macrocarpa*) extract in vitro had antibacterial potential against the growth of *Escherichia coli*. An increase in the concentration of Flavonoids in Mahkota dewa fruit (*Phaleria macrocarpa*) extract led to an increase in the diameter of the inhibition zone created in the well holes. The larger the diameter of the inhibition zone formed, the lower the growth of *Escherichia coli* bacteria.

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