



## Antibacterial activity of *Parkia speciosa* Hassk. peel to *Escherichia coli* and *Staphylococcus aureus* bacteria

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### ABSTRACT

*Parkia speciosa* Hassk. is one of edible plants that has not been cultivated as medicinal plants yet. The aim of this research was to study the antibacterial activity of its peel extract against *Staphylococcus aureus* and *Escherichia coli* bacteria using the gel diffusion (well) method. The peel was extracted using *n*-hexane, ethyl acetate, and 70% ethanol. The concentration used for each solvent was 50, 100, 150, 200, 250, and 300 mg/mL. The results showed that the ethyl acetate extract had the highest antibacterial activity with 404.51% of streptomycin's antibacterial activity against *S. aureus*, and 279.12% of streptomycin 10 mg/mL antibacterial activity against *E. coli*. TLC results showed that the ethyl acetate extract with a 93:7 toluene:ethyl acetate ratio solvent had eight spots under 254 nm UV. The variance of solvents and their concentrations also affected significantly ( $P < 0.05$ ) the inhibition areas.

**Keywords:** antibacteria, *E. coli*, *Parkia speciosa* Hassk., *S. aureus*, TLC

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### INTRODUCTION

Indonesia as a developing country has low levels of health in some of its underdeveloped areas. Lack of awareness for good sanitation caused some health problems, especially those related to bacterial infection of the gastrointestinal tract and skin. Such diseases like typhus, diarrhea, and abscess are caused by pathogenic bacterial infection that can be healed by using antibiotic drugs. But using antibiotic drugs can cause negative effects such as the emergence of bacterial resistance to the antibiotic activity. These effects can be avoided by investigating natural antibacterial compounds that can be used to reduce the negative effects of antibiotics [1].

*Parkia speciosa* Hassk. plants can be found in some of the tropical countries such as Indonesia, Malaysia, and Thailand [2]. Its seed is commonly consumed as a vegetable, complementary dish, or flavor material for cuisine. After using the seed, the peels of *Parkia speciosa* Hassk. are simply discarded. However, the peels of *Parkia speciosa* Hassk. have been used for the anti-inflammation of mosquito bites in Indonesian traditional culture. A previous research study revealed some medicinal effects of *Parkia speciosa* Hassk. The extract of *Parkia speciosa* Hassk. seed had antibacterial activity against *Escherichia coli* and *Helicobacter pylori* [3]. Besides that, the peels of *Parkia speciosa* Hassk. had antioxidant phytochemicals, such as tannin [4]. This compound is also well known as an antibacterial agent.

The objectives of this research were to study the phytochemical compounds in *Parkia speciosa* Hassk. peels and their antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. This research also found out natural antibiotic resources from unutilized commodities.

## EXPERIMENTAL SECTION

**Sample collection and preparation:** *Parkia speciosa Hassk* peels were collected in Bogor. All *Parkia speciosa Hassk* peels were chopped and dried using oven in 52°C for 72 hours to removed the water content. Dried *Parkia speciosa Hassk* peels then grinded into 80 mesh measurement to prior solvent extraction.

**Solvent extraction:** Three solvents were used for extraction process: n-hexane, ethyl acetate, and ethanol 70%. There were two methods of extraction, which were maceration and ultrasonication. For maceration method, the dried powder of *Parkia speciosa Hassk* peels were mixed with 1:5 solvent ratio for 24 hours with frequent agitation. For ultrasonication method, the dried powder of *Parkia speciosa Hassk* peels were mixed with 1:10 solvent ratio and then ultrasonitcaed it for 20 minutes. After extraction process, the solution was concentrated by evaporating solvent using rotary-evaporator and oven until the weight of rendement was fixed.

**Phytochemical Screening:** The extracts were tested for specific presence of certain phytochemicals. Analysis was done for alkaloids, saponin, triterpenes/steroid, tannins, and flavoinoid as described by Harborne [5].

**Antibacteria screening:** Two bacteria was used in this research, which were *Escherichia coli* and *Staphylococcus aureus*. One loop of each bacteria culture was enriched to 10 mL of nutrient broth and incubated for 24 hours before cultured in nutrient agar. Each extract was diluted into concentration 50, 100, 150, 200, 250, and 300 mg/mL with DMSO as solvent. About 50 µL extract was dropped into 0,6 mm diffusion well on cultured media, DMSO was used as negative control, and streptomycin 10 mg/mL as positive control. Cultured agar was incubated in 37°c for 24 hours.

**Thin layer chromatography:** The further test was determined the best extract in antibacteria screening. Put a drop of extract to 1x10 cm silica gel 60 GF<sub>254</sub> (Merck, Germany), and running it with toluen:ethyl acetat (93:7) eluen until reach the end line in saturated jam.

## RESULTS AND DISCUSSION

Yield from solvent extraction of *Parkia speciosa Hassk* peels showed a high amount of ethanol 70% extract for both maceration and ultrasonication method. The ethanol 70% yield was 12,13% for maceration method, and 11,62% for ultrasonication method. Yield for other solvents were 0,33% for n-hexane maceration and 0,35% for n-hexane ultrasonication. While for ethyl acetate solvent, the yield were 0,32% for maceration and 0,38% for ultrasonication.

**Table 1. Yield of *Parkia speciosa Hassk* peels extraction**

Method of extraction	Solvent	Yield of extraction (%)
Maceration	n-hexane	0,33 ± 0,06
	Ethyl acetate	0,32 ± 0,03
	Ethanol 70%	12,13 ± 0,06
Ultrasonication	n-hexane	0,35 ± 0,04
	Ethyl acetate	0,38 ± 0,09
	Ethanol 70%	11,62 ± 0,04

**Table 2. Qualitative phytochemical screening of *Parkia speciosa Hassk* peels**

Phytochemical test	n-hexane	Ethyl acetate	Ethanol 70%
Alkaloid	-	+	++
Saponin	+	++	+++
Flavonoid	+	+	-
Tanin	+	+	+++
Steroid dan Triterpenoid	steroid	Triterpenoid	Triterpenoid

Qualititative phytochemical screening method detected the presence of a particular phytochemical compound in *Parkia speciosa Hassk* peels extract. N-hexane extract contained saponins, flavonoids, tannin, and steroids. Ethyl acetate extract contained alkaloids, saponins, flavonoids, tannin, and triterpens. Whereas ethanol 70% extract contained alkaloids, saponins, tannin, and triperpen. Flavonoids, saponin, and tannin had a potential factor as an antibacteria agents [6][7].

Flavonoid exposed in bacteria can cause to disruption of cytoplasmic membrane permeability so it can cause a leakage of cell material [8]. Previous research about antibacteria showed that flavonoids contain in iron wood and kesum leaves has a role in interfering permeability of bacteria cell membrane [9][10]. Saponins are secondary metabolites compound with character like a detergent. These compound can be seen because of its ability to form a

foam. Saponins has a ability to inhibit the function of cell membrane so that the membrane permeability damage resulting in damage to the cell wall. Tannins mechanism of inhibition is to react with the cell membrane, inactivation of essential enzymes, and the destruction of the function of genetic material. Tannins can made a bind with proteins so cell wall formation is inhibited. Tannins are also contained in the extract were tested. Estimation of inhibition mechanism on this *Parkia speciosa* Hassk peels extract was flavonoid and saponins cause the lysis of bacteria membrane so tannin can easily get into the bacterial cell and coagulating bacteria protoplasm.

Extract of *Parkia speciosa* Hassk peels showed the potential of antibacterial activity. It was proved by the inhibitory zone made around the diffusion well of *Parkia speciosa* Hassk peel extract compare to inhibitory zone around diffusion well by streptomycin as shown in Fig 2 and 4 .

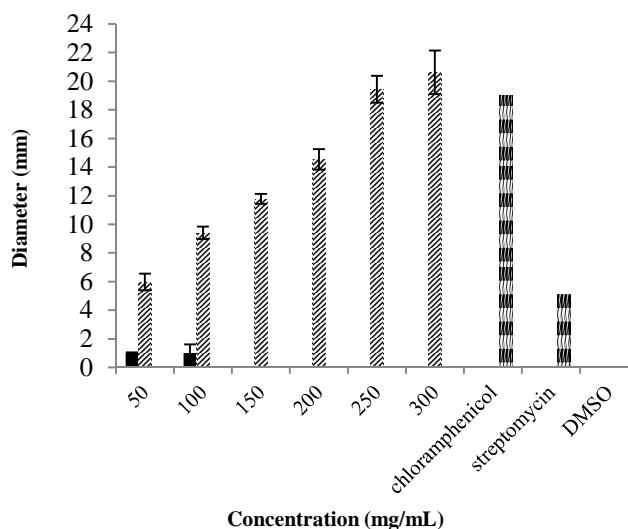


Figure 2 Inhibitory zone of Petai peels extract to *Staphylococcus aureus*

■ n-hexane    ▨ ethyl acetate    ▩ control +

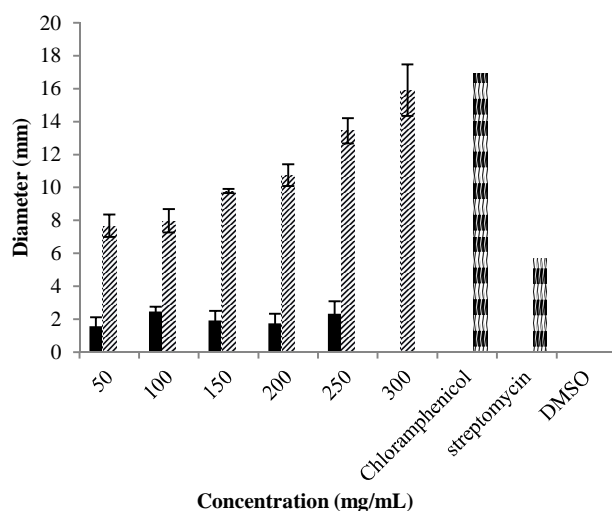


Figure 4 Inhibitory zone of Petai peels extract to *Escherichia coli*

■ n-hexane    ▨ ethyl acetate    ▩ control +

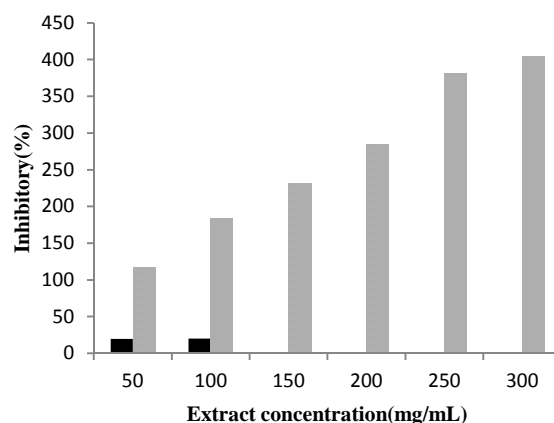
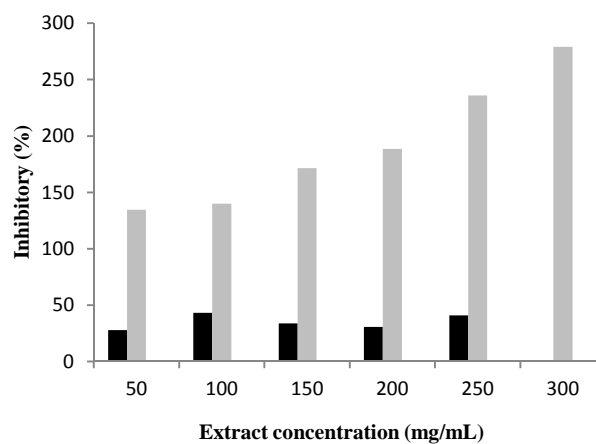


Figure 3 Inhibitory percentage of Petai peels extract to *Staphylococcus aureus*

■ n-hexane    ▨ Ethyl acetate



Picture 5 Inhibitory percentage of Petai peels extract to *Escherichia coli*

■ n-hexane    ▨ Ethyl acetate    ▩ Ethanol 70%

*Parkia speciosa* Hassk peels extract with ethyl acetate as the solvent had the highest value in inhibitory percentage, it's about four times inhibitory ability of streptomycin for *S.aureus* bacteria as shown in Fig. 3 and about three times for *E. coli* bacteria as shown in Fig. 5. The *Parkia speciosa* Hassk peels extract from n-hexane had a inhibitory ability too, but its not bigger than 50% of streptomycin inhibitory ability for both bacteria. Ethanol 70% extract of *Parkia speciosa* Hassk peels had no ability in inhibition of *S.aureus* and *E. coli*.

Ethyl acetate extract from *Parkia speciosa* Hassk peels showed higher both inhibitory zone or inhibitory percentage. From the qualitative phytochemical screening result, ethyl acetate extract showed positive results whether for alkaloid, saponin, flavonoid, or tannin. Whereas n-hexane extract and ethanol 70% extract showed negative result in alkaloid and flavonoid, respectively.

Based on statistical analysis performed on the antibacterial activity, treatment with different concentrations of the tested solvents and significant effect on the diameter of the clear zone obtained at the level of 95%. The results of this analysis further strengthened by the Duncan test gave significantly different results between ethyl acetate solvent or concentration used.

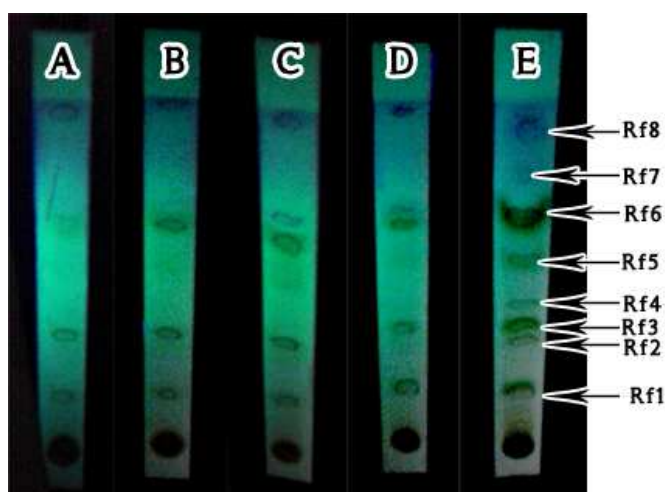


Figure 4. Ethyl acetate extract of *Parkia speciosa* Hassk peels with toluene:ethyl acetate (93:7) chromatogram under 254 nm UV light. (A) 100 mg/mL; (B) 200 mg/mL; (C) 300 mg/mL; (D) 400 mg/mL; (E) 500 mg/mL

Ethyl acetate extract of *Parkia speciosa* Hassk peels fractionated by thin layer chromatography (TLC) using a combination of eluent toluene: ethyl acetate (93: 7). TLC is a separation method that uses two phases, stationary phases which are in the form of plates with a layer of an inert adsorbent and mobile phase eluent can be selected based on the polarity of the compound. Eluent polarity significantly affects the value of the retention factor (Rf). The more non polar compound, the more distance non polar compound move up in silica plate [11].

The concentration of different extracts were tested by TLC to obtain the best separation, 100, 200, 300, 400, 500 mg/mL concentration used in this research. Ethyl acetate extract of *Parkia speciosa* Hassk peels at a concentration of 500 mg/mL showed eight spots after observed under UV light with a wavelength of 254 nm. The result were Rf1 = 0,2; Rf2 = 0,35; Rf3 = 0,41; Rf4 = 0,48; Rf5 = 0,6; Rf6 = 0,73; Rf7 = 0,84 dan Rf8 = 0,95. The larger the Rf value, the smaller the polar value of ethyl acetate extract fraction. So Rf1 was the most polar francement, and Rf8 was the least polar fraction of *Parkia speciosa* Hassk peels extracts.

## CONCLUSION

Ultrasonication method rendement was not significantly different with maceration method, but it takes shorter time compared with maceration. The results of phytochemical analysis of *Parkia speciosa* Hassk peels extracts showed that *Parkia speciosa* Hassk peels extracts contains alkaloids, saponins, flavonoids, tannins, steroids, and triterpenoids. *Parkia speciosa* Hassk peels has potential as an antibacterial agents. Ethyl acetate extracts of *Parkia speciosa* Hassk peels had the greatest potential as an antibacterial. Fractionation of ethyl acetate extract of *Parkia speciosa* Hassk peels by TLC showed eight spot under UV light with 254 nm wavelength that means it contained eight compound in the extract.

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