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Antimicrobial Activity of Ethanol Extract of Akar Kaik-kaik (*Uncaria cordata* (Lour.) Merr.) Leaves Against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*

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ABSTRACT
Akar kaik-kaik (Uncaria cordata) or known as hook vine is one of the medicinal plants whose leaves are used in traditional medicine as an antidiabetic by the people of Riau. Other than that, this plant also has potential as an antimicrobial agent due to the dominant of alkaloid content possessed by members of the genus Uncaria. This research aims to study the antimicrobial activity from the ethanol extract of akar kaik-kaik leaves against Staphylococcus aureus (Gram-positive bacteria), Escherichia coli (Gram-negative bacteria), and Candida albicans (yeast). The extraction was carried out using the maceration method and a solvent of 70% ethanol. The extract then tested for phytochemical screening to find out the secondary metabolite. After that, the extract was tested for antimicrobial activity. The test was done by Kirby-Bauer Method at Nutrient Agar (NA) for bacteria, and Sabouraud Dextrose Agar (SDA) for yeast. The extract contained alkaloid, saponin, and tannin according to the phytochemical analysis. The antimicrobial activity showed that the extract can inhibit the growth of S. aureus at a concentration of 10%, 20%, and 40% respectively about 6.91 ± 0.04 mm, 8.51 ± 0.14 mm, and 10.89 ± 1.09 mm. Meanwhile, the extract cannot inhibit the growth of E. coli and C. albicans.

Keywords: *Akar kaik-kaik, Antimicrobial, Ethanol, Leaves extract, Uncaria cordata*

1. INTRODUCTION

The use of plants as traditional medicine in Indonesia is an ancestral tradition that has been passed down from one generation to another. Every region in Indonesia may have different medicinal plants to treat one disease due to the high diversity of potential plants in this country. The use of plants as natural medicine is inseparable from raw materials that are easy to obtain, cheap, and less effects compared to synthetic drugs [1,2].

One of the medicinal plants known by the local people is from *Uncaria* genus. *Uncaria* is believed to be able to heal wounds, fevers, headaches, gastrointestinal ailments, and fungal or bacterial infections. The most common chemical compounds found in *Uncaria* are alkaloids, triterpenes, and flavonoids. The existence of those compounds is likely what makes *Uncaria* have antioxidant, antidiabetic, and antimicrobial roles. One of the *Uncaria* species that has been studied to have antimicrobial activity was *Uncaria tomentosa* against pathogenic microbes such as *Streptococcus mutans*, *Pseudomonas aeruginosa*, *Staphylococcus* sp., and *Candida albicans* [3–5]. Another species is *Uncaria gambir* which has antimicrobial activity against *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, and *Bacillus cereu*s [6].

Akar kaik-kaik (*Uncaria cordata*) or known as hook vine is another species from *Uncaria* genus which potential as medicinal plant. Local people in Riau used this plant for diabetic treatment, while people in Jambi used it for diarrhea and dysentery treatment [7]. The research about the potential of antidiabetic from akar kaik-kaik (*Uncaria cordata*) leaves has been reported by Ahmad et al. [8].

However, the research about antimicrobial activity from this species was lack of report.Therefore, the goal of this study was to investigate the antimicrobial activity of an ethanol extract of akar kaik-kaik (*Uncaria cordata*) leaves against *S. aureus* (Gram-positive bacteria), *E. coli* (Gram-negative bacteria), and *C. albicans* (yeast). The results of this study were expected to serve as a reference and source of information regarding the antimicrobial activity from akar kaik-kaik leaves.

2. MATERIALS AND METHODS

2.1. Chemicals and Reagents

Nutrient Agar (NA), Sabouroud Dextrose Agar (SDA), Aquadest (Brataco), 70% Ethanol (Brataco), $FeCl_3$ (Merck), Wagner reagent, Mayer reagent, Dragendorff reagent, Ammoniak (Merck), Acetic acid anhydride (Merck), $NaNO_2$ (Merck), $AlCl_3$ (Merck), HCl (Merck), Chloroform (Merck), H_2SO_4 (Merck), DMSO, immersion oil, Crystal violet (Merck), Safranin, Lugol's iodine, 0.9% NaCl, Blank disc (Oxoid), the antibiotic disk of Nystatin and Amoxicillin, analytical balance (Excellent), oven (Memmert), blender (Phillips), aluminium foil (Klin Pak), autoclave, incubator, vacuum rotary evaporator, Hot plate, and Laminar Air Flow.

2.2. Microbial Strains

The microorganisms tested were *S. aureus* ATCC 25923 (representative of Gram-positive bacteria), *E. coli* ATCC 25922 (representative of Gram-negative bacteria), and *C. albicans* ATCC 10231 (representative of yeast). The *S. aureus* and *E. coli* were incubated for 24 hours, and *C. albicans* was incubated for 48 hours.

2.3. Preparation and Extraction of Akar Kaik-kaik Leaves

Akar kaik-kaik leaves were obtained from Larangan Adat forest, Rumbio Village, Kampar, Riau. The sample was identified in the Research Center for Plant Conservation and Botanic Gardens, Indonesian Institute of Sciences (LIPI), Bogor. The fresh leaves of akar kaik-kaik were cleaned with fresh water from the tap and dried for 14 days with air-dried method [9–11]. The dried leaves then powdered using blender and homogenized by sieving using mesh 60. The sieving produces simplicia of akar kaik-kaik leaf powder [12,13].

The akar kaik-kaik leaf powder was weighed as much as 80 g and then extracted with a maceration method using 70% ethanol as a 1:10 ratio solvent [14]. The maceration was performed for 24 hours and re-macerated twice with the same procedure [15]. The maceration results were filtered through filter paper, and the filtrate was evaporated in a vacuum rotary evaporator until a thick extract was obtained.

2.4. Phytochemical Screening

The phytochemical screening tests were carried out at Lux Chemicals Laboratory (Chemicals Product and Chemical Analysis Service), Depok. The screening test included alkaloid (using Mayer, Bouchardat, Dragendorff reagents), flavonoid, saponin, tannin, and steroid/triterpenoid [16,17].

2.5. Antimicrobial Activity Tests

The extract was tested for antimicrobial activity using Kirby-Bauer disk diffusion method in Testing Laboratory of Biotechnology Center, Agency for the Assessment and Application of Technology (BPPT), Serpong. The microorganisms tested were *S. aureus* ATCC 25923 (representative of Gram-positive bacteria), *E. coli* ATCC 25922 (representative of Gram-negative bacteria), and *C. albicans* ATCC 10231 (representative of yeast). The *S. aureus* and *E. coli* were incubated for 24 hours, and *C. albicans* was incubated for 48 hours.

3. RESULT AND DISCUSSION

3.1. Sample and Yield Extract

The dried leaves that obtained in this research were about 700 g from 2 kg wet leaves. The akar kaik-kaik leaves were dried using an air-dried method to avoid the loss of thermolabile compounds by sunlight [12]. The air-dried method could also retain the chlorophyll content in the sample [18]. However, this method has limitation such as time-consuming [18–20]. The air-dried method can consume about 3-7 days to months, or even a year depending on the types of samples dried [12].

The extraction was done using maceration methods with 70% ethanol as a solvent. Maceration is a general technique to extract medicinal plants. Maceration was chosen because it is a simple and easy to apply method. Besides that, maceration is a cold extraction method, so it can avoid the loss and damage of some active substances which are not heat resistant [12,21]. During the maceration process, the sample was stirring occasionally. Stirring aims to facilitate solvent contact in the cavity of plant cells

and gave circulation in it, so that the extraction occurs optimally [22].

The 70% ethanol was chosen because it is a polar solvent which is capable of extracting secondary metabolites maximally because of the presence of sugars that are bound to secondary metabolites such as flavonoids, glycosides, saponins, tannins, and some alkaloids. Solvents can diffuse into solid plant material and dissolve compounds of similar polarity. Furthermore, it is also a recommended solvent by the Ministry of Health, Republic of Indonesia, because its low toxicity [22–25].

Table 1. Yield Extract of Ethanol Extract of Akar Kaik-kaik Leaves.

Simplicia/Akar Kaik-kaik leaf powder (g)	Thick extract (g)	Yield (%)
80	48.3	60.37

The ethanol extract of akar kaik-kaik leaves yielded approximately 60.37% (Table 1). This indicated that the chemical compounds attracted to the extraction were quite high. Yield extract showed an amount of active compounds that are trapped during the extraction process [19,26]. The high content of the active compounds in a sample is indicated by the high percentage yield [16]. The higher the polarity of the solvent, the yield will also increase [27]. The more polar the solvent, the better the process of extraction. 70% ethanol has high polarity, so it was efficient to attract active compounds in akar kaik-kaik leaves [24,28].

3.2. Phytochemicals Screening

Table 2. Phytochemicals Screening Results of Ethanol Extract of Akar Kaik-kaik Leaves

Chemical Compounds		Results
Alkaloid	Wagner	(+)
	Mayer	(-)
	Dragendorf	(+)
Flavonoid		(-)
Tannin		(+)
Saponin		(+)
Steroid		(-)
triterpenoid		(-)

(-): not contain the tested compound; (+): contain the tested compounds

Phytochemical's screening was done qualitatively by the change color reaction method using several reagents [27]. The results of the phytochemicals screening showed positive results to alkaloid, tannin, and saponin. Meanwhile, for flavonoid, steroid, and triterpenoid showed negative results (Table 2).

The positive results of alkaloid showed an agreement with literature which stated that *Uncaria* genus was known for their alkaloid constituents [4,29,30]. According to Wardhani et al. [31], ethanol can be used to extracted the alkaloid from kemuning leaf. The statement was corresponded with Liu & Liu [32] which proved that the 70% ethanol was best solvent to extract alkaloid from *Actinidia arguta* fruits compared to 60% ethanol and 80% ethanol. Those previous researches confirmed that 70% ethanol can also extracted alkaloids from the akar kaik-kaik leaves.

Besides alkaloid, the data at Table 2 also showed the presence of saponin in ethanol extract of akar kaik-kaik leaves. Saponins are triterpene glycosides which have polar tendencies in its glycosidic bonds [27]. According to the law of similarity and intermiscibility (like dissolves like), a solvent with a polarity close to the polarity of the solute is likely to perform and vice versa [21]. That's why ethanol as a polar solvent can attract saponin from akar kaik-kaik leaves. Ahmad et al. [8] also reported that the methanol extract of stems and leaves *Uncaria cordata* were containing saponin.

Another compound that found in ethanol extract of akar kaik-kaik leaves was tannin. This was also in agreement with Ahmad et al. [8], who found tannin in the methanol extract of *Uncaria cordata* stems and leaves. The presence of tannin indicates that the ethanol extract of akar kaik-kaik leaves contained polyphenol compounds. Alcohol solvents are suitable to extract the polyphenol compounds, such as tannin and flavonoid [24,33]. However, the extract showed negative results to flavonoid (Table 2). This was a rare condition, since flavonoids can be found in the epidermis of leaves and the skin of fruits.

Flavonoids play a role in plant pigmentation (fruits, flowers or seeds) and as a UV protector [34,35]. From the results, it may be assumed that the polyphenol compounds in akar kaik-kaik leaves were in small amount, because it only positive at tannin test and negative in flavonoid. According to Syafitri et al. [36] the differences of total phenolic and flavonoid content in an extract were affecting by the polarity of a solvent. The total phenolic was found highest in high polarity solvent such as 70% ethanol, while total flavonoid content found highest in 96%

Table 3. Antimicrobial Activity of Ethanol Extract of Akar Kaik-kaik Leaves Against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*

Sample	Concentration	Inhibition Zone (mm)		
		S. aureus	***E. coli***	***C. albicans***
Ethanol extract of akar kaik-kaik leaves	5%	-	-	-
	10%	6.91 ± 0.04	-	-
	20%	8.51 ± 0.14	-	-
	40%	10.89 ± 1.09	-	-
Positive Control	Amoxicillin/Nystatin	41.22 ± 0.08	24.44 ± 1.35	7.39 ± 0.02
Negative Control	10% DMSO	-	-	-

(-): no activity; positive control: amoxicillin (bacteria), nystatin (*C. albicans*); negative control: 10% DMSO

ethanol. Flavonoids were more dissolved in 96% ethanol rather than at 70% ethanol [36,37]. This assumption was also supported by the result of Rachmatiah et al. [38], which showed a positive result in flavonoid screening in 96% ethanol extract of akar kaik-kaik leaves.

3.3. Antimicrobial Activity

The antimicrobial activity was done using a Kirby-Bauer disk diffusion method. This method is used to determine whether pathogenic microorganisms are sensitive or resistant to various antimicrobial compounds. The clear zone appears around the disk was measured as inhibition zone [39-41]. The results of antimicrobial activity of akar kaik-kaik leaves were served in Table 3.

The data in Table 3 showed that the extract had no affect on the growth of *E. coli* and *C. albicans*. The extract only had an effect on *S. aureus* growth at concentrations of 10%, 20%, and 40%. The research about the antimicrobial activity from akar kaik-kaik leaves was very limited. The only study regarding antimicrobial activity was reported by Rachmatiah et al. [38] which using 96% ethanol as a solvent. They reported that 96% ethanol extract of akar kaik-kaik leaves have activities against *S. aureus* and *Salmonella typhi*.

Salmonella typhi is a member of Gram-negative bacteria like *E. coli*. They have same structure of cell wall components. The difference results between *E. coli* and *S. typhi* against the akar kaik-kaik leaves extract probably due to the presence of flavonoid in extract. Our results showed no flavonoid content, while Rachmatiah et al. [38] showed positive in flavonoid test. Flavonoid could interrupt the bacterial cell wall and membrane of microorganisms. Hence, in 70% ethanol extract which showed no flavonoid content, the cell wall of *E. coli* was remains stable and undamaged. This prevents the extract from penetrating bacterial cell wall and damaging the cell components of *E. coli.* In the meantime, the antimicrobial activity against *S. aureus* was probably due to the synergistic mechanisms of the chemical compounds found in the ethanol extract of the akar kaik-kaik leaves, viz. alkaloid, tannin, and saponin. Saponin worked by disrupting the bacterial cell membrane. It makes the cell became unstable and ruptured. Tannin acted by disturbing the protein synthesis of the bacterial cell, while alkaloids could damage the DNA synthesis [42–46]. These mechanisms of collaborations may inhibit bacterial growth or even cause bacterial cell death.

The other microorganism that we tested against the extract was *C. albicans*. The result revealed that the ethanol extract of akar kaik-kaik leaves neither can inhibit the *C. albicans* growth. *Candida albicans* cell wall was made up of chitin, glucan, and mannoprotein. The cell wall is divided into two layers, with mannoproteins on the outside and chitin on the inside. Glucans are found in the inner layer and serve as a link between the inner and outer layers. Since the mannoproteins in the outer layer have low permeability and porosity, some compounds, such as antifungal agents, cannot pass through them easily. This structure confers resistance to antifungal drugs or a host defense mechanism on *C. albicans* [47,48]. This statement corresponded to Lima et al. [49] who stated that higher mannan structure in fungi could lead to *Candida* resistance to antimicrobial agents.

4. CONCLUSION

The ethanol extract of akar kaik-kaik leaves can inhibit the growth of *S. aureus*, but had no effect on *E. coli* and *C. albicans.*

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