

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

ABSTRACT

Akar kaik-kaik (*Uncaria cordata*) or known as hook vine is one of the traditional medicinal plants whose leaves are used as **an** anti-diabetic 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 done by maceration method using 70% ethanol as a solvent. The extract then **tested testing** for phytochemical screening to find out the secondary metabolite **it-has**. 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 phytochemical screening showed that the extract **was** contained of alkaloid, saponin, and tannin. 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*

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 (Sari, 2006; Sholikhah, 2016).

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* (Heitzman et al., 2005; Martins & Nunez, 2015; Zhang et al., 2015). Another species is *Uncaria gambir* which has antimicrobial activity against *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, and *Bacillus cereus* (Magdalena & Kusnadi, 2015).

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 (Nursanti et al., 2018). The research about the potential of antidiabetic from akar kaik-kaik (*Uncaria cordata*) leaves has been reported by Ahmad et al. (2011). However, the study about antimicrobial activity from this species has not been reported yet. Therefore, this research aimed to study the antimicrobial activity from the ethanol extract of akar kaik-kaik (*Uncaria cordata*) leaves against *S. aureus* (Gram-

positive bacteria), *E. coli* (Gram-negative bacteria), and *C. albicans* (yeast).

METHODS

Chemicals and Reagents. Nutrient Agar (NA), Sabouroud Dextrose Agar (SDA), Aquadest (Brataco), 70% Ethanol (Brataco), FeCl₃ (Merck), Wagner reagent, Mayer reagent, Dragendorff reagent, Ammoniak (Merck), Acetic acid anhydride (Merck), NaNO₂ (Merck), AlCl₃ (Merck), HCl (Merck), Chloroform (Merck), H₂SO₄ (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.

Preparation and Extraction of Sempur 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 wind-dried method (Sulasmi et al., 2017; Yuningtyas et al., 2018; Putra et al., 2019). The dried leaves then powdered using blender and homogenized by sieving using mesh 60. The sieving produces simplicia of akar kaik-kaik leaf powder (Azwanida, 2015; Sa'adah & Nurhasanawati, 2015).

The akar kaik-kaik leaf powder was weighed as much as 80 g then extracted with maceration method using 70% ethanol as a solvent with ratio 1:10 (Depkes RI, 1995). The maceration was done for 24 hours and re-macerated twice with the same procedure (Depkes RI, 2008). The maceration results were filtered with filter paper and the filtrate was evaporated using the vacuum rotary evaporator until it produces a thick extract.

Phytochemical Screening. The extract was tested for phytochemical screening in Lux Chemicals Laboratory (Chemicals Product and Chemical Analysis Service), Depok. The screening test included alkaloid (with Mayer, Bouchardat, Dragendorff reagents), flavonoid, saponin, tannin, steroid/triterpenoid (Harborne, 1987; Agoes, 2007).

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, while *C. albicans* about 48 hours.

RESULT AND DISCUSSION

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 (Azwanida, 2015). The air-dried method also could retain the chlorophyll content in the sample (Luliana et al., 2016). However, this method has limitation such as time-consuming (Rivai et al., 2010; Luliana et al., 2016; Priamsari et al., 2019). The air-dried method can take time about 3-7 days to months and up to a year depending on the types of samples dried (Azwanida, 2015).

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 (Azwanida, 2015; Zhang et al., 2018). 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 (Velavan, 2015).

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 the solid plant material and solubilize compounds with similar polarity. Furthermore, it is also a recommended solvent by the Ministry of Health, Republic of Indonesia, because its low toxicity (Depkes RI, 2000; Tiwari et al., 2011; Velavan, 2015; Joshi & Adhikari, 2019).

The yield of ethanol extract of akar kaik-kaik leaves was about 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 (Hasnaeni et al., 2019; Priamsari et al., 2019). The high content of the active compounds in a sample is indicated by the high percentage yield (Harborne, 1987). The higher of the solvent polarity, the yield obtained will also increase (Parbuntari et al., 2018). The more polar of a solvent, the better the extraction process. 70% ethanol has high polarity, so it was efficient to attract active compounds in akar kaik-kaik leaves (Tiwari et al., 2011; Truong et al., 2019).

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 |

Phytochemicals Screening

Phytochemicals screening was done qualitatively by the change color reaction method using several reagents (Parbuntari et al., 2018). 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).

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

The positive results of alkaloid showed an agreement with literature which stated that *Uncaria* genus was known for their alkaloid constituents (Martins & Nunez, 2015; Abdullah et al., 2016; Olivar et al., 2018). According to Wardhani et al. (2019), ethanol

can be used to extracted the alkaloid from kemuning leaf. The statement was corresponded with Liu & Liu (2015) which proved that the 70% ethanol was best solvent to extract alkaloid from *Actinidia arguta* fruits compared to 60% ethanol and 80% ethanol.

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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 (Parbuntari et al., 2018). Based on the law of similarity and intermiscibility (like dissolves like), a solvent with a polarity near the polarity of the solute is likely to perform and vice versa (Zhang et al., 2018). That's why ethanol as a polar solvent can attract saponin from akar kaik-kaik leaves. Ahmad et al. (2011) also reported that the methanol extract of stem and leaves *Uncaria cordata* were containing saponin.

Another compound that found in ethanol extract of akar kaik-kaik leaves was tannin. This was also alignment with Ahmad et al. (2011) which reported that the methanol extract of stem and leaves *Uncaria cordata* were containing tannin. 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 (Tiwari et al., 2011; Sadeek & Abdallah, 2019). 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 (Crozier et al., 2006; Panche et al., 2016). 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. (2014) 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% ethanol. Flavonoids were more dissolved in 96% ethanol rather than at 70% ethanol (Stankovic et al., 2011; Syafitri et al., 2014). This assumption was also supported by the result of Rachmatiah et al. (2020 in press), which showed a positive result in flavonoid screening in 96% ethanol extract of akar kaik-kaik leaves.

Antimicrobial Activity

The antimicrobial activity was done using a Kirby-Bauer disk diffusion method. This method used to determine the sensitivity or resistance of pathogenic microorganisms to various antimicrobial compounds. The clear zone appears around the disk was measured as inhibition zone (Pratiwi, 2008; Dafale et al., 2016; Hudzicki, 2016). The results of antimicrobial activity of akar kaik-kaik leaves was served in Table 3.

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

| Sample | Concentration | Microorganisms | | |
|--|----------------------|------------------|----------------|--------------------|
| | | <i>S. aureus</i> | <i>E. coli</i> | <i>C. albicans</i> |
| 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

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The data in Table 3 showed that the extract did not affect the growth of *E. coli* and *C. albicans*. It only affected *S. aureus* growth at concentration 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. (2020 in press) 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. (2020 in press) 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 causes the extract unable to penetrate the bacterial cell wall and damage the cell components of *E. coli*.

Meanwhile, the antimicrobial activity occurred against *S. aureus* was probably due to the synergistic mechanisms among chemical compounds found in ethanol extract of akar kaik-kaik leaves, viz. alkaloid, tannin, and saponin. Saponin was worked by disrupting the bacterial cell membrane. It makes the cell became unstable and ruptured. Tannin was acted by disturbing the protein synthesis of the bacterial cell, while alkaloid could damage the DNA synthesis (Cowan, 1999; Cushnie et al., 2014; Gokhale et al., 2015; Khameneh et al., 2019; Othman et al., 2019). Those collaborations of mechanisms may inhibit

bacterial growth or even causes bacterial cell death.

Another microorganism that we tested against the extract was *C. albicans*. The result showed that the ethanol extract of akar kaik-kaik leaves neither can inhibit the *C. albicans* growth. *Candida albicans* cell wall was composed of chitin, glucan, and mannoprotein. The cell wall is forming a two-layer structure with mannoproteins in the outer, while chitin in the inner layer. Glucans lie in the inner layer and connecting the inner and outer layer. The mannoproteins in outer layer have low permeability and porosity, so it cannot easily pass by some compounds including antifungal agents. This structure made the *C. albicans* resistance to antifungal drugs or host defence mechanism (Malanovic et al., 2015; Garcia-Rubio, 2020). This statement was corresponding with Lima et al. (2019) which stated that a more mannan structure in fungi can develop the resistance in *Candida* against antimicrobial agents.

CONCLUSION

The ethanol extract of akar kaik-kaik leaves can inhibit the growth of *S. aureus*, while for *E. coli* and *C. albicans* showed no activity.

ACKNOWLEDGEMENT

We thank the Ministry of Research and Technology/National Research and Innovation Agency, The Republic of Indonesia for financing research funds through PDP Grant year 2020.

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