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Antibacterial Activity of Ethanol Extracts of Sempur Air (*Dillenia suffruticosa*) and Akar Kaik-kaik (*Uncaria cordata*) Leaves Against *Shigella dysentriae* and *Salmonella typhimurium*

Vilya Syafriana[1,*], Lidia Anggita Ramadhani[1], Fitria Helma[1], Wirna Ningsih[1], Suyatno[2]

[1]Faculty of Pharmacy, National Institute of Science and Technology, Jakarta, Indonesia
[2]RSUD Depati Hamzah, Pangkal Pinang, Kepulauan Bangka-Belitung, Indonesia
*Corresponding author: v.syafriana@istn.ac.id

Abstract

Sempur air (*Dillenia suffruticosa*) and akar kaik-kaik (*Uncaria cordata*) are two plants that can be found on Sumatra, Indonesia. The locals employ those herbs as traditional medicine in variety of ways, including treating diarrhea. This study sought to confirm the efficacy of sempur air and akar kaik-kaik leaf extracts as traditional diarrhea remedies. Leaves were obtained in Bangka-Belitung and Riau, Sumatra. They were extracted with 70% ethanol as a solvent and then evaluated for the phytochemical constituents and antibacterial properties. The phytochemical screening found that the ethanol extract of akar kaik-kaik leaf contains alkaloids, tannins, and saponins, but no flavonoids or steroids/triterpenoids. The ethanol extract of sempur air leaf included alkaloids, flavonoids, tannins, and saponins; but neither steroids or triterpenoids. The ethanol extract of akar kaik-kaik leaf showed no impact on *Shigella dysentriae* and *Salmonella typhimurium*, whereas the ethanol extract of sempur air leaf could inhibit *S. dysentriae* but not *S. typhimurium*.

Keywords: akar kaik-kaik, antibacterial, ethanol, leaves, sempur air

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1 Introduction

Sumatra is one of Indonesia's major islands with the highest plant diversity after Papua and Borneo [1]. Sempur air (*Dillenia suffruticosa*) and akar kaik-kaik (*Uncaria cordata*) are two plants found on this island. Those plants are empirically used by the local people as traditional medicine, one of which is to treat diarrhea. The treatment is carried out by boiling the plant's leaves, as done by the Bangka-Belitung who use sempur air leaf and the Jambi who use akar kaik-kaik leaf [2],[3],[4].

Diarrhea is a condition in which a person defecates frequently and in liquid form. This disease is caused by the presence of toxins secreted by Gram-negative bacteria, such as *Shigella* and *Salmonella* in the gastrointestinal tract. Infections caused by these two pathogens are varying severity. *Shigella* most commonly causes bloody diarrhea in the stool, known as bacillary dysentery, whereas *Salmonella* causes non-bloody gastroenteritis [5],[6],[7],[-8].

Scientific research should be conducted to ensure the efficacy of plants as alternative medicinal ingredients. The research on sempur air and akar kaik-kaik leaves against *Shigella* and *Salmonella* species is still very limited. A study on the antibacterial activities of akar kaik-kaik leaf against *Salmonella typhi* was reported only by Rachmatiah et al. [3] and the sempur air leaf against *Shigella dysentriae* was reported only by Syafriana et al. [9]. The study revealed that the infusion of sempur air leaf had a negative effect on *S. dysentriae*, whereas the ethanol extract of akar kaik-kaik leaf showed potent inhibition of *S. typhi* growth.

The purpose of this study was to inform and confirm the efficacy of sempur air and akar kaik-kaik leaves extracts as traditional diarrhea treatments. Based on previous research, the antibacterial activity of sempur air and akar kaik-kaik leaves against *Salmonella* and *Shigella* was investigated further. The extraction solvent used in this study was 70% ethanol, as recommended by the Indonesian Herbal Pharmacopoeia [10].

2 Methods

2.1 Chemicals and reagents

Nutrient Agar (Oxoid), Aquadest (Brataco), 70% Ethanol (Brataco), 95% ethanol, $FeCl_3$ (Merck), Wagner reagent, Mayer reagent, Dragendorff reagent, Lieberman-Burchard reagents, Mg (Merck), HCl (Merck), DMSO, 0.9% NaCl, Blank disc (Oxoid), the antibiotic disk of Amoxicillin (Oxoid), analytical balance (Excellent), oven (Memmert), blender (Phillips), aluminium foil (Klin Pak), autoclave, incubator, vacuum rotary evaporator, hot plate, and Laminar Air Flow.

2.2 Preparation and extraction of sempur air and akar kaik-kaik leaves

Sempur air leaves were obtained from Pedindang Village, Pangkalan Baru, Central Bangka District, Bangka-Belitung Island, while akar kaik-kaik leaves were obtained from Larangan Adat Forest, Rumbio Village, Kampar, Riau. Akar kaik-kaik leaves were thoroughly washed and then dried using the air-dried method. The same procedure was followed for the sempur air leaves samples, but due to the large shape of the leaves, they were cut into 2-3 cm pieces before drying. The drying time of the two leaves was 14-15 days. The dried leaves were then powdered and homogenized in size by sieving through mesh 60 [4],[11].

The akar kaik-kaik leaf powder weighed up to 80 g, while the sempur air leaf powder up to 100 g. Each powder was extracted using a maceration method with a 70% ethanol solvent in a 1:10 ratio. Maceration lasted 24 hours and was repeated twice with the same procedure. The maceration results were filtered and then

evaporated using a vacuum rotary evaporator until it became a thick extract [10],[12],[13].

2.3 Phytochemicals screening

The screening tests for phytochemicals were conducted at the Lux Chemicals Laboratory in Depok (Chemicals Product and Chemical Analysis Service). The screening tests were based on the color change reaction using various reagents. The tests included alkaloids, flavonoids, saponins, tannins, and steroid/triterpenoids [14],[15].

2.4 Antibacterial activity

The antibacterial activity test of the extracts was performed against the *Salmonella typhimurium* and *S. dysentriae*. The test was conducted at the Testing Laboratory of Biotechnology Center, Agency for the Assessment and Application of Technology, Serpong. The method used was disc diffusion with a bacterial suspension of 10^6 CFU/mL and incubated for 24 hours. The inhibition zone was determined by measuring the diameter of the clear zone formed around the disc [16].

3 Results and Discussion

3.1 Yield extracts

Extraction is the separation of active compounds using standard procedures and solvents, one of the methods is maceration. Maceration is the process of extracting simplicial by shaking or stirring it several times at room temperature. Apart from being easy to use in terms of equipment and procedure, this method was chosen because there is no heating involved, preventing loss or damage to the thermolabile compounds [17],[18]. Maceration was done for 1×24 hours while being stirred. Stirring helps the solvent come into contact with the plant cells' cavities so that the solvent may draw out the compounds contained therein. Stirring can also enable the solvent to circulate, which helps extraction proceed as effectively as possible. The yield of the extract from each sample is listed in Table 1. Yield calculation can determine the levels of secondary metabolites extracted by the solvent but not the type of compound involved [19],[20].

Table 1. Yield extracts of the ethanolic extracts of sempur air and akar kaik-kaik leaves

Samples	Weight of powder (g)	Weight of extract (g)	Yield (%)
Akar kaik-kaik leaf powder	80	48.3	60.37
Sempur air leaf powder	100	65.6	65.6

According to the data in Table 1 the two extracts have high yields since both were above 50% [4]. Yield extract demonstrated some of the active compounds that were captured throughout the extraction process. A high percentage yield indicates that a sample contains a high concentration of active compounds. The high yield of the two extracts was most likely due to the solvent used. The higher the polarity of the solvent, the greater the yield percentage. The solvent for the extraction in this research was 70% ethanol. It is a universal and polar solvent which capable of extracting secondary metabolites such as flavonoids, glycosides, saponins, tannins, and several alkaloids more efficiently than pure ethanol or other solvents. Besides that, 70% ethanol was also recommended due to its low toxicity [4],[10],[11].

Our previous study in akar kaik-kaik leaf extract using 96% ethanol as a solvent showed that the yield extract was 18% [3]. These results were in line with the findings of an ethanol extract of *Psidium guineense* leaves and parijoto fruit, which revealed that the extract with 70% ethanol solvent produced a higher yield than the extract with 96% ethanol solvent [21],[22]. Other research in sempur air leaf extracts showed that yield extracts using methanol was about 9.2% [23] and 21.8% [24]; while using aqueous as a solvent was about 9.2% [24] and 4.02% [25]. Those data support our findings that using 70% ethanol solvent yielded a higher extract yield percentage than other solvents.

3.2 Phytochemicals screening

To determine the content of secondary metabolites, phytochemical screening was performed, which included tests for alkaloids, flavonoids, saponins, tannins, and steroids/triterpenoids. Table 2 showed the phytochemical screening results of ethanol extracts of akar kaik-kaik and sempur air leaves.

Table 2. Phytochemical screening of ethanol extracts of akar kaik-kaik and sempur air leaves

Chemical Compounds		Results	
		akar kaik-kaik leaf extract	sempur air leaf extract
Flavonoid		(-)	(+)
Alkaloid	Wagner	(+)	(+)
	Mayer	(-)	(-)
	Dragendorf	(+)	(+)
Tanin		(+)	(+)
Saponin		(+)	(+)
Steroid		(-)	(-)
Triterpenoid		(-)	(-)

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

Data in Table 2 showed that the phytochemical screening of the two leaf extracts differ. In the flavonoid test, the akar kaik-kaik leaf extract did not perform well, whereas the sempur air leaf extract did. Other phytochemical tests showed the same results, namely positive for alkaloids, tannins, and saponins, while steroids and triterpenoids were negative in both extracts. The phytochemical screening for akar kaik-kaik leaf extract differed from the results of Rachmatiah et al. [3], who found positive results in the steroid/triterpenoid test and negative in the alkaloid test. Differences in phytochemical results were also found in sempur air leaf extract compared to previous researchers, specifically in the results of alkaloids [25], steroids/triterpenoids [23],[24], and tannins [26]. The solvent used will influence the extraction of secondary metabolites from plants. According to the law of like dissolves like, secondary metabolites will be attracted based on the polarity of the solvent used [18],[27].

Aside from the effect of the solvent, environmental factors such as temperature, air quality, light intensity, soil condition, and fertility influence the content of medicinal plant secondary metabolites. Plants may produce specific compounds in response to changing seasons or environmental conditions in order to survive and avoid extinction [28],[29],[-30]. These conditions may have contributed to the variations in phytochemical test results.

3.3 Antibacterial activity

The antibacterial activity of *S. typhimurium* and *S. dysentriae* was investigated. Antibacterial activity was tested using the disc diffusion method. The antibacterial activity was evidenced by the formation of a clear zone around the disc [16]. Table 3 displays the results of the antibacterial activity.

Table 3. Antibacterial activity of ethanol extracts of akar kaik-kaik and sempur air leaves

Extract	Bacteria	Inhibition Zone (mm)					
		5%	10%	20%	40%	K+	K-
Akar kaik-kaik leaf	*Shigella dysentriae*	-	-	-	-	23.82 ± 0.01	-
	Salmonella typhimurium	-	-	-	-	28.31 ± 0.12	-
Sempur air leaf	*Shigella dysentriae*	-	-	6.59 ± 0.03	7.12 ± 0.13	23.90 ± 0.08	-
	Salmonella typhimurium	-	-	-	-	27.63 ± 0.12	-

Note: bacteria incubated for 24 hours; (-): no inhibition; K+: positive control (Amoxicillin); K-: negative control (DMSO 10%)

The antibacterial test results in Table 3 demonstrated that the sempur air leaf extract could inhibit bacterial growth, albeit only for *S. dysentriae* and not for *S. typhimurium*. The ethanol extract of akar kaik-kaik leaf did not appear to inhibit either microorganism. The presence of an inhibitory effect on *S. dysentriae* by sempur air leaf extract demonstrates the empirical application of sempur air leaf, which is utilized as an antidiarrheal herb by indigenous Sumatran people. However, greater investigation into the effectiveness of this leaf against *S. dysentriae* should be undertaken since the inhibition zone values observed were rather low. Despite the limited inhibitory zone, these results outperformed the sempur air leaf infusion, which exhibited no inhibition at any concentration (5%, 10%, 20%, and 40%) [9].

The inhibitory zone was considered to be formed by active compounds contained in sempur air leaf extracts, such as alkaloids, flavonoids, tannins, and saponins, all of which have antibacterial effects. Alkaloids are known to inhibit bacterial growth by blocking nucleic

acid synthesis [31]. Flavonoids have the ability to limit bacterial growth by denaturing proteins, resulting in the halting of bacterial cell metabolic activity. Tannins are compounds that can affect bacterial cell membranes by shrinking cell walls and interfering with cell permeability. Saponins are compounds that can lyse bacterial cell walls because their surface-active substances are similar to detergents. Saponins have the potential to lower bacterial cell wall surface tension and membrane permeability [32]-[33],[34],[35],[36].

4 Conclusions

The ethanol extract of akar kaik-kaik leaf had no effect on *S. dysentriae* or *S. typhimurium*. The ethanol extract of sempur air leaf inhibited *S. dysentriae* while being negative for *S. typhimurium*. More research on the activity of the two leaves is required to substantiate the empirical beliefs as a diarrhea traditional medicine. Several parameters related to the optimization of the drying process, the use of solvents, the extraction method, and the concentration of the extract utilized can be analyzed and investigated further because these factors can affect the extract's ability to prevent bacterial growth.

5 Declarations

5.1 Acknowledgements

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5.2 Author Contribution

Vilya Syafriana, Suyatno and Lidia Anggita Ramadhani designed the study. Suyatno was in charge of the fieldwork, while Fitria Helma and Wirna Ningsih handled the laboratory work. Vilya Syafriana and Lidia Anggita Ramadhani examined the data and prepared the paper.

5.3 Conflict of interest

The authors declare no conflict of interest.

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