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4 Phytochemical Screening and Antimicrobial Activity of *Cordyline fruticosa* Leaf Infusion and Ethanol Extract Against *Shigella dysenteriae* and *Candida albicans*

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Abstract. *Cordyline fruticosa*, also known to Indonesians as andong, is one of the simplest plants to grow. This plant is commonly used as an ornamental plant in yards, gardens, cemeteries, as well as a road barrier. Although it is primarily grown for ornamental purposes, the leaves of this plant empirically have been used to treat diarrhea and dysentery. The goal of this study was to determine what chemicals are present in andong leaves, as well as to see if an extract derived from the leaves has any activity against microbes that cause diarrhea, such as *Shigella dysenteriae* and *Candida albicans*. Andong leaves were extracted using two different methods, namely maceration with 96% ethanol as a solvent and infusion with distilled water heated to 90 °C. Phytochemicals screening test was conducted qualitatively using the color-change reaction method. While antimicrobial activity test was performed using the disk diffusion method and continued with the Minimum Inhibitory Concentration (MIC) test using the solid dilution method. Phytochemicals screening revealed that the ethanol extract and infusion of andong leaves contained flavonoids, tannins, saponins, and phenols. The antimicrobial activity results showed that the ethanol extract and infusion of andong leaves inhibited the growth of *S. dysenteriae* but had no effect on *C. albicans*. These findings suggest that andong leaves have the potential to treat diarrhea caused by *S. dysenteriae*, but not by *C. albicans*.

Keywords: Andong leaves · Diarrhea · Ethanol · Infusion · Maceration

1 Introduction

Cordyline fruticosa or also known as andong in Indonesia, is an easy-to-grow plant that thrives in a variety of soil types. Due to its striking color, this plant is commonly used as an ornamental plant in yards, gardens, cemeteries, as well as a road barrier. However, some Indonesian's have actually used this plant to treat diarrhea and dysentery. In the community, the treatment is usually done by boiling andong leaves and drinking the filtrate as medicine [1–3].

Diarrhea is a state of defecating with lots of fluids and is a symptom of certain disease or disorders. The majority of these cases occur in developing countries with low

living standards. The cause of diarrhea is a toxin released by bacteria, particularly Gram-negative bacteria such as *Escherichia coli*, *Salmonella* sp., and *Shigella* sp. Diarrhea can also be caused by *Candida albicans*, a type of fungus [1, 4, 5].

Several previous studies have found that andong leaves extract contain secondary metabolites of phenolic groups, such as flavonoids, tannin, and saponins [1, 6, 7]. These compounds are known to act as antimicrobial agents [8]. Some reports proved that andong leaves extract has antibacterial activity against *Enterococcus faecalis*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Salmonella typhimurium*, and *Shigella dysenteriae* [1, 3, 7, 9, 10]. However, the antifungal activity of andong leaves has not been discovered.

Based on the above description, a study was conducted to determine the antimicrobial activity of andong leaves against the bacteria *S. dysenteriae* and fungus *C. albicans*, both of which cause diarrhea. Andong leaves were extracted with two techniques, namely hot infusion with distilled water as a solvent and maceration with 96% ethanol as a solvent. The presence of specific chemicals in the powder, infusion, and ethanol extract of andong leaves was investigated using phytochemical screening. The findings of the study are expected to aid the development of andong leaves as a medicinal plant, apart from being an ornamental plant.

5 Materials and Methods

2.1 Chemicals and Reagents

Distilled water (Brataco), 96% ethanol, 70% ethanol, blank disc (Oxoid), ciprofloxacin disk 5 µg (Oxoid), ketoconazole, Bouchardat reagent, Mayer reagent, Dragendroff reagent, chloroform (Merck), ammoniac (Merck), HCl (Merck), FeCl₃ (Merck), NaNO₂ (Merck), ether, H₂SO₄ (Merck), anhydrate acetic acetate (Merck), 0.9% NaCl, Nutrient Agar (NA) (Oxoid), Sabouraud Dextrose Agar (SDA) (Oxoid), blender (Maspion), vacuum rotary evaporator (BUCHI), waterbath, oven (Mettler), autoclave (Hirayama), incubator (Mettler), Laminar Air Flow (LAF), analytical balance (Excellent), microscope (Olympus), hot plate stirrer (B-One), vortex (Barnstead), aluminium foil (Klin Pak), Petri dish, test tube (Pyrex), Beaker glass (Iwaki), Erlenmeyer (Iwaki), volumetric flask (Iwaki), micro pipette (VWR dan Pippette), infusion pot, calipers (Kenmaster).

2.2 Microbial Strains

The microorganisms used were *Shigella dysenteriae* and *Candida albicans* obtained from Microbiology Laboratory, Faculty of Pharmacy, Institut Sains dan Teknologi Nasional (National Institute of Sciences and Technology).

2.3 Sample Preparation and Simplicia Production

Andong leaves (*Cordyline fruticosa* (L.) A. Chev.) were obtained from Indonesian Medicinal and Aromatic Crops Research Institute (IMACRI). The samples were determined in Herbarium Bogoriense, Research Center for Biology, Indonesian Institute of Sciences (LIPI).

Four kg of andong leaf were sorted and washed under running water. To hasten drying, the leaves were divided about 2–3 cm after being cleaned. The leaves were then dried in an oven at 30–45 °C. The dried leaves were blended, then sieved through a 60-mesh cloth to obtain a homogenous powder.

2.4 Sample Extraction

The extraction was carried out by two methods, namely infusion and maceration. Infusion used distilled water as a solvent while maceration used 96% ethanol.

The infusion process was carried out by weighing 150 g of andong leaf powder, placing it in an infusion pan, and filling it with enough water (until it soaked). The sample was heated to 90 °C. The heating process was carried out for 15 min with stirring occasionally. While the infusion of andong leaves was still hot, it was filtered through filter cloth and enough hot water was added through the dregs to achieve the desired volume of infusion.

Maceration was carried out by weighing 150 g of andong leaf powder and placed in a maceration vessel, the added 1.5 L of 96% ethanol solvent. The maceration vessel was covered to keep it out of the sun and left to soak for 24 h with stirring occasionally every 6 h. The obtained macerate was filtered and the pulp was remacerated twice with the same treatment. All the filtrate obtained from maceration and remacerations was concentrated using a vacuum rotary evaporator, then evaporated over a waterbath to produce a thick extract. The thick extract was ethanol-free tested to ensure that there was no more ethanol in it.

2.5 Phytochemicals Screening

Phytochemicals screening was conducted based on several references, namely *Materia Medika Indonesia* [11], Endarini [12], and Ensamory et al. [13]. Mayer, Dragendorff, and Bouchardat reagents were used to test alkaloids; saponins were tested by the formation of foam stable; flavonoids were tested with a solution of 5% NaNO₂, 10% AlCl₃, and 1 N NaOH; tannins were tested with 1% FeCl₃ solution, while phenol using 3% FeCl₃ solution; and steroids/terpenoids with Liebermann-Burchard reaction.

2.6 Antimicrobial Activity Test

The antimicrobial test was conducted using the disk diffusion method to determine the diameter of Inhibition Zone (IZ) of the extract against *Shigella dysenteriae* and *Candida albicans*. Minimum Inhibitory Concentration (MIC) test was performed to determine the MIC value based on the IZ value. The MIC was carried out using the solid dilution method.

2.6.1 Bacterial and Fungal Suspension Preparation

S. dysenteriae aged 24 h was taken 3–4 oses, then placed in a test tube containing 9 mL of 0.9% NaCl, then vortexed until homogeneous. The bacterial suspension was adjusted to 9×10^6 CFU/mL using McFarland no. 3 (9.0×10^8 CFU/mL) as the turbidity standard.

The appropriate suspension was used as the test inoculum. The same procedure was performed on *C. albicans*, but the fungus was 48 h old.

2.6.2 Extract Concentrations Preparation

The ethanol extracts and infusions of andong leaves were prepared at four different concentrations: 5%, 10%, 15%, and 20%. Distilled water was used as a negative control. The antibiotic ciprofloxacin was used as a positive control for bacteria, and ketoconazole was used as a positive control for fungi.

2.6.3 Diameter of Inhibition Zone (IZ) Test

Each of microbial suspension was pipetting 0.1 mL and put into a petri dish containing the growth media (NA for *S. dysenteriae*, SDA for *C. albicans*). The suspension then being spread using drygalski to evenly distribute. After the media and the microbial suspension have dried, a sterile paper disk was placed onto the agar. The extract from each concentration was dripped for about 20 μ L and then incubated at 37 °C for 24 h (*S. dysenteriae*) and 48 h (*C. albicans*). The clear zone formed around the disk was observed and measured as the Inhibition Zone (IZ) using a caliper.

2.6.4 Minimum Inhibitory Concentration (MIC) Test

In a sterile petri dish, 15 mL NA or SDA was mixed with 1 mL microbial suspension (9×10^6 CFU/mL) and 1 mL extract. The mixture was homogenized by spinning around the dish to form the number 8. The mixture then being incubated for 24–48 h at 37 °C. The presence or absence of microbial growth in the media was observed. Media with microbial growth indicated that the concentration of the extract was unable to inhibit the microbial growth, whereas in media without microbial growth indicated that the concentration was able to inhibit the microbial growth.

3 Results and Discussion

3.1 Simplicia Results

Andong leaves were dried in an oven at a temperature of 30–45 °C. Simplicial material can be dried at temperatures ranging from 30–90 °C, but the best temperature is below 60 °C to prevent heat-sensitive or volatile compounds from being damaged [14]. The oven was chosen because it keeps the temperature constant and allows for faster drying. The drying process aims to produce simplicial that is resistant to damage and can be stored for a long time and avoid mold contamination. The drying process can also reduce the amount of water in the sample, which can inhibit the enzymatic process and protect the sample from degradation or damage [15–17].

Drying fresh andong leaves resulted in a yield of 1.020 g of simplicial from a starting weight of 4 kg. The andong leaves were sifted before extraction to create a uniform powder, which increased the efficiency of the contact between the simplicial and solvent [18].

3.2 Extraction Process

In this study, two extraction methods were compared: the cold method by maceration with 96% ethanol as a solvent and the hot method by infusion with water as a solvent. The infusion was chosen because it is adapted to empirical conditions in the community where andong leaves were used by boiling. However, the infusion has the drawback of being unable to be stored for an extended period of time, as the extract will become easily damaged and contaminated [12]. The maceration method was chosen because it is easy to use, requires simple and inexpensive tools, and extracts at least 50% of the compounds found in plants. Another advantage of maceration is that it prevents the destruction of thermolabile compounds. This is due to that the maceration takes place at a temperature of 25–30 °C [17, 19].

The solvent used in the maceration was 96% ethanol because it has high polarity, so it is very effective at attracting active compounds from plants. The OH group in ethanol helps dissolve polar molecules, whereas the ions and alkali groups can bind non-polar materials. As a result, ethanol can dissolve both non-polar and polar compounds. Furthermore, ethanol is non-toxic, neutral, and requires less heat to concentrate, ensuring that the substance being extracted is not damaged [19, 20].

The maceration filtrate was concentrated and evaporated to remove the remaining solvent in the extract. This was done because the ethanol solvent can inhibit microbial growth, so the antimicrobial activity of the extract may be biased if there is still residual ethanol in the extract [19, 21]. Ethanol-free result showed that the sample had no odor of Iodoform and did not form a yellow precipitate. It denotes the absence of ethanol in the extract (ethanol-free) [22]. Based on these findings, an ethanol extract of andong leaves can be used to test for antimicrobial activity.

The total weight of the thick extract obtained was 37.22 g from 150 g of simplicial powder. According to this result, the yield of the extract obtained was 24.8%. This result showed a higher yield than previous research (22.30%) that used 95% ethanol as a solvent [1]. The yield determines the amount of secondary metabolites extracted by the solvents, but the compounds contained are unknown [23]. The higher the yield value, the greater the number of chemical compounds attracted [17].

3.3 Phytochemicals Screening

The chemical compounds contained in the sample, such as alkaloids, flavonoids, saponins, phenols, tannins, and steroids/triterpenoids were determined through phytochemicals screening. The test was done qualitatively by looking at the color change reaction in the sample [20]. The experiment was conducted on three samples of andong leaves, namely simplicial powder, infusion, and ethanol extract. The results of phytochemicals screening can be seen in Table 1. Data in Table 1 showed that flavonoid, phenols, tannins, and saponins were detected in the powder, infusion, and ethanol extract of andong leaves. These results were in agreement with other studies that have used polar solvents like 95% ethanol and methanol [1, 6, 7]. Ethanol was known to be the best solvent for extracting polyphenolic compounds from plants such as flavonoids, tannins, and phenols. Saponins, on the other hand, were likely to be drawn to polar solvents like 96% ethanol due to their polar glycosidic bonds [24].

Table 1. Phytochemicals screening of simplicial powder, infusion, and ethanol extract of andong leaves (*Cordyline fruticosa*)

Chemicals compounds		Andong leaf samples			Description
		Powder	Infusion	Ethanol extract	
Alkaloid	Mayer	(–)	(–)	(–)	No white precipitate was formed
	Dragendorff	(–)	(–)	(–)	No brown precipitate was formed
	Bouchardat	(–)	(–)	(–)	No brick red precipitate was formed
Flavonoid		(+)	(+)	(+)	The powder and infusion showed red color, while the ethanol extract showed orange color
Tannin		(+)	(+)	(+)	Showed blackish green color
Phenol		(+)	(+)	(+)	Showed black color
Saponin		(+)	(+)	(+)	Stable foam formed > 1 cm
Steroid/ Triterpenoid		(–)	(–)	(–)	No ring formed and no discoloration

(+): contains the tested compounds; (–): does not contain the tested compounds

3.4 Antimicrobial Activity

3.4.1 Diameter of Inhibition Zone (IZ) Test

The disk diffusion method was used to test the antimicrobial activity of an infusion and ethanol extract of andong leaves against *S. dysenteriae* and *C. albicans*. There were two kinds of positive control used, namely for *S. dysenteriae* using 5 mcg ciprofloxacin, while for *C. albicans* using 15 mcg of ketoconazole. As a negative control, distilled water was used in this study. The diameter of the clear zone formed around the disc was measured as part of this antimicrobial activity test. The clear zone showed the amount of inhibition (Inhibition Zone = IZ) caused by the sample on the growth of the test microbes. Figures 1 and 2 showed the results of the IZ formation.

The data at Fig. 1 illustrated the IZ of the ethanol extract was greater compared to infusion. This was probably due to the ethanol extract could attract more active compounds from the andong leaves than the infusion. The more active substances extracted, the more antimicrobial compounds were produced, resulting in a higher IZ value [25]. In addition, because the infusion in this study was done by heating, it's possible that some thermolabile compounds were lost or evaporated. As a result, the infusion contained less active substance than the ethanol extract.

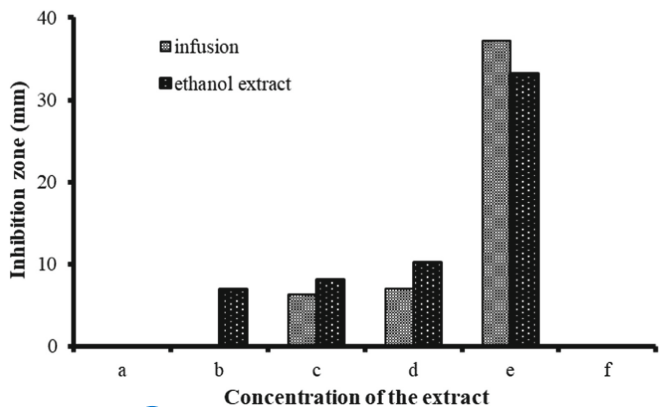


Fig. 1. Inhibition Zone (IZ) of infusion and ethanol extract of *Cordyline fruticosa* (L.) A. Chev leaf (a = 0,05%; b = 0,1%; c = 0,15%; d = 0,2%; e = ketoconazole, f = distilled water) against *Shigella dysenteriae*.

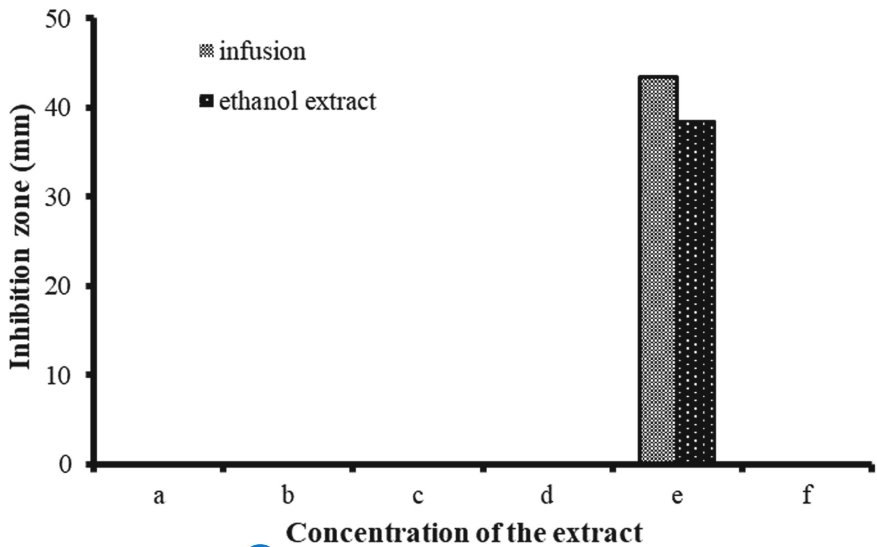


Fig. 2. Inhibition Zone (IZ) of infusion and ethanol extract of *Cordyline fruticosa* (L.) A. Chev leaf (a = 0,05%; b = 0,1%; c = 0,15%; d = 0,2%; e = ketoconazole, f = distilled water) against *Candida albicans*.

The data at Fig. 2 showed the antimicrobial activity of infusion and ethanol extract against *C. albicans*. Figure 2 illustrated that at all concentrations, neither the infusion nor the ethanol extracts inhibited *C. albicans* growth. These findings were in line with previous research which found that an ethanol extract of *Uncaria cordata* and *Dillenia suffruticosa* leaves were also unable to inhibit the growth of *C. albicans* [20, 26]. This phenomenon was presumably due to the fact that *C. albicans* as a member of fungus has

Table 2. Minimum Inhibitory Concentration (MIC) of infusion and ethanol extract of andong leaves (*Cordyline fruticosa* (L.) A. Chev.) against *Shigella dysenteriae*

Concentrations of Andong leaves Infusion	Results	Concentrations of Andong leaves Ethanol Extract	Results
15%	—	10%	—
14%	+	9%	—
13%	+	8%	+
12%	+	7%	+
11%	+	6%	+

+ : bacterial growth discovered; — : no bacterial growth discovered

a mannoprotein structure in its cell wall. Because of this structure, certain compounds, including antifungal agents, have difficulty penetrating the cell walls of fungi [27, 28].

3.4.2 Minimum Inhibitory Concentration (MIC) Test

The MIC test was only performed on *S. dysenteriae*, because the *C. albicans* did not show antimicrobial activity. The method used for the MIC test was the solid dilution method, which involved observing the presence or absence of bacterial growth in the media. The goal of this test was to find the sample's lowest concentration that could still inhibit bacterial growth. The concentrations used in the infusion MIC test were 15%, 14%, 13%, 12%, and 11%, while the ethanol extract concentrations tested were 10%, 9%, 8%, 7%, and 6%. The results of the test were shown in Table 2.

Table 2 showed that the minimum concentration of andong leaves infusion that could still inhibit the growth of *S. dysenteriae* was at 15%, while the ethanol extract was shown to be at a concentration of 9%. The inhibition of bacterial growth by the sample may be due to the presence of chemical compounds in the infusion or ethanol extract. Both infusion and ethanol extract showed the presence of polyphenolic compounds such as flavonoids, tannins, and phenols, as well as saponin compounds. These compounds were known to disrupt cell wall stability, cell membrane permeability, and interfere with protein and nucleic acid synthesis [29–31].

Flavonoids, tannins, saponins, and phenols were found in andong leaves powder, infusion or ethanol extract. The ethanol extract and infusion of andong leaves inhibited the growth of *S. dysenteriae* but had no effect on *C. albicans*. These findings suggest that andong leaves have the potential as an antibacterial against *S. dysenteriae*.

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research, phytochemicals screening and antimicrobial assay. All authors read and approved the final manuscript.

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