The difference of inhibitory zone between *katuk* (*Sauropus androgynous* L. merr.) leaf infusion and roselle (*Hibiscus sabdariffa* L.) petals towards oral *Candida albicans*

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ABSTRACT

Introduction: *Katuk* (*Sauropus androgynus* L. Merr.) leaf and roselle (*Hibiscus sabdariffa* L.) are part of plants that have antifungal activity against *Candida albicans*. The purpose of this study was to examine the inhibitory zone of *katuk* leaf's infusion compared to roselle petals towards the growth of oral *Candida albicans*. **Methods:** The research methods was experimental laboratory. Each of *katuk* leaf and roselle's infusion were respectively made in four concentration, i.e 5, 10, 20, and 40%. The positive control was 2% ketoconazole, while negative control was sterile aquadest. **Results:** The result of the study showed that the mean of inhibitory zones of *katuk* leaf 5%, was 17.90±0.8 mm, *katuk* leaf of 10% was 19.67±1.78 mm, *katuk* leaf of 20% was 19.67±1.78 mm, and *katuk* leaf consentration of 40% was 22.93±1.00 mm, meanwhile the mean of inhibitions zones of roselle petals infusion of 5% shows its mean of 18.53±0.67 mm, roselle petals of 10% was 22.40±1.28 mm, roselle petals of 20% was 26.20±0.87 mm, and roselle petals of 40%was 29.47±2.87 mm. **Conclusion:** It can be concluded that the mean of inhibitions zones of *katuk* leaf's infusion was smaller than roselle petals toward the growth of oral *Candida albicans*.

Keywords: Inhibitory zones, Katuk leaf infusion, Roselle petal infusion, *Candida albicans*.

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INTRODUCTION

Nowaday, the use of traditional medicine is on rise, both in developing and developed countries. This phenomenon occurs because traditional medicines have pharmacological effects that almost resemble to potent medicines. In addition, its natural ingredient has low side effects. Leaves of *katuk* and roselle are traditional medicines that are favorites and easy to find for medical doctors who are assigned in the rural area due to cheap and not a seasonal crop.[1]

Katuk leaf has long been used as a traditional medicine by Indonesian people. This leaf or known by the Latin name as *Sauropus androgynus* L. Merr. is a local Southeast Asian plant that has been widely used as an alternative medicine.[2] *Katuk* leaf is a potential alternative

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medicine because it is rich in vitamins, nutrients and has an effectiveness in its active compounds such as carbohydrates, proteins, glycosides, phenols, steroids, alkaloids, terpenoids, tannins, saponins, and flavonoids. Various studies suggest that the ingredients contained in *katuk* leaf are efficacious as antidiabetic, anti-obesity, anti-inflammatory, induces lactation, anti-oxidant, and anti-microbial activity.[3] As an antimicrobial, Selvi *et al*.[4] proved that *katuk* leaf content is able to produce zone inhibition against the growth of *Candida albicans* better than the flower and the root part. Heroor's[5] research proved that the antifungal activity of methanol extract of *katuk* leaves were significantly different from control at concentration of 20 µg/ml.

Other plants that have also been used extensively as alternative treatments of various diseases are roselle petals known as latin *Hibiscus sabdariffa L*. Roselles have pharmacological effects such as anticancer, antihypertensive, anticholesterol, antidiabetic, antiplasmodic, and antimicrobials due to saponin, gossypeptin (hydroxyl flavone), anthocyanin (red substance), hibicin glucoside, carotene, thiamine, niacin, ascorbic acid, and flavonoids. The study of Tanjong[6] proves that the ethanol extract of 96% of roselle petals at concentrations of 40%, has an inhibition zone similar to ketoconazole 200 g against *Candida albicans*. The dentistry field utilizes a stew of roselle petals' infusion of 5% as a soak liquid for acrylic removable denture so that bacterial growth and *Candida albicans* are inhibited.[7]

Candida albicans is a normal flora in the oral cavity that could be a pathogen for the body if there is a situation that allows for it's replication, including changes in the oral environment caused by many things such as antibiotics usage, corticosteroids, xerostomia, systemic diseases, nutritional deficiencies, and immunosuppression.[8] However, in some cases, it was reported that *Candida albicans* may be resistant to the antifungal of the azole group, so it is necessary to develop an alternative antifungal from herbs.[9-11]

Based on the description above, the author was interested to study the difference of inhibition zone between *katuk* leaf infusion and roselle toward *Candida albicans*.

METHODS

The research method used was experimental.[12] The research was conducted by Kirby-Bauer method. Sterile discs papers was immersed in *katuk* leaf and roselle infusion at each concentration of 5, 10, 20 and 40%. Then, it were placed on medium agar that had been inoculated by 0.1 ml of *Candida albicans*. The positive control used was 2% ketoconazole and the negative control was used 1 ml sterile aquadest. After being incubated for 1x24 hours, the clear zones around the discs were measured.

Katuk leaf was obtained from Sukabumi, West Java and roselle was obtained from Wonodadi, Blitar, East Java. Plants are washed and dried, then boiled at 90°C in water for 15 minutes. The *katuk* leaf infusion was prepared for 4 concentrations of 5, 10, 20, and 40%.

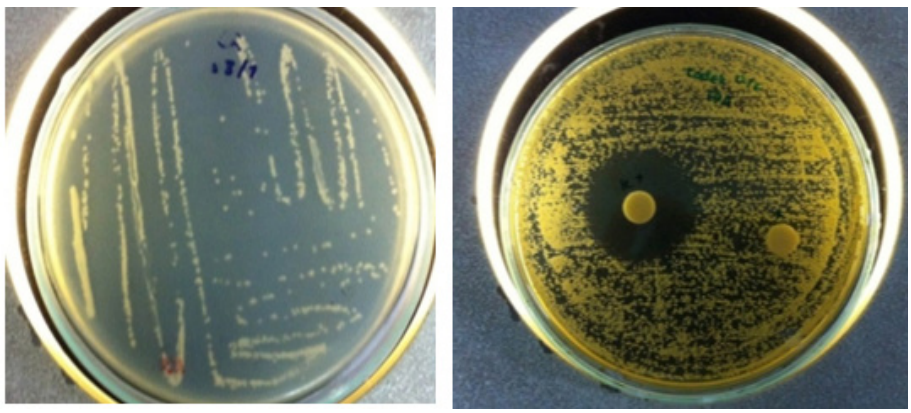
Oral *Candida* was swab from the tongue of healthy person and not taking drugs or antiseptic by using sterile cotton bud. The species was determined according to it's characteristics such as yellowish-white colony, smooth, slippery and distinctive smell like yeast when cultured on Saboraud Dextrose Agar media (SDA).[9] The smear of microorganism was planted on SDA and put into the exsicator, subsequently incubated in temperature of 37°C for 18-24 hours. Next, the growing colony was tested to its carbohydrate fermentation and performed gram staining for *Candida*. Identified *Candida albicans* colony were taken by using ose subsequently was scraped on SDA agar medium in a tube, and incubated for 24 hours at 37°C. After incubation, it was stored in the refrigerator at 4°C until used. The grew of *Candida albicans* was diluted into Mueller Hinton liquid medium, homogeneously shaken, until it was equal to 10 Mc Farland's standard. This solution will be used as a sample stock.[5,9]

Candida albicans suspension standard was inoculated in SDA plate, and incubated at room temperature of approximately 10 minutes. Each disc paper containing *katuk* leaf infusion (50 µL) or ketonazole (50 µL) as well as sterile aquadest (50 µL) were placed on top of the surface of SDA by using tweezers with the distance between the paper one to another 3 cm and from the edge of the media 2 cm.[13] Each petri dish, was eventually

incubated at 37°C for 1x24 hours. The inhibition zone were seen as the clear zones around the discs papers. These procedures were repeated for 3 times. Inhibitions zones of various concentration of infusions and controls were measured by using a ruler. The data were analyzed using independent sample *t* test.

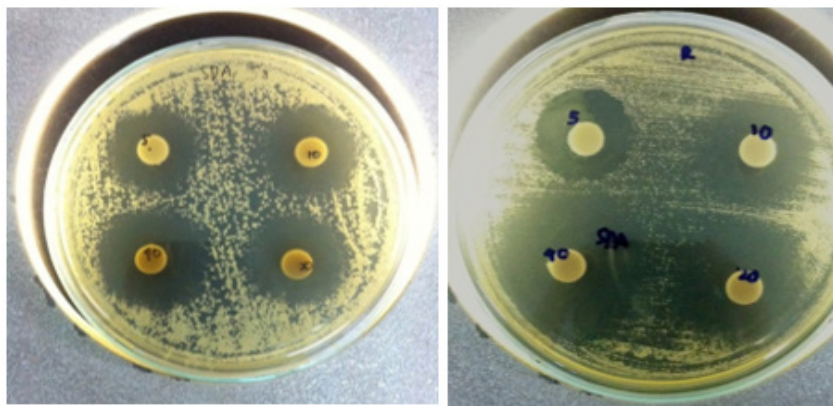
RESULT

Figure 1A shows characteristics of *Candida albicans* colonies in SDA plate. The colony is yellowish-white, smooth, and slippery. The ketoconazole disc paper is an antifungal used as a positive control correlated to the formed inhibition zone



A B

Figure 1. (A) *Candida albicans* colonies on Sabouraud Dextrse Agar; (B) Positive control zone (ketoconazole) and negative control zone (aquades) toward oral *Candida albicans*



A B

Figure 2. A. *Katuk* leaf inhibitions zones against *Candida albicans*; B. Inhibitions zones of roselle infusion against oral *Candida albicans*

Table 1. Inhibitions zone of *katuk* leaf infusion, *roselle* infusion, positive control and negative control against oral *Candida albicans*

Repetition	Inhibitions Zone of *Sauropus androgynus* (mm)				Inhibitions Zone of *Hibiscus sabdariffa* (mm)			
	5%	10%	20%	40%	5%	10%	20%	40%
1	18.5	19.3	20.1	23.7	18.1	22.1	25.2	26.2
2	16.9	18.1	20.1	21.8	18.2	23.8	26.8	30.6
3	18.3	21.6	23.2	23.3	19.3	21.3	26.6	31.6
Max	18.5	21.6	23.2	23.7	19.3	23.8	26.8	31.6
Min	16.9	18.1	20.1	21.8	18.1	21.3	25.2	26.2
Mean	17.90	19.67	21.13	22.93	18.53	22.40	26.20	29.47
DST	0.87	1.78	1.79	1.00	0.67	1.28	0.87	2.87

around its disc meanwhile aquades used as a negative control does not form an inhibition zone (Fig. 2b).

The inhibitions zones of the *katuk* leaf and the roselle infusion against oral *Candida albicans* were presented in the Figure 1. The Table 1 shows that the mean of inhibition zone of roselle infusions of 5, 10, 20, and 40% is larger than the mean of inhibitions zones of *katuk* leaf infusion against oral *Candida albicans* at the same concentration.

DISCUSSION

The diameter of clear area that represents an inhibitory activity produced by the roselle infusion was bigger than *katuk* leaf infusion. This may due to the active compound in both types of the plants were different. Phytochemical examination proved that *katuk* leaf contain alkaloid group compounds, terpenoids, saponins, tannins, polyphenols, glycosides, and flavonoids. On the other hand, the roselle contains active compounds such as tannins, saponins, phenols, glycosides, flavonoids, and anthocyanins. Among these compounds, some have a good antifungal effect; those are alkaloids, saponins, tannins, polyphenols, flavonoids, anthocyanins, and terpenoids.[2,14] Terpenoid compound seems to be more in *katuk* leaf, while anthocyanin is in rosselle petals.

The study, conducted by Ayerdi *et al*, showed that 66% of the roselle content was polyphenols. The high phenol compound effectively binds microtubule proteins in the cells and disrupts the spindle mitotic function resulting in inhibition of fungus growth.[2] Anthocyanin is the largest component of roselle, which is 60.26%.[15] Anthocyanin is a compound that has a strong antifungal properties by denaturing proteins and cause disorders in protein formation or impaired function of protein molecules in fungi.[1] This leads to changes in protein structure resulting in the occurrence of cytoplasmic membrane protein coagulation. The disturbance of the cytoplasmic membrane may lead to increased cell permeability so that intracellular components such as pyrolyine nucleotides, pyrimidines, and proteins will come out of the cell. This results will lead in inhibiting cell growth and may even lead to cell death. Anthocyanins can also interfere the process of food diffusion into *Candida albicans* cells so that the cell's growth stops. This process is consistent with the Schaefer, Rentzsch, and Breuer studies which show that anthocyanins have a strong anti-inflammatory effect.[1]

Terpenoids compounds can inhibit the growth of fungi, spores, and mycelium by interfering permeability of cytoplasm's membrane.[12] It can inhibit the action of certain enzymes that causes fungal cell metabolism is disrupted, hence the elongation process of fungal hyphae becomes inhibited and hyphae fragmentation is distracted thus causes fungal cell can not multiply within a certain time.[16] Zore *et al*. proved that the antifungal activity of terpenoids against cell morphology of *Candida albicans* at different phases of the cell cycle is causing apoptosis in cells of *Candida albicans* which will lead to inhibition of more than 50% of germs cultured in experimental tubes.[17]

Terpenoids is the dominant compound in the *katuk* leaf and have fungistatic effect against *Candida albicans*, whereas athocyanin is dominant compound in the roselle and have effect as fungistatic and fungicide as well against *Candida albicans*. It is already known that fungicide (kill colony) was more potent than fungistatic (inhibit the grow of colony). Hence, it can be explained that the inhibition zone of roselle infusion was wider than *katuk* leaf infusion.

CONCLUSION

The mean of inhibitions zones of *katuk* leaf infusion is smaller than roselle toward the growth of oral *Candida albicans*.

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