Phytochemical Screening and Antimicrobial Activity of *Limonia acidissima* Ethanol Extract against Microbes from Clinical Isolates

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Abstract: The aim of this study is to determine the content of secondary metabolites ethanol fraction of fruit peel of *Limonia acidissima* qualitatively and test the activity of antimicrobial on *Escherichia coli, Staphylococcus aureus,* and *Candida albicans.* Phytochemical screening was carried out by running for separating the extract in silica gel by eluent n-hexane: ethyl acetate. The Thin Layer Chromatography (TLC) plate results then sprayed with a stain viewer; Alkaloid stain viewer: Dragendorff reagent; Terpenoids: Anisaldehyde-sulfuric acid reagents. The plate is heated to a temperature of 100°C; Flavonoids: H2 SO4 10% reagents; Polyphenols and tannins: FeCl 3 reagents 10%; Anthraquinone: 10% KOH reagent in methanol. The antimicrobial test was carried out using disk diffusion methods with concentrations of 50 mg/ml, 75 mg/ml and 100 mg/ml with control of nystatin and chloramphenicol. Phytochemical screening results of ethanol fraction *Limonia acidissima* can be detected in the content of alkaloids, terpenoids, anthraquinones, and saponins. Antimicrobial test results obtained data that the ethanol fraction *Limonia acidissima* has an increasing inhibitory activity of 50 mg/ml, 75 mg/ml, and 100 mg/ml.

1 INTRODUCTION

Infectious disease is a serious threat in the field of medicine. Coupled with the development of antimicrobial resistance is very high from year to year (Gyles 2011). This is exacerbated by the high nosocomial infections that occur in health installations (Brusselaers, Vogelaers, and Blot 2011). The rate of increase in antimicrobial resistance increases significantly from year to year which causes fewer antibiotic treatment options and more expensive medical costs (Bingyun Li and Thomas J. Webster 2018).

The use of antibiotics with new variants and increasing the dose of antibiotic therapy are some of the ways taken so far to deal with infectious diseases. But the two ways above are of course not enough to overcome this problem. One way that needs to be taken is to explore various natural resources that are potentially used as an antimicrobial alternative. Several amounts of research show that there are many potential sources from plants as antimicrobial agents. Twelve plant extracts which are extracted using acetone solvent has good antibacterial activity against multidrug-resistant bacteria that cause diarrhea, one of which is *Staphylococcus aureus* (Bisi-johnson et al. 2017).

In another study the extract of the *Myrtus communis* and *Cinnamomum zeylanicum* plant extracted using ethanol solvent had antibacterial activity against several multidrug-resistant bacteria tested (*S. aureus, E. coli, P. aeruginosa and S. enteric*). It is even known that *M. communis* extract has better antibacterial activity than penicillin (Zandi 2015). Likewise in several plant extract tests on *Aeromonas hydrophila* it was found that extracts from several plant species namely *Olea europea, Myrtus communis, Thymus vulgaris, Rosmarinus officinalis,* and *Achillea falcata*, were known to have better antibacterial activity than some antibiotics (Anwar et al. 2014).

Microbiologists have several reasons for making plants as hope as antimicrobial agents because various research results on plant phytochemical compounds are proven to have potential as antimicrobials (Rios and Recio 2017), (Mostafa et al. 2018), (Tchinda et al. 2017). Plants have the potential as an alternative source of natural antimicrobials which have a different mechanism of action than commercial antibiotics, some even have competitive

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effects compared to some commercial antibiotics that exist today (Abdallah 2011).

Kaemferia pandurata extract (256 micrograms/ml) and Senna alata (512 micrograms/ml) are known to have inhibitory activity against the growth of methicillin-resistant Staphylococcus aureus (MRSA), extended spectrum beta-lactamase and resistant-carbapenemase bacteria (Wikaningtyas and Sukandar 2016). In addition, due to increasing public awareness about the importance of sources of natural remedies made from plants (Cowan, 1999).

This research will be tested the anti-microbial activity of ethanol extract of *Limonia acidissima fruit peel*. Limonia acidissima is a plant that has various potentials. Among other things, this plant has a high antioxidant activity, has a protective activity against body tissues (Balamuruganvelu et al. 2015). This plant also has antitumor activity. Tests using male model mice with ascitic lymphoma obtained information that *Limonia acidissima* fruit extracts could increase the life span of experimental animals, significantly suppressing tumor growth compared to the control group (Eluru, Taranalli, and Kawatra 2015).

The fruit also has wound healing activity, speed up the process of epithelialization of the wound, accelerating wound closure. It is monitored to increase the activity of antioxidant enzymes such as Superoxide Dismutase (SOD) and catalase enzymes (Ilango and Chitra 2010). Another potential of this plant is as an antimicrobial, it is known that *Limonia acidissima* has antibacterial activity. Some research records show the antimicrobial activity of *Limonia acidissima*, among others, petroleum ether extract of *L acidissima* leaf inhibits the growth of *E. coli* and *S. aureus* (Harshali et al., 2015). The ethanolic extract of *Limonia acidissima* leaves has the potential to inhibit the growth of Gram-positive and negative bacteria (Neelamadhab et al., 2013). The ethanol extract of *L. acidissima* leaf has anti dermatophyte activity in fungi such as *Trichophyton mentagrophytes, Microsporum canis* and *Epidermatophyton floccosum* (Buvanaratchagan, 2016).

Various types of Indonesian plant extracts are known to have antimicrobial activity with various levels (Pratiwi et al., 2015). Therefore, research on antimicrobial activity on various plant extracts always needs to be intensified in order to find alternative solutions to the problem of resistance to microorganisms in various infectious diseases. Medicinal plants need to be explored antimicrobial activity because the content of chemical compounds in various extracts, has a variety of physiological potential for humans. In addition, few compounds in plants are toxic to humans (Zubaır et l., 2011). In this study, the antimicrobial activity of *Limonia acidissima* fruit peel is tested against *Staphylococcus aureus, Escherichia coli,* and *Candida albicans* from clinical samples and the chemical contents from extracts and fractions of some of these plants are observed. The three microbes were chosen because they were general infectious agents and represented a class of Gram-positive, Gram-negative bacteria and fungal pathogens.

2 METHODS

2.1 Plant Simplisia Extraction

Plants *Limonia acidissima* L. obtained from the district. Rasanae Barat Kota Bima, NTB. Plant identification was carried out at UPT Materia Medika Batu. Fruit flesh is washed with water until clean and carried out drying at a temperature of 40 ° C. After drying, then crushed with a grinding machine, so that obtained fine powder. The fine powder is then sieved using a *SieveShaker* with a certain degree of fineness.

Limonia acidissima fruit meat powder was extracted stratified using three types of solvents (n-hexane which is non-polar, ethyl acetate which is semi-polar, and ethanol which is polar). 1000 grams of powdered fruit peel of *Limonia acidissima* is macerated using the first (n-hexane) at a ratio of 1: 4 (mass/volume), after 24 hours residue screening results maceration first macerated with a second solvent (ethyl acetate) in comparison with the similarly, 24 then a filter is made and the residue is macerated again using a third solvent (ethanol). The filtrate from the third maceration (using ethanol solvent) was concentrated with a rotary evaporator until it was concentrated and the weight was stable.

2.2 Phytochemical Screening

Phytochemical screening was carried out according to the method carried out by [21] by removing the extract in silica gel with the eluent n-hexane: ethyl acetate. TLC plate results then sprayed with stain viewer to detect the compound, the Alkaloid: Dragendorff Reagent (produce staining orange); Terpenoids: Anisaldehyde-sulfuric acid reagents. The plate is heated to 100°C (produces a purplestain); Flavonoids: H_2 SO_4 10% reagent (produce yellow stain); Polyphenols and tannins: Reactionof 10% $FeCl_3$ (produces a black stain); Anthraquinone: 10%

KOH reagent in methanol (produces yellow or brownish-yellow stains).

2.3 Antimicrobial Activity Test

The bacteria *Escherichia coli, Staphylococcus aureus,* and the fungus *Candida albicans* are medical samples obtained from the Biomedical Laboratory of the Faculty of Medicine, University of Muhammadiyah Malang. The antimicrobial activity test is carried out by the disc diffusion method. The ethanol fraction of *Limonia acidissima* fruit with a concentration of 5 mg/ml, 7.5 mg/ml, and 10 mg/ml was absorbed on paper discs. Then tested on microbial samples *Escherichia coli, Staphylococcus aureus,* and *Candida albicans*. The preparation was incubated in an incubator for 24 hours, at 37 ° C then the clear zone that appeared was measured.

2.4 Data Analysis

Anti-microbial test data in the form of inhibitory zones were analyzed for the significance of differences between treatments using ANOVA followed by the Tukey test with a significance of $P < 0.05$ using SPSS program ver 23.

3 RESULTS AND DISCUSSION

Phytochemical screening results of ethanol fraction *Limonia acidissima* can be detected in the content of alkaloids, terpenoids, anthraquinones, and saponins qualitatively as shown in Table 1.

While the results of the antimicrobial test of the fraction gave a positive inhibitory growth on *Staphylococcus aureus* and *Escherichia coli* but not on *Candida albicans* molds from clinical samples as listed in Table 2.

Table 1. Phytochemical screening results show Alkaloid, Terpenoid, Anthraquinone and Saponin content in the ethanol fraction of *Limonia acidissima*

No	Compound	Stain Viewer	Results	Rf value	Information
1	Alkaloids	Dragendorff	(+)	0.225	Orange color is formed
2	Flavonoids	H2SO4 10%	-	-	-
3	Polyphenols and Tannins	FeCl3 10%	-	-	-
4	Terpenoids	anisaldehyde-sulfuric acid	(+)	0.0625; 0.1125; 0.175; 0.325; 0.4625	Purple color
5	Anthraquinone	KOH 10% in methanol	(+)	0.05	Yellow formed
6	Saponin	Froth test	(+)		Formed stable froth more than 3 cm above the liquid surface.
		Salkowski Test	(+)		The formation of a red ring in the test solution given concentrated H2SO4.

Table 2. Test results for the antimicrobial activity of the ethanol fraction *Limonia acidissima*

The concentration of test material	Inhibitory zone diameter (mm)		
	Candida albicans	*Staphylococcus aureus*	*Eschericia coli*
5 mg / ml	-	19.50 ± 2.50	13, 77 ± 1.72
7.5 mg / ml	-	23.63 ± 1.89	15.47 ± 2.08
10 mg / ml	-	26.76 ± 1.79	16.63 ± 2.11
Nystatin (Control +)	17.13 ± 1.35	-	-
Chloramphenicol 30 µg / ml (control +)	-	15.30 ± 1.44	16.67 ± 1.05

Phytochemical screening results that show positive on alkaloid compounds, terpenoids, anthraquinone, and saponins that are in the polar fraction. This means that only the more polar active compounds are extracted in the ethanol fraction according to the nature of the ethanol solvent compared to the other two solvents that have been used to extract *Limonia acidissima* powder, n-hexane and ethyl acetate. The content of secondary metabolites from plants have levels in different polarity. It is possible in a group of compounds such as flavonoids, saponins, phenol hydroquinone, alkaloids, tannins, steroids can be extracted with solvents that have different polarities. Polar flavonoids will be extracted with polar solvents such as ethanol, semi-polar flavonoids will be extracted with semi-polar solvents such as ethyl acetate, and non-polar flavonoids will be extracted with non-polar solvents such as n-hexane, as well as other secondary metabolite compounds (Widyawati et al. 2014). Based on the antimicrobial activity test of marigolds (*Tagetes erecta*) with various solvents (non-polar, semi-polar and polar) it is known that antimicrobial activity is best obtained in marigold extract with polar solvents (Padalia and Chanda 2015).

It was found that the antimicrobial activity of ethanol fraction of *L. acidissima* fruit against *E. coli* in this study was higher than all parts of the fruit extracted using ethanol in other studies (Audia Anda Rini, Supriatno 2017). This fact shows the activity of *L. acidissima* fruit flesh metabolite compounds works better when extracted stratified as this research method compared with the results of other studies that use one-time maceration (not multilevel maceration). Based on research exploration (Vijayvargia and Vijayvergia 2014), (Vijayvargia, Choudhary, and Vijayvergia 2014) [25] [26] overall *L. Acidissima* plant extracts contain Alkaloids, flavonoids, phenols, terpenoids, tannins, fats steroids, saponins, glycosides, gum, mucilage, and essential oils.

The results of the detection of the presence of *L. acidissima* metabolite compounds in this study are known to be minus the presence of flavonoids, polyphenols, and tannins. It turns out that the absence of flavonoid compounds, polyphenols, and tannins in this fraction increases the anti- mic r o ba activity of *E. coli*.

The results of antimicrobial activity against *S. aureus*, *Limonia acidissima* fruit *extract* of this study looked the same as the study (Vijayvargia et al. 2014). These results appear that fractionation with n-hexane, ethyl acetate and ethanol did not affect the strength of the antimicrobial activity of *L. acidisima* fruit peel.

4 CONCLUSIONS

Ethanol *fraction* from three factions (n-hexane, ethyl acetate, and ethanol) *Limonia acidisima* showed antimicrobial activity against *E. Coli* and *S. aureus* but not *Candida albicans*. And the content in the fraction contained alkaloids, terpenoids, anthraquinone, and saponins.

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