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The comparative study of the fruit and leaf extract of *Ficuslyrata*Warb on antibacterial activities

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Abstract. The extraction process of anti-microbial compounds from F.lyrataWarb fruit and leaf had been identified. The study employed a maceration extraction method used water as solvent agents, to extract seconder metabolite, example phenolic, flavonoid, tannin as an anti-microbial agent. The compound which is known to have an inhibition effect to decline the growth of the microorganism of food quality decreasing triggers. Its effects were subjected to 3 tested bacterias, i.e.; Pseudomonasaeruginosa, Escherichia coli, and Bacillus subtilis. This study purpose to determine and compare the content of phytochemicals and antimicrobial activity in leaf and fruit extracts. Experimental laboratorium was conducted and the data analyzed by descriptive analysis in this research. The statistical test used is two sample tests, which aims to know the difference in mean value between leaf extractwith fruit, which is not mutual related. Results indicated the formation of the inhibited zone in the tested media which proved by the Kirby-Bauer testing method are different between fruit and leaf extract.

1. Introduction

One of the natural medicinal plants have been used for antimicrobial resources is a Ficus plant. In Indonesia, the most important species of *Ficus* are *F. lyrata*Warb, *F. elastica*, and *Ficuscarica.* Various parts of the plant like bark, leaves, stem, fruits, seeds, and fruit are medicinally important [1]. Ficus usually grows in subtropical and tropical climate areas. Fruits and leaf of ficus trees are widely used in the fresh or dried form. They are an excellent source of minerals, vitamins, amino acids, crude fibers as well as phenolic compounds [1]. This natural material extracted on the skin of the roots and stems to produce antimicrobial [2]. Compounds contained in the root bark and stems are flavonoids, alkaloids, tannins, phenolic compounds and terpenoids compounds that can inhibit the growth of pathogenic bacteria. The fruit extract of *Ficussycomorous L, Ficusbenjamina, Ficusbengalensis L* and*Ficusreligiosa* showed antibacterial and antitumor activity [3]. Due toextract of *F. lyrataWarb* fruits and leaf could show antimicrobial.

The increased of usage the chemical preservative is dangerous to human health. Literature reports and ethnobotanical records suggest that plants are potential of the pharmaceutical industry. They may provide a natural source of antimicrobial agents that willor provide novel or lead compounds that may be employed in controlling some infections

globally[1]. The fruit and leaf extract of *F. lyrata*Warb contain the bioactive compounds, especially phenolic compounds, flavonoids, triterpenoids, and tannins [2]. The application of natural preservatives in the food industry, especially in the preservation of meat can improve food safety. The aim of this study is to compare the potential utilization of *F. lyrata* fruit and leaf extract as antimicrobial agents. The predicted result of this research is a difference the concentration of compound bioactive and antimicrobial activity between fruit and leaf extract.

2. Material and Methods

2.1. Raw materials

Fresh fruits and leaves of *F. lyrata*Warb or plant parts were collected randomly from Universitas Padjadjaran, Indonesia. Fresh fruits leaf materials were washed under running tap water, air dried and then ground to fine powder and stored in airtight bottles.

2.2. Crude extraction

The Dried Powder of fruit and leaf of 200 g of was extracted in 1600 mL of distilled water for 48 h at room temperature[1]. Extraction was conducted in sealed flasks, and then it was filtered through vacuum filtered. The supernatant was collected. It was then rotavaped to result in the concentration of extract up to 50 % at 40 ºC. All experiments were performed in duplicate to check the reproducibility.

2.3 Phytochemical screening

The extracts were screened for phytochemicals like alkaloids, flavonoids, triterpenoids, phenolic compounds,and tannins, following the procedure of Harborne[4].

Test for phenols (Ferric chloride test) [4], 2 mL of sample was treated with aqueous ferric chloride 5% and observe for the formation of deep blue or black color. **Test for tannins (Ferric chloride test)** [5], 2 mL of sample was treated with aqueous ferric chloride 1% and observe for the formation of deep blue or black color. **Test for flavonoids (NaOH 10% test)** [5], 1 mL of sample was treated with NaOH 10% and observed for formation of brownish orange. **Test for triterpenoids (Lieberman-Burchard test)** [6], 1 mL ofthe sample was treated with 2 mLof acetic anhydride and 2 mL of H_2SO_4. The formation of dark red indicates the presence of triterpenoids. **Test for alkaloids (Mayer's reagent)** [5], 2 mL ofthe sample was treated with 2 mL of Mayer's reagent. The formation of dark red indicates the presence of triterpenoids. **Test for saponins (Foam's test)** [4], 5 mL of sample in the test tube was heated for 1 minute in 55-60ºC. The test tube was shaken vertically for around 5 minutes. The foam that formed indicated the presence of saponins.

Total phenols (Folin-Ciocalteau method) [7], about 3 g sample was weighted and put inside 50 mL volumetric flask. 0.5 ml sample was put inside a 25 mL volumetric flask then was added 0.5 ml FolinCiocalteau and 10 mL of Na_2CO_3 7.5%. The absorbance was measured in 750 nm with gallic acid as standard. **Total Tannins (Folin-Denis method**) [8], About 3 g sample was weighted and put inside 50 mL volumetric flask. 0.5 ml sample was put inside a 25 mL volumetric flask then was added 1.25 mL ofFolin Denis and 2.5 ml Na_2CO_3. The absorbance was measured in 760 nm with tannic acid (0, 2,0 ; 4,0 ; 6,0 ; 8,0 ; 10,0 ppm) as standard. **Total Flavonoids**[9], abouts 3 g sample was weighted and put inside 50 mL of volumetric flask. 0.5 ml sample was put inside 25 ml volumetric flask then was added 0.30 mL of $NaNO_2$ 5%, 0.30 mL of $AlCl_3$ 10%, and 2 mL of NaOH 1M. The

absorbance was measured in 510 nm with quercetin (0, 2,0 ; 4,0 ; 6,0 ; 8,0 ; 10,0 ppm) as standard.

2.4. Analysis antibacterial activity

Antibacterial activity for the fruit and leaf extracts was evaluated by disc diffusion method Kirby Bauer[10]. The bacteria used in the study include *Pseudomonasaeruginosa*, *Escherichia coli*, and *Bacillus subtilis*were obtained from the Pharmacy Faculty, Universitas Padjadjaran, Indonesia. The bacterial isolates were first sub cultured in a nutrient broth and incubated at 37°C for 36 h. Amoxycillin (250 mg) was used as a standard antibiotic for comparison of the results. Muller-Hinton agar medium was loaded into Petri dish, stored until freezing and then inoculated tested bacterial to medium. The diameter of the zone of inhibition was measured in millimeter (mm). The experiment was repeated in triplicates and the average values were calculated. Phytochemical content data and antimicrobial activity were analyzed using two-sample t-test to determine whether there was a difference between leaf and fruit extract[11].

3. Results and Discussion

3.1. Phytochemical Screening

Phytochemical compound screening of *F. lyrata* Warb fruit and leaf extracts was conducted using a qualitative method including flavonoids, phenolic compounds, triterpenoids and tannins from the dried fruit, but the triterpenoid tobe absent in leaf extract. Phytochemicals such as alkaloids, triterpenoids, and saponin were found to be absent in both extracts. The observed results the value of phytochemical screening were presented in table 1.

Table 1. Phytochemical screening the various extracts of *FicuslyrataWarb*

Phytochemical compound	Result	
	Fruits	Leaf
Phenolic	+	+
Flavonoid	+	+
Alkaloid	-	-
Triterpenoid	+	-
Saponin	-	-
Tanin	+	+

Note : (+) : Present
(-) : Absent

Many of active compound like phenolic compounds in plants comprise a comparatively large class of secondary metabolites with varies potential bioactivities, such as antioxidant, antibacterial, and anti-inflammatory[12,13]. The amounts of total bioactive compounds in the water extract was huge. It is generally known that water extract a better influence on the extractability of phenolic compounds, moreover, water is more suitable for the food industry than the other organic solvent. The comparative phytochemical compound of extracts of *Ficuslyrate*Warb fruit and leaf as shown in table 2.

Table 2. Various Phytochemical compound of extracts of fruit and leaf *Ficuslyrata* Warb

Phytochemical compound	Value (mg/g)	Value (mg/g)
	Fruit	Leaf
Phenolic	0.38^{a}±0.04	1.67^{b}±0.21
Flavonoid	0.13^{a}±0.01	1.22^{b}±0.25
Tanin	0.39^{a}±0.17	0.95^{b}±0.13

Table 2 show that the bioactive compounds found in both types of the extract are phenol, flavonoid,and tannin. The content of phenol, flavonoid and tannin compounds on leaves is significantly different from the compounds contained in the fruit. Where the bioactive component is higher. This suggests that the leaf extract is more potentially used as an antimicrobial agent.This is similar with the research reported Rajiv[12], *Ficusreligiosa* fruit extract gave positive results on phytochemical qualitative tests of phenolic compounds, flavonoids, terpenoids,andglycosides. In addition, Adebayo[14], reported the results of qualitative tests performed on *ficus exasperate* leaves gave positive results on tannin, flavonoid, and saponin components.

3.2. Antibacterial activity

The antimicrobial activity test aims to explore the antimicrobial potential resulting from an ingredient by looking at the clear zone formed. In order to see the clear zone formed by the diffusion method[15]. This is based on antimicrobial activity can be seen from the clear zone formed around it. The clear zone shows the resistance of microbial growth in the solid layer in the petri dish.

In the evaluation of antibacterial activity of the fruit and leaf extract, zone of inhibition was observed in the extracts of water. Microbial growth was determined by measuring the diameter of the zone of inhibition. For each bacterial strain, controls were maintained in which pure solvents were used instead of the extract. The antibacterial activity of aqueous extracts against of 3 bacteria is shown in figure 1.

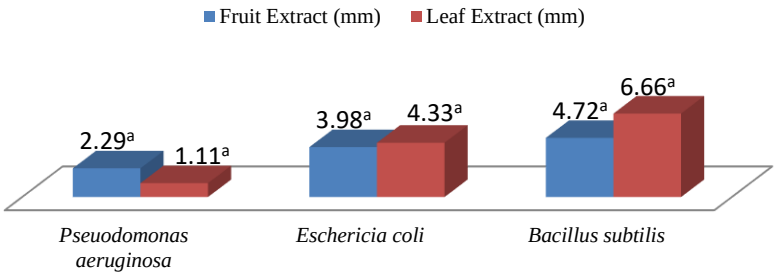

Figure 1. Inhibition zone of extracts against of the different bacteria and different extract

Maximum antibacterial activity was shown by *Bacillus subtilis* from leaf extract while minimum activity was shown by *P. Aeroginosa* from leaf extract. From the results of phytochemical screening analysis and antibacterial activity, it is clear that the presence of phytochemicals compound including flavonoids, phenolic and tannins are essential material

for the inhibition of bacterial growth. Inhibition zone of *B.subtilis and E. coli* in leaf extract is higher than in fruit extract. On the other hand, in *P. Aeroginosa* bacteria, the inhibitory zone of the fruit is higher than leaf extract. This result indicates that the antimicrobial activity of leaf extract is more potentially use as biopreservative to against the *B.subtilis and E. Coli.* This is because the content of bioactivities such as phenolic, flavonoid,and tannin is higher in leaf extract than in fruit. When compared with similar research conducted on ficus plants such as Ficusexasperata extracted using methanol solvent yielding a clear zone diameter on *P. aeruginosa* bacteria is between 3-6 mm[14]. This value is close to the result of research where the clear zone of *P. aeruginosa* bacteria on ethyl acetate extract is 2.97 mm. Meanwhile reported that the inhibitory zone of *Ficusreligiosa* plant using aquadest as the solvent is 5-9 mm with control is tetracycline with 13 mm inhibitory zone value[13].

Flavonoids and tannins are included in the phenol compounds that have an important role in the biochemical process. Such compounds may cause major disruption because of their ability to form protein complexes through hydrogen bonds. When the plant cell content mixes with the membrane becomes damaged during the isolation process, the phenol compound rapidly reacts to form a complex with proteins. As a result, there is often an enzyme inhibition of plant extracts [4]. The mechanism of inhibition of phenol compounds that inhibit protein synthesis and damage the action of enzymes[16]. This causes cell metabolism disorders and destroys cell membranes by dissolving fat in cell walls. Factors affecting antimicrobial activity are extract type, amount, a microbial source, antimicrobial agent concentration, temperature, contact time, and physical properties of substrate chemistry (pH, moisture content, surface tension, type and amount of solute, colloids,and compounds other compounds). This mechanism occurs so that the *F. Lyrata*fruit and leaf extractshave antimicrobial activity.

4. Conclusion

This research showed that extracts of *F. lyrata*Warbfruit and leaf could use as antimicrobial resources. The phytochemical screening resulted in the water fruit leaf extract presence of secondary metabolites like flavonoids, phenolic, and tannin. Antimicrobial activity was observed by the disc diffusion method against bacterial pathogens including *Pseudomonasaeruginosa, Escherichia coli, dan Bacillus subtilis.* The result showed that the leaf extract has the higher of bioactive compounds than fruit extract,on the other side is antimicrobial activityis not a significant difference.

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6. References

[1] Jagathambal, M, K.;Madhavan.; Parvathi.;Karunakaran,:Phytochemical Screening Antimicrobial activity of Seven Solvent Extracts of Dried Fruit of *Ficuscarica* Against Five Human Pathogenic Bacteria G.:Journal of Pharmacy Research, **4**(12) (2011), ,4498-4500

[2] Bidarigh, S, P.; Khoshkholgh, A.; Massiha,;Issazadeh, K,: . Proceeding International Conference on Biotechnology and Environment Management IPCBEE, vol 18 (2011)

[3] Sirisha. N, M.; Sreenivasulu, K.; Sangeeta, C. M.; Chetty.: Antioxidant Properties of Ficus Species-A Review, International Jurnal of pharmTech Research, **2**(4), 2010.
[4] Harbone J B., "Phytochemical methods: A guide to modern techniques of plant analysis", 3rd Edn, Chapman and Hill, London, 1998
[5] Noor, H. Al Atraqchi., Wafaa. M. A., and Al Shaikh. H. 2014. Preliminary Phyochemical Creening and In Vitro Evaluation of Antioxidant Activity of Iraqi Species of *Silybummarianum*Seeds. International Research Jounral of Pharmacy, 2014 5 (5). ISSN 2230-8407
[6] Kokate, C.K., A.P. Purohit, dan S.B. Gokhale. 2008. Pharmacognosy. NiraliPrakashan, Maharashtra
[7] Sagu, Sorel Tchewonpi, Sankha Karmakar, Emmanuel Jong Nso, and Sirshendu De. 2014. Primary Clarification of Banana Juice Extract by Centrifugation and Microfiltration. Department of Chemical Engineering, Indian Institute of Technology, Kharagpur, India
[8] AOAC. 2000. Official of Analysis. Association of Official Analytical Chemist. EUA
[9] Tambe, V. D., and R. S. Bhambar. 2014. Estimation of Total Phenol, Tannin, Alkaloid and Flavonoids in *Hibiscus tillaceus*Linn. Woos Extracts. Journal of Pharmacognosy and Phytochemistry Volume 2, Issue 4. ISSN : 2321-6182.
[10] Capuccino, J.G., N. Sherman., "Microbiology: A Laboratory Manual", Sixth Edition, Benjamin Cummings, San Fransisco, 2001
[11] Skaik, Y: The Bread and butter of Statistical Analysis "T-test: Uses and Misuses, Pak J Med Sci, 2015 Nov-des; 31(6): 1558-1559
[12] Rajiv, P,; R. Sivaraj.: Screening For Phytochemicals And Antimicrobial Activity Of Aqueous Extract Of Ficusregilosa Linn, Int. Jurnal of Pharmacology and Pharmaceutical Sciences, 4 (5), (2012).
[13] Rizvi W., Rizvi M., Kumar R., Kumar R., Shukla I., Parveen M.: Antimicrobial Activity of Ficuslyrata-An In Vitro Study, Int. J. Pharmacol, **8**(2).7 (2010).
[14] Adebayo, E. A., O. R. Ishola, O. S. Taiwo, O. N. Majolagbe, B. T. Adekeye. 2009. Evaluations of The Methanol Extract of *Ficus exasperate* Stem Bark, Leaf and Root fot Phytochemical analysis and Antimicrobial Activities. African Journal of Plant Science Vol. 3 (12).
[15] Jawetz, E., J. Melnick and E. Adelberg. 2004. Medical Microbiology 23rd ed. Mc-Graw Hill Publ. Co. Ltd. New York.
[16] Davidson, P.M. (1993) Parabens and phenolic compounds. antimicrobials in Foods, 2nd edn. ed. Davidson, P.M. and Branen,A.L. pp. 263–306. New York: Marcel Dekker Inc.