Antimicrobial Activity of Some Indonesian Plant Species from The Genus *Ficus*

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ABSTRACT

Aim: The purpose of this research was to examine the antimicrobial activity of 10 Indonesian *Ficus* species that had not been subjected to previous pharmacological testing.
Methods: Organic extracts of plants from the genus *Ficus* were filtered to test antimicrobial activity against bacteria and fungi. The antimicrobial activity of extracts was assessed against *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Candida albicans*, and *Aspergillus niger* by utilizing agar diffusion and micro broth dilution methods.
Results: The results of the preliminary study using the paper disc diffusion method showed that all methanol leaves extracts of *Ficus* species had antibacterial activity against at least 2 of 4 tested bacteria (at concentrations of 0.8, 0.4 and 0.2 g/mL) and none of the extracts showed activity against fungi or yeast. Extracts of *Ficus* species were showed selectivity against bacteria with *Minimum Inhibitory Concentration* (MIC) and *Minimum Bactericidal Concentration* (MBC) in the range of 250-1000 µg/mL.
Conclusion: Plant extracts are a rich source of antibacterial potential to combat bacterial diseases. Extracts from *Ficus consociate* and *Ficus ribes* have promising antibacterial activity, with potential applications in the treatment of bacterial diseases.

Keywords: *Ficus*, antibacterial, antimicrobial, *Ficus consociate, Ficus ribes*

INTRODUCTION

Ficus is a quite diverse plant, including epiphytes, small shrubs, climbing plants, lithophytes, rheophytes, and high trees. In general, the types are known as fig, fig tree or fig wood. Species of fig are identified by their unique inflorescence and typical pollination pattern, which involves a kind of wasp from the family Agaonidae to pollinate its closed flowers [1]. Plant species of the genus *Ficus* (Moraceae) are widely grown in tropical and sub-tropical regions including in Indonesia. Genus *Ficus* is recognized by more than 800 different species and subspecies [2].

Plants of the genus *Ficus* are also known to play an important role in the treatment and culture of Indonesian people. In Indonesia, several kinds of genus *Ficus* are also used and entered into Indonesian culture, for example in the center of the city in the Javanese tradition is always planted with beringin trees (*Ficus benjamina*). The Gayo community uses steaming Tabat Barito (*Ficus deltoidea*) plant for aphrodisiacs, likewise by Sundanese people who use

this plant as medicine [3]. In Balinese people, the boiled water of the plant Uyah-uyahan (*Ficus quercifolia*) is taken to treat urinary stones and this plant is mixed together with salt to treat skin diseases and is used also to treat bloating. Getah benying plants (*Ficus fistulosa*) are used to treat wounds by sharp objects by applying them to the wound dry 1-2 times a day. Whereas in Sumba, fig trees are pounded, boiled to treat intestinal worms are also used for mothers who have just given birth and, on the other hand, the Tolaki community is used for abortion [3] as well as research on the pharmacological activities of *Ficus* plants has been well documented, among them are analgesics [4], antioxidants [5], anti-inflammatory [6,7], antiangiogenesis [8], antidiabetic [9], and antimicrobial [10–12].

Some research on the antimicrobial activity of *Ficus* plant reported that *Ficus benjamina* has antimicrobial activity against *Salmonella typhimurium, Candida albicans, Staphylococcus aureus, Escherichia coli, Aspergillus niger,* and *Aspergillus brassicola* [13], *Ficus chlamydocarpa*, against *Mycobacterium smegmatis* [14], *Ficus cordata*, against *Bacillus subtilis* and *Escherichia coli* [15], *Ficus drupacea*, against *Staphylococcus aureus* and *Escherichia coli*, and *Bacillus cereus Aspergillus versicolor* and *Aspergillus ochraceous* [16]. While a small number of *Ficus* species are used as traditional medicines, their pharmacological and antimicrobial activity has been tested, most of about 850 species and sub-species have not undergone any pharmacological activity.

This study was conducted as a preliminary stage of the research program to systematically study the antimicrobial activity of the genus *Ficus*. This report was described screening the antimicrobial activity of a number of *Ficus* species that has never been the subject of pharmacological and antimicrobial activity test previously.

MATERIALS AND METHODS

Plant samples

Ten plants of Indonesian *Ficus* were collected from Bogor Botanic Garden, Indonesia. Those were *Ficus callosa, Ficus consociate, Ficus drupacea, Ficus geocarpa,, Ficus glabella, Ficus grandis, Ficus melinocarpa, Ficus nekbudu, Ficus pubinervis*, and *Ficus ribes*. The leaves were washed, dried and grounded to small pieces.

Extraction

About 100 g of powder of the dried leaves were macerated with methanol (1:10) for 24 hours. Maceration process was repeated for three times. The methanolic extracts were concentrated using a rotary evaporator.

Phytochemical analysis

The powder of the dried leaves were screened for the presence of saponins (frothing test), tannins (ferric chloride test and lead acetate test) alkaloids, (Dragendroff's test, Meyer's test, Hager's test, and Wagner's test), steroids (Salkowski test and Lieberman-Burchard's test) and flavonoids (Shinoda's test, ferric chloride test, and Pew test).

Test Microbes

Test microbes in this study were *Staphylococcus aureus* (American Type Culture Collection (ATCC 6538), *Bacillus subtilis* (ATCC 6636), *Escherichia coli* (ATCC 8939), *Pseudomonas aeruginosa* (ATCC 9027), *Candida albicans* (ATCC 10231), and *Aspergillus niger* (ATCC 16404).

Antimicrobial Activity Test

Antimicrobial activity test of the extract was carried out by the agar diffusion method using 6 mm diameter paper discs. Determination of the *Minimum Inhibitory Concentration (MIC)* and *Minimum Bactericidal Concentration (MBC)* using microdilution broth method based on National Committee for Clinical Laboratory Standard (2012). The microorganisms used in the antibacterial assay are maintained at the of Department microbiology Bandung Institute of Technology, West Java, Indonesia. The inoculum was prepared by culturing organism in Mueller Hilton Broth (MHB, Oxoid) at 37°C to a turbidity equivalent to McFarland 0.5 standard (1.5×10^8 CFU/mL). Amoxicillin and ketoconazole were used as positive control.

RESULTS AND DISCUSSION

Plants that produce natural products (flavonoids, terpenoids, steroids, etc.) get more attention in the past decade because these plants have various activities including antimicrobial, antioxidant, antitumor and anti-inflammatory activities. In this study, *Ficus* plant phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, and steroids/triterpenoids [Table 1]. This showed that *Ficus* plants can potentially have antimicrobial, anti-oxidant, anti-cancer and anti-inflammatory activities in the future.

Table 1: The Results of Phytochemical Screening From Some Ficus Species

No.	Sample	Alkaloid	Flavonoid	Tannin	Steroid/ triterpenoid	Saponin
1	*Ficus callosa*	+	+	-	+	-
2	*Ficus consociate*	+	+	+	+	+
3	*Ficus drupacea*	-	+	-	+	-
4	*Ficus geocarpa*	+	+	+	+	-
5	*Ficus glabella*	-	+	-	+	-
6	*Ficus grandis*	-	+	-	+	-
7	*Ficus melinocarpa*	+	+	-	-	+
8	*Ficus nekbudu*	-	+	+	+	+
9	*Ficus pubinervis*	+	+	+	+	-
10	*Ficus ribes*	+	+	+	+	-

Note : +: detected
: -: not detected

Flavonoids from *Ficus* plants have several biological activities and have been proven in experiments, they have cytotoxic activity [17], antioxidant [18, 19], anti-inflammatory [20], and antimicrobial [21]. Triterpenoid and steroid from *Ficus* plant are important for antimicrobial activity[13] and cytotoxic [22].
Antimicrobial compounds can be produced from various metabolites such as flavonoids [23], alkaloids [24], terpenoids [13], and tannins. for example conrauiflavonol dan thonningiisoflavone metabolites from *Ficus thoningii* [23], triterpenoids from *Ficus benjamin* [13].[NMS1]

Antimicrobial screening test performed by the agar diffusion method. Determination of MIC by the micro broth dilution was done to extract selectively. In this study, the extracts with MIC values <1000 µg/mL were potential to be developed for antimicrobial compounds.
A preliminary test of antimicrobial activity of ten *Ficus* species using paper disc diffusion test showed that nine extracts had activity against *Eschericia coli* except for *Ficus melinocarpa*.

Ficus grandis and *Ficus pubinervis* had activity against *Pseudomonas aeruginosa*. Nine extracts had activity against *Staphylococcus aureus* except for *Ficus nekbudu*, and all extracts *Ficus* test has activity against *Bacillus subtilis*, in the concentration range 0.2-0.8 g / mL. All *Ficus* extracts do not inhibit the growth of *Candida albican* and *Aspergillus niger*. Table 2 was showed the results of inhibition zones in the agar diffusion assay.

Table 2: Inhibition Diameter of Various *Ficus* Extracts against Some Tested Bacteria

No	Extracts	Concentration (g/mL)	*E.coli*	*P. aeruginosa*	*S.aureus*	*B. subtilis*	*C. albicans*	*A. niger*
1	*Ficus callosa*	0.8	16.00	-	14.53±0.51	10.13±0.23	-	-
		0.4	11.83±0.42	-	9.37±0.70	7.67±0.92	-	-
		0.2	13.63±0.32	-	9.97±0.90	-	-	-
2	*Ficus consociate*	0.8	17.07±0.31	-	16.87±0.25	15.50±0.62	-	-
		0.4	11.30±0.50	-	14.33±0.75	11.50±0.82	-	-
		0.2	9.30±0.36	-	14.23±0.49	8.60±0.26	-	-
3	*Ficus drupacea*	0.8	11.60±0.70	-	12.30±0.44	11.30±0.50	-	-
		0.4	8.47±0.47	-	9.00±0.62	9.56±0.15	-	-
		0.2	-	-	7.07±0.97	7.20±1.08	-	-
4	*Ficus geocarpa*	0.8	10.73±0.51	-	12.4 ±0.10	9.70±0.36	-	-
		0.4	9.83±0.84	-	12.23±0.50	8.40±0.26	-	-
		0.2	8.30±0.61	-	10.70±0.30	-	-	-
5	*Ficus glabella*	0.8	11.80±0.87	-	15.63±0.49	9.70±0.36	-	-
		0.4	10.60±0.30	-	14.60±0.80	8.73±0.40	-	-
		0.2	9.20±0.72	-	12.80±0.76	7.50±0.85	-	-
6	*Ficus grandis*	0.8	14.10±0.35	9.77±3.28	8.96±5.14	11.80±0.26	-	-
		0.4	10.97±0.91	6.57±5.17	7.23±2.14	7.67±0.31	-	-
		0.2	7.87±3.23	6.17±3.82	-	-	-	-
7	*Ficus melinocarpa*	0.8	-	-	12.07±0.57	8.13±0.06	-	-
		0.4	-	-	12.90±0.20	-	-	-
		0.2	-	-	8.83±0.91	-	-	-
8	*Ficus nekbudu*	0.8	14.03±0.47	-	-	11.93±0.21	-	-
		0.4	11.13±0.68	-	-	9.50±0.46	-	-
		0.2	8.80±0.26	-	-	8.20±0.44	-	-
9	*Ficus pubinervis*	0.8	14.73±0.15	7.43±1.24	9.30±0.44	12.83±0.59	-	-
		0.4	13.73±0.25	6.67±0.70	9.20±0.30	12.83±0.61	-	-
		0.2	12.63±0.60	6.37±0.35	7.87±0.38	9.60±0.95	-	-
10	*Ficus ribes*	0.8	15.77±0.06	-	20.40±0.85	12.73±0.47	-	-
		0.4	13.77±0.60	-	16.03±0.49	11.33±0.81	-	-
		0.2	11.83±0.42	-	15.63±0.64	9.27±0.21	-	-
	DMSO 5%		-	-	-	-	-	-
	Amoxicillin		10.23±1.41	9,0±0,16	12,77±0,73	7.77±0.31	-	-
	Ketokenazole						14.45±0,39	18.83±0,40

Noted: - not active

Table 3 was showed the MIC values of plant extracts against all tested microbes, while Table 4 was showed the MBC values of them.

Table 3: Minimum Inhibitory Concentration of Various *Ficus* Extracts

No	Extract	MIC (µg/mL)			
		E. coli	*P. aeruginosa*	*S. aureus*	*B. subtilis*
1	*Ficus callosa*	>1000	-	250	>1000
2	*Ficus consociate*	500	-	500	1000
3	*Ficus drupacea*	>1000	-	>1000	>1000
4	*Ficus geocarpa*	>1000	-	>1000	>1000
5	*Ficus glabella*	1000	-	500	>1000
6	*Ficus grandis*	>1000	>1000	>1000	>1000
7	*Ficus melinocarpa*	-	-	>1000	-
8	*Ficus nekbudu*	>1000	-	-	>1000
9	*Ficus pubinervis*	>1000	>1000	>1000	>1000
10	*Ficus ribes*	1000	-	500	1000
	Amoxicillin	1	32	0.25	4

Noted: - not active

Table 4: Minimum Bactericidal Concentration of various *Ficus* Extracts

No	Extract	MBC (µg/mL)			
		E coli	*P aeruginosa*	*S aureus*	*B subtilis*
1	*Ficus callosa*	>1000	-	>250	>1000
2	*Ficus consociate*	>500	-	>500	>1000
3	*Ficus drupacea*	>1000	-	>1000	>1000
4	*Ficus geocarpa*	>1000	-	>1000	>1000
5	*Ficus glabella*	>1000	-	>500	>1000
6	*Ficus grandis*	>1000	>1000	>1000	>1000
8	*Ficus Melinocarpa*	-	-	>1000	-
9	*Ficus nekbudu*	>1000	-	-	>1000
10	*Ficus pubinervis*	>1000	>1000	>1000	>1000
11	*Ficus ribes*	>1000	-	>500	>1000
	Amoxicillin	1	32	0.25	4

Noted: - not active

The results of this study were consistent with several previous studies. where in a previous study stated that some *Ficus* had antimicrobial activity, including *Ficus septica* which contain ficuseptine and antofine which are active against *Penicillium oxalicum*, *Bacillus subtillis*, *Micrococcus luteus* and *Eschericia coli* [25]. Methanol extract of *Ficus religiosa* showed antibacterial activity against *Eschericia coli* [26], The methanol extract from the roots *Ficus polita* had antimicrobial activities against *Salmonella typhi*, *Escherichia coli*, and *Candida albicans* with the range MIC value of 32-64 µg/ml [27], Lutaoside and benjaminamide a new ceramide glycoside was isolated from the woods of *Ficus lutea* showed some antimicrobial activities against *Chlorella vulgaris*, *Scenedesmus subspicatus*, *Chlorella sorokiniana*, *Mucor miehei*, *Bacillus subtilis*, and *Candida albican* [28].

In this study, we observed that four extracts i.e *Ficus callosa*, *Ficus consociata*, *Ficus glabella*, and *Ficus ribes* had MIC ranging from 250-100 µg/mL. *Ficus consociata* and *Ficus*

ribes have the broadest spectrum, which can inhibit three tested bacteria, namely *Eschericia coli*, *Staphylococcus aureus,* and *Bacillus subtillis*. The three tested bacteria can cause various diseases, so this study shows that *Ficus callosa* have the potential to be used for infections caused by *Staphylococcus aureus*. *Ficus consociata* and *Ficus ribes* can potentially be used for infections caused by *Eschericia coli*, *Staphylococcus aureus,* and *Bacillus subtillis,* while *Ficus glabella* has the potential to be used for infections caused by *Bacillus subtillis* and *Eschericia coli.*

Ficus consociata and *Ficus ribes* extracts have the best antimicrobial activity compared to other extracts. Both extracts are promising to be developed to overcome the problem of infection caused by *Eschericia coli*, *Staphylococcus aureus,* and *Bacillus subtillis*. Both extracts of *Ficus* can be used in the form of extracts, fractions or in isolate of active compounds that have antibacterial activity. Our study was the first time that reported the antimicrobial effects of these plants. Subsequent research can be directed to the isolation and characterization of compounds from *Ficus consociata* and *Ficus ribes* which are responsible for the antimicrobial activity.

CONCLUSION

The present study suggest that *Ficus consociata* and *Ficus ribes* may be potential sources of new and selective agents for the treatment of important infectious diseases.

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