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In Vitro Antimicrobial Potential of Impatiens Balsamina Flower Against Staphylococcus Aureus, Escherichia Coli and Candida Albicans

Aghnia Fuadatul Inayah, Siti Rofida*, Lilik Yusetyani, Muhammad Erfan, Lintang Alhaj Faulina Dinasty, Selvia Mufidah, Hafidhah Shabrina, Nur Wazilah, Yenny Endah Sari, Nindya Alfa Dicha Agustina Wati, Parista Adellina, Dinda Oni Tsarah Fatimawati Girsang

Department of Pharmacy, Faculty of Health Sciences, University of Muhammadiyah Malang

ORCID
Siti Rofida: https://orcid.org/0000-0002-2541-9698

Abstract.
The search for new drugs to treat cases of infection continues. This is because the microorganisms that cause infection continue to mutate as a form of self-defense which causes resistance to antibiotics. One strategy for finding new drugs is through the exploration of active ingredients derived from plants that have been used empirically by the community. *Impatiens balsamina* is a plant that has been shown to have antibacterial, antifungal, antipruritic, and anti-allergic characteristics. The ethanol extract of the *Impatiens balsamina* flower contains secondary metabolites such as naphthoquinones, coumarin derivatives, flavonoids, steroids, quinones, and saponins. This study aimed to obtain the active ingredient of the *Impatiens balsamina* flower which is used as an herbal medicine in the treatment of infection. A multilevel extraction process was carried out using solvents of different polarity, so that fractions containing nonpolar compounds, semipolar compounds, and polar compounds were obtained. Then, each fraction was tested for antimicrobial potency. Antimicrobial testing in vitro was carried out using the disc diffusion method. The results showed that the secondary metabolites contained in the hexane fraction were terpenoids, flavonoids, polyphenols, and anthraquinone compounds. Secondary metabolites contained in the ethyl acetate fraction were alkaloids, terpenoids, flavonoids, polyphenols, and anthraquinone compounds. Secondary metabolites contained in the ethanol fraction were terpenoids, flavonoids, polyphenols, and anthraquinone compounds. The hexane fraction had the best percentage of inhibition and percentage of effectiveness against *Staphylococcus aureus*, *Escherichia coli,* and *Candida albicans*.

Keywords: antimicrobial, fractionation, *Impatiens balsamina* L flower, disc diffusion

Corresponding Author: Siti Rofida; email: rofida79@umm.ac.id

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1. Introduction

Infection is a pathological condition caused by microorganisms such as bacteria, viruses, fungi and protozoa, and can occur in the community or in hospitals. Patients who are being treated in a hospital have a greater risk of contracting an infection than outside

the hospital. This can occur as a result of interactions between patients, the environment and microbes. According to Wikansari et al[1]shows that 10 of the 16 inpatient rooms at the ”X” hospital in Semarang City have airborne germ numbers exceeding the total germ threshold in the inpatient room. In one hospital in the Sidoarjo area, it was stated that in 2016-2017, the map of germs in the intensive care unit showed the dominance of gram-negative bacteria, namely Proteus vulgaris, Yersinia enterocolitica, Enterobacter aerogenes, Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa [2].

Infections that occur in hospitals and attack patients who are in the process of being treated are known as nosocomial infections or Healthcare Associated Infections (HAIs). The prevalence of nosocomial infections in hospitalized patients is 15%, of which 75% of cases occur in Southeast Asia and Africa [3]. Various efforts have been made by hospitals in overcoming nosocomial infections, namely by washing hands before and after contact with patients; use personal protective equipment such as gloves, masks and other personal protective equipment; carry out decontamination of tools after use in service; sharp tool management; medical and non-medical waste management [4].

Handling in cases of infection is the administration of antibiotic therapy. However, currently some antibiotics are no longer able to overcome cases of infection because there has been antibiotic resistance. This antibiotic resistance occurs because of the free use of antibiotics, so that microorganisms become more resistant to antibiotics. The resistance of microorganisms to antibiotics occurs because they are too often exposed to antibiotics, so that microorganisms undergo mutations to form a layer of biofilms, so that their cell walls are thicker and cannot be penetrated by antibiotics. This mechanism is a defense mechanism for microorganisms to survive[5]. The highest antibiotic resistance against bacteria is found in antibiotics with a beta lactam ring, namely the first and third generation penicillins and cephalosporins [6–8].

This antibiotic resistance if not treated can cause cases of infection to become out of control. The increase in the incidence rate will be very fast, because infection is a contagious disease. Efforts are being made to prevent antibiotic resistance by establishing a policy on the use of antibiotics only for diseases which, according to laboratory data, have been proven to be caused by microorganisms [2, 7, 9].

Another effort is to explore new drugs with plant sources that have been empirically proven to be efficacious as antimicrobials. How many secondary metabolites have been proven to have antimicrobial properties are phenolic compounds such as simple phenols, phenolic acids, quinones, flavones, flavonoids, tannins, coumarins; group of terpenoid compounds and essential oils; and group of alkaloid compounds[10].

One of the plants that has been empirically proven to be an antimicrobial is plants *Impatiens balsamina* L. This plant contains coumarin compounds, flavonoids, quinones, saponins, and steroids [11]. Based on the research conducted by Kusuma et al (2014), that the ethanolic extract of *Impatient balsamina* L. showed inhibitory activity against Aeromonas hydrophila bacteria with an inhibition zone diameter of 21.4 mm. This antimicrobial activity was higher than that of the ethanol extract of leaves and stems, where the diameter of the inhibition zones were 11.2 mm and 13.7 mm, respectively [12]. According to Budiana et al (2015), thatflower ethanol extract *Impatient balsamina* L. at concentrations of 20%, 40% and 80% inhibited the growth of Staphylococcus aureus, Pseudomonas aeruginosa, and Eschercihia coli bacteria with inhibition zone diameters of 10.16-17.16 mm, respectively; 10.33-17.16 mm; and 10.16-19.00 mm [13]. According to Sugiati (1996) the test showed the inhibitory power of Impatiens balsamina L. flower infusion on the growth of Candida albicans at a concentration of 60% equivalent to a ketoconazole solution of 4.8927 g/ml, a concentration of 70% equivalent to a ketoconazole solution of 7.1865 g/ml, and a concentration of 7.1865 g/ml. 80% equivalent to 9.6876 g/ml ketoconazole solution.

2. Method

The type of research used in this research is experimental research. The research will be conducted for 2 consecutive years. The stages of activities carried out in the first year include:

1. Preparation of dried simplicia from *Impatient balsamina* L.
2. Graded extraction of from the flower *Impatient balsamina* L.
3. In vitro antimicrobial activity testing

2.1. Tools and Materials

2.1.1. Plant Material

Flower Impatiens balsamina L. used in this study is a purple flower. Interest simplicia obtained fromTrenggana market, Penatih village, Denpasar city, Bali. The interest has been terminated at UPT Materia Medika, East Java Provincial Government with number 074/117/102.7/2017.

2.1.2. Chemicals

Hexan, ethyl acetate, Ethanol, DMSO, dragendorf, anisaldehyd-sulfuric acid, FeCl3, KOH, and H2SO4 10%, Erythromycin, chloramphenicol, Nystatin, Aquadest, TLC plate silica gel F254

2.1.3. Bacterial Strains and Growth Media

Staphylococcus aueus, *Escherichia coli*, and *Candida albicans* which has been identified in the biomedical and microbiology laboratory Faculty of Medicine University of Muhammadiyah Malang, Mueller Hinton broth, Mueller Hinton Agar.

2.2. Work procedures

2.2.1. Metabolite profiling of Impatiens balsamina L.

The metabolite profile of hexsan, ethyl acetate, and ethanol fraction from *Impatiens balsamina* L. was carried out using thin layer chromatography. The stationary phase used silica gel F254 and mobile phase of ethyl acetate:chloroform = 3:7. Chromatogram profiles were observed with UV wavelength 254 nm and 365 nm. Metabolite compounds were detected by dragendorf, anisaldehyd-sulfuric acid, FeCl3, KOH, and H2SO4 10% reagent.

2.2.2. Antibacterial Assay

Antibacterial activity of the hexane, ethyl acetate, and ethanol fraction from *Impatiens balsamina* L. was tested using the disc diffusion method. *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* were grown on Mueller Hinton Agar. Solution of the substance with a concentration of 80 mg/mL, 120 mg/mL and 160mg/mL each of 50 microliters was absorbed onto the paper disc. Samples on paper discs were diffused on bacterial growth medium and incubated at 37°C for 24 hours. The clear zone around the paper disc indicates inhibition of bacterial growth. The inhibition activity was calculated by equation :

$$Inhibition\ activity\ (\%) = \frac{d2 - d1}{d1} x\ 100\% (1)$$

where d1=diameter of paper disc (6mm); d2=diameter of inhibition zone ofhexsan, ethyl acetate, and ethanol fraction of *Impatiens balsamina* L. (mm)

The effectiveness of hexsan, ethyl acetate, and ethanol fraction of *Impatiens balsamina* L. as antibacterial on *Staphylococcus aueus*, *Escherichia coli*, and *Candida albicans* compared to the positive control control Erythromycin, chloramphenicol, Nystatin, can be calculated by the equation:

$$Antibacterial\ effectiveness(\%) = \frac{d2}{d3} x100\% (2)$$

Where d2= diameter of inhibition zone of hexsan, ethyl acetate, and ethanol fraction of *Impatiens balsamina* L. (mm); d3=diameter of inhibition of positive control Erythromycin, chloramphenicol, Nystatin, (mm)

3. Result

3.1. The results of making dried simplicia flowers Impatiens balsamina L.

Flower *Impatiens balsamina* L. used in this study is a purple flower. Interest simplicia obtained from Trenggana market, Penatih, Denpasar, Bali. The interest has been terminated at UPT Materia Medika, East Java Provincial Government with number 074/117/102.7/2017. Fresh flower simplicia, flower simplicia that has gone through the drying process using an oven at 40˚C for 3 days, and the mashed simplicia can be seen in Figure 1.

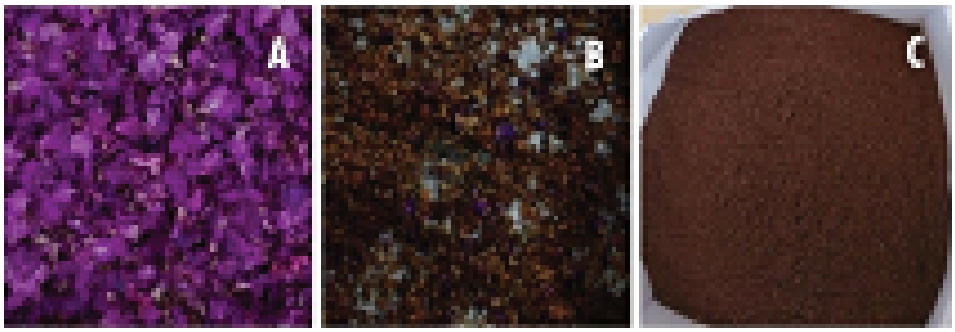

Figure 1: Flower *I.balsamina*L. wet (A), flower*I.balsamina*L. dry (B), flower simplicia powder*I.balsamina*L. (C).

3.2. The results of graded extraction from the flower Impatiens balsamina L.

Extraction of chemical compounds from powder flower *Impatiens balsamina* L. using the kinetic maceration method for 4 hours at a speed of 400 rpm. Extraction is carried

out in stages using solvents that have different polarities, namely by using hexane, ethyl acetate, and ethanol as solvents. The results of the multilevel extraction of *Impatiens balsamina* L. flower simplicia powder can be seen in Table 1.

Table 1: Flower fractionation yield *Impatiens balsamina* L.

No	Test Material	% Randemen
1	N-hexane fraction	5.53
2	Ethylacetate Fraction	31.08
3	Ethanol Fraction	21.20

3.3. Chemical compound screening results Fraction of Impatiens balsamina L.

Chemical compounds extracted in each fraction were identified using the TLC technique. Hexane fraction using fsilica gel stationary ase F254; mobile phase hexane:ethyl acetate+formic acid in a ratio of 7.5:2.5+1 drops. Ethyl acetate fraction using fsilica gel stationary ase F254; mobile phasehexane:ethyl acetateby comparison2:8. Ethanol fraction using fsilica gel stationary ase F254; mobile phaseethyl acetate:ethanolby comparison8:2. The results of chemical screening for each fraction can be seen in Table 2.

3.4. Inhibitory activity and antibacterial effectiveness Farction of Impatiens balsamina L.

Some microbes such as bacteria, fungi, and viruses have pathogenic properties in humans. Some of these microbes are *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*. Infections from these microbes are given therapy in the form of antibiotics or antifungals [14–17]. Disk diffusion antibiotic susceptibility testing are the qualitative evaluation of tolerance that can be done by the number of colonies inside a typical inhibition zone [18, 19]. The results of the disk diffusion measurement showed that some fractions have good inhibition. This can be seen from the results of the inhibition zone as shown in Table 3 [20, 21]. The data then obtained the inhibitory activity and antibacterial effectiveness. In Table 4, it can be seen that based on the percentage of the hexane fraction, it appears to have better inhibition. Thus, *Impatiens balsamina* L. interest in the hexane fraction can be maximized to develop the discovery of potent antimicrobial compounds.

Table 2: Screening of chemical compounds from the flower fraction *Impatiens balsamina* L.

Test Material	Compound	Stain Viewer	Stain Color	Rf	Information
Hexan Faction	Alkaloids	Dragendor	-	-	-
	Terpenoids	Anisaldehyde-sulfuric acid	Purple	0.37	+
				0.64	
				0.91	
	Flavonoids	Sulfuric acid 10%	Intensive Yellow	0.51	+
	Polyphenol	FeCl3	Black	0	+
	anthraquinone	KOH 10%	Orange/red	0.3	+
Ethyl acetate fraction	Alkaloids	Dragendor	orange	0.46	+
			orange	0.06	
	Terpenoids	Anisaldehyde-sulfuric acid	Purple	0.96	+
				0.85	
				0.41	
	Flavonoids	Sulfuric acid 10%	Intensive Yellow	0.69	+
	Polyphenol	FeCl3	Black	0.84	+
				0.13	
	anthraquinone	KOH 10%	Green	0.84	+
			Yellow brown	0.84	
			Red	0.37	
			Yellow brown	0.09	
Ethanol Fraction	Alkaloids	Dragendor	-	-	-
	Terpenoids	Anisaldehyde-sulfuric acid	Purple	0.31	+
	Flavonoids	Sulfuric acid 10%	Intensive Yellow	0.43	+
				0.87	
	Polyphenol	FeCl3	Black	0.32	+
	anthraquinone	KOH 10%	Yellow	0.50	+

4. Conclusion

In the study of antimicrobial testing of the *Impatiens balsamina* L. flower fraction, it can be concluded that The secondary metabolites contained in the hexane fraction are terpenoids, flavonoids, polyphenols and anthraquinone compounds. The secondary metabolites contained in the ethylacetate fraction are alkaloids, terpenoids, flavonoids, polyphenols and anthraquinone compounds. The secondary metabolites contained in

Table 3: Impatiens balsamina L. Antimicrobial Test Results flower fraction.

Test Material	Microorganisms	Concentration (mg/disc disc)	Inhibition Zone Diameter (mm)			Average±SD
			1	2	3	
Hexan Faction	*Staphylococcus aureus*	4	12.0	10.5	10.0	10.8±0.9
		2	9.0	8.0	8.3	8.4±0.4
		1	7.0	7.0	6.5	6.8±0.2
	Escherichia coli	4	8.83	9.2	9.2	9.1±0.2
		2	8.3	8.3	8.3	8.3±0
		1	6.8	6.8	6.2	6.6±0.3
	Candida albicans	4	21.0	21.75	19.5	20.8±0.9
		2	11.2	11.5	10.8	11.2±0.3
		1	10.8	10.5	9.00	10.1±0.8
KN	Tween 80	5%	-	-	-	
Ethyl acetate fraction	*Staphylococcus aureus*	4	11.7	10.7	9.8	10.7±0.8
		2	10.0	8.3	8.3	8.9±0.8
		1	9.0	8.2	8.2	8.5±0.4
	Escherichia coli	16	9.5	10.5	10.9	10.3±0.6
		8	8.2	8.9	8.8	8.6±0.3
		4	7.2	8.4	7.9	7.8±0.5
	Candida albicans	16	19.3	19.3	17.3	18.6±0.9
		8	18.5	19.0	16.3	17.9±1.2
		4	10.2	10.2	7.7	9.4±1.2
KN	Tween 80	5%	-	-	-	
Ethanol Fraction	*Staphylococcus aureus*	40	19.5	19.0	19.5	19.3±0.2
		20	14.8	17.2	16.0	16.0±0.9
		10	10.8	13.8	12.4	12.3±1.2
	Escherichia coli	40	7.6	7.5	7.5	7.5±0.1
		20	7	6.8	7	6.9±0.1
		10	6.7	6.7	6.7	6.7±0
	Candida albicans	1	13.8	11.0	12.5	12.4±1.1
		0.5	10.3	10.5	10.2	10.3±0.1
		0.25	6.7	9.8	8.3	8.3±1.2
KN	DMSO	1%	-	-	-	
Erythromycin	*Staphylococcus aureus*	15 g	27.7	26.0	27.2	26.9±0.7
			24.7	23.8	24.0	24.2±0.4
			24.5	25.2	26.5	25.4±0.8
Nystatin	*Candida albicans*	2000 IU	16.5	15.0	17.4	16.3±0.9
			11.0	14.8	12.7	12.8±1.6
			16.2	14.3	15.5	15.3±0.8
Chloramphenic	*Escherichia coli*	30 g	23.3	22.7	22.2	22.7±0.4
			25.4	26.5	29.8	27.2±1.9
			26.0	25.8	26.7	26.2±0.4

the ethanol fraction are terpenoids, flavonoids, polyphenols and anthraquinone compounds. The hexane fraction has the best percentage of inhibition and percentage of effectiveness against bacteria *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*.

Table 4: Percentage of inhibitory activity and antimicrobial effectiveness of flower fraction Impatiens balsamina L.

Sample	Microorganisms	Concentration	Inhibition Percentage	Antimicrobial Effectiveness Percentage
Hexan Faction	*Staphylococcus aureus*	4	80.6	44.8
		2	40.6	34.8
		1	13.9	28.2
	Escherichia coli	4	51.3	34.6
		2	38.3	31.7
		1	10.0	25.2
	Candida albicans	4	245.8	135.6
		2	86.1	73.0
		1	68.3	66.0
Ethyl acetate fraction	*Staphylococcus aureus*	4	78.9	44.4
		2	47.8	36.6
		1	41.1	35.0
	Escherichia coli	16	71.7	39.3
		8	43.9	33.0
		4	30.6	29.9
	Candida albicans	16	210.6	121.8
		8	198.9	117.2
		4	56.1	61.2
Ethanol Fraction	*Staphylococcus aureus*	40	222.2	79.9
		20	166.7	66.1
		10	105.6	51.0
	Escherichia coli	40	25.6	28.8
		20	15.6	26.5
		10	11.7	25.6
	Candida albicans	1	107.2	81.3
		0.5	72.2	67.5
		0.25	37.8	54.0

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