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Evaluation of antimicrobial activity and phytochemical screening of red Kamboja (*Plumeria rubra* L.) extracts

Ni Kadek Yunita Sari[1*], Ni Wayan Deswiniyanti[1], Putu Angga Wiradana[1]
[1]Faculty of Health, Science, and Technology, Universitas Dhyana Pura
Jl. Raya Padang Luwih, North Kuta, Badung, Bali, Indonesia. 80351
*Email: yunitasari@undhirabali.ac.id

ABSTRACT. Natural antimicrobial sources such as red Kamboja (*Plumeria rubra* L.) flower extract can be utilized to treat infectious disorders caused by *Staphylococcus aureus, Escherichia coli*, and *Candida albicans*. This study aims to determine evaluate the effectiveness of methanol and ethanol extracts of *P. rubra* floral against *E. coli, S. aureus*, and *C. albicans* growth, as well as the amount of secondary metabolites in *P. rubra* extract. The study's findings indicate that the highest DIZ value of *P. rubra* methanol extract was 7.40 mm, 7.36 mm, and 7.30 mm for *S. aureus* ATCC25923 at 5%, 10%, and 20%, respectively, while the highest DIZ value for *C. albicans* ATCC10231 at 10%, 10%, and 20% was 25.08 mm, and 25.04 mm, respectively. The DIZ value of the *P. rubra* flower ethanol extract against *E. coli* strain was 5.26 mm at 5%, and 7.30 mm at 20%. Secondary metabolites of saponins, tannins, alkaloids, flavonoids, steroids, and phenols were present in the methanol and ethanol extracts of *P. rubra* flowers. In summary, our findings highlight the use of *P. rubra* flower extract as a biological source with antibacterial properties for the control of human infectious illnesses.

Keywords: antimicrobial potency; bioactivity compounds; natural products; red frangipani; traditional medicine

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INTRODUCTION

Indonesia is one of the Southeast Asian regions physically surrounded by a high level of tropical biodiversity. The tropical habitat may facilitate the spread of infectious diseases that cause various human health problems (Tambo *et al*., 2014; Lindahl & Grace, 2015). Because infectious diseases are caused mainly by pathogens (bacteria, fungi, parasites, and viruses), they spread rapidly and easily (Solo-Gabriele *et al*., 2016). Most infectious microorganisms are caused by *Candida albicans* (Dou *et al*., 2015; Zuza-Alves *et al*., 2017), gram-positive bacteria such as *Staphylococcus aureus*, and gram-negative bacteria such as *Escherichia coli* (de Kraker *et al*., 2011; Plano *et al*., 2013). Respiratory tract infections caused by *S. aureus* and *Candida* spp., as well as digestive pathologies caused by *E. coli*, are instances of severe global public health burdens (Mellata, 2013).

The antibiotic discovery was one of the twentieth century's most significant medical and pharmacological breakthroughs (Rai *et al*., 2013; Mohr, 2016; Hutchings *et al*., 2019). On the other hand, the excessive practice may significantly increase bacterial strain resistance, resulting in a treatment failure ratio. Bacterial resistance can develop due to changes in the antibiotic binding site, the production of enzymes capable of degrading or altering the structure of antibiotics, mutations in genes encoding transport proteins resulting in impaired cell wall permeability, or antibiotic molecule pumping (Guilhelmelli *et al*., 2013; Blair *et al*., 2015; Aldulaimi, 2017). Numerous studies conducted over the last few decades have emphasized using natural chemicals derived from plants as antibacterial treatments (Zacchino *et al*., 2017). Since ancient times, plants have been utilized as an alternative to conventional medicine, particularly in numerous Asian and Pacific regions (Bihani, 2021), including Indonesia. The world health organization (WHO) is progressively emphasizing the use of traditional medicinal substances due to their low toxicity, environmental friendliness, and potential to reduce the amount of synthetic pharmaceutical components (Sofowora *et al*., 2013). Most

indigenous communities in Indonesia's varied regions continue to utilize natural resources and natural goods (Widhiantara & Jawi, 2021).

Plumeria spp. is a genus of plants that may be utilized in traditional medicine, consisting of numerous species, the bulk of which are lactiferous trees and deciduous shrubs found worldwide. Notably, 11 of the total species are found worldwide in tropical and subtropical climates (Zumbroich, 2013; Ahaotu *et al*., 2020; Bihani, 2021). Locals, particularly in Java (Indonesia), refer to this plant as Kamboja, while it is referred to as Jepun in Bali (Indonesia) (Oliveira *et al*., 2019; Sanjaya *et al*., 2020). In several events, the Balinese largely use red Kamboja, particularly the flower organ, as a method of culture. Additionally, spa product entrepreneurs employ red Kamboja flowers as raw materials to create body lotions, bath soaps, aromatherapy candles, scents, and body scrubs (Paranatha *et al*., 2013; Martida & Pharmawati, 2016). Furthermore, dried Kamboja flowers are used as raw materials in the manufacture of incense, air freshener, and beverages (Sholeha *et al*., 2014; Patrisia *et al*., 2017).

Local people still utilize red Kamboja flowers for aesthetic, cosmetic, and religious purposes, and their use as a source of natural medicine is not widely acknowledged. The study aims to determine the phytochemical activity of a red Kamboja flower extract (*Plumeria rubra* L.) against a variety of human pathogenic bacteria. Our findings highlight the interest of antibacterial potential of local plants. Efforts to develop new therapeutic agents by using indigenous plants that are both environmentally friendly and effective at protecting the body against infectious diseases are urgently needed.

MATERIALS AND METHODS

Plant material and extracts preparation. A total of 1000 g of *Plumeria rubra* L. flowers were taken, and wet sorting was carried out by separating the dirt and dust attached. Washing was carried out with running water, then dried in an oven at 50°C for 24 h. The dried samples were ground using a blender to form a powder. The methods was following our previous sutdies (Sari & Sumadewi, 2021). The maceration method was used to make methanol and ethanol extracts of *P. rubra* flowers, which involved weighing 100 g of simplicia powder, then placed it in a maceration tube, methanol solvent (Merck, Germany) and 750 ml of ethanol (Merck, Germany) was added in each tube then homogenized, and tightly close it. The extracted material was stored in a dark place for three days. Furthermore, the mixture of simplicia and solvent was filtered using filter paper to obtain the results of maceration I. The remaining maceration I pulp was then soaked with 250 ml of methanol and ethanol solvent for one day. The extract was filtered with filter paper to obtain maceration II. The results of maceration I and II were mixed in one sterile bottle. The extract was concentrated by evaporation using a rotary evaporator at 40°C to obtain thick methanol and ethanol extracts.

Preparation of microbial suspensions. Pure cultures of *Staphylococcus aureus* strain ATCC 25923, *Escherichia coli* strain ATCC 8739, and *Candida albicans* strain ATCC 10231 were obtained from the Laboratory of Microbiology, Faculty of Medicine, Universitas Udayana. All cultures were put into sterile test tubes filled with 3 ml of 0.9% NaCl solution (Merck, Germany) and then homogenized. The culture suspensions were then compared with the turbidity level of the McFarland 0.5 standard (Zapata & Ramirez-Arcos, 2015).

Antimicrobial activity assay. Test for antimicrobial activity using the well diffusion method (Balouiri *et al*., 2016). A total of 0.1 ml of microbial suspension was dropped into a sterile petri dish, then 10 ml of Sabouraud dextrose agar (SDA) media was added for the growth of *C. albicans* and nutrient agar (NA) media for the growth of *S. aureus* and *E. coli*. The media was homogenized by shaking until solid. The solidified media was then drilled with a cork drill with a diameter of 0.6 cm. Each well in each medium was added with *P. rubra* flower extract, 50 µl of positive control (ketoconazole and gentamicin), and negative control (methanol and ethanol). Incubation was carried out by placing all treatments in an incubator at 37°C for 24 h. The inhibition zone

formed around the wellbore was measured for its vertical and horizontal diameters using a caliper (Jaidka *et al*., 2017).

Determination of the qualitative phytochemical content. Phytochemical screening of samples of *P. rubra* flower extract was carried out using a qualitative test (Awaad *et al*., 2017). Identification of alkaloid compounds using 2% HCl and Mayer reagent, flavonoid compounds were identified by 5 drops of concentrated Mg and HCl powder, saponin compounds were identified by foam test, steroid and terpenoid compounds were identified with the Liberman-Burchard reagent, while phenol and tannin compounds were identified with $FeCl_3$ reagent.

Data analysis. This study uses both quantitative and qualitative data. The diameter of antimicrobial inhibition was quantified at each *P. rubra* flower extract concentration using ANOVA at the 95% level, followed by Duncan's test. Qualitative data in phytochemical test findings were analyzed, specifically the presence or absence of a specific group of bioactive chemicals in the extract.

RESULTS AND DISCUSSION

Antimicrobial activities of the *Plumeria rubra* L. with methanol extract. The *P. rubra* floral parts extract was tested *in vitro* for antimicrobial activity using the well diffusion method. The diameter of the inhibitory zone (DIZ) was used to represent the results (Table 1). Concentration-dependent antibacterial activity was shown for the methanol extract against all microbial strains. According to the DIZ value, methanol extracts of *P. rubra* at concentrations of 5, 10, 15, and 20% w/v inhibited the growth of *E. coli*, *S. aureus*, and *C. albicans*. The examination of *P. rubra* methanol extract at 10% on the *E. coli* strain revealed the highest DIZ value of 6.30 mm. Similarly, the methanol extracts of *P. rubra* at 5%, 10%, and 20% all had a high DIZ value of 7.40, 7.36, and 7.30 mm, respectively. Meanwhile, the maximum DIZ values for *P. rubra* extract on *C. albicans* growth were observed at 10% and 20%, notably 25.08 mm and 25.04 mm, respectively. Indeed, our findings indicated that methanol extract possessed a significantly higher DIZ value ($p<0.05$) than gentamicin (positive control).

Table 1. Antimicrobial activities of the *Plumeria rubra* methanol extracts.

Microorganism strain	DIZ (mm) Gentamicin (+ control)	Methanol (- control)	Extract concentration (mg/ml) 5%	10%	15%	20%
Escherichia coli ATCC 8739	5.30±.14[a]	0.00±.00[b]	3.20±.16[c]	6.30±.14[d]	4.30±.14[e]	3.18±.10[c]
Staphylococcus aureus ATCC25923	5.48±.08[a]	0.00±.00[b]	7.40±.10[c]	7.36±.05[c]	4.46±.05[d]	7.30±.14[c]
Candida albicans ATCC10231	6.28±.20[a]	0.00±.00[b]	14.20±.17[c]	25.08±.10[d]	7.02±.04[e]	25.04±.08[d]

Notes: DIZ= diameter inhibition zone; Different letter notations in the same column show a significant difference ($P<0.05$).

Antimicrobial activities of the *P. rubra* with ethanol extract. The DIZ value of the *P. rubra* flower ethanol extract against the highest *E. coli* strain was shown at 5%, which was 5.26 mm and significantly different from the other three concentrations, but not with gentamicin (Table 2). The DIZ value of the highest *P. rubra* flower ethanol extract against the *S. aureus* strain was shown at 20% (7.30 mm), not significantly different from the 5% and 10% extract but significantly different from gentamicin. Interestingly, the DIZ value of the *P. rubra* flower ethanol extract at 20% was able to inhibit the growth of *C. albicans* up to 47.08 mm. These results differed significantly from the concentration and control groups ($p<0.05$).

Medicinal plants have been widely highlighted for their nutritional value and beneficial health roles. Cho *et al*. (2021) reported a positive correlation between the bioactive content of plants and their therapeutic activity. Our findings revealed that *P. rubra* flower extract has the potential to inhibit the growth of pathogenic microbial strains with a higher inhibitory value than the antibiotic gentamicin. The antibacterial activity of the ethanol and methanol extracts of *P. rubra* flowers was evaluated, these two extract solvents inhibited the growth of microbial strains such as *E. coli*, *S. aureus*, and *C. albicans.*

Table 2. Antimicrobial activities of the *Plumeria rubra* ethanol extracts.

Microorganism strain	DIZ (mm)					
	Gentamicin (+ control)	Ethanol (- control)	Extract concentration (mg/ml) 5%	10%	15%	20%
Escherichia coli ATCC 8739	5.30±.04[a]	0.00±.00[b]	5.26±.05[a]	4.28±.13[c]	4.24±.05[c]	4.14±.05[d]
Staphylococcus aureus ATCC25923	5.48±.20[a]	0.00±.00[b]	7.20±.12[c]	7.27±.04[c]	4.44±.19[d]	7.30±.12[c]
Candida albicans ATCC10231	6.28±.10[a]	0.00±.00[b]	13.08±.10[c]	13.08±.10[c]	5.08±.08[d]	47.08±.10[e]

Notes: DIZ= diameter inhibition zone; Different letter notations in the same column show a significant difference (P<0.05).

Sharma & Kumar (2012), Ali *et al.* (2013), and Ali *et al.* (2014) previously demonstrated that extracts of *P. obtusa* flowers in petroleum ether, ethyl acetate, isobutanol, chloroform, and ethanol could inhibit the growth of gram-positive bacteria (including *Bacillus atrophaeus*, *B. subtilis*, and *S. aureus*), as well as gram-negative bacteria (*E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Salmonella* spp.). Numerous factors, including the rate of microorganism growth, the ability and rate of diffusion of the active ingredient in the medium, the sensitivity of microorganisms to the active substance, and the thickness and viscosity of the medium, can all affect the size of a biocontrol material's area of inhibition (Prakash & Gunasekaran, 2011).

We assume that the difference in the zone of inhibition at each concentration is due to the active component present in the extract having a high or low concentration, which directly impacts the amount of active substance acting on microbial growth. Microbial inhibition occurs as a result of the pace at which the organic component containing secondary metabolites diffuses into the microbial growth medium (Balouiri *et al*., 2016). On the other hand, the effectiveness of an extract to suppress bacteria can be altered by their sensitivity to the concentration of the extract (Fitri *et al*., 2020). The antibacterial efficacy of *S. aureus* was shown to be higher than that of *E. coli* in our study. This was owing to variations in the cell wall components of the two strain of bacteria, which affected *P. rubra* method of action. Gram positive bacteria have highly thick peptidoglycan cell walls, which makes it more difficult for chemicals to pass through, in comparison to gram negative bacteria, which have a thinner cell wall layer (Vermassen *et al*., 2019).

Table 3. Phytochemical screening of *Plumeria rubra* flower extracts.

Compounds	Extract of *Plumeria rubra*	
	Methanol	Ethanol
Saponin	+	+
Tanin	+	+
Alkaloid	+	+
Flavonoid	+	+
Steroid	+	+
Fenol	+	+
Terpenoid	-	-

Notes: + = detected, - = not detected.

Screening for phytochemicals. Table 3 summarizes the results of a qualitative screening of phytochemical extracts from *P. rubra* flower parts. Qualitative phytochemical analysis of *P. rubra* flower extracts indicated the presence of the same metabolite chemicals in both extract types. Our findings differ slightly from those previously reported, which indicated the presence of terpenoid compounds in *P. rubra* plant extracts (Bihani, 2021). Additionally, the study confirmed the isolation of over 110 chemical constituents from *P. rubra*, including significant chemical constituents with pharmacological activity, such as fulvoplumierin, plumieride, stigmasterol, lupeol, rubrinol, rubrajalellol, oleanolic acid, taraxasterol acetate, plumieride-pE-coumarate, rubranonoside, and plumericin (Bihani, 2021; Bihani *et al*., 2021). Each compound has a specific solubility in plants, which is dictated by the type of solvent employed during the maceration process (Abubakar & Haque, 2020). As a result, when that type of secondary metabolite is dissolved in a variety of solvents, it will exhibit a variety of pharmacological actions. Ethanol and methanol are both universal polar solvents capable of extracting any secondary metabolite containing the phenyl group (phenolic acids, flavonoids, alkaloids, tannins, saponins, and lignin) (Mujeeb *et al*., 2014).

Saponins' antimicrobial activity is based on their ability to interact with sterols prevalent in microbial membranes via complex molecules, resulting in the formation of lipid bilayer defects that impair membrane integrity and increase cellular permeability (Vu *et al*., 2015; Ngo *et al*., 2017). Increased permeability results in a more concentrated intracellular fluid being drawn out of the cell, allowing nutrients, metabolic chemicals, enzymes, and proteins contained inside the cell to escape, ultimately killing the fungal cell (Wang *et al*., 2017; Cavalheiro & Teixeira, 2018). According to our previous studies (Sari & Sumadewi, 2021), white frangipani flower saponins (*P. acuminata*) are antifungal against *C. albicans* ATCC 10231. The antimicrobial mechanism of action of tannin alkaloid compounds is to damage the major components of the cell wall, which include chitin, glucans, and lipids (de Oliveira Santos *et al*., 2018). Moreover, flavonoids function by building complex compounds with extracellular proteins and are soluble chemicals that can disrupt fungal cell membranes, resulting in releasing intracellular components (Yanto *et al*., 2020). Apart from being antimicrobial, flavonoid molecules have antiviral, anticancer, anti-diabetic, and antiinsecticide properties (Zakaryan *et al*., 2017). The mechanism of action of steroids as antimicrobials is associated to membrane lipids and their susceptibility to steroid components, which results in lysosome leakage as a result of changes in membrane integrity and morphology (Miller & Zachary, 2017). The same thing occurs with phenol's mechanism, which is capable of damaging the cytoplasmic membrane and causing nucleus leakage (Miklasiska-Majdanik et al., 2018), as well as agglomerating microbial cellular proteins at greater phenol concentrations. This activity is particularly effective when bacteria are in the cleavage stage, when the phospholipid layer around the cell is extremely thin, allowing phenol to easily harm the contents of the cell (Petersen, 2017). Qualitative phytochemical analysis also revealed that *P. rubra* flower extract ingredients are considered to be a source of bioactive molecules that are effective in controlling microbial pathogens associated with infectious diseases in humans. Thus, the development of novel antimicrobial medicines based on indigenous plants is crucial for the future health of humankind.

CONCLUSION

Plumeria rubra methanol extract had the greatest DIZ values of 7.40 mm, 7.36 mm, and 7.30 mm for *Staphylococcus aureus* ATCC25923 at 5%, 10%, and 20%, respectively, whereas *Candida albicans* ATCC10231 had the highest DIZ values of 25.08 mm, 25.04 mm, and 25.08 mm, respectively. The DIZ value of the ethanol extract of *P. rubra* flowers against *Escherichia coli* strain was 5.26 mm at 5% and 7.30 mm at 20%. The methanol and ethanol extracts of *P. rubra* flowers contained secondary metabolites of saponins, tannins, alkaloids, flavonoids, steroids, and phenols. Further research is still needed, especially in evaluating other bioactive compounds and the antioxidant activity of *P. rubra* and their cytotoxicity to human cells. This approach is invaluable given the lack of knowledge of the toxicity and direct positive effects on the human health of these extracts.

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