BIOLOGY, MEDICINE, & NATURAL PRODUCT CHEMISTRY ISSN 2089-6514 (paper)
Volume 14, Number 1, April 2025 | Pages: 327-335 | DOI: 10.14421/biomedich.2025.141.327-335 ISSN 2540-9328 (online)

Phytochemicals and Antibacterial Activity of *Impatiens balsamina* L. Leaf Extracts Against Gram-Positive and Gram-Negative Bacteria

Putu Utari Fridayanthi[1], Made Dharmesti Wijaya[2,*], Desak Putu Citra Udiyani[2], Anak Agung Gede Indraningrat[3], Marta Setiabudy[3]
[1]Medical Study Program; [2]Department of Pharmacology; [3]Department of Microbiology and Parasitology,
Faculty of Medicine and Health Sciences, Warmadewa University, Jl. Terompong No 24 Denpasar 80235, Tel. +62 361 240727, Indonesia.

Corresponding author*
dharmestiwijaya@warmadewa.ac.id

Manuscript received: 23 February, 2025. Revision accepted: 27 June, 2025. Published: 01 July, 2025.

Abstract

The emergence of antibiotic resistance as one of the global public health threats makes research on new antibacterial compounds urgently needed. Among natural resources, the *Impatiens balsamina* plant has the potential to be explored as the new source of antibacterial agents. This study aimed to identify the phytochemical composition and evaluate the antibacterial activity of *I. balsamina* leaf extracts against Gram-positive and Gram-negative bacteria. Extracts were prepared using the maceration method with methanol, chloroform, and n-hexane solvents at a 1:5 sample-to-solvent ratio. Phytochemical screening was performed qualitatively, and antibacterial activity was evaluated using the disc diffusion assay. Analysis of methanol extract detected the presence of flavonoids, saponins, tannins, phenols, steroids, and glycosides, while chloroform extract consisted of tannins, phenols, and steroids. On the other hand, steroids were the only compounds detected qualitatively in n-hexane extract. Antibacterial testing revealed that methanol extract exhibited the highest activity, with zones of inhibition (ZOI) of 15.10±0.18 mm, 9.40±0.30 mm, 14.75±1.28 mm, and 8.67±0.50 mm against *Streptococcus mutans* FNCC 0405, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and *Klebsiella pneumoniae* ATCC 700603, respectively. A concentration-dependent ZOI was observed in the methanol extract, with activity increasing at higher concentrations. GC-MS analysis of the methanol extract identified 32 compounds, including n-hexadecanoic acid (12.12%), 2-acetylbenzoic acid (8.26%), 5-hydroxymethylfurfural (8.21%), and 2-methoxy-4-vinylphenol (4.67%), which are known to possess antimicrobial, antioxidant, and anti-inflammatory activities. Chloroform extract showed moderate activity against *S. mutans* (7.04±0.15 mm) and *S. aureus* (7.10±0.31 mm), while n-hexane extract exhibited no antibacterial activity. The significant antibacterial activity of methanol extract is likely due to its rich phytochemical composition, highlighting methanol as an effective solvent for extracting bioactive compounds. These findings provide a strong foundation for further exploration of *I. balsamina* leaf extracts as a source of antibacterial agents.

Keywords: Antibacterial Activity; Gram-Positive; Gram-Negative; Impatiens balsamina Leaf Extracts; Phytochemicals.

INTRODUCTION

Bacterial antimicrobial resistance (AMR) is the result of bacterial alterations that reduce the effectiveness of antimicrobial medications (Murray et al., 2022). In 2019, bacterial AMR had been associated with an estimated 4.95 million fatalities, of which 1.27 million were directly related to bacterial AMR (Murray et al., 2022). If the emergence of AMR is not handled carefully, it is predicted to cause approximately 10 million deaths per year globally by 2050 (Singh & Tandon, 2023). The development of AMR has led to the ineffectiveness of the most widely used antibiotics and consequently placed AMR as one of the top ten threats to human health worldwide (Pouran et al., 2024; World Health Organization, 2024).

One of the crucial aspects that should receive more attention to combat antibiotic resistance is the development of new antibiotics through the exploration of natural resources, notably herbal plants (Pouran et al., 2024; Seukep, Nembu, et al., 2023). Herbal plants are a prospective source of new antimicrobial drugs due to the bioactivities of their diversified secondary metabolites, including alkaloids, flavonoids, phenolic compounds, terpenes, saponins, and others (Šovljanski et al., 2023). These compounds are not only essential to plant defense systems, but also display substantial antimicrobial activities (Seukep, Mbuntcha, et al., 2023; Šovljanski et al., 2023). Scientific studies to evaluate the antibacterial activity of medicinal plant extracts against Gram-positive and Gram-negative bacteria are crucial in the quest to search and to identify novel antibiotic compounds that are effective against pathogenic bacteria (Gorlenko et al., 2020; Pandey & Agnihotri, 2015).

The plant *Impatiens balsamina* L. has been traditionally utilized to treat skin infections, wounds, and inflammation, suggesting the potential for active compounds with antibacterial activity (Seukep,

Mbuntcha, et al., 2023; Šovljanski et al., 2023). Although this plant is known to have benefits in traditional medicine (Qian et al., 2023), in-depth scientific studies on its antibacterial effectiveness, especially against Gram-positive and negative bacteria, are still very limited. Most of the previous studies are more general or limited to a few specific types of bacteria, so data on its effectiveness against a wide range of Gram-positive and negative bacteria is rather scarce. In addition, specific phytochemical profiles that contribute to antibacterial activity have not been widely described. Optimization of the type of solvent used in extracting *I. balsamina* also needs to be done to obtain the best antibacterial activity.

This study aims to identify the phytochemical content and to analyze the antibacterial activity of *I. balsamina* leaf extracts against several Gram-positive bacteria such as *Streptococcus mutans* and *Staphylococcus aureus,* as well as Gram-negative bacteria such as *Escherichia coli* and *Klebsiella pneumoniae*. In this study, *I. balsamina* was extracted using three different solvents namely methanol, chloroform, and n-hexane, to select extract with the best antibacterial activity. This research is crucial to discover new potential antibacterial compounds from *I. balsamina* leaves and contribute as one of the solutions in overcoming bacterial AMR. This study offers a novel contribution by identifying the phytochemical profile of *I. balsamina* leaf extract and evaluating its antibacterial activity comprehensively, covering clinically relevant Gram-positive and negative bacteria.

MATERIALS AND METHODS

Materials

Materials used in this study were *I. balsamina* leaves, methanol (Merck, Germany), chloroform (Merck, Germany), and n-hexane (Merck, Germany) solvents. The tested bacteria used were Gram-positive bacteria *Staphylococcus aureus* atcc 25923 and *Streptococcus mutans* fncc 0405, as well as Gram-negative bacteria *Escherichia coli* atcc 25922 and *Klebsiella pneumoniae* atcc 700603. The media used in the antibacterial activity test was Luria Bertani (LB). In the phytochemical screening, the materials used were Mg powder, HCl 2 N, concentrated HCl, $FeCl_3$, H_2SO_4, and Liebermann-Burchard reagent. In addition, the tools used were blender, jar, Whatmann No.1 filter paper, rotary evaporator, paper disc, petri dish, ose needle, test tube, bunsen burner, micropipette, laminar air flow cabinet, autoclave, and glassware.

Sample preparation

Impatiens balsamina leaves were obtained from Sembung Village, Mengwi Sub-District, Badung District, Bali Province, Indonesia. For identification purposes, whole plants (roots, stems, leaves, and flowers) were made into voucher specimens by attaching them to clean paper and then stacking them with heavy objects until they were dry and stiff. The voucher specimens were identified at the Research Center for Plant Conservation, Botanical Gardens, and Forestry, Bedugul, Bali. The leaf samples used have been selected that are neither too young nor too old, by taking the fourth leaf from the top and the second leaf from the bottom. About 5 kg of fresh leaves were washed and chopped into smaller pieces, then dried in the oven at 45° for 24 hours. The dried simplisia was then tested for moisture content using the oven gravimetric method, with a temperature of 105°C for 5 hours until the moisture content was less than 10%. The dried simplisia is then blended into coarse powder, then stored at room temperature in a closed and airtight container (Wijaya & Indraningrat, 2021).

Extraction

A total of 100 grams of dry simplisia powder was macerated using 500 mL of each solvent (methanol, chloroform, and n-hexane) for 2 x 24 hours. The results of the first 24 hours of maceration were filtered with Whatmann No. 1 filter paper, then the extract was stored in the refrigerator (4°C) while the pulp was macerated again for 24 hours. The extracts from the first and second maceration were combined and then the solvent was evaporated using a rotary evaporator at 50°C. The concentrated extract that was obtained was stored in a closed and airtight vial at 4°C until ready to be used for further experiments (Wijaya & Indraningrat, 2021).

Phytochemical screening

Phytochemical screening of *I. balsamina* extracts was carried out using the qualitative method with colour reagents as a preliminary test to determine the presence of secondary metabolites, namely flavonoids, tannins, saponins, steroids, glycosides, and phenols. The extract with the wider diameter zone of inhibition (ZOI) was selected and further be analysed using the gas chromatography coupled to mass spectrometry (GC-MS) method.

Antibacterial activity test

Luria Bertani (LB) agar media was used for isolate maintenance. This media was made by mixing 5 grams of yeast extract, 10 grams of peptone, 5 grams of NaCl, and 20 grams of agar. The solution was then homogenized and put into an autoclave for sterilization at 121°C for 15 minutes. The solution was then poured into petri dishes and allowed to solidify. Meanwhile, each pure culture on agar media of Gram-positive bacteria *Staphylococcus aureus* atcc 25923 and *Streptococcus mutans* fncc 0405, as well as Gram-negative bacteria *Escherichia coli* atcc 25922 and *Klebsiella pneumonia* atcc 700603 were inoculated using a sterile Ose needle and then in sterile liquid LB tubes, and incubated at 37°C for 24 hours.

The antibacterial activity test of each concentrated extract was conducted against four tested bacteria using the disc diffusion method. First of all, each bacterial isolate was rejuvenated on liquid LB media, then 200 µL suspension of each test bacterium with an optical density (OD) of 0.5 was taken and put into a petri dish containing LB agar media with the spread plate technique using a sterile cotton swab. Next, paper discs with a size of 6 mm were prepared for each extract. A total of 20 µL of extract was pipetted on the disc paper, then dried for 30 minutes. Each paper disc was transferred using tweezers to LB agar media that already contained the test bacteria. The petri dishes were incubated for 24 hours at 37°C in an upside-down position. The repetition was carried out three times.

Antibiotics ampicillin, streptomycin, and nalidixic acid were used as positive controls while the three solvents used during the extraction process became negative controls. Antibacterial activity was calculated based on the average diameter of the inhibition zone formed from the extract using a caliper (Wijaya & Indraningrat, 2021). The diameter of ZOI formed was grouped based on 4 categories, namely: weak (0-5 mm), moderate (5-10 mm), strong (10-20 mm), and very strong (>20 mm) (Davis & Stout, 1971). Extract that displays the best ZOI were then made into three different concentrations, namely 75%, 50%, and 25%, to determine whether there are significant differences in ZOI at different concentrations.

Data Analysis

A descriptive analysis was used for the phytochemicals detected in the extracts. For the antibacterial activity, the average value ± standard deviation of the ZOI diameter for each extract was calculated using Microsoft Excel 2019. One Way Analysis of Variance (ANOVA) test was then conducted using IBM SPSS 25 to test the similarity of the means of populations of three or more. Then the post-Hoc test was carried out using the Tukey method to determine which samples had significant differences.

Ethical clearance

The experiment reported in this paper has received ethical approval from the Health Research Ethics Commission, Faculty of Medicine and Health Sciences, Warmadewa University, Denpasar-Bali under ethics number: 81/Unwar/FKIK/EC-KEPK/IX/2022 on 21 September 2022.

RESULTS AND DISCUSSION

Sample identification

Impatients balsamina leaf samples were obtained from Sembung Village, Mengwi District, Badung Regency, Bali. In addition to leaves, whole plant samples consisting of roots, stems, leaves, fruits, and flowers were also taken and made into voucher specimens (Figure 1) for plant identification purposes. Plant identification was conducted at the Research Center for Plant Conservation, Botanical Gardens, and Forestry, Bedugul, Bali. The identification results showed that the samples used in this study were indeed *I. balsamina* L. species (Table 1).

Figure 1. Voucher specimen of the *Impatiens balsamina* sample.

Table 1. Result of sample plant identification.

Kingdom	Plantae
Division	Magnoliophyta
Class	Magniioliopsida
Ordo	Ericales
Family	Balsaminaceae
Genus	Impatiens L.
Species	*Impatiens balsamina L.*

***Impatiens balsamina* extraction with different solvents**

About 5 kg of *I. balsamina* leaves were harvested, then dried and pulverized to become 616.3 grams of dried powder. The water content was then calculated using the oven gravimetric method and a value of 4.86% was found. After the extraction using three different solvents and solvent evaporation processes were carried out, it was found that the extract yields obtained with methanol, chloroform, and n-hexane solvents were 9.8%, 5.1%, and 4.8%, respectively. The difference in the amount of yield from the extraction of *I. balsamina* leaves macerated with different solvents is due to the polarity value of each solvent. The amount of extract obtained is influenced by several things such as the amount of simplisia powder used, the fineness of the simplisia, and the type of solvent used (Andriyani et al., 2017; Dirar et al., 2019; Zhang et al., 2021). The type of solvent can affect the yield of active substances and the use of the best solvent will ensure an optimal extraction process. The highest extract yield was obtained using methanol solvent because it has polar-protic properties. Polar solvents can dissolve phytochemical compounds more optimally because of their hydrogen bonding and dipole-dipole interactions that facilitate the dissolution of the compounds, and thus, enhancing their extraction efficiency (Chatepa et al., 2024; Ghaffar & Perveen, 2024).

Antibacterial activity of *Impatiens balsamina* leaf extracts

The antibacterial activity of methanol, chloroform, and n-hexane extracts from *I. balsamina* leaves was tested using the disc diffusion method. Negative controls (the solvents) confirmed no antibacterial effects, while positive controls (streptomycin, nalidixic acid, and ampicillin) were used to compare the antibacterial activity. The test results indicated that methanol extract has the strongest antibacterial activity, effective against both Gram-positive and Gram-negative bacteria, with the largest ZOI of 15.10 ± 0.18 mm on *S. mutans* (Table 2). Chloroform extract was effective only against Gram-positive bacteria (*S. aureus* and *S. mutans*), while n-hexane extract showed no activity. Methanol, a polar solvent, excels at extracting diverse bioactive antibacterial compounds compared to chloroform or n-hexane (Aziz et al., 2018; Neamah et al., 2021; Singh et al., 2010). These findings suggest that methanol is the most suitable solvent for isolating antibacterial compounds from *I. balsamina* leaves, offering potential for therapeutic applications.

Table 2. Antibacterial activity test results.

Bacteria	Treatment	Zone of Inhibition Mean (mm) ± SD
Streptococcus mutans	Methanol extract	15,10 ± 0,18
	Chloroform extract	7,04 ± 0,15
	N-hexane extract	0
	Control (-)	0
	Control (+) Streptomicin	17,15 ± 0,29
	Control (+) Nalidixic acid	15,45 ± 0,26
	Control (+) Ampicilin	6,74 ± 0,04
Staphylococcus aureus	Methanol extract	9,40 ± 0,30
	Chloroform extract	7,10 ± 0,31
	N-hexane extract	0
	Control (-)	0
	Control (+) Streptomicin	15,01 ± 0,29
	Control (+) Nalidixic acid	15,16 ± 0,10
	Control (+) Ampicilin	7,84 ± 0,16
Eschericia coli	Methanol extract	14,75 ± 1,28
	Chloroform extract	0
	N-hexane extract	0
	Control (-)	0
	Control (+) Streptomicin	14,94 ± 0,09
	Control (+) Nalidixic acid	15,53 ± 0,96
	Control (+) Ampicilin	8,79 ± 0,15
Klebsiella pneumoniae	Methanol extract	8,67 ± 0,50
	Chloroform extract	0
	N-hexane extract	0
	Control (-)	0
	Control (+) Streptomicin	16,04 ± 0,27
	Control (+) Nalidixic acid	15,89 ± 0,85
	Control (+) Ampicilin	8,73 ± 0,40

The normality and homogeneity test showed that all treatment data were normally distributed and homogeneous ($p>0.05$). ANOVA test revealed that solvent types significantly affected ZOI in *S. mutans, S. aureus, E. coli,* and *K. pneumoniae* ($P=0.001$, $\alpha<0.05$). Moreover, Tukey test results indicated that antibacterial activity of the methanol extract was comparable to nalidixic acid against *S. mutans* and to streptomycin and nalidixic acid against *E. coli* (Tabel 3). Additionally, methanol extract showed antibacterial activity equivalent to ampicillin against *K. pneumoniae*. Methanol is highly effective in extracting antibacterial bioactive compounds due to its polarity, which enhances the solubility and extraction of phenolic compounds. The polarity of the solvent is crucial, as it directly impacts the efficiency of bioactive compound extraction and their subsequent antibacterial activity (Arya et al., 2022; Cui et al., 2020). Methanol and other polar solvents are preferred for extracting compounds with significant antimicrobial properties, making them valuable in pharmaceutical and industrial applications (Arya et al., 2022).

Table 3. Comparison of antibacterial activity (ZOI) between extracts and positive controls against selected bacterial strains.

Treatment	Tested Bacteria			
	S. mutan	***S. aureus***	***E. coli***	***K. pneumoniae***
Methanol extract	**15.108[b]**	9.403[b]	**14.750[a]**	**8.671[b]**
Chloroform extract	7.040[c]	7.100[d]	0.000[c]	0.000[c]
N-hexane extract	0.000[d]	0.000[e]	0.000[c]	0.000[c]
Control (+) Streptomicin	17.159[a]	15.019[a]	**14.948[a]**	16.044[a]
Control (+) Nalidixic acid	**15.452[b]**	15.168[a]	**15.553[a]**	15.890[a]
Control (+) Ampicilin	6.743[c]	7.840[c]	8.790[b]	**8.734[b]**

Note: Mean values followed by the same letter in the same column indicate mean values are not significantly different in the Tukey test (α = 0.05); Mean values followed by different letters in the same column indicate mean values are significantly different in the Tukey test (α = 0.05).

Further analysis was performed on the methanolic extract of *I. balsamina*, which demonstrated the strongest antibacterial activity among the other extracts. To evaluate the relationship between extract concentration and antibacterial efficacy, the extract was tested at varying concentrations of 25%, 50%, and 75%. This approach aimed to assess how changes in concentration influenced the extract's ability to inhibit bacterial growth. Table 4 shows that higher concentrations of the extract resulted in greater inhibition. Of the four test bacteria, methanol extract showed the strongest antibacterial activity against *E. coli* at 75%, while no ZOI was observed for *S. aureus* and *K. pneumoniae* at 25% concentration. Higher concentrations of plant extracts generally result in greater zones of inhibition, indicating stronger antibacterial activity (Allyn et al., 2018). This dose-dependent relationship is consistent across various plant extracts and bacterial strains (Aishah et al., 2012; Allyn et al., 2018; Hasan et al., 2013).

Table 4. Effect of methanol extract concentration on antibacterial activity against selected bacterial strains.

Bacteria	Concentrations	Zone of Inhibition Mean (mm) ± SD
Streptococcus mutans	25%	6,47 ± 0, 23
	50%	7,51 ± 0,06
	75%	8,46 ± 0,27
	Control (-)	0
	Control (+) Streptomicin	17,15 ± 0,29
	Control (+) Nalidixic acid	15,45 ± 0,26
	Control (+) Ampicilin	6,74 ± 0,04
Staphylococcus aureus	25%	0
	50%	7,06 ± 0,33
	75%	9,19 ± 0,39
	Control (-)	0
	Control (+) Streptomicin	15,01 ± 0,29
	Control (+) Nalidixic acid	15,16 ± 0,10
	Control (+) Ampicilin	7,84 ± 0,16
Eschericia coli	25%	9,59 ± 0,56
	50%	12,55 ± 0,63
	75%	12,56 ± 0,79
	Control (-)	0
	Control (+) Streptomicin	14,94 ± 0,09
	Control (+) Nalidixic acid	15,53 ± 0,96
	Control (+) Ampicilin	8,79 ± 0,15
Klebsiella pneumoniae	25%	0
	50%	7,02 ± 0,60
	75%	8,58 ± 0,34
	Control (-)	0
	Control (+) Streptomicin	16,04 ± 0,27
	Control (+) Nalidixic acid	15,89 ± 0,85
	Control (+) Ampicilin	8,73 ± 0,40

The ZOI of *I. balsamina* methanolic extracts at three concentrations were analyzed to assess the effect of concentration on antibacterial activity. The Shapiro-Wilk test confirmed normal data distribution while Levene's

test indicated homogeneity ($p>0.05$). ANOVA test results showed that extract concentration significantly affected ZOI in *S. mutans*, *S. aureus*, *E. coli*, and *K. pneumoniae* ($p<0.05$). Tukey test analysis further revealed significant differences among concentration groups, as shown in Table 5, except for *E. coli*, where 50% and 75% concentrations showed no significant difference in ZOI.

Table 5. Diameter of zone of inhibition in different methanol extracts concentrations.

Treatment	Tested Bacteria			
	S. mutan	***S. aureus***	***E. coli***	***K. pneumoniae***
25%	6.472[c]	0.000[c]	9.599[b]	0.000[c]
50%	7.511[b]	7.062[b]	12.550[a]	7.022[b]
75%	8.468[a]	9.191[a]	12.563[a]	8.581[a]

Note: Mean values followed by the same letter in the same column indicate mean values are not significantly different in the Tukey test ($\alpha = 0.05$); Mean values followed by different letters in the same column indicate mean values are significantly different in the Tukey test ($\alpha = 0.05$).

Phytochemicals of *Impatiens balsamina* leaf extracts

The phytochemical analysis showed that methanol extract contains the most secondary metabolites, namely flavonoids, tannins, saponins, phenolics, glycosides, and steroids (Table 6). Chloroform extract contains secondary metabolite compounds, namely tannins, phenolics, and steroids. Meanwhile, n-hexane extract only contains steroid compounds. The abundance of secondary metabolites in the methanol extract likely explains its superior antibacterial activity compared to chloroform and n-hexane extracts. The limited composition of bioactive compounds in chloroform and n-hexane extracts corresponds to their lower antibacterial effectiveness. These findings emphasize the importance of solvent polarity in extracting a broad range of bioactive compounds, highlighting methanol as the most effective solvent for isolating antibacterial agents from *I. balsamina*.

Table 6. Phytochemical composition of methanol, chloroform, and n-hexane extracts of *I. balsamina* leaves.

Compounds	*I. balsamina* Leaves Extracts		
	Methanol	**Chloroform**	**N-Hexane**
Flavonoid	+	-	-
Saponin	+	-	-
Tannin	+	+	-
Phenol	+	+	-
Steroid	+	+	+
Glycoside	+	-	-

The polarity of the solvent significantly influences the extraction of bioactive compounds. Polar solvents like methanol are particularly effective in extracting phenolic compounds. Studies have shown that methanol extracts generally have higher phenolic and flavonoid contents compared to non-polar solvents (Arya et al., 2022; Cui et al., 2020). Phenolic compounds including flavonoids and tannins are known for their antibacterial properties due to their ability to damage bacterial cell membranes, inhibit bacterial enzymes and toxins that are crucial for their survival and pathogenicity, as well as prevent the formation of bacterial biofilms (Chen et al., 2024; Miklasińska-Majdanik et al., 2018). Flavonoids are also able to inhibit the synthesis of bacterial DNA and RNA, thereby preventing their replication and survival (Tan et al., 2022). In addition, tannins are known to disrupt bacterial membrane proteins and lipids, affecting membrane fluidity and integrity (Olchowik-Grabarek et al., 2022).

Phytochemical screening also showed that the methanol extract of *I. balsamina* leaves was positive for saponins and glycosides, while the chloroform and n-hexane extracts did not contain these compounds. Saponins interact with bacterial membrane lipids, disrupting the lipid bilayer and leading to cell lysis (Li & Monje-Galvan, 2024). Meanwhile, glycoside compounds are able to disrupt bacterial cell membranes and lead to cell death, as well as prevent biofilm formation (Wang et al., 2024). In addition, the three extracts were found to positively contain steroids. Steroid compounds exhibit diverse mechanisms of antibacterial action, ranging from disrupting bacterial cell membranes to enhancing the efficacy of existing antibiotics (da Silva et al., 2022; Ralambondrahety et al., 2021).

GC-MS analysis of *I. balsamina* methanol extract identified 32 compounds, with the five most abundant listed in Table 7. The dominant compound, n-hexadecanoic acid (12.12%), is known to exhibit antioxidant, antimicrobial, anti-inflammatory, anticancer,

nematicidal, and pesticidal activities (Mazumder et al., 2020; Siswadi & Saragih, 2021). Another compound, 2-acetylbenzoic acid, has antimicrobial, antioxidant, and antifungal properties (Vimalavady & Kadavul, 2013). The extract also contained 5-hydroxymethylfurfural (8.12%), known for its antioxidant, antiischemic, and tyrosinase-inhibiting effects(Zhao et al., 2013). Additionally, 2-methoxy-4-vinylphenol (4.67%) was identified, with reported antimicrobial activity(Rubab et al., 2020). However, no data is available on the bioactivity of 1- [methoxy (phenyl) methyl]-4-methylbenzene.

Table 7. Five compounds with the largest peak area in methanol extract of *I. balsamina* leaves.

No	Retention Time	Chemical Compounds	Area (%)
1	29.228	n- Hexadecanoic acid	12.12
2	18.004	1- [methoxy (phenyl) methyl]-4-methylbenzene	12.03
3	18.830	2-Acetylbenzoic acid	8.26
4	11.915	5-Hydroxymethylfurfural	8.21
5	14.373	2-methoxy-4-vinyl-phenol	4.67

CONCLUSIONS

This study concludes that the methanol extract of *I. balsamina* leaves exhibits significant antibacterial activity against both Gram-positive bacteria (*Streptococcus mutans* FNCC 0405 and *Staphylococcus aureus* ATCC 25923) and Gram-negative bacteria (*Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603), with ZOI values of 15.10 ± 0.18 mm, 9.40 ± 0.30 mm, 14.75 ± 1.28 mm, and 8.67 ± 0.50 mm, respectively. The inhibition increased with higher extract concentrations. The chloroform extract shows moderate activity against *S. mutans* (7.04 ± 0.15 mm) and *S. aureus* (7.10 ± 0.31 mm), while the n-hexane extract demonstrates no antibacterial activity against either Gram-positive or Gram-negative bacteria. Phytochemical analysis reveals that the methanol extract contains flavonoids, saponins, tannins, phenols, steroids, and glycosides, which likely contribute to its superior antibacterial activity. GC-MS analysis identified 32 compounds in the methanol extract, including bioactive compounds such as n-hexadecanoic acid, 2-acetylbenzoic acid, 5-hydroxymethylfurfural, and 2-methoxy-4-vinylphenol, which are known for their antimicrobial, antioxidant, and anti-inflammatory properties. Meanwhile, the chloroform extract contains tannins, phenols, and steroids, and the n-hexane extract contains only steroids. These findings highlight the concentration-dependent antibacterial effects of the methanol extract and the potential of methanol as a solvent for extracting bioactive compounds with antibacterial properties from *I. balsamina* leaves. Future studies should focus on isolating and characterizing the specific bioactive compounds responsible for the observed antibacterial activity to understand their mechanisms of action. Additionally, broader investigations involving different bacterial strains, cytotoxicity testing, and in vivo studies are recommended to validate the extract's efficacy and safety.

Acknowledgements: This research was funded by the Faculty of Medicine and Health Sciences, Warmadewa University, under grant number 1023/Unwar/FKIK/PD-13/IX/2024.

Authors' Contributions: Putu Utari Fridayanthi, Made Dharmesti Wijaya, and Desak Putu Citra Udiyani designed the experiments. Putu Utari Fridayanthi performed the antibacterial test, compiled data analysis, and wrote the early draft of the manuscript. Made Dharmesti Wijaya provided consumables, performed data analysis on GC-MS, and reviewed the draft manuscript. Desak Putu Citra Udiyani performed qualitative phytochemical screening. Anak Agung Gede Indraningrat provided the bacterial strains. Anak Agung Gede Indraningrat and Marta Setiabudy performed analysis on antibacterial test results.

Competing Interests: The authors declare no conflict of interest.

REFERENCES

Aishah, H. N., Zaini, N. M., & Haslinda, M. (2012). Antimicrobial activity of pimpinella anisum seed extract. In *Microbes in Applied Research: Current Advances and Challenges, Malaga, Spain, 14 - 16 September 2011* (pp. 486–491). World Scientific Publishing Co. https://doi.org/10.1142/9789814405041_0098

Allyn, O. Q., Kusumawati, E., & Nugroho, R. A. (2018). Antimicrobial activity of terminalia catappa brown leaf extracts against staphylococcus aureus ATCC 25923 and Pseudomonas aeruginosa ATCC 27853. *F1000Research*, *7*. https://doi.org/10.12688/F1000RESEARCH.15998.1

Andriyani, R., Kosasih, W., Ningrum, D. R., & Pudjiraharti, S. (2017). Effect of temperature, time, and milling process on yield, flavonoid, and total phenolic content of Zingiber officinale water extract. In K. M. & N. M. (Eds.), *IOP Conference Series: Earth and Environmental Science* (Vol. 60, Issue 1). Institute of Physics Publishing. https://doi.org/10.1088/1755-1315/60/1/012012

Arya, O. P., Adhikari, P., Pandey, A., Bhatt, I. D., & Mohanty, K. (2022). Health-promoting bioactive phenolic compounds in

different solvent extracts of Curcuma caesia Roxb. rhizome from North-East India. *Journal of Food Processing and Preservation*, *46*(8). https://doi.org/10.1111/jfpp.16805

Aziz, M. A., Akter, M. I., Islam, M. R., Sajon, S. R., Hossain, M. S., Tajmim, A., Yasmen, N., Rahman, S. M. M., Hazra, A. K., & Jodder, P. (2018). Phytochemical screening and antimicrobial activity of microcos paniculata leaves. *Pharmacologyonline*, *1*, 9–14. https://www.scopus.com/inward/record.uri?eid=2-s2.0-85048035860&partnerID=40&md5=cd064f606c82bec7b90cda55e6199bdb

Chatepa, L. E. C., Mwamatope, B., Chikowe, I., & Masamba, K. G. (2024). Effects of solvent extraction on the phytoconstituents and in vitro antioxidant activity properties of leaf extracts of the two selected medicinal plants from Malawi. *BMC Complementary Medicine and Therapies*, *24*(1). https://doi.org/10.1186/s12906-024-04619-7

Chen, X., Lan, W., & Xie, J. (2024). Natural phenolic compounds: Antimicrobial properties, antimicrobial mechanisms, and potential utilization in the preservation of aquatic products. *Food Chemistry*, *440*. https://doi.org/10.1016/j.foodchem.2023.138198

Cui, N., Zhang, L., Quan, M., & Xu, J. (2020). Profile of the main bioactive compounds and: In vitro biological activity of different solvent extracts from Ginkgo biloba exocarp. *RSC Advances*, *10*(73), 45105–45111. https://doi.org/10.1039/d0ra09490k

da Silva, A. R. P., Costa, M. D. S., Araújo, N. J. S., de Freitas, T. S., de Almeida, R. S., Barbosa Filho, J. M., Tavares, J. F., de Souza, E. O., de Farias, P. A. M., Pinheiro, J. C. A., & Coutinho, H. D. M. (2022). Potentiation of Antibiotic Action and Efflux Pump Inhibitory Effect on Staphylococcus aureus Strains by Solasodine. *Antibiotics*, *11*(10). https://doi.org/10.3390/antibiotics11101309

Davis, W. W., & Stout, T. R. (1971). Disc plate method of microbiological antibiotic assay. I. Factors influencing variability and error. *Applied Microbiology*, *22*(4), 659–665. https://doi.org/10.1128/aem.22.4.659-665.1971

Dirar, A. I., Alsaadi, D. H. M., Wada, M., Mohamed, M. A., Watanabe, T., & Devkota, H. P. (2019). Effects of extraction solvents on total phenolic and flavonoid contents and biological activities of extracts from Sudanese medicinal plants. *South African Journal of Botany*, *120*, 261–267. https://doi.org/10.1016/j.sajb.2018.07.003

Ghaffar, N., & Perveen, A. (2024). Solvent polarity effects on extraction yield, phenolic content, and antioxidant properties of Malvaceae family seeds: a comparative study. *New Zealand Journal of Botany*. https://doi.org/10.1080/0028825X.2024.2392705

Gorlenko, C. L., Kiselev, H. Y., Budanova, E. V, Zamyatnin, A. A., & Ikryannikova, L. N. (2020). Plant secondary metabolites in the battle of drugs and drug-resistant bacteria: New heroes or worse clones of antibiotics? *Antibiotics*, *9*(4). https://doi.org/10.3390/antibiotics9040170

Hasan, N. A., Nawahwi, M. Z., & Malek, H. A. (2013). Antimicrobial activity of nigella sativa seed extract. *Sains Malaysiana*, *42*(2), 143–147. https://www.scopus.com/inward/record.uri?eid=2-s2.0-84872319852&partnerID=40&md5=b56dbf693b39640288742d8f5eba5fe9

Li, J., & Monje-Galvan, V. (2024). Effect of Glycone Diversity on the Interaction of Triterpenoid Saponins and Lipid Bilayers. *ACS Applied Bio Materials*, *7*(2), 553–563. https://doi.org/10.1021/acsabm.2c00928

Mazumder, K., Nabila, A., Aktar, A., & Farahnaky, A. (2020). Bioactive variability and in vitro and in vivo antioxidant activity of unprocessed and processed flour of nine cultivars of Australian lupin species: A comprehensive substantiation. *Antioxidants*, *9*(4). https://doi.org/10.3390/antiox9040282

Miklasińska-Majdanik, M., Kępa, M., Wojtyczka, R. D., Idzik, D., & Wąsik, T. J. (2018). Phenolic compounds diminish antibiotic resistance of staphylococcus aureus clinical strains. *International Journal of Environmental Research and Public Health*, *15*(10). https://doi.org/10.3390/ijerph15102321

Murray, C. J. L., Ikuta, K. S., Sharara, F., Swetschinski, L., Robles Aguilar, G., Gray, A., Han, C., Bisignano, C., Rao, P., Wool, E., Johnson, S. C., Browne, A. J., Chipeta, M. G., Fell, F., Hackett, S., Haines-Woodhouse, G., Kashef Hamadani, B. H., Kumaran, E. A. P., McManigal, B., … Naghavi, M. (2022). Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *The Lancet*, *399*(10325), 629–655. https://doi.org/10.1016/S0140-6736(21)02724-0

Neamah, R., Mohsin, H., & Kamil, H. (2021). Phytochemical Screening and Antibacterial Effect of Methanol Extracts of Suaeda aegyptiaca Leaves on Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, and Pseudomonas aeruginosa. *Archives of Razi Institute*, *76*(5), 1221–1227. https://doi.org/10.22092/ari.2021.356133.1784

Olchowik-Grabarek, E., Sękowski, S., Kwiatek, A., Płaczkiewicz, J., Abdulladjanova, N., Shlyonsky, V., Swiecicka, I., & Zamaraeva, M. (2022). The Structural Changes in the Membranes of Staphylococcus aureus Caused by Hydrolysable Tannins Witness Their Antibacterial Activity. *Membranes*, *12*(11). https://doi.org/10.3390/membranes12111124

Pandey, A., & Agnihotri, V. (2015). Antimicrobials from medicinal plants: Research initiatives, challenges, and the future prospects. In *Biotechnology of Bioactive Compounds: Sources and Applications* (Vol. 9781118733, pp. 123–150). Wiley Blackwell. https://doi.org/10.1002/9781118733103.ch5

Pouran, F., Mahdavian, A., Aghazadeh, H., & Navidinia, M. (2024). Mini Review: Antimicrobial Agents Based on Natural Compounds: The Key to Solving the Current Crisis. *Archives of Pediatric Infectious Diseases*, *12*(4). https://doi.org/10.5812/apid-146195

Qian, H., Wang, B., Ma, J., Li, C., Zhang, Q., & Zhao, Y. (2023). Impatiens balsamina: An updated review on the ethnobotanical uses, phytochemistry, and pharmacological activity. *Journal of Ethnopharmacology*, *303*. https://doi.org/10.1016/j.jep.2022.115956

Ralambondrahety, R., Couzinet-Mossion, A., Rabesaotra, V., Andriambeloson, O., Barnathan, G., Rakotovao, M., & Wielgosz-Collin, G. (2021). Antibacterial activity of steroids isolated from the madagascar marine sponge biemna laboutei: Δ7 steroids as new potential agents against pathogenic bacteria. *Natural Products Journal*, *11*(1), 57–62. https://doi.org/10.2174/2210315509666191204123011

Rubab, M., Chelliah, R., Saravanakumar, K., Barathikannan, K., Wei, S., Kim, J. R., Yoo, D., Wang, M. H., & Oh, D. H. (2020). Bioactive potential of 2-methoxy-4-vinylphenol and benzofuran from Brassica oleracea L. var. capitate f, rubra (Red Cabbage) on oxidative and microbiological stability of beef meat. *Foods*, *9*(5). https://doi.org/10.3390/foods9050568

Seukep, A. J., Mbuntcha, H. G., Zeuko'o, E. M., Woquan, L. S., Nembu, N. E., Bomba, F. T., Watching, D., & Kuete, V. (2023). Established antibacterial drugs from plants. *Advances in Botanical Research*, *106*, 81–149. https://doi.org/10.1016/bs.abr.2022.08.005

Seukep, A. J., Nembu, N. E., Mbuntcha, H. G., & Kuete, V. (2023). Bacterial drug resistance towards natural products. *Advances in Botanical Research*, *106*, 21–45. https://doi.org/10.1016/bs.abr.2022.08.002

Singh, C., Singh, S. K., Singh, K. A., Singh, A., Nath, G., & Rai, N. P. (2010). Inhibitory response of drug resistant bacteria towards methanol extract of Piper longum L. fruit. *Pharmacologyonline*, *1*, 634–643. https://www.scopus.com/inward/record.uri?eid=2-s2.0-77953190152&partnerID=40&md5=9f17c6adfa938944cd639e686b84a12b

Singh, R., & Tandon, V. (2023). Antibiotics: Past, Present, Future, and Clinical Pipeline. In *Recent Advances in Pharmaceutical Innovation and Research* (pp. 583–619). Springer Singapore. https://doi.org/10.1007/978-981-99-2302-1_24

Siswadi, S., & Saragih, G. S. (2021). Phytochemical analysis of bioactive compounds in ethanolic extract of Sterculia quadrifida R.Br. *AIP Conference Proceedings*, *2353*(May). https://doi.org/10.1063/5.0053057

Šovljanski, O., Kljakić, A. C., & Tomić, A. (2023). Antibacterial and Antifungal Potential of Plant Secondary Metabolites. In *Reference Series in Phytochemistry* (Vol. 2023, Issues 41–1, pp. 1–43). Springer Science and Business Media B.V. https://doi.org/10.1007/978-3-031-30037-0_6-1

Tan, Z., Deng, J., Ye, Q., & Zhang, Z. (2022). The Antibacterial Activity of Natural-Derived Flavonoids. *Current Topics in Medicinal Chemistry*, *22*(12), 1009–1019. https://doi.org/10.2174/1568026622666220221110506

Vimalavady, a, & Kadavul, K. (2013). Phytocomponents identified on the various extracts of stem of Hugonia mystax L. (Linaceae). *European Journal of Experimental Biology*, *3*(1), 73–80.

Wang, Z.-X., Kong, W.-Z., Guan, S.-N., Zhang, N., Yu, Y.-B., & Zhang, X.-Y. (2024). Pitsubcosides M−S: Novel antibacterial cadinane sesquiterpenoid glycoside esters from Pittosporum subulisepalum. *Industrial Crops and Products*, *208*. https://doi.org/10.1016/j.indcrop.2023.117917

Wijaya, M. D., & Indraningrat, A. A. G. (2021). Antibacterial Activity of Mangrove Root Extracts from Ngurah Rai Mangrove Forest, Denpasar-Bali. *Biology, Medicine, & Natural Product Chemistry; Vol 10, No 2 (2021)DO - 10.14421/Biomedich.2021.102.117-121*. https://sciencebiology.org/index.php/BIOMEDICH/article/view/163

World Health Organization. (2024). *Ten threats to global health in 2019*. https://www.who.int/news-room/spotlight/ten-threats-to-global-health-in-2019

Zhang, R.-B., Gao, W.-M., Dai, Z.-Y., Li, M.-Y., Chen, Z.-G., & Ren, G.-P. (2021). Study on the extraction process of dihydromyricetin from the combination of medicine and food homologous materials. *Food and Machinery*, *37*(10), 138-143and175. https://doi.org/10.13652/j.issn.1003-5788.2021.10.024

Zhao, L., Chen, J., Su, J., Li, L., Hu, S., Li, B., Zhang, X., Xu, Z., & Chen, T. (2013). In vitro antioxidant and antiproliferative activities of 5-hydroxymethylfurfural. *Journal of Agricultural and Food Chemistry*, *61*(44), 10604–10611. https://doi.org/10.1021/jf403098y

THIS PAGE INTENTIONALLY LEFT BLANK