

## Antibacterial Activities of Parijoto (*Medinilla speciosa* Blume) Fruit Extracts Against Clinical Isolates of *Salmonella typhi* and *Shigella dysenteriae*

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### Abstract

*Medinilla speciosa* (*M. speciosa*) Blume is a plant originating from Mount Muria, Kudus District, Central Java, Indonesia. Its fruit has been used by the local community to treat mouth sores, diarrhea, inflammatory, hyperlipidemia, cancer, bacterial infection and nutrients for pregnant women. However, the antibacterial activity against *Salmonella typhi* (*S. typhi*) and *Shigella dysenteriae* (*S. dysenteriae*) is yet unknown. Thus, the aim of this study was to determine the antibacterial activities of *M. speciosa* fruit extracts against clinical strain of *S. typhi* and *S. dysenteriae*. Plant determination and sample preparation were conducted. The fruits of *M. speciosa* were extracted by gradual maseration using n-hexane, ethyl acetate and methanol as solvents. Phytochemicals were screened by Fransworth method. Antibacterial activity was determined using agar well diffusion. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values and comparison study of antibacterial activity were determined. The results showed that all of the extracts exhibited antibacterial activities, with the greatest activity shown by the methanol extract. This extract was more effective against *S. dysenteriae* than *S. typhi*, as evidenced by the largest inhibition diameter and lowest MIC (25 mg/mL) and MBC (50 mg/mL) values. With regard to the results of phytochemical screening, the antibacterial activity of methanol extract could be due to the presence of alkaloids, flavonoids, polyphenols, quinones, saponins and tannins. From the comparative antibacterial activity value indicated that in order to give the same inhibition diameter with 1 ppm of chloramphenicol, 312.3 ppm methanol extract is needed. It can be conclude that *M. speciosa* fruit has a potential to be developed as natural antibacterial agent, especially to treat *bacillary dysentery*.

**Keywords** : Chloramphenicol, Minimum Inhibitory Concentration, Minimum Bactericidal Concentration, Phytochemicals

### Introduction

The therapeutic efficacies of many herbal plant used in the treatment and control of certain diseases and ailments have endeared

their use over the years.<sup>1,2</sup> The success achieved in the treatment is associated with the phytochemical constituents which have been demonstrated to have therapeutic and

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nutritional qualities.<sup>3,4</sup> Phytochemicals such as alkaloids, tannins, flavonoids, saponin, phenolic compounds, and glycosides are potent antimicrobial, antioxidant and bioactive compounds years.<sup>5,6</sup> These phytochemical constituents may function individually and or synergistically in their mode of action to inhibit the growth of pathogenic organisms and positively affect other physiological processes years.<sup>7</sup> Due to multi-resistant pathogenic bacteria to many commonly administered antibiotics years,<sup>7</sup> further study is necessary to find other possible sources of antimicrobial agents, including *M. speciosa* Blume.<sup>8</sup>

Parjoto is an endemic plant of Asian mainland with its central distribution in Philippines, Malaysia and Indonesia. In Indonesia, especially in Kudus District, Central Java, they grow in Mount Muria area at a height of 1,602 meters above sea level.<sup>9</sup> *M. speciosa* fruit contains flavonoids, saponins, tannins, and glycosides.<sup>10</sup> This fruit is widely used by the local community for treating various diseases, such as diarrhea, mouth sores, inflammatory, hyperlipidemia, cancer, and bacterial infection and nutrients for pregnant women. The other utilization of parijoto is used as the symbol of ritual and ornamental plants.<sup>9</sup>

Various studies have established the antibacterial activities of *M. speciosa* fruit. Extracts of n-hexane, ethyl acetate, and methanol extracts of this fruit showed antibacterial activities against *Pseudomonas aureginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, Extended Spectrum Betalactamase (ESBL) *Escherichia coli* and Methicillin Resistant *Staphylococcus aureus* (MRSA).<sup>11</sup> However, there is no information its antibacterial activities against *S. typhi* and *S. dysenteriae*.

Typhoid fever is an acute infectious disease of the small intestine caused by *S. typhi*, with symptoms of fever a week or more with disorders of the gastrointestinal tract with or without impaired consciousness. Most of the transmission of the disease through contaminated food and drink.<sup>12</sup> *Bacillary dysentery* is a digestive tract infection caused by *S. dysenteriae*. Initial signs and symptoms include fever, abdominal pain, and cramping followed by frequent watery stools. In some developing countries, the disease is endemic and occurs every year 80 million cases with 700,000 fatalities.<sup>13</sup>

Based on this background, we conducted the research on the antibacterial activities of n-hexane, ethyl acetate and methanol extracts from *M. speciosa* fruit against clinical strain of *S. typhi* and *S. dysenteriae*. It is expected that the study findings will be used as evidence of the potential *M. speciosa* fruit to be used as natural antibacterial agent, especially to treat typhoid fever and bacillary dysentery.

## Methods

### Materials

*M. speciosa* fruit was collected from Mount Muria, Kudus District, Central Java, Indonesia. The clinical strain of *S. typhi* and *S. dysenteriae* were obtained from Microbiology and Parasitology Laboratory, Faculty of Medicine, Universitas Padjadjaran, Jatinangor, West Java, Indonesia. All bacteria were cultured in Salmonella-Shigella Agar/SSA (Oxoid®), Mueller-Hinton Agar/MHA (Oxoid®), and Mueller-Hinton Broth/MHB (Oxoid®). Chloramphenicol, as a reference antibiotic, was obtained from PT. Kimia Farma Indonesia.

The others materials used in this research were n-hexane (Merck), ethyl acetate (Merck), methanol (Merck), ammonia (Merck), chloroform (Merck), ether (Merck),

hydrochloric acid (Merck), 96% ethanol (Bratachem), amyl alcohol (Merck), dimethyl sulfoxide/DMSO (Merck), Mayer reagent, Dragendorf reagent, Liebermann-Burchard reagent, magnesium powder (Bratachem), potassium hydroxide (Merck), iron (III) chloride (Merck), gelatin (Bratachem), vanillin (Merck), normal saline solution (Otsu-NS), 0.5 McFarland standard solution and distilled water.

#### *Plant Determination and Sample Preparation*

The plant was determined at Plant Taxonomy Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Jatinangor, West Java, Indonesia. The fresh fruit was washed with running tap water, followed by sterile distilled water. After washing, the fruit was air dried under shade and pulverized to coarse powder using electric mixer grinder.

#### *Extracts Preparation*

The sample was extracted by gradual maceration using three types of solvents in the decreasing of polarity, starting from non-polar (n-hexane), semi-polar (ethyl acetate) and polar (methanol) solvents according to the method of Niswah (2014),<sup>14</sup> with some modifications. The dried fruit powder (192.97 g) was macerated with 1 L of n-hexane for 5 x 24 hours at room temperature. The residual sample was dried, then macerated in the same way using ethyl acetate and methanol as solvents. The filtrate was concentrated at 45°C using a rotary evaporator and later subjected to air drying to give dried crude extracts. The extracts were observed organoleptically, continued by yields determination.

#### *Phytochemicals Screening*

The n-hexane, ethyl acetate, and methanol extracts of *M. speciosa* were used for qualitative phytochemicals screening. Alkaloids, flavonoids, quinones, polyphenols,

saponins, tannins, steroids, triterpenes, monoterpenes and sesquiterpenes were analyzed according to the previous standard method.<sup>15</sup>

#### *Preparation of Bacterial Suspensions*

The pure cultures of clinical strain of *S. typhi* and *S. dysenteriae* were streaked onto SSA plate and incubated at 37°C for 18-24 hours. The well-isolated colonies were aseptically transferred to 2 mL of sterile normal saline solution. The turbidity of each bacterial suspension was adjusted to the 0.5 McFarland standard solution, which equivalent to  $1.5 \times 10^8$  CFU/mL.<sup>16</sup>

#### *Antibacterial Activity Assay*

*M. speciosa* fruit extracts were tested on MHA against both of bacterial test using the agar well diffusion method and it was evaluated by measuring the zone of inhibition.<sup>16</sup> The extracts were serially diluted using dimethyl sulfoxide (DMSO) to prepare concentration of 100, 200, 300 and 400 mg/mL.<sup>11</sup> Bacterial suspension (20  $\mu$ L) was dropped into a sterile petri dish and suspended in 20 mL of liquid MHA. The mixture was homogenized and allowed to solidify at room temperature. The solid medium then perforated to form wells as extract storages. Each well was filled with 50  $\mu$ L of the various concentration of the extracts. The positive (MHA inoculated with bacterial suspension) and negative control (only MHA) were also prepared to confirm the result. The plates were incubated at 37°C for 18-24 hours and diameter surrounding the wells were measured by a caliper. The test performed in triplicate to verify the results.

#### *Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) Values*

Determination of MIC and MBC values of the most potential extract of *M. speciosa* fruit against clinical strain of *S. typhi* and *S. dysenteriae* were performed using a method

described in the CSLI.<sup>17</sup> MIC was conducted in 96-well round-shaped bottom microtiter plate, using broth microdilution methods in triplicates. The extracts were dissolved in DMSO in the concentration of 100 mg/mL served a stock solution. For the assay, stock solutions of extract solution were diluted by serially two-fold dilution in the MHB to produce decreasing dilutions ranging from column 3 to 11. Column 3 of the microtiter plate contained the highest concentration (100 mg/mL), while column 11 contained the lowest concentration (0.39 mg/mL). The adjusted inoculum suspensions should be diluted in sterile normal saline solution, so that, after inoculation, each well contains approximately  $5.0 \times 10^2$  CFU/mL.

Bacterial suspension (100  $\mu$ L) was inoculated against each well containing 100  $\mu$ L of extract in the dilution series, and were mixed thoroughly. Column 1 served as negative control (only MHB), column 2 served as positive control (MHA inoculated with bacterial suspension), while column 12 served as extract control. The plates were incubated at 37°C for 18-24 hours. The MIC was defined as the lowest concentration of extract that resulted in the complete inhibition of visible growth.

The MBC was defined as the lowest concentration of antibacterial agent at which no growth occurred on the MHA plate and was determined by sub-culturing the suspension (10  $\mu$ L) from each well on MHA. The plates were then incubated at 37°C for 18-24 hours. The lowest concentration that did not produce visible growth after the incubation period was considered as MBC.

#### Comparison Study of Antibacterial Activity

The comparison study was performed in the same plate using the same procedure as antibacterial activity assay.<sup>16</sup> The various

concentrations of the extract were 50,000; 100,000; 200,000; 300,000 and 400,000 ppm in DMSO for *S. typhi* and 25,000; 50,000; 100,000; 200,000 and 400,000 ppm in DMSO for *S. dysenteriae*.<sup>13</sup> The concentrations for chloramphenicol were 20; 30; 40; 50 and 60 ppm. The plates were incubated at 37°C for 18-24 hours. Diameter of inhibition around wells were measured using a caliper.

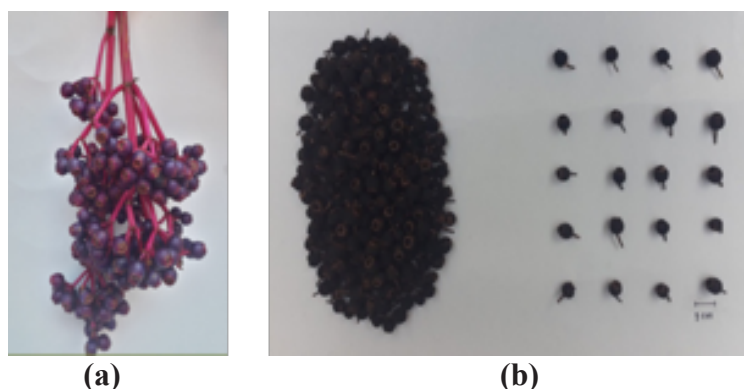
The log concentration values as x-axis were plotted against the inhibition diameter (mm) on the y-axis. The linear regression as mathematical model was obtained and used to calculate comparative antibacterial activity value. By comparing the concentration of *M. speciosa* fruit extracts with concentration of chloramphenicol producing the same inhibition diameter, comparison value can be evaluated.<sup>18</sup>

#### Results and Discussion

The plant determination showed that the plant was *M. speciosa* from Melastomaceae family, Myrtales order, Magnoliopsida class, and Traceophyta division.

*M. speciosa* fruits were extracted and obtained 92.97 g dried fruit. There were 6.08 g n-hexane extract, 2.6851 g ethyl acetate extract, and 16.0087 g methanol extract. The percentage yields of n-hexane, ethyl acetate and methanol extracts were 3.34, 1.47 and 8.79 % (w/w), respectively. The characteristics of the extract were thick, sticky, distinctive smelling with specific color (light green for n-hexane, dark green for ethyl acetate and red brown for methanol extract). Methanol extract gave highest yield, followed by n-hexane and ethyl acetate extract. The results revealed that the compounds extracted from *M. speciosa* fruit more dissolved in polar solvent than non-polar or semi-polar solvents.

The *M. speciosa* fruit extracts contained



**Figure 1.** *M. speciosa* Blume (a) fresh fruit (b) dried fruit

**Table 1. Phytochemicals in *M. speciosa* Fruit Extracts**

Phytochemicals	n-hexane Extract	Ethyl Acetate Extract	Methanol Extract
Alkaloids	-	+	+
Flavonoids	-	+	+
Quinones	+	-	+
Polyphenols	+	+	+
Saponins	-	+	+
Tannins	-	+	+
Steroid and Triterpenes	+	-	-
Monoterpenes and Sesquiterpenes	-	+	-

(+) presence (-) absence

quinones, polyphenols, steroids and triterpenes (Table 1). Non-polar compounds, such as fat, steroids, coumarin and some terpenes dissolve in n-hexane.<sup>19</sup>

Furthermore, ethyl acetate extract contained alkaloids, flavonoids, polyphenols, saponins, tannins, monoterpenes, and sesquiterpenes, while methanol extract contained alkaloids, flavonoids, quinones, polyphenols, saponins and tannins. The compounds contained in the three *M. speciosa* fruit extracts are polyphenols. Polyphenols, tannins, flavonoids, and saponins compounds are generally dissolved in polar or semi-polar solvents, such as methanol and ethyl acetate. Flavonoid compounds have polyhydroxy groups, thus, these compounds are more partitioned in semi-polar and polar

solvents.<sup>19</sup>

*M. speciosa* fruit extracts showed antibacterial activities against both of bacterial test, with the methanol extract showing the highest activity. The sensitivity clinical strain of *S. typhi* and *S. dysenteriae* increased gradually with the increased concentration of the extracts (Table 2).

With regard to the results of phytochemical screening, the antibacterial activity of methanol extract could be due to the presence of alkaloids, flavonoids, quinones, polyphenols, saponins and tannins. The finding was in line with former study which stated that alkaloids, terpenes (monoterpenes, diterpenes,



**Table 2. Antibacterial activities of *M. speciosa* fruit extracts**

Extract	Concentration (mg/mL)	Inhibition Diameter of <i>S.typhi</i> (mm)	Inhibition Diameter of <i>S. dysenteriae</i> (mm)
n-hexane	100	4.356 ± 0.512	4.800 ± 0.348
	200	4.978 ± 0.608	5.900 ± 0.208
	300	5.056 ± 0.190	5.878 ± 0.870
	400	5.644 ± 0.278	7.110 ± 0.781
Ethyl Acetate	100	4.322 ± 0.583	8.422 ± 0.222
	200	5.822 ± 0.201	9.944 ± 0.255
	300	6.022 ± 0.550	11.340 ± 0.184
	400	6.044 ± 0.989	12.601 ± 0.435
Methanol	100	9.411 ± 0.562	13.422 ± 0.019
	200	13.120 ± 0.572	16.661 ± 0.226
	300	16.920 ± 0.619	16.767 ± 0.929
	400	17.360 ± 0.186	18.889 ± 0.278

(+) presence (-) absence

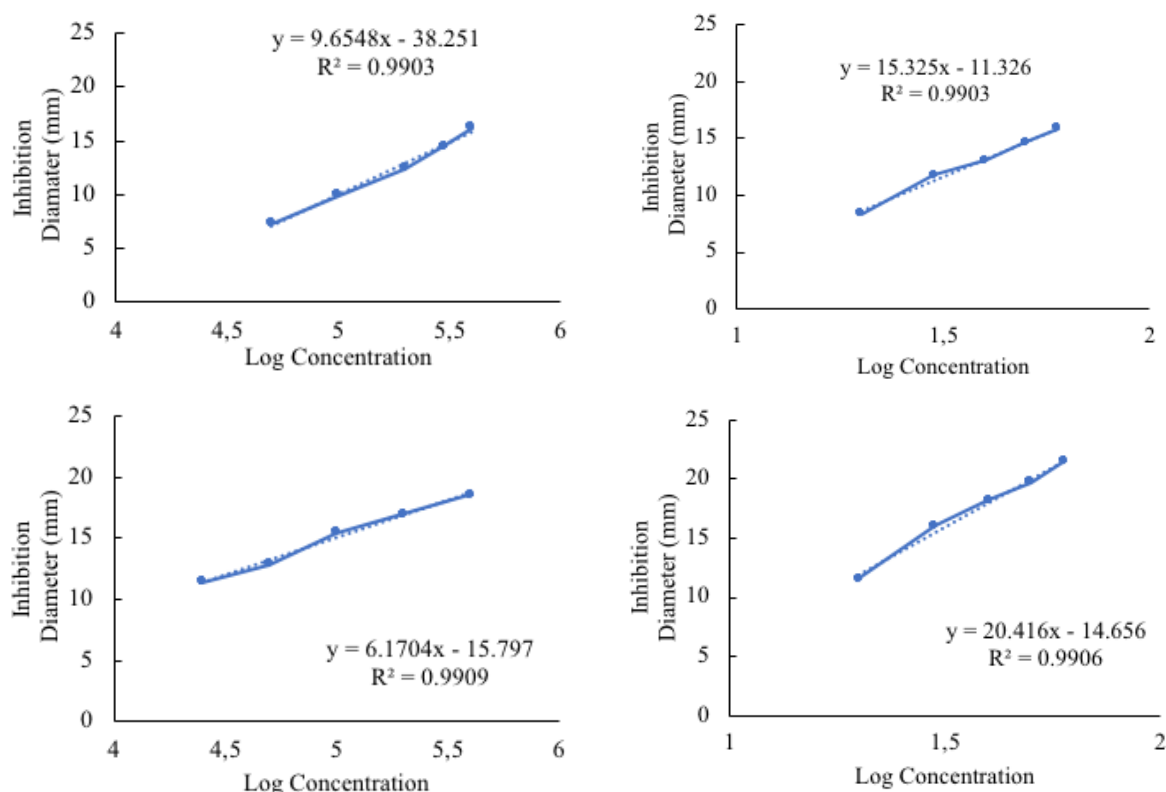
**Table 3. The MIC and MBC Values of Methanol Extract from *M. speciosa* Fruit**

Extract Concentration (mg/mL)	MIC		MBC	
	<i>S. typhi</i>	<i>S. dysenteriae</i>	<i>S. typhi</i>	<i>S. dysenteriae</i>
100	-	-	-	-
50	-	-	+	-
25	+	-	+	+
12.5	+	+	+	+
6.250	+	+	+	+
3.125	+	+	+	+
1.563	+	+	+	+
0.780	+	+	+	+
0.390	+	+	+	+

(+) presence (-) absence

triterpenes, sesquiterpenes and sterols), polyphenols, flavonoids, quinones, tannins and saponins contained in plants generally have an antimicrobial activity.<sup>19</sup> Hence, this statement need to be proven by further

study. Moreover, the presence of alkaloid and flavonoid revealed potent antimicrobial properties.<sup>20,21</sup> Several publications have documented the antibacterial activities of plant extracts.<sup>17, 22</sup> Lipophilic flavonoids can



**Figure 2. Linear regression function for prediction of the effect of concentration**  
**(a) methanol extract (b) chloramphenicol against clinical strain of *S. typhi* (c)**  
**methanol extract (d) chloramphenicol against clinical strain of *S. dysenteriae***

be related to microbial membranes. Complex compounds can be selected by flavonoids with extracellular proteins and dissolved proteins can also produce complex compounds with bacterial cell walls.<sup>23</sup> Flavonoids combined with bacterial DNA which could damage the permeability of bacterial cell walls, microsomes, and lysosomes.<sup>24</sup>

The mechanism of saponin as an antibacterial agent by cutting the function of the microbial membrane. Saponins could damage the permeability of cell walls, the prospect of content chords and death through complex hydrogen compounds produced by saponins with cell membranes.<sup>24</sup>

Tannins has the ability as an antibacterial that could reverse toxic-causing bacteria. The

antimicrobial effects of tannins were also by their ability to activate microbial adhesions, enzymes, cell membrane transport proteins, and mineral absorption. According to Andini (2014), tannins have a function to reduced accumulation in the blood by removing emissions through feces.<sup>25</sup>

Further study on determination of MIC and MBC values were conducted to methanol extract of *M. speciosa* fruit, which showed most potent antibacterial activities (Table 3). Concentrations of the extract, from third column to eleventh column were 100; 50; 25; 12.5; 6.25; 3.125; 1.563; 0.78 and 0.39 mg/mL.

The MIC value of the extract against *S. typhi* and *S. dysenteriae* are 50 mg/mL and 25 mg/

mL, while MBC value are 100 mg/mL and 50 mg/mL, respectively. The methanol extract was most effective against *S. dysenteriae* than *S. typhi*, as evidenced by the largest inhibition diameter and lowest MIC (25 mg/mL) and MBC (50 mg/mL) values (Table 3).

The result from antibacterial activities of methanol extract from *M. speciosa* fruit and chloramphenicol against clinical stain of *S. typhi* and *S. dysenteriae* are listed in Table 4

The log of concentration on the x-axis were plotted against the inhibition diameter on the y-axis. Either extract or chloramphenicol provided linear regression equations as shown in Figure 2. In conclusion, the increase of inhibition diameter resulted by particular concentration of either extract or chloramphenicol can be predicted from by simple linear regression function.

As example of calculation, from Figure 2 it is known that the linear regression equation for antibacterial activity of chloramphenicol against *S. typhi* was  $y = 15.325x - 11.326$  with  $R^2 = 0.9903$ . Applying this equation to calculate the inhibition diameter, 10 ppm chloramphenicol is predicted to give inhibition diameter approximately 3.999 mm. Employing the linear regression equation of methanol extract in order to provide same diameter of inhibition zone with that by chloramphenicol, this diameter value is applied into the linear regression equation of methanol extract,  $y = 9.6548x - 38.251$  with  $R^2 = 0.9903$ . The result of x value was 4.3758, therefore its antilog 4.3761 was 23,773 ppm. It can be conclude that the comparative antibacterial activity value of methanol extract from *M. speciosa* fruit against chloramphenicol was 2,377.3 : 1. Accordingly, it can be conclude that in order to give the same inhibition diameter with 1 ppm of chloramphenicol, 2,377.3 ppm methanol extract from *M. speciosa* fruit is needed.

With the same way, it can be conclude that the comparative antibacterial activity value of methanol extract from *M. speciosa* fruit to chloramphenicol against *S. dysenteriae* was 312.3 : 1. To give the same inhibition diameter with 1 ppm of chloramphenicol, 312.3 ppm methanol extract from parijoto fruit is needed.

### Conclusion

Methanol extract of *M. speciosa* Blume fruit showed potential antibacterial activity compared to ethyl acetate and n-hexane, due to more biologically active presence of in the extract. This result revealed that the extract can be a candidate for novel alternative antibacterial, especially to treat bacillary dysentery. The findings from current study was in agreement with traditional use of the plant in treatment in curing microbial infections. It necessitates further investigations for isolating the active antibacterial agent in pure compound form that could be used in further pharmaceutical use as alternative for current antibacterial treatments.

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### Conflict of Interest

None declared

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