



Original Article

## In vitro study of potential antibacterial *pometia pinnata* leaf extract in *Neisseria gonorrhoeae*

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### ABSTRACT

**Background:** Gonorrhea cases experiencing antibiotic resistance are increasing due to inadequate treatment or failure to contribute to an increase in antibiotic resistance cases; WHO suggests adding herbal therapy treatment methods is expected to minimize the occurrence of drug resistance as much as possible. Previous studies that have been carried out using other herbal plants have not shown any potential to inhibit the growth of *Neisseria gonorrhoeae* bacteria, seen from the amount of extract concentration and the dosage and content of the compounds in the contents monitoring the time.

**Objective:** To analyze the difference in the effect of various concentrations of Matoa Leafs Extract (*Pometia Pinnata*) compared to 500mg Levofloxacin on the increase in the growth inhibition of *Neisseria gonorrhoeae*.

**Methods:** This is true-experimental research with a post-test-only control group with a randomized design. The sample in this study was the microorganism *Neisseria gonorrhoeae* obtained through vaginal swabs in 2 females (FSW) positive for Gonorrhea by gram staining and microscopic examination and culture on Chocolate Agar Plant (CAP) media. The culture was then suspended in CAP media. Matoa Leaf extract concentration of 100%, 80%, 60%, 40%, 20%, positive control with Levofloxacin 500 mg and negative control with distilled water with four replications and monitoring in 24 hours, 48 hours, 72 hours, and 96 hours. Data analysis used Kruskal-Wallis, Mann-Whitney, and cohen's test.

**Result:** Levofloxacin 500mg in inhibiting *Neisseria Gonorrhoeae* bacteria is very effective, and Matoa Leaf extracts 60% and 40% have a strong enough potential to inhibit the growth of *Neisseria gonorrhoeae* bacteria with the same potential strength. The cohen's test 1.4 effect the levofloxacin 500mg provides a significant influence inhibiting *Neisseria Gonorrhoeae*.

**Conclusion:** Matoa Leaf extract 60%, and 40% have solid antibacterial potential, although not as strong as Levofloxacin 500mg.

### INTRODUCTION

Pharmacological treatment of Gonorrhea caused by the gram-negative bacteria *Neisseria gonorrhoeae* diplococcus, which is infected by sexual intercourse using Levofloxacin 500mg.<sup>1</sup> The CDC reports that 820,000 cases and 246,000 cases of Gonorrhea are resistant to antibiotics due to improper use of condoms and inadequate treatment or failure to contribute to an increase in cases of antibiotic resistance and the emergence of several strains bacteria that are resistant to some commonly used antibiotics.<sup>2,3</sup> This disease can infect mucosal surfaces in the urogenital organs, infect the eye membranes

in infants born vaginally.<sup>4</sup> Indonesia has the highest number of cases of sexually transmitted diseases in the highest risk group in female sex workers, as many as 8,918 cases.<sup>5-8</sup>

The highest prevalence of Gonorrhea in 2018 was in the Female Sex Worker (FSW) group, namely in the Direct sex worker group at 21.20% and in the indirect sex worker group at 9.67%.<sup>5</sup> Based on data from Semarang City in 2020, there were 3,115 FSW. In the working area of the Mangkang Health Center in 2019, the number of FSW was 546, and those experiencing Gonorrhea were 0.7%,<sup>9,10</sup> and the number of Gonorrhea cases in 2020 increased by

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5,542 cases or 78.2% in Indonesia<sup>11</sup>. The prevalence of travel-related sex activities in Indonesia in 2018 led to the prevalence of drug resistance in Gonorrhoea by 20% to 34%.<sup>11</sup>

Non-pharmacological treatment by utilizing herbal plants has long been carried out; WHO recommends using herbal remedies that are proven to be effective and safe because they have great potential to fight various diseases,<sup>12</sup> One of them is by utilizing the Matoa tree, a native plant of Indonesia where the results of previous studies examined the content of active compounds in the bark of the Matoa tree, Matoa fruit, and Matoa tree leaf. There has been no research on the specific active ingredients that can fight bacteria. Previous studies have shown that Matoa Leaf extract has potential as a treatment for HIV infection.<sup>13,14</sup>

The ethanol in the Matoa leaf has high antioxidant power, inhibiting bacterial growth.<sup>13,14</sup> Previous studies using basil leaf extract (*Ocimum sanctum Linn*) and mangosteen peel extract 100% and 80% showed 0 live colonies.<sup>15,16</sup> Another study by assessing the diameter of the inhibition zone using the extract of Putri Malu (*Mimosa Pudica*) leaf at various concentrations has not been able to inhibit *Neisseria gonorrhoeae* bacteria.<sup>17</sup> Based on the background, previous research has not used graded concentrations consistently; the purpose of this study was to analyze differences in the administration of Matoa leaf extract at various concentrations in inhibiting the growth of *Neisseria gonorrhoeae* based on the diameter of the inhibition zone and the number of live colonies, the total concentration of the extract and the dose and content of the compound in the content by monitoring the time at which the leaf extract Matoa can be used as an alternative to herbal medicine in the treatment of Gonorrhoea to slow down the occurrence of chemical antibiotic resistance.

## METHOD

### Study Design

This is a true-experimental research design with a post-test-only control group with randomization.

### Study Site

The study was conducted from April to May 2021. *Gonorrhoeae* bacteria collection was carried out on female sex workers in Mangkang Public Health Center Semarang, Matoa leaf determination test was carried out in MIPA Faculty of Biology LAB Mataram University NTB, Antibacterial activity and inhibition test were carried out in Integrated Laboratory of Diponegoro University di Semarang, Bacteriology laboratory of majoring in health analysis, Ministry of Health Poltekkes Semarang.

## Materials

The materials used were dried Leaf of Matoa, Ethanol 96% as a solvent in the manufacture of extracts, CAP (Chocolate Agar Plan) as a medium for the growth of *Neisseria Gonorrhoeae* which had been modified by adding BAP (Blood Agar Plant), Sabouraud, Plate Count Agar, Nystatin, B12, 10% blood, NaCl, Levofloxacin 500mg as a comparison antibiotic, 28 blank disks as a medium to monitor the diameter of the inhibition zone to be measured.

## Plant Extraction

The extraction of Matoa leaf was by sorting and washing as much as 7 kg of Matoa leaf were dried using room temperature 20-23°C for seven days to shrink to 1,60kg of dry leaf. Next, do the dried Matoa leaf are mashed and macerated, and 4.8 liters of 96% ethanol solvent is added until completely submerged for 2x24 hours and repeated for 24 hours by using a macerator then filtering using Whatman paper, a rotary evaporator carries out the results of the filtering with a temperature of 500C which aims to maximize the ethanol evaporation and produce ± 98.5 g thick ethanol macerate from 4800 ml of macerated matoa leaf extract.

## In Vitro Procedure

### Bacterial Preparation

*Neisseria gonorrhoeae* was rejuvenated and incubated for one day at 37°C; after the test bacteria grew, then one ose was taken to be implanted into CAP media, and four blank discs were immersed in negative control distilled water, four blank discs were dipped into positive control Levofloxacin 500mg, and each of 4 blank disks into the extract of the five concentrations. The diameter of the inhibition by observing the inhibitory zone of the extract that had been separated and made various sample concentrations (100%, 80%, 60%, 40%, 20%), Levofloxacin 500 mg (antibacterial) as a positive control and distilled water as control negative.

### Experimental Procedure

The bacteria *Neisseria Gonorrhoeae*, a gram-negative group of gonococci, were used topically in vaginal secretions as evidenced by gram staining results and microscopically showed the gonococcal *Neisseria gonorrhoeae* bacteria in the test sample. Furthermore, *Neisseria gonorrhoeae* bacteria were cultured to homogenize so as not to cause contamination from other types of bacteria or fungi. Caliper is used as a tool to measure the inhibition zone's diameter by measuring the outside, the inside, the depth, and the terraced part. For metric caliper, this major scale is indicated by units of measure cm and mm, where each part has a distance of 1mm.

Measurements were carried out for 24 hours, 48 hours, 72 hours, and 96 hours where the results of the diameter of

the inhibition area were compared with the treatment group concentration of matoa leaf 100%, 80%, 60%, 40%, 20%, the positive control group (Levofloxacin 500 mg) and the Negative group (aquades), then the measurement results were divided by 4 to assess the average number average resistance (mm). Measurement of the diameter of the inhibition zone is said to be sensitive, intermediate, and resistant<sup>18-20</sup> with weak (<5mm), medium (5-10mm), strong (10-20mm), very strong ( $\geq 20$ mm) inhibition categories.<sup>21</sup>

### Statistical Analysis

To analysis the differences in 6 groups using the Kruskal-Wallis test and to test the differences between groups in concentrations of 100%, 80%, 60%, 40%, 20%, and Levofloxacin 500mg using the Mann Whitney and Cohen's test for the magnitude of the effect

### Ethical Consideration

This research has been registered in the Research Bioethics Commission of Sultan Agung Islamic University Semarang with the number 123/IV/2021/Bioethics Commissions.

## RESULTS

Table 1 proves that matoa leaf contains active compounds of flavonoids, tannins, saponins, and triterpenoids and compounds with antibacterial abilities such as flavonoids and 100% Matoa leaf extract containing 27.57 mg of flavonoids and 345.75 mg of tannins. Table 2 shows that the inhibitory power of Levofloxacin 500mg based on monitoring time is in the robust category, while the extract concentrations of 100%, 60%, 40%, and 20% are in a strong category and the concentration of 80% in the medium category. Analysis on Figure 1, (a) concentration of 100% indicates the size of the inhibition zone is stable or not much adrift between group 1-4, in group 1 (b) it can be seen that levofloxacin 500mg has the largest inhibition zone then at a concentration of 60% & 40%, it has an inhibition zone that is not far apart and is more significant than 80% & 20% as well as in group 2 (c) and group 3 (d), (e) in group 4 there is a significant decrease.

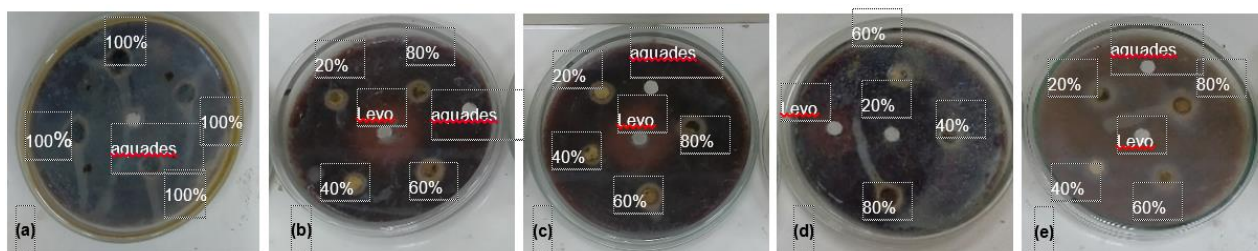
**Table 1.** Active Compounds of Matoa Leafs Extract, Qualitative and Quantitative Test

Active Compounds / Concentration	Qualitative test	Quantitative test				
		100%	80%	60%	40%	20%
Alkaloid	Negative (-)	-	-	-	-	-
Flavonoid	Positive (+)	27.57 mg	21.76 mg	15.68 mg	12.44 mg	7.25 mg
Tanin	Positive (+)	345.75 mg	275.07 mg	234.79 mg	182.36 mg	95.00 mg
Saponin	Positive (+)	-	-	-	-	-
Steroid	Negative (-)	-	-	-	-	-
Triterpenoid	Positive (+)	-	-	-	-	-

**Table 2.** The Diameter of The Inhibition Zone (mm)

Group	Diameter of The Inhibition Zone (Mean $\pm$ SD (mm))				Mean $\pm$ SD	Effect Size
	24 Hours	48 Hours	72 Hours	96 Hours		
Levofloxacin 500mg	30.35 $\pm$ 6.49	29.97 $\pm$ 7.16	28.50 $\pm$ 4.48	16.04 $\pm$ 10.20	26.21	
Concentration 100%	10.50 $\pm$ 0.57	11.27 $\pm$ 1.21	10.63 $\pm$ 1.07	9.94 $\pm$ 1.56	10.58	
Concentration 80%	9.68 $\pm$ 0.83	10.72 $\pm$ 0.73	9.89 $\pm$ 0.56	9.68 $\pm$ 1.48	9.99	
Concentration 60%	12.55 $\pm$ 1.51	11.70 $\pm$ 0.65	11.65 $\pm$ 0.61	11.17 $\pm$ 0.86	11.76	1.4 <sup>c</sup>
Concentration 40%	11.72 $\pm$ 1.90	11.70 $\pm$ 1.69	11.19 $\pm$ 1.30	12.03 $\pm$ 1.59	11.66	
Concentration 20%	10.00 $\pm$ 0.91	10.67 $\pm$ 0.23	10.55 $\pm$ 0.57	11.11 $\pm$ 1.50	10.58	
p-value	0.005 <sup>a</sup>	0.026 <sup>a</sup>	0.007 <sup>a</sup>	0.544 <sup>a</sup>	0.026 <sup>b</sup>	

<sup>a</sup> Kruskal Wallis; <sup>b</sup> Mann Whitney; <sup>c</sup> Cohen's



**Figure 1.** Picture of Inhibition Zone Diameter between groups. (a) matoa leaf extract 100%, (b) the group 1 concentration with control positive and negative, (c) the group 2 concentration with control positive and negative, (d) the group 3 concentration with control positive and negative, and (e) the group 4 concentration with control positive and negative.

## DISCUSSION

This study indicates that the administration of Matoa leaf extract in various concentrations can affect the growth inhibition of *Neisseria gonorrhoeae*  $p=0.026$ . Levofloxacin 500mg is more effective because the mean difference is higher than Matoa leaf extract with concentrations. The effect size 1,4 states that Levofloxacin 500mg and extract 60%, and 40% had a significant effect on inhibiting *Neisseria Gonorrhoea*.

The use of Levofloxacin 500mg can be used as a pharmacological treatment in the treatment of Gonorrhea, where treatment with Levofloxacin 500mg has a sensitivity in inhibiting *Neisseria gonorrhoeae*, which works by damaging the cell wall of bacteria so that it inhibits bacterial DNA gyrase (DNA Topoisomerase II) thus inhibiting DNA replication and transcription in bacteria.<sup>22</sup> The increase in the inhibition of the growth of *Neisseria gonorrhoeae* based on the time of monitoring the inhibition zone experienced a significant increase at 24 hours, 48 hours to 72 hours.

The difference in the mean increase in the growth inhibition of *Neisseria gonorrhoeae* in the variable Levofloxacin 500mg as a positive control was higher than the concentration of Matoa Leaf extract, namely 14.31mm versus 1.38mm, and this difference was significantly different. At 100% and 80%, concentrations had moderate inhibitory power because the diluted extract was thick. The active compound content in the extract was disrupted and could not optimally when soaked for 24 hours. While the 40% concentration the mean value is not much different from the 60% extract concentration, so at 40% concentration, it is necessary to increase the dose so that it can be equivalent to 60% concentration, and 20% concentration can inhibit the medium category, but the dilution results are too liquid and not concentrated; as a result, the content of the active compounds in it is disturbed, the average value at a concentration of 20% is equal to a concentration of 100%. This proves that the concentration of 20% has a strong enough inhibitory power which is equivalent to a concentration of 100%.

The antibacterial and antibiotic content of Matoa leaf extract has active compounds of flavonoids and tannins, and the flavonoid content plays a role in inhibiting energy metabolism.<sup>23</sup> These compounds will interfere with energy metabolism similarly to inhibiting the respiratory system; sufficient energy is required for the active absorption of various metabolites and the biosynthesis of macromolecules. These compounds will interfere with energy metabolism similarly to inhibiting the respiratory system; sufficient energy is required for the active absorption of various metabolites and the biosynthesis of macromolecules.<sup>14,24</sup> This research is supported by Kuspradini research that there is an antioxidant and

antibacterial activity in Matoa leaf extract, where Matoa Leaf extract has a strong enough ability to inhibit the growth of gram-negative and positive bacteria at a concentration of 100%.<sup>14,25</sup>

Previous research conducted by Tiffany stated that the content of basil extract with a concentration of 80% with 0 live colonies was effective in inhibiting the growth of *Neisseria Gonorrhoeae*<sup>15</sup>, and research conducted by Marera stated that mangosteen peel extract with concentrations of 80% effectively inhibited the growth of *Neisseria gonorrhoeae* with 0 live colonies.<sup>16</sup> However, in this study, Matoa leaf extract with practical live colony measurements at concentrations of 20% with 0 live colonies stated that Matoa leaf extract was more sensitive than basil leaf extract.

Another study conducted by Adelia et al. regarding the effectiveness of *Mimosa pudica* Leaf extract in inhibiting the growth of *Neisseria gonorrhoeae* with the average monitoring results in 24 hours at concentrations of 40% 0.5 mm, 60% 1.5 mm<sup>17</sup> and in this study based on the results of the average monitoring within 24 hours the diameter of the inhibition zone at a concentration of 40% 11.72mm, 60% 12.55mm and Levofloxacin 500mg 30.35mm stated that Matoa leaf extract was more potent in inhibiting the growth of *Neisseria Gonorrhoeae* than the *Mimosa pudica* leaf extract and 60% & 40% effective with a strong category in inhibiting the growth of *Neisseria Gonorrhoeae* for up to 72 hours.

## CONCLUSIONS AND RECOMMENDATION

The potency of Matoa Leaf extract has been shown to potentially increase the inhibition of *Neisseria gonorrhoeae* with an average monitoring time of 24 hours, 48 hours, 72 hours, and 96. However, Levofloxacin 500 mg is more sensitive than matoa leaf extract, with a strong category in inhibiting the growth of *Neisseria gonorrhoeae*. Therefore, the following study should be an *In Vitro* study test that needs to be carried out to analyze how much the antibacterial power of matoa leaf extract can be used non-pharmacological treatment of Gonorrhea.

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