

ANALGETIC EFFECT OF COMBINATION 3:2:1 GEL EXTRACT *Ageratum conyzoides* Mussaenda frondosa) AND *Curcuma domestica* of *Staphylococcus aureus* INFECTED POST PARTUM MICE

*Ara Aulia Nada*¹, *Noor Hujjatusnaini*¹, *Jumrodah*¹, *Ridha Nirmalasari*¹, *Astuti Muh Amin*²

¹*Program Studi Tadris Biologi, Fakultas Tarbiyah dan Ilmu Keguruan, IAIN Palangka Raya*

²*Program Studi Tadris Biologi, Fakultas Tarbiyah dan Ilmu Keguruan, IAIN Ternate*

*Corresponding Email: *noor.hujjatusnaini@iain-palangkaraya.ac.id*

About the Author

1. 1st Author : Ara Aulia Nada
Affiliation : Program Studi Tadris Biologi, FTIK, IAIN Palangka Raya
Mailing address : Jl. G. Obos Komplek Islamic Centre IAIN Palangka Raya - Indonesia
Email of author : araulian27@gmail.com
Orcid ID : -
Google Scholar URL : -
Phone number : 085248518740
- 2nd Author : Noor Hujjatusnaini
Affiliation : Program Studi Tadris Biologi, FTIK, IAIN Palangka Raya
Mailing address : Jl. G. Obos Komplek Islamic Centre IAIN Palangka Raya - Indonesia
Email of author : noor.hujjatusnaini@iain-palangkaraya.ac.id
Orcid ID : 0000-0002-7522-7475
Google Scholar URL : wA2gAAAAj&hl=id
Phone number : 081251136304
- 3rd Author : Jumrodah
Affiliation : Program Studi Tadris Biologi, FTIK, IAIN Palangka Raya
Mailing address : Jl. G. Obos Komplek Islamic Centre IAIN Palangka Raya - Indonesia
Email of author : jumrodah@iain-palangkaraya.ac.id
Orcid ID : 0000-0003-4741-6954
Google Scholar URL : https://scholar.google.com/citations?user=_gSHph4AAAAJ&hl=id
Phone number : 085222976725
- 4th Author : Ridha Nirmalasari
Affiliation : Program Studi Tadris Biologi, FTIK, IAIN Palangka Raya
Mailing address : Jl. G. Obos Komplek Islamic Centre IAIN Palangka Raya - Indonesia
Email of author : ridhanirmalasari@iain-palangkaraya.ac.id
Orcid ID : 57214722631
Google Scholar URL : https://scholar.google.com/citations?view_op=list_works&hl=id&user=N-PjsksAAAAJ
Phone number : 081242878804
- 5th Author : Astuti Muh Amin
Affiliation : Program Studi Tadris Biologi, FTIK, IAIN Ternate
Mailing address : Jl. Lumba-Lumba No. 8, IAIN Ternate - Indonesia
Email of author : astutimuhamin@iain-ternate.ac.id
Orcid ID : 0000-0001-7269-0299

ABSTRACT

The lack of knowledge about Ageratum conyzoides, Mussaenda frondosa, and Curcuma domestica as alternative medicines for treating postpartum infections, because there is still little research and documentation being carried out. Therefore, this research is expected to prove in vivo in order to know the analgesic effect of the combination formulation 3:2:1 extracts of Ageratum conyzoides, Mussaenda frondosa, and Curcuma domestica as therapy for post-partum infections. The research method used a true experimental post-test only control-group design, with 36 pregnant female mice as the test object. Ageratum conyzoides leaves, Mussaenda frondosa, and Curcuma domestica were prepared in a 3:2:1 formulation, with design levels of 40%, 60% and 80%. Analgesic-effect data were analyzed using the ANAVA statistical method and Duncan's test with a significant level of 1%. The results of the analgesic effect were obtained from the average number of S.aureus colonies and the vaginal mucosal fluid secretion index of mice infected with S.aureus. The results showed that the combination formulation of 3:2:1 extracts with a concentration of 30% had a significant effect on growth in S.aureus at a significant level of 1%. It was concluded that the 3:2:1 combination formulation can be recommended as the most effective in inhibiting infection S.aureus.

Keywords: Analgetic, Combination, *Ageratum conyzoides*, *Mussaenda frondosa*, *Curcuma domestica*

ABSTRAK

Minimnya pengetahuan pada tanaman *Ageratum conyzoides*, *Mussaenda frondosa*, dan *Curcuma domestica* sebagai obat alternatif untuk mengatasi infeksi pasca melahirkan, sebab masih sedikitnya penelitian dan pendokumentasian yang dilakukan. Oleh karena itu, penelitian ini diharapkan menjadi pembuktian secara in vivo agar diketahui efek analgetik formulasi kombinasi 3:2:1 ekstrak *Ageratum conyzoides*, *Mussaenda frondosa*, dan *Curcuma domestica* sebagai terapi herbal infeksi post partum. Metode penelitian menggunakan true experimental post test only control group design, dengan objek uji berupa mencit. Daun *Ageratum conyzoides*, daun *Mussaenda frondosa*, dan *Curcuma domestica* dibuat dalam formulasi 3:2:1, dengan desain taraf 40%, 60% dan 80%. Data efek analgetik dianalisis dengan metode statistik ANAVA dan uji Duncan dengan taraf signifikan 1%. Hasil data efek analgetik diperoleh dari rerata jumlah koloni *S. aureus* dan indeks sekresi cairan mukosa vagina mencit terinfeksi *S. aureus*. Hasil penelitian menunjukkan bahwa formulasi kombinasi 3:2:1 ekstrak *Ageratum conyzoides*, *Mussaenda frondosa*, dan *Curcuma domestica* dengan konsentrasi 30% berpengaruh nyata terhadap pertumbuhan pada *S. aureus* taraf nyata uji Duncan 1%. Disimpulkan bahwa formulasi kombinasi 3:2:1 dapat direkomendasikan paling efektif dalam menghambat infeksi *S. aureus*.

Kata kunci: Analgetik, Kombinasi, *Ageratum conyzoides*, *Mussaenda frondosa*, *Curcuma domestica*

INTRODUCTION

On practically all of its islands, including one in the province of Central Kalimantan, Indonesia possesses abundant natural resources in the form of medicinal plants. Plants have been used as therapeutic substances by Indonesians for a very long period of time (Susanti et al., 2018; Novaryatiin, et al., 2018). *Ageratum conyzoides*, *Mussaenda frondosa*, and *Curcuma domestica* are only a few examples of plants that are quite simple to discover, often neglected, on the sides of highways, in yards, and along riverbanks.

Radam, et al. (2016) claim that the leaves of *Ageratum conyzoides* can be utilized as a blood thinner for puerperium, vaginal discharge, and ulcer medicine in addition to treating stomach ulcers. When applied to white rats, *Mussaenda frondosa* leaf extract promotes wound healing and functions as an antibiotic against *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, and *Staphylococcus albus* (Widyastuti, et al., 2021). As a traditional medicine, *curcuma domestica*'s advantages include the ability to cure wounds, function as an antibiotic, decrease intestinal motility, get rid of body odor, lower fever, and treat diarrhea. The presence of curcuminoids, a class of phytochemicals found in *Curcuma domestica*, has an impact on this potential (Prabowo et al., 2019).

Because there hasn't been much research done on the components of these plants used as medicines, people tend to think of them as simple wild plants or herbs with no attempt to cultivate them more extensively, which contributes to the lack of knowledge about these plants as medicines among the general public. The public is aware that *Ageratum conyzoides* and *Mussaenda frondosa* work as alternative medicinal plants to treat postpartum infections, and previous researchers have thoroughly examined specific combination formulations in vitro (Ardiansyah, 2021; Emeilia, 2021; Bunga, 2021; Widyastuti, 2021). Given that *Staphylococcus aureus* is one of the microorganisms that cause postpartum vaginal infections based on its antibacterial ability on the growth of *S. aureus*, Ardiansyah (2021) reported that the combination formulation of *Ageratum conyzoides* leaves, *Mussaenda frondosa*, and *Curcuma domestica* was effective in suppressing the growth of *S. aureus* in vitro.

The results of this investigation supported the possibility of *Staphylococcus aureus* growth inhibition by the 3:2:1 combination formulation of *Ageratum conyzoides* leaves, *Mussaenda frondosa*, and *Curcuma domestica*. The results of these tests still need to be confirmed in vivo, though, in order to pinpoint the starting dose that will have the most human-like effects.

This study demonstrates the possibility of testing the ideal formulation in vivo on test animals. Research on medicinal plants is typically conducted in part, and it is only sometimes validated in specific combination formulations. The maximum effect on curing the ailment will come from combining numerous medicinal plants. Additionally, according to Kholifah (2018), a blend of many medicinal herbs has a higher level of potential as an antibiotic. In the expectation that the analgesic efficacy of the combination formulation of *Ageratum conyzoides*, *Mussaenda frondosa*, and *Curcuma domestica* as a herbal therapy for postpartum infections can be understood, this opinion served as the foundation for the in vivo evidence in this investigation..

According to Panday et al. (2013), the analgesic effect is a therapeutic response to substances manufactured from natural ingredients or synthetic medications at specific levels that are capable of suppressing or lowering pain without making the user pass out. Accordingly, the final results of this study suggest that further research is required to determine the analgesic activity of the 3:2:1 formulation of *Ageratum conyzoides* leaf extract, *Mussaenda frondosa* leaf, and *Curcuma domestica* in order to determine its efficacy as a postpartum infection therapy.

METHOD

A true experimental post-test only control group design was used in the research, and 36 pregnant female Balb/c strain mice weighing 20–28 grams served as the test subject. *Ageratum conyzoides*, *Mussaenda frondosa*, and *Curcuma domestica* leaves were combined in a 3:2:1 formulation with the 40%, 60%, and 80% design dilution levels of the research therapy. Counting *Staphylococcus aureus* colonies and measuring vaginal mucosal fluid production in postpartum mice treated with the bacteria yielded observational data. Then weigh the urogenital tract of the test animals both before and after giving them RPMI medium. One-way analysis using the SPSS 22 program is the data analysis technique utilized to evaluate the hypothesis.

Autoclaves, surgical supplies, operating tables, pins, 1000-ml beakers, 500-ml beakers, 250-ml beakers, 100-ml beakers, 50-ml beakers, 500-ml Erlenmeyer flasks, 250-ml Erlenmeyer flasks, and stirrers were among the equipment utilized in this investigation. Iron, glass stirrer, glass funnel, petri dish, magnetic stirrer, micropipette, microtubule, volumetric, pipette pump, dropping pipette, digital balance, scissors, tray, gas burner, office supplies, cloth napkins, cupboard ice, buckets, stomach sonde, drinking bottles, animal cages, and feeders.

The materials used in this research phase included extraction of *Ageratum conyzoides* leaves, *Mussaenda frondosa* leaves, *Curcuma domestica* rhizomes, nutrient agar (NA), nutrient broth (NB), RPMI, pure culture of *Staphylococcus aureus*, chloramphenicol, distilled water, 70% alcohol, methanol, cotton, vaseline, filter paper, cover paper, gauze, label paper, blotting paper, rubber bands, drinking water and animal feed, lysol, laundry soap, cotton buds, and aluminum foil, and mice.

Combination formula preparation and extract phytochemical testing

Ageratum conyzoides leaves, *Mussaenda frondosa* leaves, and *Curcuma domestica* are processed into simplicia as the first step in creating the combination formula. This is done by gathering the raw materials, sorting them using water, washing, and drying the raw materials until they are ready for processing. subsequently comes the stage of phytochemical testing.

To demonstrate the presence of secondary metabolites in this study, flavonoid compounds with orange, brick red, pink precipitate parameters, and crimson were tested in the phytochemical test phase using extracts from *Ageratum conyzoides* leaves, *Mussaenda frondosa* leaves, and *Curcuma domestica*. Additionally, three test methods—Meyer's test, Dragendroff's test, and Bouchardat's test—have been developed to determine the amount of alkaloid chemicals present in extracts of *Ageratum conyzoides* leaves, *Mussaenda frondosa* leaves, and *Curcuma domestica*. Following that, a test for the presence of tannin and phenolic compounds using black-brown and black-blue color criteria was conducted as part of the extract's phytochemical analysis. The terpenoid content test also includes a triterpenoid test with orange and brownish orange parameters as well as a steroid test with bluish green parameters. The final phytochemical experiment involved measuring saponins, which are characteristics of persistent foam..

Analgesic Potency Analysis of Combination Formulation 3:2:1

Ageratum conyzoides leaves, *Mussaenda frondosa* leaves, and *Curcuma domestica* were combined in a 3:2:1 formulation using the research treatment's design dilution levels of 40%, 60%, and 80%. Use a 1 cc syringe to inject up to 5×10^7 cfu of *Staphylococcus aureus* into each mouse once between 0 and 12 hours after delivery or right away, and then collect samples using the vaginal lavage method to calculate the postpartum infection index based on the number of *Staphylococcus aureus* colonies in the mice. In all study units, the infection index was measured from the first day after giving birth to the eleventh day following treatment. Sampling by the vaginal lavage method was carried out by collecting secretions from the genital tract of mice. Infection of the vaginal mucosa using the lavage technique can be done by collecting vaginal fluids (Azizah, 2017). The vaginal lavage method was performed without anesthesia by placing the mouse on the palm of the hand and bending its neck between the thumb and forefinger, while the tail of the mouse was placed between the fourth and fifth fingers. The mice were allowed to urinate, and then the vulva of the mice was cleaned using cotton buds that had been moistened with sterile PBS. Next, insert 50 μ l of saline or PBS into the vagina of the mice and then reinsert it repeatedly, four to five times, using a pipette.

RESULTS AND DISCUSSION

Test Results for Combination Formulation Extract 3:2:1

The first stage of testing the 3:2:1 combination formulation of *Ageratum conyzoides* leaf extract, *Mussaenda frondosa* leaf, and *Curcuma domestica* against *Staphylococcus aureus* was testing the secondary metabolite results of the three natural ingredients, which are shown in Table 1.

Tabel 1
Phytochemical Screening Test Results for *Ageratum conyzoides* Leaves, *Mussaenda frondosa* Leaves, and *Curcuma domestica*

No.	Compound identification	Parameter	Result		
			<i>Ageratum conyzoides</i>	<i>Mussaenda frondosa</i>	<i>Curcuma domestica</i>
1.	Flavonoid	Orange, Brick Red, Pink, Dark Red	(+)	(+)	(+)
2.		Alkaloid			
	Meyer	White Precipitate	(+)	(-)	(+)
	Dragendorff	Orange deposit	(-)	(-)	(+)
	Bouchardat	Chocolate Precipitate	(+)	(-)	(+)
3.	Tanin/Fenol	Dark Brown, Dark Blue	(+)	(+)	(+)
4.		Terpenoid			
	Steroid	Bluish Green	(-)	(-)	(-)
	Triterpenoid	Orange, Brownish Orange	(+)	(+)	(+)
5.	Saponin	Permanent Foam	(+)	(+)	(+)

The criteria for the development of orange, brick red, pink, and dark red precipitates are indicators of the flavonoid compounds in the leaves of *Ageratum conyzoides*, *Mussaenda frondosa*, and *Curcuma domestica*, according to data from secondary metabolite studies in Table 1 above. These three natural substances contain flavonoid components that may serve as antibacterial agents. This is based on research by Odeleye et al. (2014), who discovered that flavonoids may have a therapeutic antibacterial effect by evaluating the methanol extract of *Ageratum conyzoides* leaves against *E. coli*, *S. dysenteritae*, *S. aureus*, and *P. aeruginosa*. *Ageratum conyzoides* leaves, *Mussaenda frondosa* leaves, and *Curcuma domestica* leaves all passed additional content tests for tannins, triterpenoids, and saponins, indicating that these three natural ingredients contain secondary metabolites, one of which is known to have medicinal properties. several human diseases of various kinds. According to Titis et al. (2013), secondary metabolite chemicals that plants possess are bioactive components connected to the chemical makeup of these plants, allowing for the use of these plants as therapeutic ingredients for a variety of ailments. According to (Zaini, et al., 2022), bioactives found in natural foods have a significant potential for use in the food sector or as therapeutic agents for other metabolic illnesses. (Kalita, et al., 2016; Nguyen, et al., 2017; Indrawati, et al., 2022; Abdel-Naime et al., 2020).

Additionally, a 3:2:1 formulation of *Ageratum conyzoides* leaves, *Mussaenda frondosa* leaves, and *Curcuma domestica* was used in the laboratory experiment stage to examine the antibacterial effectiveness in vitro. This study's main objective was to assess how these three natural components' secondary metabolites affected *Staphylococcus aureus*. The number of colonies before and after treatment was one of the metrics this study examined to assess antibacterial inhibition. *Ageratum conyzoides* leaves, *Mussaenda frondosa* leaves, and *Curcuma domestica* were combined in a 3:2:1 ratio, and the combination's effectiveness was tested both before and after *Staphylococcus aureus* infection.

Analgesic Effects on Staphylococcus aureus Infected Postpartum Mice

According to research done by Azizah (2017), the analgesic effect of *Ageratum conyzoides* leaf extract, *Mussaenda frondosa* leaf, and *Curcuma domestica* was tested orally using the stomach sonde method in postpartum mice at a dose of 0.4 ml/mice 3 times per day for 10 days. Chloramphenicol served as the study's positive control. Because it is an antibiotic that can be used to treat bacterial infections, chloramphenicol is utilized.

Based on the growth of the number of *Staphylococcus aureus* colonies on NA medium, the treatment data of the combination of *Ageratum conyzoides* leaf extract, *Mussaenda frondosa* leaf, and *Curcuma domestica* formulation on the number of *Staphylococcus aureus* colonies in the 3:2:1 combination was calculated. The data is then presented in the form of a recapitulation table as necessary. noticed in Table 2.

Tabel 2

Data on Analgesic Effect of 3:2:1 Combination Formulation of *Ageratum conyzoides* Leaf Extract, *Mussaenda frondosa* Leaf, and *Curcuma domestica* in Postpartum Mice Infected with *Staphylococcus aureus*

Treatment	Standard deviation of <i>S. aureus</i> colony counts			Mice's typical vaginal mucosal fluid secretion index		
	Before	After	Mean Difference	Before	After	Mean Difference
P1 (+)	371,2	346,2	25	0,15	0,25	0,09
P2 (-)	379,4	378,4	1	0,16	0,31	0,16
P3 (40%)	344,8	179,6	165,2	0,17	0,21	0,05
P4 (60%)	367,8	180,8	187	0,16	0,21	0,06
P5 (80%)	382,4	164	218,4	0,18	0,26	0,07

Data on the analgesic effect of the 3:2:1 combination formulation of *Ageratum conyzoides* leaf extract, *Mussaenda frondosa* leaf, and *Curcuma domestica* in post partum mice infected with *Staphylococcus aureus* in Table 2. After administration of the 3:2:1 combination formulation, it was then analyzed statistically using One Way ANAVA to determine the significance of the effect of the variables in the study, namely by the ANOVA statistical test as shown in Table 3. The table of results of the analysis of variance on the number of *S. aureus* colonies shows the Fcount value with p-value = 0.008, where the p-value < α ($\alpha = 0, 05$) or at a significance level of 1%. Based on these data it can be interpreted that the treatment of the 3:2:1 combination formulation on *Staphylococcus aureus* had a significant effect. Then the results of the analysis of variance on the vaginal mucosal fluid secretion index of mice infected with *Staphylococcus aureus* had an Fcount with a p-value = 0.000, where the p-value was < α ($\alpha = 0.05$) or at a significance level of 1%. Based on these data it can be interpreted that the treatment of the 3:2:1 combination formulation on *Staphylococcus aureus* had a significant effect.

Tabel 3

ANOVA Test Results Data on Analgesic Effects

	Between Groups		Within Groups	
	Colonies of <i>S. aureus</i>	Fluid secretion index	Colonies of <i>S. aureus</i>	Fluid secretion index
Sum of Squares	214.408.000	.037	38.198.000	.040
df	4	4	20	20
Mean Square	53.602.000	.009	1.909.900	.002

F	28.065	4.710
Sig.	.000	.008

To find the most suitable and efficient concentration for reducing the growth of the number of *Staphylococcus aureus* colonies, the analysis was furthered with the 1% Duncan test. The Duncan test was used, as shown in Table 4, to determine the value of the difference in significance at each level of study therapy. When P1 (Chlorampenicol) was used as a positive control study, the results of the 1% Duncan test for the combination formulas P3, P4, and P5 revealed a significant difference. In other words, the 3:2:1 combination formulation treatment concentration was more successful at reducing the quantity of *Staphylococcus aureus* colonies. This demonstrates that the concentration of P3 (40%) is ideal and effective for preventing *Staphylococcus aureus* growth in the

Tabel 4
Results of Duncan's Test 1% After being given a Combination Formulation on the Number of Colonies
Staphylococcus aureus

Treatment	N	Subset for alpha = 0.05
P5 (80%)	5	164.00 _a
P3 (40%)	5	179.60 _a
P4 (60%)	5	180.80 _a
P1 (+)	5	346.20 _b
P2 (-)	5	378.40 _b

Based on the analysis of Duncan's test results in Table 4 with a significant level of 1%, it is clear that all treatment levels of the extract concentrations of the 3:2:1 combination formulation of *Ageratum conyzoides* leaves, *Mussaenda frondosa* leaves, and *Curcuma domestica* differ significantly from both the positive and negative controls of the study, whereas P3 (40%), P4 (60%), and P5 (80%) have no significantly different effects. This means that the *Staphylococcus aureus* colony index in the vaginal mucosa of infected mice can be suppressed at a concentration of 40%. The results of the data analysis are shown in Table 3. It can be inferred that a concentration of 40% is more effective than a greater concentration at preventing *Staphylococcus aureus* colonies from growing in infected mice's vaginal mucosa.

Moreover, Duncan's 1% test was used to carry out further investigation to establish the ideal and efficient concentration in the vaginal mucosal fluid secretion index of postpartum mice infected with *Staphylococcus aureus*. The Duncan test is used, and the results are shown in Table 5, to determine the significance of the difference at each level of study therapy.

Tabel 5
Results of Duncan's Test 1% After being given a Combination Formulation on the Number of Colonies
Staphylococcus aureus

Treatment	N	Subset for alpha = 0.05
P3 (40%)	5	.0460 _a
P4 (60%)	5	.0580 _a
P5 (80%)	5	.0740 _a
P1 (+)	5	.0940 _a
P2 (-)	5	.1560 _b

Based on the results of Duncan's test analysis with a significant level of 1% in Table 5 above, it shows that the treatment with the largest extract concentration, namely P3 (40%) is the effective concentration in observation, while the concentration of the combination formulation 3:2:1 of

Ageratum conyzoides leaves, leaves *Mussaenda frondosa*, and *Curcuma domestica*, which have the optimum ability to secrete vaginal mucosal fluid in mice infected with *Staphylococcus aureus*, was at a concentration of P5 (80%).

According to the analysis results above, when compared to chloramphenicol, the study's positive control, the 3:2:1 formulation significantly slowed the growth of the colonies and the vaginal mucosal fluid index for *Staphylococcus aureus* infection. In general, it is understood that the 30% concentration in the 3:2:1 formulation is both the most ideal and the most effective concentration. This is demonstrated by the decreased growth of colonies and the vaginal mucosal fluid's resistance to *Staphylococcus aureus* infection.

Comparison of the analgesic effect of the combination extract of *Ageratum conyzoides* leaves, *Mussaenda frondosa* leaves, and *Curcuma domestica* can be seen descriptively in Figure 1.

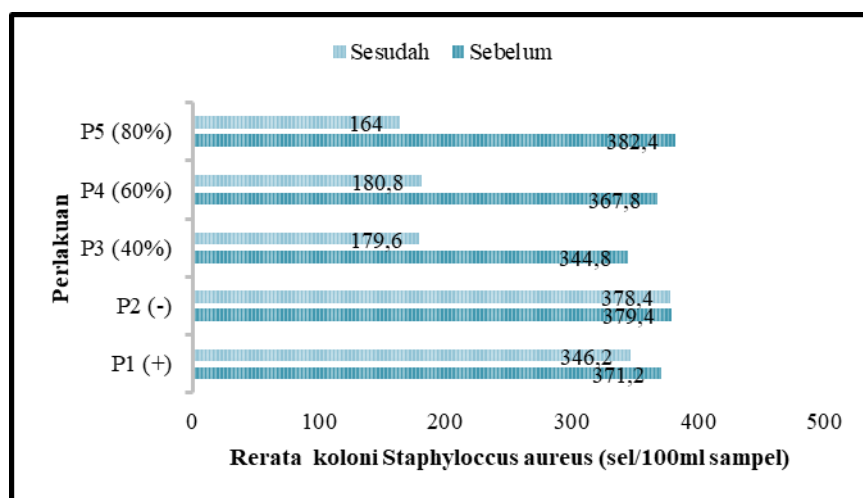


Figure 1. Mean Comparison of the Number of *Staphylococcus aureus* Colonies

In comparison to other doses, the 40% extract of the combination formulation had analgesic effects on robust bacterial inhibition in postpartum mice infected with *Staphylococcus aureus*. The ability of a 40% concentration of the combination formulation, which was stronger in reducing the growth of *Staphylococcus aureus* colonies after treatment, was what this study's analgesic impact was characterized by. Except for P2 as a negative control, all treatments resulted in a decrease in the overall number of *Staphylococcus aureus* colonies. This suggests that the secondary metabolites examined in this work may provide proof of the potent antibacterial properties of the leaves of *Ageratum conyzoides*, *Mussaenda frondosa*, and *Curcuma domestica*. *Ageratum conyzoides* leaves are more prevalent in the 3:2:1 formulation, and the tannins in these leaves induce bacterial cells to lyse. Due to tannins' targets on the polypeptide wall of the bacterial cell wall, the cell wall cannot fully form, which results in the death of the bacterial cell (Suwarni et al., 2016). Tannins have the power to stop bacterial enzymes from working and to obstruct the flow of proteins in the inner lining of cells, according to Ngajow et al. (2013). As a result, it is clear that this substance is among the most crucial ones for preventing bacterial development in this study. According to the findings of numerous studies, various antimicrobial mechanisms exist in metabolite compounds (Wertz & De Szalay, 2020). On target cells, they all generally have the same analgesic action. (Abdel-Naime et al., 2020; Sanjay et al., 2016; Jørgensen et al., 2018; Lu, et al., 2022).

Antibacterials contained in the leaves of *Ageratum conyzoides*, *Mussaenda frondosa* leaves, and *Curcuma domestica* in the phytochemical tests also have flavonoid compounds. The mechanism of action of flavonoid compounds as antibacterials is thought to be by forming complex compounds with extracellular and dissolved proteins, which can damage the bacterial cell membrane, followed

by the release of intracellular compounds. (Amalia et al., 2017). According to the claim (Manik et al., 2017), flavonoid chemicals that restrict bacterial growth damage bacterial cell walls, impair the function of enzymes, and subsequently bind to adhesins to cause bacterial cell membrane destruction. Data showing less *Staphylococcus aureus* colonies overall in the vagina of infected post-partum mice and fewer colonies overall in the test material sample of 100 ml further supported this. Comparative data on the typical reduction in vaginal mucosal fluid flow in post-partum infected mice following therapy (Figure 2) provide support for these findings.

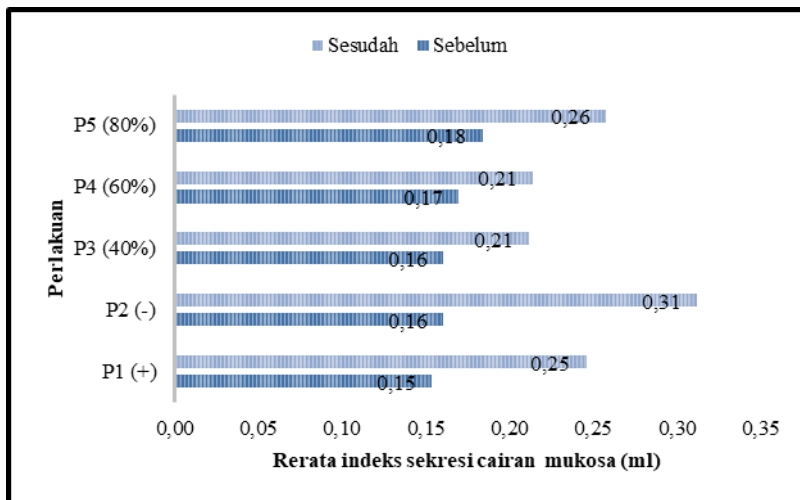


Figure 2. Mean Comparison of Vaginal Mucosal Fluid Index infected with *Staphylococcus aureus*

According to Figure 1, where a concentration of 40% exhibits a bigger average decrease in mucosal fluid secretion than other concentrations, equivalent to 0.05 ml, the data in Figure 2 demonstrate the applicability of the analgesic effect. *Staphylococcus aureus*-conditioned postpartum mice will become inflamed in response to infection or tissue damage after 24 hours (Phadmacanty et al., 2016). Antimicrobial activity, according to Wertz & De Szalay (2020), is a sign of which microorganisms are more adapted to particular mucosal tissues. The same is true for the antibacterial ingredients in this study's combination formulation. Additionally, according to Phadmacanty et al. (2016), *Staphylococcus aureus* is a bacterium with a respectable capacity for adaptation. It is also known to possess an exotoxin, which, when administered, can result in inflammation, cell cariorexis, and tissue necrosis. Amniotic fluid is present in vaginal infections at the time of delivery, and *Staphylococcus aureus* growth in this investigation spread to the uterine and paravaginal tissues. Because bacterial toxins produce an active transport system in the vaginal mucosa, mucous cells that are irritated will secrete more fluid.

Therefore, it is clear that the combination formulation of *Ageratum conyzoides*, *Mussaenda frondosa*, and *Curcuma domestica* at doses of 40%, 60%, and 80% has analgesic efficacy. Because they contain flavonoids, alkaloids, tannins, triterpenoids, and saponins, the chemicals in the leaves of *Ageratum conyzoides*, *Mussaenda frondosa*, and *Curcuma domestica* have analgesic qualities. Because of this, it has a strong ability to stop the growth of *Staphylococcus aureus* germs, which is consistent with Irani et al.'s (2019) assertion that the saponins and flavonoids in *Ageratum conyzoides* leaves can prevent bacterial infections. Protein denaturation is the means by which saponins work to kill bacteria. In order to disrupt the bacterial cell membrane, flavonoids work as antibacterials by forming complex compounds with extracellular proteins and dissolved proteins. This damage is then followed by the release of intracellular chemicals. The bacterium *Staphylococcus aureus* is one of these. It is known from this that the dominant *Ageratum conyzoides* leaf extract was coupled with *Mussaenda frondosa* and *Curcuma domestica* leaf extracts, which had an impact on the growth of *Staphylococcus*

aureus bacteria. Additionally, because *Ageratum conyzoides* leaves and *Mussaenda frondosa* leaves both contain flavonoids, this is also possible.

According to a number of sources, the presence of inflammation indicates whether antigens were successful in infiltrating tissues (Belkaid & Hand, 2014). Vaginal mucosal tissue invasion is one example of this (Brannon et al., 2020; Hashimoto et al., 2021). When the vaginal mucosal tissue secretes mucus, it is a sign of toxic shock and a marker for the presence of antigens that have managed to get past the vaginal mucosal tissue's non-specific defense system (MacPhee et al., 2013; Kumamoto & Iwasaki, 2012; Balakrishnan et al., 2012). Enzymes associated with their hazardous characteristics are produced under these circumstances (Akhrass et al., 2019). In this study, *Staphylococcus aureus* infection in the vagina infiltrated the uterine tissue and persisted in the paravaginal region, which in turn caused adhesion and colonization of the mucosal tissue. When invasion occurs, the antibodies stimulate the secretion of vaginal mucosal fluid as a form of resistance to the antigens. Mucous fluid secreted by vaginal mucosal tissue was reported by Chase & Kaushik, 2019 to contain s_IgA, where IgA is an isotype of immunoglobulin (Brandtzaeg, 2009; Zheng et al., 2020). Furthermore, IgA binds to the polymeric immunoglobulin receptor (pIgR) carrying out an antibody transporter (Turula & Wobus, (2018)), which is expressed on the basolateral surface of vaginal mucosal tissue (Bakema & Van Egmond, 2011).

Due to the greater *Ageratum conyzoides* composition in the 3:2:1 formulation, the secondary metabolites, such as alkaloids and other phenolic compounds, are also more potent. According to Tungmunnithum et al. (2018), flavonoid phenolic chemicals function as protein coagulants, whereas tannin polyphenols alter cell permeability until cell lysis and necrosis take place (Bouhlali et al., 2020). Reduced mucus secretion during infection, which results from enzyme inactivation and genetic material function loss, is a hallmark of the analgesic effect.

Ageratum conyzoides leaf extract, *Mussaenda frondosa* leaf, and *Curcuma domestica* rhizome were combined in a 3:1:2:1 ratio and were shown to have analgesic activity in postpartum mice infected with *Staphylococcus aureus*. This formulation was also successful at 40%, which contributed to the research's conclusions.

LIMITATION OF THE STUDY

This study was limited to a number of things, including, namely, Balb/c mice with the sex of a pregnant female, \pm 3-4 weeks old, \pm 25 gr body weight. Then, this study was limited to efforts to determine the potential effect of the combined extract of *Ageratum conyzoides* leaves, *Mussaenda frondosa* leaves, and *Curcuma domestica*, the optimal concentration level for inhibiting the growth of *Staphylococcus aureus* bacteria, and the length of time needed for infection healing..

CONCLUSIONS AND SUGGESTIONS

In accordance with the study's findings, postpartum mice infected with *Staphylococcus aureus* respond analgesically to a combination formulation of *Ageratum conyzoides* leaf extract, *Mussaenda frondosa* leaf, and *Curcuma domestica* in a ratio of 3:1:2, which is also effective in the 40% combination formulation.

ETHICAL CONSIDERATIONS

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Conflict of Interest Statement

With regard to this article, we certify that there are no actual or possible conflicts of interest.

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