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Fluid Secretion of Fallopian Tube Epithelial Cells of Mice (*Mus musculus*) BALB/C with *Pelvic Inflammatory Disease* (PID) Infected with *Candida albicans* Post Therapy with *Myrmecodia sp*. Jack Extract

Pratisa Delfiera Ajiza(*), Noor Hujjatusnaini, Ayatussa'adah Ayatussa'adah

Program Studi Biologi Fakultas Tarbiyah Dan Ilmu Keguruan
Institut Agama Islam Negeri Palangka Raya
Jl. Damang Salilah I, Palangka Raya, Kalimantan Tengah, Indonesia, Postcode 73112

***Corresponding Author:** ajizapratisa@gmail.com

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Abstract

This study examines the therapeutic potential of Myrmecodia sp. extract in treating Pelvic Inflammatory Disease (PID) induced by Candida albicans in Balb/c mice. The study used a true experimental design with a post-test group. The method involved administering Myrmecodia sp. extract with various concentrations (from 0.4 to 3.2 %) to female Balb/c mice suffering from PID. Observations included clinical indicators of infection, the number of bacterial colonies, and histopathological changes in the epithelial cells of the fallopian tube mucosa. The results showed that Myrmecodia sp. contains flavonoids, tannins, terpenoids, and saponins, which have antimicrobial and anti-inflammatory properties. The mice that were infected with Candida albicans had a lot fewer bacterial colonies after being treated with Myrmecodia sp. extract, but it did not completely get them back to how they were before the infection. The ANOVA test showed that there were important differences between the treatment groups, which shows that Myrmecodia sp. works as an extra treatment for PID. These results confirm the potential of Myrmecodia sp. as an alternative herbal therapy for PID, although further research is needed to improve its effectiveness and understand its mechanism of action.

Keywords: *Antimicrobial; Candida albicans; Myrmecodia sp; Pelvic Inflammatory Disease; Herbal Therapy*

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INTRODUCTION

The island of Borneo is home to a wide variety of plants. People have used traditional medicine to treat ailments caused by plants found in Borneo's forests. The process of

utilizing medicinal plants commences with the collection of plants from their natural habitats, followed by the subsequent processing until they are prepared for medical use. Medicinal plants are typically harvested directly from their natural habitats and subsequently processed until they are suitable for use (Aryadi & Fithria, 2014).

Typically, the plant's roots, leaves, or fruit are employed. Nevertheless, this has not been adequately documented. Currently, people solely rely on the inherited local wisdom from their ancestors, which they continue to use today (Umaternate et al., 2022). The Sarang Semut *Myrmecodia sp.* plant is one of the numerous plant species that are frequently encountered in Central Kalimantan but have yet to be extensively introduced as references or direct learning sources. This plant is recognized for its potential therapeutic benefits in the treatment of a variety of conditions, including cancer, gout, liver disorders, strokes, heart problems, hemorrhoids, back pain, and allergies. Researchers led by Dr. Ir. M. Ahkam Subroto M.App.Sc., who focuses on studying ant nest plants for human health, found that this plant has 14 chemicals from the flavonoid and tannin groups (Astuti et al., 2021; Lisnanti & Fitriyah, 2017). These chemical compounds, which include triterpenoid, flavonoid, saponin, quinone, tannin, carbohydrate, and glycoside compounds, as well as numerous other minerals, have also been identified by numerous other researchers (Rana et al., 2014).

Antibacterial compounds that can serve as therapeutic agents for Pelvic Inflammatory Disease (PID) are believed to be present in *Myrmecodia sp.* ant nests, according to a literature review (Rumaolat, 2021). The PID often caused by infection with microorganisms such as *Candida albicans*, the PID is an infection of the female reproductive organs, including the cervix, uterus, and ovaries, in the fallopian tubes. Infection of the vagina or cervix by bacteria results in the PID (Ellen et al., 2024; Sudiono, 2019). 750,000 women were diagnosed with the acute PID in 2016, as indicated by WHO data (Oktarina et al., 2024). Around 40 – 45 % of the Indonesian populace is affected by the PID. The number of patients increases by approximately 70 % annually. Sexually transmitted infections are one of the most prevalent causes of the PID. The PID is frequently the result of a bacterial infection that progresses from the vagina or cervix to the uterus, fallopian tubes, and ovaries (Han et al., 2020; Yagur et al., 2021). Antibacterial and anti-inflammatory properties have been demonstrated in numerous prior studies regarding ant nests (Myrmecodia sp., Jack). The potential of ant nests in the treatment of the PID caused by *Candida albicans* infection has not been specifically examined in any study. A fungus known as *Candida albicans* is responsible for a variety of inflammations, including mucosal candidiasis and opportunistic inflammation (Ghojoghi et al., 2024; Ibe & Pohl, 2024). *Candida albicans* fungus proliferation can result in infection, and if it is excessive in the vagina, it can contribute to the PID, which typically occurs in small quantities (Camacho et al., 2024). For example, long-term illnesses like AIDS, diabetes, thyroid problems, and taking corticosteroids and cytostatics can also lead to *Candidiasis* (Hujjatusnaini et al., 2024; To et al., 2015).

There is still limited research on how ant nests influence the secretion of epithelial cell fluid in the fallopian tube mucosa of Balb/c mice with Pelvic Inflammatory Disease (PID) caused by *Candida albicans* infection following

Myrmecodia sp. therapy. Therefore, this study aims to examine the potential of antibacterial compounds in ant nests as a therapy for the PID. Specifically, researchers have explored the activity of the *Myrmecodia sp.* species in the healing process of PID caused by *Candida albicans* (Lisnanti et al., 2024). By understanding medicinal plants that are typical of the region, the community can utilize them without damaging the existing natural potential wisely and sustainably to improve health and welfare.

METHOD

The research method used a true experimental posttest group design. The Microbiology Laboratory of Tadris Biologi, IAIN Palangka Raya, conducted this research. The materials used in this study were simplisia. We obtained ant nests of the *Myrmecodia sp.* type from Murung Raya Regency, Central Kalimantan. The manufacture of simplisia and pure extracts of ant nests began with selecting healthy, clean ant nests, then cleaning them with clean water and drying them in the shade or using an oven at a temperature of 40 – 50 °C. Furthermore, the ant nests were cut into small pieces so the drying process was faster and stored in an airtight container in a dry place, with a total weight of 2500 grams.

We then used the maceration method with 96 % ethanol for 24 hours at concentrations of 0.4 %, 0.8 %, 1.6 %, and 3.2 %. We used 24 female Balb/c mice that were about 3 – 4 weeks old and weighed ± 28 grams each. We also used 70 % and 96 % alcohol, gauze, cotton buds, cover paper, tissue rubber, label paper, Lysol, and *Candida albicans* from the IAIN Palangka Raya Laboratory. The chemicals used for the analysis included chloroform, standard gallic acid solution, Folin-Ciocalteu reagent, sodium carbonate solution (Na_2CO_3), gallic acid, Vaseline, SDA, NB, and amoxicillin.

Figure 1. Documentation of the manufacture of pure ant nest extract (*Myrmecodia sp*)

The equipment used in this study were 15 cm petri dishes, test tubes, probes, 1 mm syringes, analytical scales, 500 ml measuring flasks, beakers, micropipettes, 2 ml microtubes, colony counters, centrifuges, measuring cylinders, Erlenmeyers, autoclaves, incubators, LAF (Laminar Air Flow), spirit lamps, matches, magnetic stirrers, and hot plates.

Qualitative Test of Secondary Metabolite Compound Content

The results of phytochemical analysis showed that the water extract from the ant nest contained flavonoids, tannins, saponins, and terpenoids. The quantitative test in this study included antioxidant test. Antioxidant activity was measured using a spectrophotometric method with DPPH (2,2-diphenyl-1-picrylhydrazyl) free radicals. *Myrmecodia sp.* extracts were prepared in various concentrations (0.4 %, 0.8 %, 1.6 %, and 3.2 %). We mixed a total of 1 mL of each extract concentration with 3 mL of 0.1 mM DPPH solution, which we had dissolved in methanol. The mixture was then incubated in the dark for 30 minutes. We measured the absorbance at a wavelength of 517 nm using a spectrophotometer.

Quantitative Test of Bioactive Compounds

The quantitative test in this study included antioxidant activity test calculated in the form of DPPH inhibition percentages. An extract of *Myrmecodia sp.* at a concentration of 1 mg/mL was used for the qualitative secondary metabolite compound content tests to find active compounds, such as flavonoids, tannins, alkaloids, and saponins. The flavonoid test was carried out by adding 1 mL of extract to 1 mL of concentrated HCl and some magnesium powder. If an orange-red color appeared, it indicated the presence of flavonoids. Using Dragendorff or Mayer reagents, 1 mL of extract was mixed with 2 mL of reagent to make an alkaloid test. If a precipitate formed, it meant that alkaloids were present. The saponin test was carried out by shaking 5 mL of extract for several minutes; if stable foam was formed, it indicated the presence of saponins. For the tannin test, 1 mL of extract was mixed with 2 mL of 1 % $FeCl_2$ solution. If the color changed to dark green or blue, it meant that tannins were present (Widyastuti & Sundaryanto, 2017).

Sample or Participant

We induced a *Candida albicans* infection in mice by dissolving 1 ml in a 0.1 % peptone solvent at pH 4.5. We conditioned mice to experience the Pelvic Inflammatory Disease (PID) with *Candida albicans* infection for 4 days before treating them with *Myrmecodia sp.* extract. On the fifth day, clinical observations were made on the surface of the mice's vagina to detect symptoms of infection. The mice's vagina showed clinical signs such as reddish-white color, irritation, and swelling, as well as a total colony of *Candida* fungi infection of more than 10^3 CFU/mL, indicating the PID. We quantitatively recorded clinical observation data using a Likert scale (Table 1) (Breyer et al., 2017; Hubbard, 2010).

A gastric tube was used to give each group 400 mg/kg BW of *Myrmecodia sp.* extract orally in Group I got 21 mg/day (400 mg/kg BW = 6 mg), Group II got 27 mg/day (800 mg/kg BW = 12 mg), Group III got 50.4 mg/day (1600 mg/kg BW = 39 mg), and Group IV got 63 mg/day (3200 mg/kg BW = 48 mg). The extract was given 3 times a day at a dose of 0.6 mL for 7 days. All of the test animals had surgery on the eighth day to see how much morphological irritation there was in mice with pelvic inflammation after treatment, using the MLIL (Modified Light Indicator Lamp) method. This method used the principle of lighting or indicator lamps that provided

visualization related to changes in tissue morphology in mice, such as to see signs of irritation or inflammation.

Table 1. Quantitative Indicators of Clinical Observation of Infection

Indicator	Scale	Information
Vagina Color	1	Very red
	2	Red
	3	A bit ed
	4	Normal
Vaginal Irritation	1	Very swollen
	2	Swollen
	3	A bit swollen
	4	Normal

Phytochemical Test of *Myrmecodia sp.* Extract

The phytochemical test aimed to identify active compounds that had the potential to be used in pelvic inflammatory therapy. This study explored secondary metabolite compounds in the extract that might have antimicrobial effects on fallopian tube infections in vivo. Table 2 shows the results of this test which are expected to be a solid basis for the development of effective herbal therapies.

Tabel 2. Compound of Secondary Metabolite

Secondary Metabolite Group	Result
Flavonoid Test	+
Alkaloid Test	-
Tannin Test	+
Terpenoid Test	+
Saponin Test	+

Information:
(+) : Detected to contain the tested compound
(-) : Detected not containing the tested compound

Table 2 shows the results of phytochemical tests on the extract. It indicates that it contained several secondary metabolite compounds that had the potential to have therapeutic effects. Tests for flavonoids, tannins, terpenoids, and saponins came back positive. These compounds were found in the extract. These compounds were known to kill microbes, reduce inflammation, and protect cells from damage. Conversely, the alkaloid test yielded negative results, indicating the absence of alkaloid compounds in the extract. The presence of these compounds showed the potential of the extract as a herbal therapy ingredient for the treatment of infection and inflammation.

Antioxidant Content Test of *Myrmecodia sp.* Extract

Figure 2 shows that the antioxidant test of the extract was carried out to strengthen the data on the antimicrobial potential of the *Myrmecodia sp.* extract, according to the research concentration level.

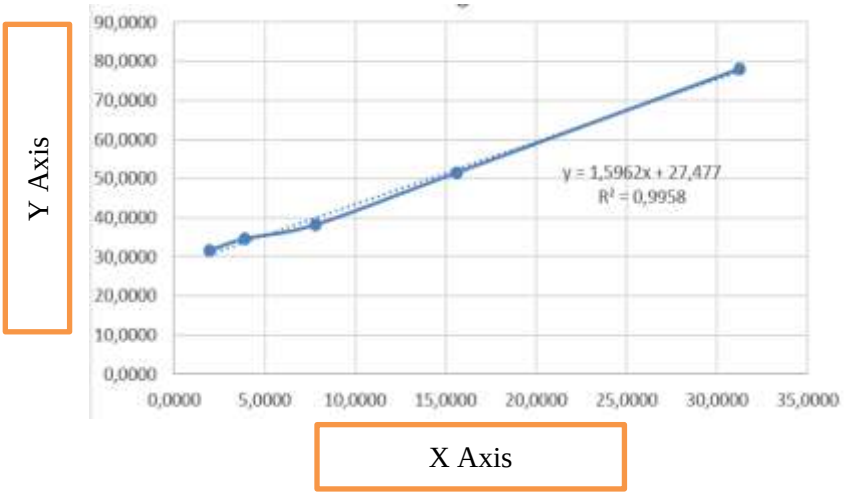

Figure 2. Antioxidant Content

Figure 2 shows that the graph shows a positive linear relationship between the two variables. The regression equation obtained was y = 1.5962x + 27.477. Every 1 unit increased, the variable (x) increased the variable (y) by 1.5962 units, with an initial value of (y) 27.477 when x = 0. The coefficient of determination (R^2 = 0.9958) shows that this model was very good at explaining the relationship, with 99.58% of the variation in data (y) being explained by it. The data pattern was clearly depicted follows the regression line so it was used for predictions to strengthen the strong linear relationship between the two variables.

Data analysis

Tabel 3. Normality Test of *Candida albicans*. Fungal Colony Data in PID Mice's Vagina

Treatment		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
Treatment Data	Before infected with *Candida albicans.*	255	6	200*	846	6	.147
	After infected with *Candida albicans.*	164	6	200*	940	6	.659
	After being given *Myrmecodia sp.* extract	154	6	200*	946	6	.707

*. This is a lower bound of the true significance.
Lilliefors Significance Correction

The Kolmogorov-Smirnov and Shapiro-Wilk tests for data normality show that the data were not all distributed the same way in all three treatment conditions. In the condition before being infected with *Candida albicans.*, the significance value of the Shapiro-Wilk test was 0.147, which meant that the data was not normally distributed. However, after being infected with *Candida albicans.*, the significance value increased to 0.659, indicating that the data was normally distributed. The condition after being

given ant nest extract also showed a significance value of 0.707, confirming that the data was normally distributed. Based on these results, it can be concluded that most of the data (two of the three treatments) were normally distributed, namely in the condition after being infected and after being given the ant nest extract treatment, while the data before being infected showed an abnormal distribution.

Table 4. Therapeutic Effect of PID Extract of *Myrmecodia sp.* based on Data of Vaginal Colonies of Mice with PID using Anova

Anova Treatment_Data	**Sum of Squares**	**df**	**Mean Square F**		**Sig.**
Between Groups	148265.333	2	74132.667	12.276	.001
Within Groups	90584.417	15	6038.961		
Total	238849.750	17			

The one-way ANOVA test showed that PID therapy with extract from *Myrmecodia sp.* had a big impact on the data of mouse vaginal colonies. The F-statistic value of 12.276 with a significance value (Sig.) of 0.001 (<0.05) indicated a significant difference between treatment groups. Variation between groups (Between Groups) had a Sum of Squares of 148265.333 with a Mean Square of 74132.667, while variation within groups (Within Groups) had a Sum of Squares of 90584.417 and a Mean Square of 6038.961. Based on data from mice's vaginal colonies, these results show that *Myrmecodia sp.* extract had a big impact on PID therapy (with a total variation of 238849.750).

Table 5. Therapeutic Effect of PID Extract of *Myrmecodia* sp. Based on Data of Vaginal Colonies of Mice with PID using Anova

Duncan[a]		**Subset for α = 0.05**	
Treatment	**N**		
Before being infected with *Candida albicans.*	6	9.2500	
After being given *Myrmecodia* sp. extract	6	2.9167	
After being infected with *Candida albicans.*	6		253.5833
Sig.		.936	1.000

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 6.000.

The results of Duncan's post hoc analysis showed that the average *Candida albicans.* colony in mice before infection was 59.25, while after being given ant nest extract, it increased slightly to 62.92. These two conditions did not show a significant difference with a significance value of 0.936. However, the average *Candida albicans*. colony in infected mice increased sharply to 253.58, which was significantly higher than the other two conditions (significance value = 1.000). These results indicated that *Candida albicans*. infection significantly increased the number of colonies, while administration of ant nest extract was able to reduce the number of colonies approaching the initial conditions before infection.

Table 6. Normality Test of Data Differences in PID Mice Vaginal Colonies

		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Treatment	**Statistic**	**df**	**Sig.**	**Statistic**	**df**	**Sig.**
Treatment_Data	Before soaking in Rockwell Solution	.254	5	200*	.818	5	.112
	After soaking in Rockwell solution	.250	5	200*	.939	5	.661

*. This is a lower bound of the true significance.
a. Lilliefors Significance Correction

The results of the normality test on the treatment data before and after being soaked with Rolwell solutions using the Kolmogorov-Smirnov and Shapiro-Wilk methods showed that all data were normally distributed. The data was normally distributed prior to the soaking process. The results of the Kolmogorov-Smirnov test provided a statistical value of 0.254 with a degree of freedom (df) of 5 and a significance value (Sig.) of 0.200. Meanwhile, the results of the Shapiro-Wilk test showed a statistical value of 0.818 with a df of 5 and a significance value of 0.112. For data after being soaked, the Kolmogorov-Smirnov test provided a statistical value of 0.250 with a df of 5 and a significance value of 0.200. The Shapiro-Wilk test showed a statistical value of 0.939 with a df of 5 and a significance value of 0.661. Both test methods had significance values greater than 0.05, indicating that data before and after Rockwell solution soaking were normally distributed. This showed that the data met the assumption of normality as an important requirement in advanced statistical analysis.

Table 7. Therapeutic Effect of PID Extract of *Myrmecodia sp.* based on Data on the Difference in Vaginal Colonies of Mice with using Anova

ANOVA Treatment_Data	**Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Between Groups	29241.056	1	29241.056	16.434	.004
Within Groups	14234.625	8	1779.328		
Total	43475.681	9			

The results of the ANOVA analysis showed that there were significant differences between the treatment groups. The variation of data between groups had a sum of squares value of 29241.056 with a degree of freedom (df) of 1, while the variation of data within groups had a sum of squares value of 14234.625 with a df of 8. The total sum of squares was 43475.681 with a df of 9. The mean square between groups was 29241.056, while the mean square within groups was 1779.328. The resulting F ratio value was 16.434, with a significance value of 0.004. Because the significance value was less than 0.05, the differences between the treatment groups were statistically significant. The treatment given had a significantly different effect.

RESULT AND DISCUSSION

Therapeutic Effect of *Myrmecodia sp.* extract on Mice Suffering from PID

A total of 24 female Balb/c mice with Pelvic Inflammatory Disease (PID) were treated for 4 days. As a positive control, ciprofloxacin was used, a synthetic drug that has been proven effective in reducing the impact of infection in patients with pelvic inflammatory disease. The antimicrobial effect test of *Myrmecodia sp*. Jack extract was carried out after the mice showed signs of pelvic inflammation, which were indicated by changes in vaginal color, vaginal irritation, and the number of bacterial colonies in the vagina of mice after infection with *Candida albicans* culture suspension. The purpose of this test was to assess the potential of *Myrmecodia sp*. extract in overcoming infection and inflammation that occurs in mice with PID. Figure 3 shows the pelvic inflammatory infection indicators in mice suffering from PID. Comparison of clinical features based on the number of vaginal bacterial colonies before and after infection (Figure 3), the difference in vaginal bacterial colonies before and after being given Rockwell solution (Figure 4)

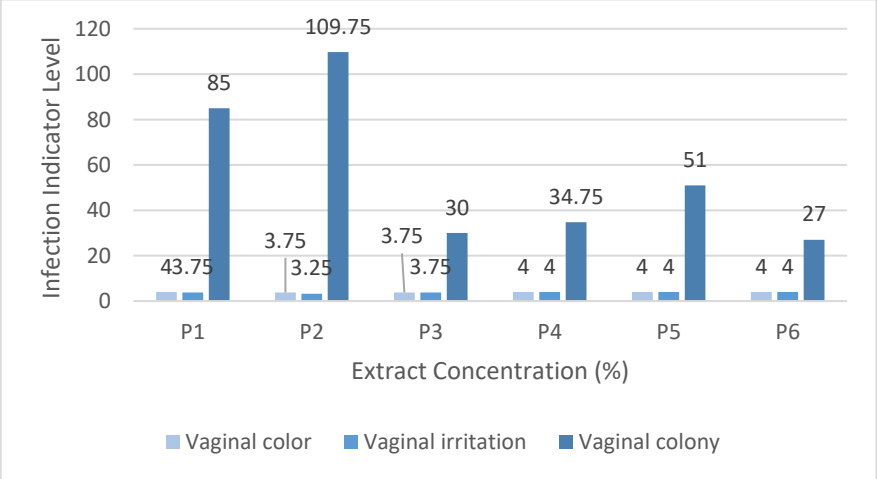

Figure 3. Pelvic Inflammatory Infection Indicators in Mice Suffering from PID

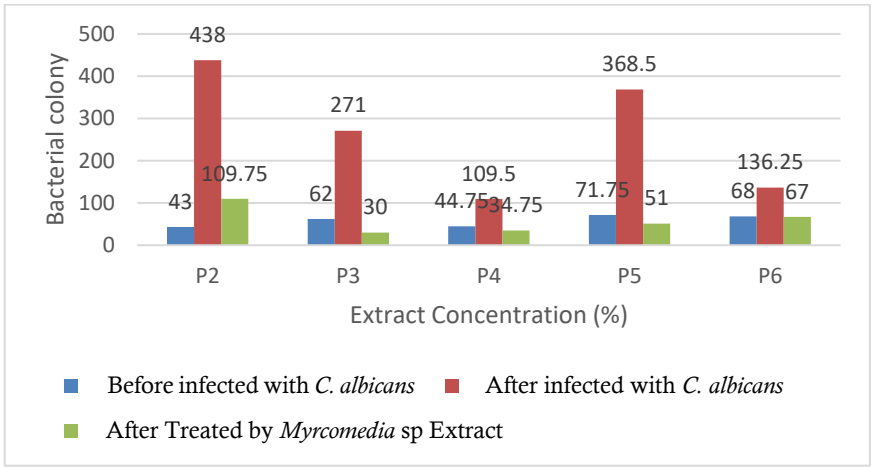

Figure 4. Vaginal Bacterial Colonies Before and After Infection, After Being Treated by *Myrcomedia* sp. Extract

Figure 4 above shows the effect of *Candida albicans.* infection and the effect of Ant Nest extract administration on several sample groups (P2, P3, P4, P5, and P6). This data provides important information about the differences in conditions before infection, after infection, and after administration of Ant Nest extract. Prior to *Candida albicans.* infection, the initial values in the sample groups indicated normal conditions. These values are relatively small and stable, such as in P2 (43 %), P3 (30 %), P4 (44 %, 75 %), P5 (71 %, 75 %), and P6 (68 %). This condition signifies a physiological state that remains unaffected by infection. After contracting *Candida albicans.*, the infection significantly increased the values in all groups. For example, in P2, the value increased drastically from 43 to 438 %, while P3 increased from 30 to 271 %, and P5 from 71.75 to 368.5 %. This spike in value indicates the negative impact caused by *Candida albicans.* infection. After administration of Ant Nest extract, the value decreased in each group compared to the condition after infection. For example, in P2, the value decreased from 438 to 109.75 %; in P3, from 271 to 62 %; and in P5, from 368.5 to 51 %. However, despite the significant decrease, the condition has not completely returned to the pre-infection state, except in group P6, where the value approached the initial state (68 before infection and 67 after administration of the extract).

These results indicate that *Candida albicans.* infection has a significant negative impact on the sample group, marked by a significant increase in the value (Kumamoto et al., 2020). Administration of Ant Nest extract has been shown to be effective in reducing the impact of infection, although it does not completely restore conditions to their initial values. The response varies in each group, which is likely due to differences in biological factors or immune responses. Ant Nest extract has the potential as a therapeutic agent to reduce the impact of *Candida albicans.* infection. Further research is needed to understand the mechanism of action of this extract and to increase its effectiveness so that it can completely restore conditions to pre-infection (Crisnaningtyas & Rachmadi, 2010). Research also needs to evaluate the factors that influence differences in response between groups.

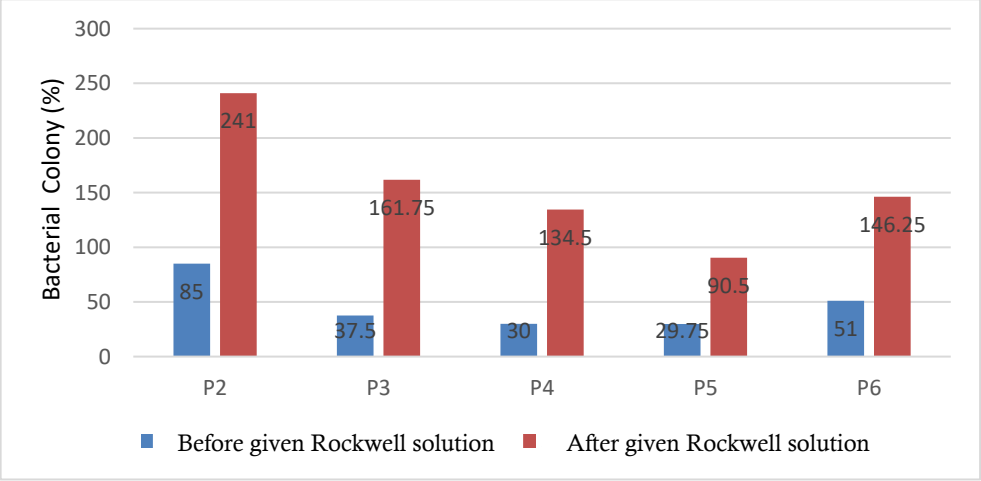

Figure 5. vaginal bacterial colonies before and after being given rockwell solution

The image above is a bar chart showing the number of vaginal bacterial colonies before and after being given Rockwell solution in five observation groups, namely P2, P3, P4, P5, and P6. The vertical axis (Y) represents the number of bacterial colonies, while the horizontal axis (X) shows the observation group. The blue color depicts the number of bacterial colonies before being given Rockwell solution, while the orange color shows the number of bacterial colonies after. The observation results indicate a significant increase in the number of bacterial colonies in all groups following the application of Rockwell solution. In group P2, the number of colonies increased from 85 to 241 %, which was the highest increase among all groups. Group P3 experienced an increase from 37.5 to 161.75 %, while group P4 increased from 30 to 134.5 %. Group P5 had an increase from 29.75 to 90.5 %, which was the smallest increase. Finally, group P6 showed an increase from 51 to 146.25 %. Overall, the administration of the Rockwell solution resulted in a significant increase in the number of bacterial colonies in all observation groups, with the highest increase occurring in group P2. These results indicate that Rockwell's solution has a consistent effect in increasing the number of bacterial colonies.

This study aims to evaluate the potential of *Myrmecodia sp.* extract as an alternative therapy for pelvic inflammatory disease (PID) due to *Candida albicans* infection. The phytochemical tests on *Myrmecodia sp.* extract showed that it has terpenoids, flavonoids, tannins, and saponins. These chemicals are known to kill microbes, reduce inflammation, and protect cells from damage (Roslizawaty et al., 2013). For example, flavonoid compounds can stop the production of pro-inflammatory cytokines like TNF-α and damage the cell membranes of microorganisms, which stops the growth of harmful microorganisms like *Candida albicans* (Lisnanti et al., 2018; Rusli et al., 2016).

Researchers in this study used antimicrobial tests to show that giving *Myrmecodia sp*. extract to mice with PID greatly decreased the number of *Candida albicans* colonies. The findings are similar to those of Efendi & Hertiani (2013), who discovered that an ethanol extract of *Myrmecodia tuberosa* can kill *Candida albicans*, *Escherichia coli*, and *Staphylococcus aureus* very effectively (Kurnilia et al., 2020; Natasya et al., 2020).

Statistical analysis using ANOVA showed a significant difference in the number of colonies before and after treatment, with a significance value of $p < 0.05$. This significant decrease in colonies strengthens the claim that *Myrmecodia sp.* extract has effective antimicrobial activity. What this means is that the active ingredients in the extract, like flavonoids and tannins, can damage microorganisms' cell membranes and stop enzymes that are needed for growth (Alkowni et al., 2023; Xiang et al., 2024). However, this study has limitations because the effectiveness of the extract is still lower than ciprofloxacin as a positive control. Further research is necessary to optimize the dosage and assess the safety of *Myrmecodia sp.* extract in humans. Additionally, larger-scale clinical trials are required to confirm its effectiveness in treating PID. Overall, this study shows that *Myrmecodia sp.* has the potential as an alternative therapeutic agent to overcome PID caused by *Candida albicans*. These findings also reinforce the urgency to further explore local herbal plants such as *Myrmecodia* sp. as a source of sustainable drug development.

CONCLUSION

Ant Nest Extract (*Myrmecodia sp.*) showed potential as an alternative therapy with various concentrations (0.4 %, 0.8 %, 1.6 %, and 3.2 %) for 24 female Balb/c mice suffering from PID to overcome infection and inflammation due to *Candida albicans* in PID mice. Although the administration of the extract reduced the number of bacterial colonies, the condition of the mice did not completely return to normal. The results of the study showed a significant effect in reducing bacterial colonies, although its effectiveness was lower than ciprofloxacin. While this study endorses the use of ant nest extract, more research is necessary to comprehend its mechanism and enhance its efficacy.

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