



## Effect Of Ethanol Centella Asiatica Extract On IL-10 Levels And Bacteria Colony In Rattus Norvegicus Endometritis Due To Infection Of Streptococcus Agalactiae

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### Abstract:

**Introduction:** Endometritis is an infection of the uterus which is characterized by inflammation of the lining of the uterus. Streptococcus was the most detected bacteria (47%) in the uterus in cases of endometritis. *Streptococcus agalactiae* has a polysaccharide capsule that is antiphagocytic, anticapsular antibodies are protective, and have the ability to induce an inflammatory response to secrete pro-inflammatory and decrease anti-inflammatory cytokines such as IL-10. *Streptococcus agalactiae* is a vaginal commensal bacterium that turns into a pathogen in utero due to an increase in the number of bacterial colonies and pH which stimulates increased biofilm production as protection. Gotu kola has a triterpene compound that can increase IL-10 which is useful for immune balance, suppressing tissue damage and as an antibacterial. This study was aimed to determine the effect of gotu kola (*Centella asiatica*) ethanol extract on increasing IL-10 and decreasing the number of *Streptococcus agalactiae* bacterial colonies.

**Material and Methods:** This research was designed using True Experimental research using randomized posttest-only control group design. The sample of this study was female white rats (*Rattus norvegicus*) which were inoculated with *Streptococcus agalactiae* after being treated with several doses of ethanol extract of gotu kola (*Centella asiatica* (L.) Urb.). This study consisted of six groups with 4 replications to evaluate the increase in IL-10 and the number of bacterial colonies.

**Result:** The results of the Shapiro-Wilk test on the IL-10 data variable and bacterial colonies showed p-value  $> \alpha = 0.05$ . The results of the homogeneity test of IL-10 levels and the number of bacterial colonies p-value  $> = 0.05$ . One Way Anova test of IL-10 levels in the six treatment groups showed significant differences with p-Value = 0.008  $\leq 0.05$ . There was a significant difference. Likewise, the number of Streptococcus agalactiae bacterial colonies between the treatment groups of female rats modeled with endometritis decreased significantly as indicated by the ANOVA p-Value = 0.000; correlation coefficient = 0.521;  $\alpha \leq 0.05$ .

**Conclusion:** *Centella asiatica* ethanol extract in endometritis female rats model can increase IL-10 levels and can reduce the number of *Streptococcus agalactiae* bacterial colonies in female white rats endometritis due to *Streptococcus agalactiae* infection.

**Keywords:** gotu kola (*Centella asiatica*); IL-10; number of colonies; Streptococcus agalactiae

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### Introduction

Endometritis is an infection of the characterized by inflammation of the lining of the uterus due to infection by microorganisms of the lower genital tract that ascend to the uterine cavity and form colonies. Streptococcus species that cause endometritis based on culture results include *Streptococcus agalactiae* (77.8%), *Streptococcus mitis* (11.1%), and *Streptococcus gallolyticus* (11.1%) Buzzaccarini *et al.*, (2020). The prevalence of *Streptococcus agalactiae* infection in the uterus worldwide is 15%, in Southeast

Asia (12%), and maternal *Streptococcus agalactiae* colonization in Indonesia reaches 30% (Safari *et al.*, 2021). Uterine infection by *Streptococcus agalactiae* impacts severe conditions such as postpartum endometritis. (Bobadilla *et al.*, 2021 *Streptococcus agalactiae* turns into a pathogen in the uterus due to the increased number of bacterial colonies and the pH that stimulates the production of biofilms to increase as protection. Shabayek and Spellerberg, 2018). *Streptococcus agalactiae* has an antiphagocytic polysaccharide capsule; anticapsular antibodies are protective and can induce an inflammatory response. TNF, IL-1, IL-6, and IL-8 are



examples of pro-inflammatory cytokines that are activated in macrophages. The IL-10 cytokine functions to maintain tissue homeostasis during infection and inflammation by limiting the exaggerated inflammatory response, upregulating innate immunity, and promoting tissue repair mechanisms (Hansen, 2019). Treatment of *Streptococcus agalactiae* infection was carried out with penicillin, a combination of penicillin and cephalosporin as prevention, but the results showed that there was a trend of rapidly increasing multidrug resistance. One of the traditional antimicrobial drugs in Indonesia is *Centella asiatica* which is widely used (BPOM, 2016; Widiyastuti, Wahjoedi and Januwati, 2016). Many antimicrobial compounds are contained in plants, one of which is the Asiaticoside compound an antimicrobial active substance from gotu kola (*Centella asiatica*). Asiaticoside can inhibit pro-inflammatory mediators and increase serum IL-10 levels (Wan *et al.*, 2013; Wahdany, Pradopo and Moeharyon, 2020). The mechanism of action of Asiaticoside is by forming bonds between asiaticoside membraneproteins and bacterial membrane proteins so that the target cell is lysed (Wahdany, Pradopo and Moeharyon, 2020). This study aims to evaluate the effect of ethanol extract of gotu kola (*Centella asiatica*) on increasing IL-10 levels and number of bacterial colonies in female white rats (*Rattus norvegicus*) with endometritis due to *Streptococcus agalactiae* infection.

### Material and Methods

*Streptococcus agalactiae* was obtained from the Microbiology Laboratory, Faculty of Medicine, Universitas Brawijaya and has been re-identified by the CAMP-test method, female white rat (*Rattus norvegicus*) aged 6-8 weeks, scales, oven, ethanol storage flask, erlenmayer tube, buncher funnel, blender evaporator, rotary evaporator, water bath, water pump, water pump hose, vacuum pump, bottle for extraction results, freezer, Buffer, ELISA kit, ELISA Reader, Minor surgery set, syringe 1 cc, plain vacutainer. gotu kola (*Centella asiatica* (L.) Urb.) was obtained from Materia Medika Batu Malang in a simplicia form. Examination of the number of colonies of *Streptococcus agalactiae* bacteria used Brain Heart Infusion Broth media and coloni counter.

### Study Setting

This research was designed using True Experimental research using randomized posttest only control group design.

### Sample size

This study was divided into 6 sample groups,

each consisting of 4 samples as follows: Negative control group (without treatment) Positive control group (inoculated with *Streptococcus agalactiae*  $1 \times 10^8$  intravaginally) P1: inoculated with *Streptococcus agalactiae*  $1 \times 10^8$  intravaginally + given ethanol extract of gotu kola (*Centella asiatica* (L.) Urb.) 100mg/kgBB

P2: inoculated with *Streptococcus agalactiae*  $1 \times 10^8$  intravaginally + given ethanol extract of gotu kola (*Centella asiatica* (L.) Urb.) 200mg/kgBB

P3: inoculated with *Streptococcus agalactiae*  $1 \times 10^8$  intravaginally + given ethanol extract of gotu kola (*Centella asiatica* (L.) Urb.) 400mg/kgBB

P4: inoculated with *Streptococcus agalactiae*  $1 \times 10^8$  intravaginally + given ethanol extract of gotu kola (*Centella asiatica* (L.) Urb.) 800mg/kgBB

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### Ethanol Extract of Gotu Kola (*Centella asiatica* (L.) Urb.)

The gotu kola material (*Centella asiatica* (L.) Urb.) was obtained from Materia Medika Batu Malang in a certified simplicia form. 100 grams of gotu kola simplicia has been put in an elemeyer tube and soaked with 900 ml of 90% ethanol, stirred for about 30 minutes until homogeneous, allowed to stand for 24 hours to form a precipitate. The results of the gotu kola immersion are put in the evaporation tube on the evaporator as much as 1 liter. The separation of the active substance with the ethanol solution in the container tube has been going on for 1.5 - 2 hours. After that, the extraction results were weighed and stored in the freezer. Gotu kola (*Centella asiatica* (L.) Urb.) extract is in liquid form, administered once a day in the afternoon orally through a syringe with a probe for 7 days of administration.

### Animal Specimens Used

The samples used in this study were experimental animals in the form of female white rats (*Rattus norvegicus*) obtained from the Wistar Farm Experimental Animal Distributor. Sample selection was done randomly.

### Inclusion And Exclusion Criteria

The inclusion criteria for the rat sample used were:

1. Female white rat (*Rattus norvegicus*) aged 6-8 weeks
2. Healthy is marked by active movement
3. Body weight between 250-300 grams
4. Haven't received any treatment before

The exclusion criteria for the mice sample used



were:

1. Rats die from treatment
2. Rats have been given previous treatment
3. Mice are sick, disabled, or die before the research treatment is completed

### Laboratory Methods

This research was carried out by the Pharmacy Laboratory of the Faculty of Medicine, Brawijaya University of Malang as a place for extracting the ethanol extract of gotu kola (*Centella asiatica* (L.) Urb.), Microbiology Laboratory, Faculty of Medicine, Brawijaya University of Malang as a place to obtain *Streptococcus agalactiae* bacterial isolates, re-identification, and calculation of the number of bacterial colonies, Bioscience Laboratory of Brawijaya University for preliminary studies, maintenance, strain staining and uterine histopathology, and Biochemistry Laboratory, Faculty of Medicine, Brawijaya University

Malang as a place to calculate the levels of IL-10 using an ELISA kit.

### Experimental Procedure

*Streptococcus agalactiae* was isolated from the Microbiology Laboratory, Faculty of Medicine, Brawijaya University and was cultured and used Vitek. Inoculation was carried out in the estrus phase. Mice were injected with dexamethasone intraperitoneally at a dose of 0.1 ml once per day for 3 days. Female rats were inoculated intra-vaginally with a concentration of *Streptococcus agalactiae*  $1 \times 10^8$  CFU in 0.8 ml of PBS. Inoculation was carried out 3 times with a distance of 1 day. The treatment with gotu kola (*Centella asiatica*) ethanol extract was carried out for 7 days with a certain dose given 1 time per day starting on day 11.

Uterus collection of female white mice (*Rattus norvegicus*) after treatment through several stages. Retrieval of the uterus of female white mice (*Rattus norvegicus*) after treatment through several stages. The rats were terminated by decapitation by skilled officers, waited until the rats were completely dead, then dissected to take the uterus. The rat uterus was taken carefully, then cleaned of blood and ligaments using 0.9% NaCl, drained on filterpaper, put in a plastic clip and put in the refrigerator (freezer). Uterine organs that will be used for ELISA examination must be prepared in the form of a solution/supernatant so that it needs to be

homogenized first in the laboratory. Rat IL10 antibody has been used to pre-coat the plate. When IL10 from the sample is added, it binds to the antibodies that have been coated on the wells. After that, rat IL10 antibody that has been biotinylated is added, and it binds to the sample's IL10. The biotinylated IL10 antibody is then bound by the addition of streptavidin-HRP. Unbound Streptavidin-HRP is removed during a washing step after incubation.

Following the addition of the substrate solution, color develops in proportion to the Rat IL10 concentration. The addition of an acidic stop solution puts an end to the reaction, and an ELISA reader

is used to quantify absorbance at 450 nm. Examination of the number of colonies of *Streptococcus agalactiae* bacteria were taken from uterine fluid cultured on Mannitol Salt Agar (MSA) and incubated at 37°C for 12 hours until the time of observation. Petri dishes containing bacterial colonies to be counted are placed on a colony counter equipped with a scale. The number of colonies was calculated by the colony counter method, namely using the formula.

### Dependent and Independent Variables

The independent variable in this study was the ethanol extract of gotu kola (*Centella asiatica* (L.) Urb.) with different doses in female white rats (*Rattus norvegicus*) chronic endometritis due to *Streptococcus agalactiae* infection. The dependent variable of this study was uterine IL-10 levels and the number of bacterial colonies in female white rats (*Rattus norvegicus*) with endometritis due to *Streptococcus agalactiae* infection.

### Statistical Analysis

Prerequisite test data for ANOVA analysis using the Shapiro Wilk normality test and homogeneity test with the p-value test results showing a value greater than the significant level = 0.05. One Way Anova test was used to compare the group mean measured between the control group and the treatment group by measuring the levels of IL-10 and the number of bacterial colonies. If the One Way Anova test has a probability value (sig) < 0.05, then there is a significant difference in this study. Data analysis was performed using SPSS 22.0 software.

### Ethical Clearance

Health Research Ethics Committee, Faculty of



Medicine, Brawijaya University, Malang, Indonesia, has authorized all procedures used in this study (No. 46/EC/KEPK-S2/03/2022), which were carried out in accordance with the required instructions and regulations.

## Results

### Endometritis Model Rat

The diagnosis of endometritis was carried out by conducting a preliminary study of making a female white rat model (*Rattus norvegicus*) of endometritis by uterine histopathology with HE staining on the rat uterus. Characteristics of endometritis found the presence of inflammatory cell infiltration which is dominated by eosinophils, neutrophils, and non-dominant lymphocytes.

### Analysis Result

The results of the Shapiro-Wilk test on the IL-10 data variable showed a p-value of 0, 200 which was greater than the significant level of  $\alpha = 0, 05$ . The results of the homogeneity test for IL-10 levels showed a p-value of 0.083 and the number of bacterial colonies showed a p-value of 0.064. The research data variables have met the prerequisites for the parametric test so that the One-Way Anova test can be carried out.

### Comparative Test between Control Groups against IL-10

One Way Anova test results demonstrated that there was a significant difference in the mean levels of IL-10 in the negative control group (113.10±18.75) and the positive control group (rats inoculated with *Streptococcus agalactiae*) (95.91±10.41) (table 1). Inoculation treatment with *Streptococcus agalactiae* showed a decrease in IL-10 levels.

**Table 1.** Results Comparison of IL-10 levels in the control group

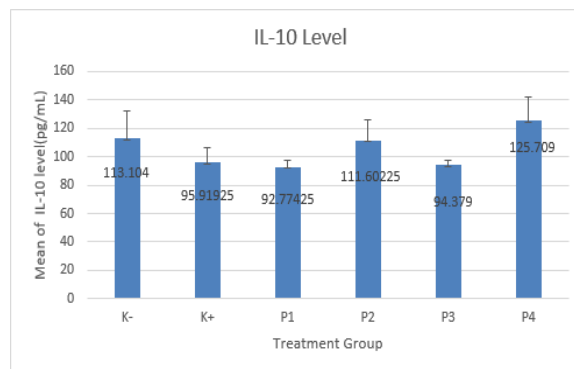
Variable	Control (-) Average±Stand. Dev	Control (+) Average±Stand. Dev	p-Value
Kadar IL-10	113.10±18.75	95.91±10.41	0.008

p-Value >  $\alpha = 0.05$  means there is no significant difference and if p-Value <  $\alpha = 0.05$  means there is a significant difference.

### Comparative Test of IL-10 Levels between Treatment Groups

The results of the One Way Anova test on IL-10 levels in the six treatment groups showed a significant difference as indicated by the p-Value = 0.008 <  $\alpha = 0.05$ . The results of the Post Hoc test of IL-10 level data variables showed a significant difference between the mean levels of IL-10 in the positive control group (95.91±10.41) and the P4 treatment group (*Streptococcus agalactiae* and *Centella asiatica* inoculation 800mg/KgBW) (125.70±16.25) with p-Value <  $\alpha = 0.05$ . The mean significant difference can also be shown from the mean IL-10 concentrations in the treatment group P1 (inoculated *Streptococcus agalactiae* and *Centella asiatica* 100mg/KgBW) (92.77±4.43) compared to the treatment group P4 (inoculated *Streptococcus agalactiae* and *Centella asiatica* 800mg/KgBW) (125.70±16.25) with p-Value <  $\alpha = 0.05$ . In addition, there was a significant difference in IL-10 levels between the P4 treatment group (*Streptococcus agalactiae* and *Centella asiatica* inoculation 800mg/KgBW) (125.70±16.25) compared to the P3 treatment group (*Streptococcus agalactiae* and *Centella asiatica* inoculation 400mg/KgBW) (94.37±3.11) with p-Value <  $\alpha = 0.05$ . Treatment of ethanol extract of *Centella asiatica* on female rats with endometritis model at a dose of 800 mg/KgBW can increase IL-10 levels compared to female rats of endometritis models that were not given *Centella asiatica* ethanol extract.

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**Figure 1** Histogram of Average IL-10 Levels in Endometritis Model Rats

The histogram shows the standard deviation and mean IL-10 levels in the negative control group, the positive control group (*Streptococcus agalactiae* inoculation), P1 (*Streptococcus agalactiae* and *Centella asiatica* inoculation 100mg/KgBW), P2 (*Streptococcus agalactiae* and *Centella asiatica* inoculation 200mg/KgBW), P3 (inoculation of *Streptococcus agalactiae* and



*Centella asiatica* 400mg/KgBW), and P4 (inoculation of *Streptococcus agalactiae* and *Centella asiatica* 800mg/KgBW).

### Comparison of Number of Colonies in Female White Mice of Endometritis Model

The analysis's findings demonstrated a significant difference in the number of colonies of *Streptococcus agalactiae* bacteria between the treatment groups of female endometritis model rats which was shown with  $p$ -Value = 0,000 <  $\alpha$  = 0,05 (table 2).

**Table 2.** Results Comparison of colony count (CFU/mL) in female white mice endometritis model

Group	N	Average ± Stand. Dev	ANOVA p-Value
Negative control	4	0,00±0,00	
Positive control ( <i>S. Agalactiae</i> )	4	202582.25±12465.25	
P1 ( <i>S. Agalactiae</i> + <i>Centella asiatica</i> 100mg/KgBB)	4	2350.75±1762.06	0.000
P2 ( <i>S. Agalactiae</i> + <i>Centella asiatica</i> 200mg/KgBB)	4	2777.75±427.50	
P3 ( <i>S. Agalactiae</i> + <i>Centella asiatica</i> 400mg/KgBB)	4	3205.25±51279.77	
P4 ( <i>S. Agalactiae</i> + <i>Centella asiatica</i> 800mg/KgBB)	4	43201.83±77222.22	

$p$ -Value > =0.05, it means that there is no significant difference and if  $p$ -Value < =0.05, it means that there is a significant difference.

The table shows the average number of colonies of *Streptococcus agalactiae* bacteria between treatment groups. The mean smallest number of colonies was indicated by the number of colonies in the negative control group (rats not inoculated for *Streptococcus agalactiae* bacteria) which was 0, 00 ± 0, 00, while the average number of colonies most compared to other treatment groups was shown in the positive control group (rats inoculated bacteria *Streptococcus agalactiae*) that is 202582.25 ± 12465.25. The mean number of colonies in the treatment groups 1 to 4 showed a decline in the number of colonies compared to the positive control group (rats inoculated with *Streptococcus agalactiae* bacteria). These results indicate that the administration of *Centella asiatica* ethanol extract at a dose of 100mg/KgBW, 200 mg/KgBW, 400 mg/KgBW, and 800 mg/KgBW can reduce the mean number of bacterial colonies in female white rats endometritis due to *Streptococcus agalactiae* bacterial infection compared with not given *Centella asiatica* ethanol extract. Similarly, the results of the Post Hoc test variable number of colonies showed a significant difference between the groups.

### Discussion

#### IL-10 levels in endometritis rats due to *Streptococcus agalactiae* infection

The One-Way Anova test results comparison between the adverse control group (healthy mice) and the constructive control group (rats inoculated with *Streptococcus agalactiae*) on IL-10 level data (pg/mL) showed that there was a significant difference, which could mean that there was a decrease in IL-10 levels. Chang's research (2014) that the induction of *Streptococcus agalactiae* can lead to a reduction in IL-10 secretion along with an increased production of inflammatory cytokines. Decreased IL-10 secretion or IL-10 deficiency aims to increase resistance to *Streptococcus agalactiae* thereby maximizing neutrophil recruitment to the site of infection. The IL-10 cytokine is an anti-inflammatory mediator that is useful for ensuring the protection of the host from excessive pathogen and microbiota responses (Saraiva et al., 2020).

CD4+ and antigen-activated T cells can activate macrophages and secrete increased amounts of interferon- $\gamma$  (IFN- $\gamma$ ) so that they can activate phagocytosis. Activation of macrophages results in the production of IL-12 which induces CD4+ T cells to differentiate into Th1 which then secretes IFN- $\gamma$ , IL-12, TNF- $\alpha$ , and IL-1 resulting in bacterial clearance and inflammatory reactions as an immune response against bacteria. The differentiation of CD4+ T cells into Th2 due to antigen presentation secretes IL-4, IL-5, IL-10, and IL-13. Several cytokines produced by Th2 cells such as IL-4, IL-10, and IL-13 have a function as an inhibitor of macrophage activity, thus cytokines secreted by IL-10 inhibit the immune response activity of Th1 cells. This occurs as a homeostatic effort of the immune response activated by bacterial antigens (Abbas, Lichtman and Pillai, 2016).

The mean levels of IL-10 in the P1 (*Streptococcus agalactiae* and *Centella asiatica* inoculated 100mg/KgBW) and P3 (*Streptococcus agalactiae* and *Centella asiatica* inoculated 400mg/KgBW) groups were lower than the positive control group (*Streptococcus agalactiae* inoculation). Treatment at P1 and P3 experienced inhibition of IL-10 secretion due to high levels of proinflammatory cytokines in the uterine circulation. The antagonistic function of IL-10's anti-inflammatory effects during bacterial infection may lead to two distinct roles namely the presence of infection with pro-inflammatory



bacteria or extracellular bacteria providing a powerful immunological reaction regulated by IL-10 thereby allowing slow bacterial clearance with tissue damage, limited host, thereby creating homeostasis (Peñaloza *et al.*, 2018).

The One Way Anova test results on IL-10 levels in the six treatment groups showed significant differences. The Asiatic acid content in the gotu kola (*Centella asiatica*) ethanol extract exhibits strong anti-inflammatory and antioxidant effects, may be associated with suppression of activation of the NLRP3 inflammasome and the NF- $\kappa$ B pathway (Kong *et al.*, 2019). The main mechanism of gotu kola (*Centella asiatica*) ethanol extract in reducing inflammation is a reduction in the production of inflammatory factors (IL-1b, IL-6, TNF- $\alpha$ ) and NLRP3 through NF- $\kappa$ B signaling pathway blockage as a regulator of inflammatory factor production, but there is an increase in levels IL-10. Administration of ethanol extract of pegagag (*Centella asiatica*) can increase IL-10 levels by increasing transcription of Treg derivatives which are anti-inflammatory IL-10 and TGF- $\beta$  mediators and reduce levels of IL-2 which is a pro-inflammatory cytokine. Upregulation of FoxP3 as the main transcription factor that regulates Tregs was observed in the administration of gotu kola (*Centella asiatica*) ethanol extract in vivo (Tawinwung *et al.*, 2021). The optimal dose of *Centella asiatica* ethanol extract in female endometritis model rats with a dose of 800 mg/KgBW can increase IL-10 levels compared to endometritis female white rats that were not given *Centella asiatica* ethanol extract, *Centella asiatica* ethanol extract with analysis showing significant differences.

The results of this study are in line with other studies showing that *Centella asiatica* extract can increase IL-10 (Zahara, 2018). Administration of *Centella asiatica* ethanol extract at a dose of 750 mg/kg can reduce tissue damage due to immune reactions due to inflammation through the expression of the enzyme matrix metalloproteinase-1 (MMP-1) and the enzyme tissue-specific matrix metalloproteinase-1 inhibitor (TIMP-1) (Arifa M, Rahayu A, 2014).

### Number of Bacterial Colonies

According to the culture data, there were more colonies in the positive control group than in the negative control group. This means that the inoculation of *Streptococcus agalactiae* bacteria causes the growth of bacterial colonies in the rat uterus. Cellular adherence and invasion is

mediated by the interaction of *Streptococcus agalactiae* with host extracellular matrix components (ECM) so as to increase pathogen resistance to mechanical clearance, avoid immune surveillance, and activate paracellular transmigration (Vornhagen *et al.*, 2018). *Streptococcus agalactiae* colonization can last for 1-2 weeks and then will experience bacterial clearance by the immune system, but there are also those that are persistent for more than 2 weeks in certain GBS strains (Patras *et al.*, 2015). Bacterial biofilms represent well-known virulence factors with important roles in persistence and chronic infection. Fibrinogen-binding protein (Fbs), laminin-binding protein (Lmb), group B streptococcal C5a peptidase (ScpB), streptococcal fibronectin-binding protein A (SfbA), GBS immunogenic bacterial adhesins (BibA), and hypervirulent adhesins are the main adhesives that mediate the interaction of GBS with host cells (HvgA) (Shabayek and Spellerberg, 2018).

The results of this study showed a decrease in the mean number of colonies in all treatment groups P1 (inoculation of *Streptococcus agalactiae* and *Centella asiatica* 100mg/KgBW), P2 (Inoculation of *Streptococcus agalactiae* and *Centella asiatica* 200mg/KgBW), P3 (Inoculation of *Streptococcus agalactiae* and *Centella asiatica* 400mg/KgBW), and P4 (inoculation of *Streptococcus agalactiae* and *Centella asiatica* 800mg/KgBW) compared with the positive control group (inoculation of *Streptococcus agalactiae*). Gotu kola extract has the capability of preventing the development of gram-positive and gram-negative bacteria (Yunita and Sari, 2020). asiaticoside in gotu kola functions as an antibacterial with a docking mechanism, which binds to bacterial membrane proteins, causing lysis. Asiatic acid in the ethanol extract of gotu kola (*Centella asiatica*) as an antimicrobial mechanism causes disruption or disruption of the bacterial plasma membrane (Meeran *et al.*, 2018). Antibacterial activity can be in the form of changes in the structure and function of bacterial cell structures (cell membranes, adhesins), cell morphology, gene expression, and the process of adhesion and biofilm formation (Sycz, Tichaczek-Goska and Wojnicz, 2022).

The One-Way Anova test results revealed a substantial variation in dosages in the number of *Streptococcus agalactiae* bacterial colonies between the treatment groups of endometritis rats due to *Streptococcus agalactiae* infection.

Another study using ethanol extract of gotu kola (*Centella asiatica* (L) Urb.) leaves at concentrations of 60%, 80%, and 100% had antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* (Widiastuti *et al.*, 2016). The administration of gotu kola extract (*Centella asiatica*) also showed antimicrobial activity with oral administration aily dosages of 50 and 250 mg/kg weight (Idris and Nadzir, 2021).

Treatment group P4 (inoculation of *Streptococcus agalactiae* and *Centella asiatica* 800mg/KgBW) showed a lower mean of bacterial colony reduction than the other treatment groups. ral administration of *Centella asiatica* has been shown to reduce the frequency of T lymphocytes in mice, particularly CD8+ T cells. As evidenced by strong CD25+ expression and overexpression of the FoxP3 gene, *Centella asiatica* can enhance the proportion of regulatory T cells. Oral treatment of *Centella asiatica* increased the percentage of CD4+ T cells and decreased the percentage of CD8+ T cells, according to phenotypic characterization of the T cell subset (Nochi and Kiyono, 2015; Abbas, Lichtman and Pillai, 2016; Tawinwung *et al.*, 2021).

## Conclusion

Administration of gotu kola (*Centella asiatica*) ethanol extract has an effect on increasing IL-10 levels in female white rats (*Rattus norvegicus*) endometritis due to infection with *Streptococcus agalactiae*. Gotu kola (*Centella asiatica*) ethanol extract was effective to increase IL-10 levels by suppressing several pro-inflammatory cytokines and increasing anti-inflammatory mediators through inhibition of signaling pathways in endometritis female rats due to *Streptococcus agalactiae* infection. The ethanol extract of gotu kola (*Centella asiatica*) orally can affect the decrease in the number of bacterial colonies in female white rats (*Rattus norvegicus*) endometritis due to infection with *Streptococcus agalactiae*

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## Conflict of Interest

The author declares that there is no conflict of interest.

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