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Original Research Article

Effects of Ethanol Extract of *Centella asiatica* on Acidity (pH) Levels of Rats Model Aerobic Vaginitis

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Abstract: Aerobic vaginitis (AV) is an infection in the vagina with a high inflammatory response, decreased Lactobacillus sp., increased pathogenic bacteria, increased parabasal cells, and increased acidity levels (pH). Increased pH levels in AV conditions are associated with reduced Lactobacillus in the vagina due to the dominance of aerobic or anaerobic bacteria that cause AV, including Streptococcus agalactiae, which can cause an increased risk of infection. Antibiotics are the mainstay of AV treatment but are resistant to several antibiotics, causing the need for other alternatives, one of which is the use of medicinal plants. The medicinal plant in this study is Centella asiatica plant which has been proven to have an anti-bacterial property. This study aims to compare pH levels before and after administration of ethanol extract of *Centella asiatica* on the acidity (pH) levels of Rats in AV model. This research is an experimental study use post-test control group only, consists of six groups Rattus norvegicus female. The first group was the negative control group (K-) rats. The second group was positive control rats (K+). The next are four groups of rats of AV models given various doses of ethanol extract of Centella asiatica, and each group consists of four rats, namely P1 (100 mg/kg). P2 group (200 mg/kg), P3 (400 mg/kg), and P4 (800 mg/kg). Paired T-Test showed results P-value is 0.000 (<0.005), stating that there was a significant difference between pH levels both before and after administration of ethanol extracts of Centella asiatica plant in rats model AV.

Keywords: Centella asiatica, pH, Aerobic vaginitis, Streptococcus agalactiae, ethanol extract, antibiotic resistance.

INTRODUCTION

The World Health Organization (WHO) predicts ± 295,000 women who die during pregnancy, the puerperium, and childbirth due to preventable causes, one of which is infection [1]. Disruption of the microbiota balance in the vagina caused by several types of bacteria can lead to infections that result in various disorders, one of the conditions is AV [2]. AV is an infection with increased aerobic and enteric bacteria in the vaginal microflora that can cause inflammation [3]. A study in Bulgaria has found that the prevalence of AV, in general, was 11.77%, in pregnant women was 13.08%, and in non-pregnant women was 4.34%, with the most age group being 21-30 years old (32.3%) [4]. This kind of condition is quite concerning because AV can cause various secondary infections that lead to severe conditions such as spontaneous abortion, chorioamnionitis, membranes rupture early, early labor, infertile, also PID [5].

AV has characteristics such as reduced or lost Lactobacillus, redness, edema, vaginal ulceration, yellow discharge, dyspareunia, and increased acidity levels (pH) [3]. A healthy vagina is characterized by a pH level of 4-4.5. It is the ideal level to fight various pathogenic microbes [6]. In AV conditions there is an increase in pH levels > 4.5 and even > 6 [7, 8]. This increase in acidity certainly has a negative impact, because normal pH levels function as microbicides, and as protection from microbes that cause Sexually Transmitted Diseases (STDs) [6]. Research also proves Lactobacillus colonization in the vagina and low vaginal pH levels can prevent the occurrence of Bacterial Vaginosis (BV), maintain a healthy pregnancy outcome, and reduce the risk of preterm labor [9]. Increased pH levels in AV conditions are associated with reduced Lactobacillus in the vagina due to the dominance of aerobic or anaerobic bacteria that cause

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AV, including E. Coli, Streptococcus aureus, E. Faecalis, and Streptococcus agalactiae [8].

Streptococcus agalactiae is a bacterium found in many cases of AV [3, 10]. WHO (2017) stated that Streptococcus agalactiae colonization in pregnant women amounted to about 18% (range 11-35%), with a total of 21.7 million people from 195 countries [11]. However, the prevalence of Streptococcus agalactiae disease in Southeast Asia is relatively low due to the lack of further in-depth research in this region [12]. Pregnant women with Streptococcus agalactiae colonization have a risk ratio of 1.21 preterm delivery compared to pregnant women without Streptococcus agalactiae colonization [13]. In non-pregnant adults, Streptococcus agalactiae infection can cause various health problems with typical clinical manifestations, such as bladder infections, pneumonitis, bacteremia, osteomyelitis and other conditions [14, 15].

Diagnosis of AV is often difficult to distinguish from Bacterial Vaginosis (BV); this can lead to errors in administering inappropriate therapy and medication; it aggravates AV conditions and leads to various complications [16]. Diagnosis of AV is based on Donders criteria using an AV score microscopically by evaluating the degree of lactobacilli, white blood cells, toxic leukocyte count, vaginal flora background, and the number of parabasal cells. The outcome criteria are if the AV score is <3 there are no signs of AV; a score of 3-4 is mild AV, 5-6 is moderate AV, and > 6 is severe AV [8]. Treatment of AV usually uses antibiotics with a cure rate of up to 93.3%. Although not all types of antibiotics are appropriate for treating AV conditions, for example, quinolones tend to show a lower impact on the vaginal microbiota than ampicillin [17].

Streptococcus agalactiae, one of the bacteria that causes AV, is resistant to several types of antibiotics such as tetracycline, ertapenem, penicillin, vancomycin, erythromycin, and clindamycin [18]. Research on pregnant women in Vietnam found GBS colonization have susceptibility to ampicillin, ceftriaxone, vancomycin, quinupristin, penicillin, cefotaxime [11]. Studies in China found that 80% of Streptococcus agalactiae have antibiotic resistance to fluoroquinolone [18]. Another study found that more than 90% of certain strains of Streptococcus agalactiae were resistant to levofloxacin [20]. One study in the United States found an increase in macrolide and lincosamide antibiotic resistance against Streptococcus agalactiae, which detecting around the world [21, 22]. Streptococcus agalactiae has also been shown to be resistant to linezolid [23].

It was estimated by WHO in 2050, there will be at least 10 million deaths per year due to drug-resistant bacterial infections, which are estimated to have an impact on economic losses [24]. Cases of antibiotic resistance lead to a twofold increase in adverse infection outcomes from resistant bacteria. The greater the increase, the more severe the disease, strain virulence, and vulnerability of the host, which can lead to higher morbidity and mortality cases [25]. In addition, the use of antimicrobials is also often associated with a decrease in the concentration of Lactobacilli, which can negatively impact the balance of the microbiota in the vagina [26]. This condition certainly requires early anticipation, one of which is to look for alternative substitutes that act as antibacterial and antimicrobial agents by maximizing the potential use of medicinal plants [27].

One of the medicinal plants well-known for its potential benefits, especially as a natural source of antimicrobial agents, is Centella asiatica [28]. This plant is a small herb that can be eaten cognate to the Apiaceae family originating from the East including India, Sri Lanka, Indonesia, Malaysia, and Vietnam [29, 30]. This plant does sell in several local markets, especially those that have not been processed and are used as primary ingredients in making herbal teas or tonic drinks [31-33]. Several studies have found that the leaves and roots of Centella Asiatica can act as antibacterial and antifungal agents that are effective against microbes [34]. The ability to fight bacteria is due to the main chemical components of the Centella asiatica, namely triterpene, which is dominated by asiaticoside, Asiatic acid, madecassoside, madecassic acid, and other compounds [35]. In several studies, triterpenoids can prevent infections caused by pathogens [35, 36]. Flavonoids and tannins in the Centella asiatica plant also have antimicrobial activity [35, 37]. The Asiatic acid substance has shown intense antibacterial activity against bacteria [38]. In addition, one study found that the ethanol extract of the Centella asiatica plant at doses of 100, 200, 300, 400 to 500 mg had good activity against Streptococcus pyrogens [39].

Fig 1: Centella asiatica plant [40]

This study aims to assess differences in acidity (pH) levels in the vagina of rats in the negative control group (non AV model), and the positive control group (AV model), which formed after the injection of

Streptococcus agalactiae. The positive control group consisted of four groups with different doses of ethanol extract of the Streptococcus agalactiaeplant (100 mg/kg, 200 mg/kg, 400mg/kg, 800 mg/kg). The main difference to be assessed is the pH level before and after administration ethanol extract of Centella asiatica in each group. The novelty of the research in this experimental study is that this is the first study to examine the manufacture of an AV model mouse using the bacterium Streptococcus agalactiae, and then given ethanol extract of Centella asiatica to see the comparison of pH levels before and after administration.

MATERIAL AND METHODS

Plant Source and Extraction Process

All parts of the Centella asiatica plant were obtained from the Herbal Materia Medika Batu Malang laboratory in powder form. Tools used Scales, oven, bottles containing ethanol solution, Erlenmeyer, buncher funnel, blender evaporator, rotary evaporator, water bath, water pump, water pump hose, vacuum pump, extract storage container, and refrigerator, ethanol solvent, filter paper, and aluminum foil paper. Five hundred grams of Centella asiatica powder were put into an Erlenmeyer tube, soaked with 4,500 ml of 90% ethanol solution, and stirred until mixed for 30 minutes. After mixing, it is allowed to settle for 24 hours. Next, the top layer of the soaked mixture of ethanol solution and the active substance is taken, filtered three times with filter paper or called a buncher funnel. Then enter the soaking results into the evaporator. The extraction results are then weighed, put in a particular container, wrapped in aluminum foil, and stored in the freezer.

Identification of Chemical Compounds

The identification process was at the Chemical Engineering Laboratory, State Polytechnic of Malang. The process of identifying plant compounds using two methods, qualitatively with LC-MS/MS method to identify the triterpene content and quantitatively using the UV-Vis method to determine the flavonoid and phenolic content.

Animal Laboratory

Rats were obtained from certified research animal distributors and had their health checked by a veterinarian in the Biosciences Laboratory of Brawijaya University. The rats in this study were Rattus norvegicus, female, aged 8-10 weeks, numbered 24, and divided into six groups. The first was the negative control group (K-) rats, four healthy rats without Centella Asiatica extract. The second was positive control rats (K+), consisting of four Aerobic vaginitis model rats without Centella asiatica extract. The third was group P1, four rats of Aerobic vaginitis model given C. Asiatica extract at a 100 mg/kg dose. The fourth was the P2 group, four AV model rats with a 200 mg/kgdose. The third was four AV model rats with a dose of 400 mg/kg, and the sixth was four AV model rats at a dose of 800 mg/kg rats birth weight. Rats were treated in the bioscience laboratory of Brawijaya University according to laboratory standards. Seven-day acclimation was performed before made into AV models

AV Models

The bacteria used Streptococcus agalactiae bacteria with a density of $1x10^8$ CFU/ml. After seven days of acclimation, rats were injected with dexamethasone sodium phosphate 5 mg/ml dose of 0.1 ml for three days. On the fourth day, the rats were given a solution of 0.4 ml of Streptococcus agalactiae bacteria intravaginally using a one cc syringe by stretching the rat's vagina by hand and slowly inserting it, after which the rat was allowed to walk with two front legs on a flat and hard surface for one minute. Bacterial solution was administered three times. The first 24 hours after the last dexamethasone administration, 48 hours, and 96 hours later. AV models forming on the seventh day after being diagnosed using Donders Score obtained from the results of gram staining to evaluate the degree of Lactobacillus and flora background in vaginal, and Giemsa staining to determine the number of leukocytes, the proportion of toxic leukocytes, and parabasal cells using a 400x magnification microscope.

Administration of Ethanol Extract

After the AV model mouse was formed, the treatment groups P1, P2, P3, and P4 were given Centella asiatica ethanol extract using a gastric probe. Extracts were given each group based on respective doses, delivered once a day for seven days.

Measurement of Vaginal pH Levels in Rats

The pH level was measured using a Macherey-Nagel brand pH test strip. Measurements were made by opening the rat's vagina and taking the vaginal secretions using a sterile cotton swab by rotating it clockwise three times. First, the swab results are smeared on the indicator strip, waiting for the color change, and measured by comparing the strip's and the indicator's colors; then, the results are recorded.

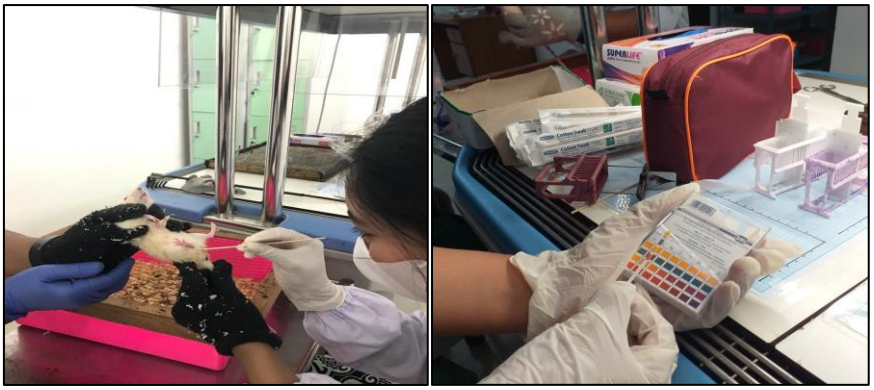

Figure 1: Vaginal swab and pH measurement

Research and Statistic Analysis

This research is a true-experimental study with a post-test-only control group design and has received approval from the Health Research Ethics Commission of the Faculty of Medicine Brawijaya University, with letter number 46/EC/KEPK-S2/03/2022, following Helsinki Declaration Guidelines. Statistical analysis used the T-test, the data distribution was tested using the Shapiro Wilk test, and the result was normal. Analysis continued using the paired T- Test. The analysis was carried out on the SSPS 2020 software.

RESULTS AND DISCUSSION

The results of quantitative analysis using the UV-Vis technique, the ethanol extract of C.asiatica kola in this study had a total phenolic content of 121,384,20 mg/kg and a total flavonoid of 6,306.15 mg/kg. Qualitatively (LC- MS/MS), the plant extracts positive contain Asiaticoside, Madecassoside, Asiatic acid, and Madecassic acid. The results of the qualitative test using the LC-MS/MS method are shown in Figure 1 and identified by the following literature [41]. The parameters used to analyze the triterpene content in Streptococcus agalactia eusing the LC/MSMS method, one of which is the Q1 moleculer ion value. Molecular ions 976.4 for Asiaticoside and the results in this study are 976.5 (red color) , 992.3 for Madecassoside, in this study are 992.0 (green color), 506.5 for Asiatic acid, in this study are 506.0 (blue color), 522.2 for identification of Madecassic acid, and in this study are 522.0(yellow color). The triterpene qualitative test results in this study were close to the values in the reference literature.

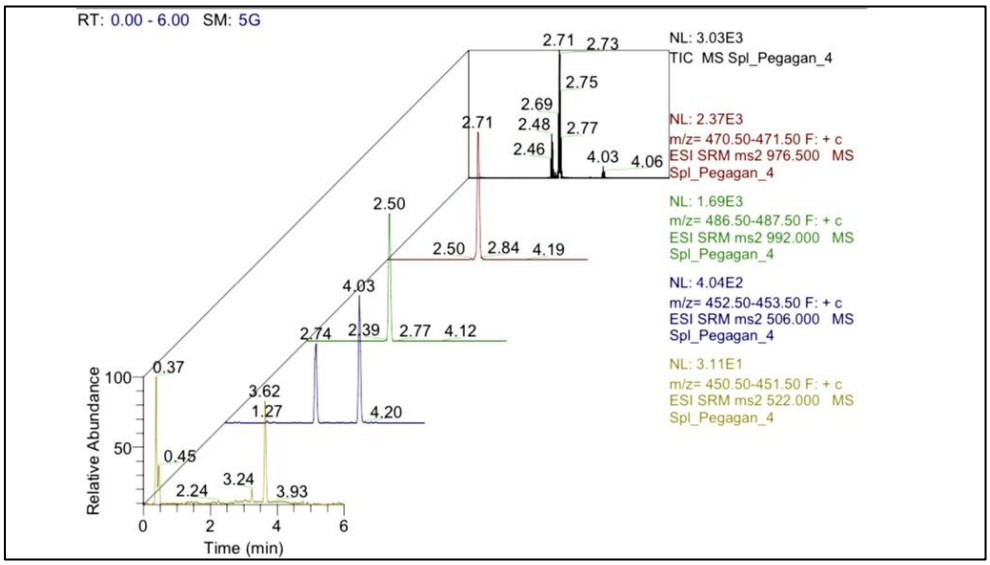

Figure 2: LC-MS/MS Test Results (Qualitative)

The results of the identification of Centella asiatica compounds in this study are in accordance with the results of research which states that the Centella asiatica extract is known to contain large amounts of asiaticoside, Asiatic acid, madecassoside, and madecassic acid, which are the main chemical components responsible for the pharmacological activity of this plant [31, 33, 42]. Other compounds identified in addition to triterpenes in this study are flavonoids and phenolics, which are, by one study, also found to have the same content in the Centella asiatica plant extract [43]. The high levels of flavonoids in this study were associated with the use of ethanol as a solvent known to be one of the solvents that give good extraction results, with the exponential equation yielding only a 12.21% error percentage [44]. Other

studies have also proven that ethanol solvent can be one of the best solvents that used to obtain extracts rich in compounds with an antimicrobial activity [45], which are needed to treat Streptococcus agalactiae bacteria as the cause of the AV model. In This study, the AV model was made by adopting the patented AV model mouse manufacturing method by Bengbu Medical College China [46]. The dose of bacteria inoculated in this study obtaining after a preliminary study.

Statistically, there was a difference in the mean and standard deviation of the vaginal pH levels of rats before administration of the ethanol extract of Streptococcus agalactiaeplant, the results are shown in Table 1:

Table 1: Paired Sample Statistics

	Mean	N	Standar Deviation
pH Pra	7.71	24	0.955
pH Post	6.25	24	0.608

The high level of vaginal pH in AV rats before administration of the ethanol extract of the Centella asiatica in this study proves that the decrease in the number of Lactobacillus, which is one of the signs of AV, is associated with an increase in vaginal pH, which is similar to the results of several other studies [47, 48]. One study stated that although it was difficult to clearly distinguish simple AV and mixed AV based on pH levels, there was a pH level higher than 4.5 in AV patients due to lower lactobacillus levels [5, 49]. This result is supported by statistical data on the average pH levels of the negative control group and the positive control group's pH levels which are depicted in the diagram below:

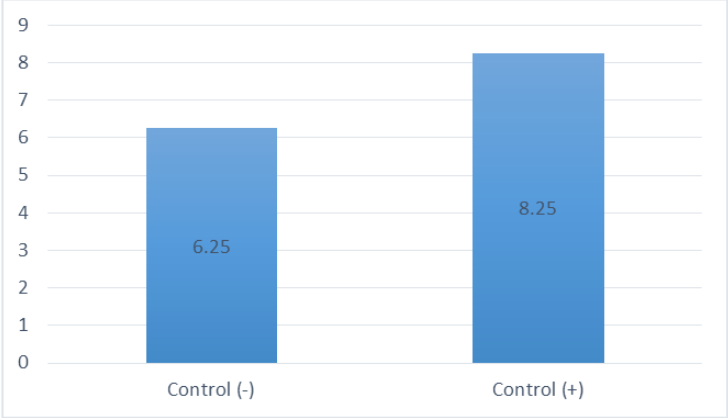

Fig 2: Comparison of pH Levels in Control Group

In Fig.2, the average level of healthy mice is lower than the pH level of AV model mice with a p-value = 0.004, which means there is a significant difference between the two. The increase in pH levels in the positive control group was associated with the discovery of a background of gram-positive flora in the form of coccus suspected of Streptococcus agalactiae bacteria inoculated in positive control mice because the invasion of AV-causing bacteria on the vaginal mucosa can cause a decrease in Lactobacillus which can lead to AV conditions characterized by Vaginal pH >4.5, purulent discharge, significant inflammation, and epithelial disorders [50].

Test results using Paired T-Test showed a decrease in the average vaginal pH of rats by 1.458 with a P-value = 0.000 (<0.005), where a decrease of this size stated that there was a significant difference between pH levels both before and after administration of plant ethanol extracts Gotu kola. The Confidence Interval of the Difference in statistical test results in this study was 95%. The presence of a significant decrease in pH levels before and after administration of ethanol extract of Centella asiatica shows the potential of the plants, and especially it is potential in the future to overcome the pathogenic bacteria that cause AV. The decrease in pH levels may be related to the ability of Triterpenoids in Centella asiatica, which can prevent infections caused by pathogens so can restore normal flora in the vagina [35, 36]. The flavonoid compounds in the Centella asiatica plant have also been shown to have good antimicrobial activity against bacteria [37]. Asiatic acid, part of the triterpenoids in the Centella asiatica, also acts as a broad-spectrum antibacterial against gram-positive and gram-negative bacteria [38].

CONCLUSION

Based on the results of this study, there was a significant difference in the decrease in pH levels in AV model rats before and after administration of the ethanol extract of the Centella asiatica at various doses. This finding is expected to be a reference for developing the potential of Streptococcus agalactiae in the future,

especially in becoming an alternative solution to antibiotics in treating bacteria that cause AV, especially Streptococcus agalactiae bacteria.

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REFERENCES

1. WHO. New report calls for urgent action to avert antimicrobial resistance crisis. World Health Organization. Published 2019. https://www.who.int/es/news/item/29-04-2019-new-report-calls-for-urgent-action-to-avert-antimicrobial-resistance-crisis
2. Kaambo, E., Africa, C., Chambuso, R., & Passmore, J. A. S. (2018). Vaginal microbiomes associated with aerobic vaginitis and bacterial vaginosis. *Frontiers in public health*, *6*, 78. doi:10.3389/fpubh.2018.00078
3. Donders, G. G., Bellen, G., Grinceviciene, S., Ruban, K., & Vieira-Baptista, P. (2017). Aerobic vaginitis: no longer a stranger. *Research in Microbiology*, *168*(9-10), 845-858. doi:10.1016/j.resmic.2017.04.004
4. Dermendjiev, T., Pehlivanov, B., Hadjieva, K., & Stanev, S. (2015). Epidemiological, clinical and microbiological findings in women with aerobic vaginitis. *Akusherstvo i Ginekologiia*, *54*(9), 4-8.
5. Zhang, T., Xue, Y., Yue, T., Xiong, L., Wang, X., Wang, W., ... & An, R. (2020). Characteristics of aerobic vaginitis among women in Xi'an district: a hospital-based study. *BMC Women's Health*, *20*(1), 1-7. doi:10.1186/s12905-020-00997-5
6. Aldunate, M., Tyssen, D., Johnson, A., Zakir, T., Sonza, S., Moench, T., ... & Tachedjian, G. (2013). Vaginal concentrations of lactic acid potently inactivate HIV. *Journal of Antimicrobial Chemotherapy*, *68*(9), 2015-2025. doi:10.1093/jac/dkt156
7. Donders, G. G., Vereecken, A., Bosmans, E., Dekeersmaecker, A., Salembier, G., & Spitz, B. (2002). Definition of a type of abnormal vaginal flora that is distinct from bacterial vaginosis: aerobic vaginitis. *BJOG: an international journal of obstetrics and gynaecology*, *109*(1), 34-43.
8. Laboratories, M. D. (2012). *Aerobic Vaginitis*, (5), 8-11. www.medlab.com
9. Mirmonsef, P., Hotton, A. L., Gilbert, D., Burgad, D., Landay, A., Weber, K. M., ... & Spear, G. T. (2014). Free glycogen in vaginal fluids is associated with Lactobacillus colonization and low vaginal pH. *PloS one*, *9*(7), e102467. doi:10.1371/journal.pone.0102467
10. Ma, X., Wu, M., Wang, C., Li, H., Fan, A., Wang, Y., ... & Xue, F. (2022). The pathogenesis of prevalent aerobic bacteria in aerobic vaginitis and adverse pregnancy outcomes: a narrative review. *Reproductive Health*, *19*(1), 1-11. doi:10.1186/s12978-021-01292-8
11. Van Du, V., Dung, P. T., Toan, N. L., Van Mao, C., Bac, N. T., Van Tong, H., ... & Viet, N. T. (2021). Antimicrobial resistance in colonizing group B Streptococcus among pregnant women from a hospital in Vietnam. *Scientific Reports*, *11*(1), 1-7. doi:10.1038/s41598-021-00468-3
12. Kobayashi, M., Schrag, S. J., Alderson, M. R., Madhi, S. A., Baker, C. J., Sobanjo-ter Meulen, A., ... & Vekemans, J. (2019). WHO consultation on group B Streptococcus vaccine development: report from a meeting held on 27–28 April 2016. *Vaccine*, *37*(50), 7307-7314. doi:10.1016/j.vaccine.2016.12.029
13. Bianchi-Jassir, F., Seale, A. C., Kohli-Lynch, M., Lawn, J. E., Baker, C. J., Bartlett, L., ... & Rubens, C. E. (2017). Preterm birth associated with group B Streptococcus maternal colonization worldwide: systematic review and meta-analyses. *Clinical Infectious Diseases*, *65*(suppl_2), S133-S142. doi:10.1093/cid/cix661
14. High, K. P., Edwards, M. S., & Baker, C. J. (2005). Group B streptococcal infections in elderly adults. *Clinical Infectious Diseases*, *41*(6), 839-847. doi:10.1086/432804
15. Loubet, P., Koumar, Y., Lechiche, C., Cellier, N., Schuldiner, S., Kouyoumdjian, P., ... & Sotto, A. (2021). Clinical features and outcome of Streptococcus agalactiae bone and joint infections over a 6-year period in a French university hospital. *Plos one*, *16*(3), e0248231. doi:https://doi.org/10.1371/journal.pone.0248231
16. Krauss-Silva, L., Almada-Horta, A., Alves, M. B., Camacho, K. G., Moreira, M. E. L., & Braga, A. (2014). Basic vaginal pH, bacterial vaginosis and aerobic vaginitis: prevalence in early pregnancy and risk of spontaneous preterm delivery, a prospective study in a low socioeconomic and multiethnic South American population. *BMC Pregnancy and Childbirth*, *14*(1), 1-10.
17. Tempera, G., & Furneri, P. M. (2010). Management of aerobic vaginitis. *Gynecologic and obstetric investigation*, *70*(4), 244-249. doi:10.1159/000314013
18. Shrestha, K., Sah, A. K., Singh, N., Parajuli, P., & Adhikari, R. (2020). Molecular characterization of Streptococcus agalactiae isolates from pregnant women in Kathmandu City. *Journal of tropical medicine*, *2020*. doi:10.1155/2020/4046703
19. Wang, H., Zhao, C., He, W., Zhang, F., Zhang, L., Cao, B., ... & Ni, Y. (2013). High prevalence of fluoroquinolone-resistant group B streptococci among clinical isolates in China and predominance of sequence type 19 with serotype III. *Antimicrobial agents and chemotherapy*, *57*(3), 1538-1541. doi:10.1128/AAC.02317-12
20. Wang, P., Tong, J. J., Ma, X. H., Song, F. L., Fan, L., Guo, C. M., ... & Yang, Y. H. (2015).

Serotypes, antibiotic susceptibilities, and multi-locus sequence type profiles of Streptococcus agalactiae isolates circulating in Beijing, China. *PLoS One*, *10*(3), e0120035. doi:10.1371/journal.pone.0120035

21. Francois Watkins, L. K., McGee, L., Schrag, S. J., Beall, B., Jain, J. H., & Pondo, T. (2018). Epidemiology of invasive group B streptococcal infections among nonpregnant adults in the United States, 2008–2016. *JAMA Intern Med. 2019;* 179: 479–88.
22. Tulyaprawat, O., Pharkjaksu, S., Shrestha, R. K., & Ngamskulrungroj, P. (2021). Emergence of multi-drug resistance and its association with uncommon serotypes of Streptococcus agalactiae isolated from non-neonatal patients in Thailand. *Frontiers in microbiology*, 2627. doi:10.3389/fmicb.2021.719353
23. Zheng, J. X., Chen, Z., Xu, Z. C., Chen, J. W., Xu, G. J., Sun, X., ... & Qu, D. (2020). In vitro evaluation of the antibacterial activities of radezolid and linezolid for Streptococcus agalactiae. *Microbial pathogenesis*, *139*, 103866. doi:10.1016/j.micpath.2019.103866
24. Mahon, C. R., Lehman, D. C. *Textbook Of Diagnostic Microbiology, American Speech*. Elsevier Saunders; 2019. doi:105.10.2307/486972.
25. Friedman, N. D., Temkin, E., & Carmeli, Y. (2016). The negative impact of antibiotic resistance. *Clinical Microbiology and Infection*, *22*(5), 416-422. doi:10.1016/j.cmi.2015.12.002
26. Westbroek, M. L., Davis, C. L., Fawson, L. S., & Price, T. M. (2010). Interactions of Lactobacilli with pathogenic Streptococcus pyogenes. *Infectious diseases in obstetrics and gynecology*, *2010*. doi:10.1155/2010/289743
27. Suresh, M., Alfonisan, M., Alturaiki, W., Al Aboody, M. S., Alfaiz, F. A., Premanathan, M., ... & Alsagaby, S. A. (2021). Investigations of bioactivity of Acalypha indica (L.), Centella asiatica (L.) and croton bonplandianus (Baill) against multidrug resistant bacteria and cancer cells. *Journal of Herbal Medicine*, *28*, 100359. doi:10.1016/j.hermed.2020.100359
28. Harun, N. H., Septama, A. W., Ahmad, W. A. N. W., & Suppian, R. (2019). The potential of Centella asiatica (Linn.) urban as an anti-microbial and immunomodulator agent: A review. *Natural Product Sciences*, *25*(2), 92-102. doi:10.20307/nps.2019.25.2.92
29. Loc, N. H., & Nhat, N. T. D. (2013). Production of asiaticoside from centella (Centella asiatica L. Urban) cells in bioreactor. *Asian Pacific Journal of Tropical Biomedicine*, *3*(10), 806-810. doi:10.1016/S2221-1691(13)60159-8
30. Roy, A., Krishnan, L., & Bharadvaja, N. (2018). Qualitative and quantitative phytochemical analysis of Centella asiatica. *Nat. Prod. Chem. Res*, *6*(4), 323. doi:10.4172/2329-6836.1000323
31. Hashim, P. (2011). Centella asiatica in food and beverage applications and its potential antioxidant and neuroprotective effect. *International Food Research Journal*, *18*(4), 1215-1222.
32. Chandrika, U. G., & Kumara, P. A. P. (2015). Gotu Kola (Centella asiatica): nutritional properties and plausible health benefits. *Advances in food and nutrition research*, *76*, 125-157. doi:10.1016/bs.afnr.2015.08.001
33. Ramli, S., Xian, W. J., & Abd Mutalib, N. A. (2020). A Review: Antibacterial activities, antioxidant properties and toxicity profile of centella asiatica. *EDUCATUM Journal of Science, Mathematics and Technology*, *7*(1), 39-47.
34. Nasution, M. Y., Restuati, M., Pulungan, A. S. S., Pratiwi, N., & Diningrat, D. S. (2018). Antimicrobial activities of Centella asiatica leaf and root extracts on selected pathogenic micro-organisms. *Journal of Medical Sciences*, *18*(04), 198-204. doi:10.3923/jms.2018.198.204
35. Idris, F. N., & Mohd Nadzir, M. (2021). Comparative studies on different extraction methods of Centella asiatica and extracts bioactive compounds effects on antimicrobial activities. *Antibiotics*, *10*(4), 457. doi:10.3390/antibiotics10040457
36. Soyingbe, O. S., Mongalo, N. I., & Makhafola, T. J. (2018). In vitro antibacterial and cytotoxic activity of leaf extracts of Centella asiatica (L.) Urb, Warburgia salutaris (Bertol. F.) Chiov and Curtisia dentata (Burm. F.) CA Sm-medicinal plants used in South Africa. *BMC complementary and alternative medicine*, *18*(1), 1-10.
37. Jayaprakash, S. B., & Nagarajan, N. (2016). Studies on the bioactive compounds and antimicrobial activities of medicinal plant Centella asiatica (Linn). *Journal of Medicinal Plants Studies*, *4*(5), 181-185.
38. Harun, N. H., Septama, A. W., Ahmad, W. A. N. W., & Suppian, R. (2019). The potential of Centella asiatica (Linn.) urban as an anti-microbial and immunomodulator agent: A review. *Natural Product Sciences*, *25*(2), 92-102.
39. Vaishnavi, S. (2015). ANTIMICROBIAL ACTIVITY OF GOTUKOLA LEAVES AND NEEM LEAVES-A COMPARATIVE INVITRO STUDY. *Journal of Ayurveda and Holistic Medicine (JAHM)*, *3*(3).
40. Bhattacharya, R. D., Parmar, K. M., Itankar, P. R., & Prasad, S. K. (2017). Phytochemical and pharmacological evaluation of organic and non-organic cultivated nutritional Centella asiatica collected after different time intervals of harvesting. *South African Journal of Botany*, *112*, 237-245. doi:10.1016/j.sajb.2017.06.003
41. Gajbhiye, N. A., Makasana, J., Saha, A., Patel, I., & Jat, R. S. (2016). LC-ESI-MS/MS Method for Simultaneous Determination of Triterpenoid Glycosides and Aglycones in Centella asiatica L. *Chromatographia*, *79*(11), 727-739.

doi:10.1007/s10337-016-3089-x

42. Sun, B., Wu, L., Wu, Y., Zhang, C., Qin, L., Hayashi, M., ... & Liu, T. (2020). Therapeutic potential of Centella asiatica and its triterpenes: A review. *Frontiers in pharmacology*, *11*, 568032. doi:10.3389/fphar.2020.568032
43. Seevaratnam, V., Banumathi, P., Premalatha, M. R., Sundaram, S. P., & Arumugam, T. (2012). Functional properties of Centella asiatica (L.): a review. *Int J Pharm Pharm Sci*, *4*(5), 8-14.
44. Dzik, S., Murphy, M. F., & Blajchman, M. A. (2020). Editorial Board. *Transfus Med Rev*, 34(3), 2. doi:10.1016/s0887-7963(20)30039-0
45. Flaherty, R. A., Borges, E. C., Sutton, J. A., Aronoff, D. M., Gaddy, J. A., Petroff, M. G., & Manning, S. D. (2019). Genetically distinct Group B Streptococcus strains induce varying macrophage cytokine responses. *PloS one*, *14*(9), e0222910.
46. Cn PM, Patents G. Patents The method for establishing rat aerobic bacteria property vaginitis model CN108283651A. :1-6.
47. Murdiyansah, S., Rasmi, D. A. C., & Mertha, I. G. (2020). Centella asiatica Activities towards Staphylococcus aureus and Escherichia coli Growth. *Jurnal Biologi Tropis*, *20*(3), 499-506.
48. S, F. (1996). Vaginitis: diagnosis and management. *Int J Fertil Menopausal Stud*, 41, 115-123.
49. Frobenius, W., & Bogdan, C. (2015). Diagnostic value of vaginal discharge, wet mount and vaginal pH–an update on the basics of gynecologic infectiology. *Geburtshilfe und Frauenheilkunde*, *75*(04), 355-366. doi:10.1055/s-0035-1545909
50. Donders, G. G., Bellen, G., Grinceviciene, S., Ruban, K., & Vieira-Baptista, P. (2017). Aerobic vaginitis: no longer a stranger. *Research in Microbiology*, *168*(9-10), 845-858. doi:10.1016/j.resmic.2017.04.004

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