Unnes Journal of Public Health 9 (1) (2020)

Unnes Journal of Public Health

http://journal.unnes.ac.id/sju/index.php/ujph

Effectiveness of Soursop Leaf Extract (*Annona muricata l.*) on IL-6 Levels in Mammary *Sprague dawley* Female Rats Induced by *Staphylococcus aureus*

Feby Purnamasari[1]✉, Risfah Yulianty[2], Syamsa Latief[3]

[1,3]Midwifery Department, Postgraduate Faculty, Universitas Hasanuddin Makassar
[2]Pharmacy Department, faculty of Pharmacy, Universitas Hasanuddin Makassar

Article Info

Article History:
Submitted June 2019
Accepted October 2019
Published January 2020

Keywords:
Extract Leaf Soursop; Levels IL-6; Rat Sprague Dawley Females

DOI
https://doi.org/10.15294/ujph.v0i0.30904

Abstract

Staphylococcus aureus is a Gram-positive bacterium that causes inflammation such as mastitis in the breast. Breast mastitis can occur in nursing mothers and requires first aid or initial treatment. Herbal ingredients can be utilized because they are easily obtained and can be prepared by the community themselves. The study aimed to analyze the effectiveness of soursop leaf extract on IL-6 levels in mammae of female rats Sprague dawley induced by S. aureus. The study was divided into four groups consisting of five rats that were induced by S. aureus 2 x 107 CFU/ml. Group I was given 0.5% Na.CMC, Group II gave 45 mg/kgBW cefadroxil antibiotics, group III administered 100 mg / kgBW soursop leaf extract, and group IV administered soursop leaf extract combined with cefadroxil. Each group was given treatment twice a day for 5 days. IL-6 levels were measured on days 3 and 6. The results of this study showed IL-6 levels in group III were 7.50 ± 1.74 pg/ml on the 3rd day, and 4.51 ± 1.47 pg/ml on the 6th day. The results showed there were differences in IL-6 levels in group III (p = 0.037). Soursop leaf extract can be used as a therapy for mastitis caused by S. aureus.

INTRODUCTION

Mastitis is an inflammatory process in one or more breast segments that may be accompanied by infection or without infection (Amir, 2014; Alasiry, 2012). Mastitis due to infection is caused by bacteria. *Staphylococcus aureus* is one and the main cause of infectious mastitis (Fursova et al, 2018; Lai & Perg, 2017; Jahanfar, 2013; Chen, 2013).

There were 876,665 women diagnosed with mastitis in Indonesia. Recent studies have shown that mastitis cases increase by 12-35%. The incidence of mastitis in breastfeeding women is reported to be around 33% and occurs within the first 6 weeks after the baby is born (most often in the 2nd and 3rd weeks postpartum) (Anasari, & Sumarni, 2014). The occurrence of mastitis is caused by a bacterial infection, one of which is *Staphylococcus aureus* (*S. aureus*) which is a gram-positive bacterium (Samantha et al, 2018; Choi et al., 2015; Bauer et al, 2015).

The immune response to *S. aureus* aims to eliminate bacteria and can be used to detect inflammation (Zou et al., 2018; Silva, 2017). Macrophages produce various cytokines such as TNF α, IL-1, IL-6, and IL-8. Interleukin-6 (IL-6) is a pro-inflammatory cytokine that plays an important role in the immune response whose chan-

✉ Corespondence Address:
Midwifery Study Program, Graduate School of Hasanuddin University, Makassar, 90245
E-mail: febypurnamasari934@gmail.com

pISSN 2252-6781
eISSN 2548-7604

ges in levels can be used to detect inflammation and anti-inflammatory (bacterial elimination). An increase in IL-6 levels indicates inflammation due to infection, while a decrease in IL-6 levels indicates an anti-inflammatory process (Han et al., 2016; Sugimoto, 2015; Kruspe et al., 2014; Ishartadiati, 2009). Cytokines IL-6 is different from other cytokines because it can be used as a pro-inflammatory and anti-inflammatory at the same time.

Soursop (Annona muricata L.) is one of the native tropical fruit plants originating from the Caribbean, Central America, North America, and South America (Oloyede et al, 2015; Moreno et al, 2014; Lutchmedial et al, 2014). In Indonesia, soursop fruit is known by various names or different names for each region such as nangka sabrang, nangka landa (Java), soursop (Sunda), sirikaya balanda (Bugis and Makassar), and naka walanda (Ternate) (Maramis et al, 2014; Rosmayanti, 2014). Soursop leaves contain bioactive compounds that are useful as anti-cancer, anti-tumor, anti-virus, anti-fungal, anti-inflammatory, anti-depressant, anti-diabetic, anti-seizure, analgesic, anti-bacterial and lowering blood pressure (Swarnakar, 2014; Mulia et al., 2015; Zuhud, 2011; Sardi, 2009). At present more than 200 acetogenin compounds have been identified and isolated from soursop plants (Telez et al., 2016). Chemical compounds contained in soursop leaves include: flavans, flavonoids, alkaloids, tannins, saponins, and essential oils (Adewole & Ajewole, 2009). The analysis shows that soursop leaf ethanol extract has potential as an anti-inflammatory (Rahman & Mustari, 2012; Estela et al, 2016; Friska et al, 2017).

Breast mastitis can occur in nursing mothers and requires first aid or initial treatment. Herbal ingredients can be utilized because they are easily obtained and can be prepared by the community themselves. Soursop leaves are widely available in the tropics, including in Indonesia, and have been shown to contain anti-inflammatory ingredients. IL-6 can be used as an indicator of inflammation. Research on the anti-inflammatory effects of soursop leaves on mastitis has never been done before. This study aims to determine the effect of soursop leaf extract on inflammation by testing the effect of giving soursop leaf extract on IL-6 levels.

METHODS

Research design

This type of research used in this study is a pure experiment in the laboratory, with a Post-Test Only Control Group Design. The type of rats in this study was rat strain of *Sprague dawley* with a weight of 200-250 grams and age of rats 3-4 months as many as 24 individuals. This study has received a recommendation on ethics approval on January 3, 2019 with No. 45/UN4.6.4.5.31/PP36-KOMETIK/2019 and amended on March 20, 2019.

Population and sample techniques

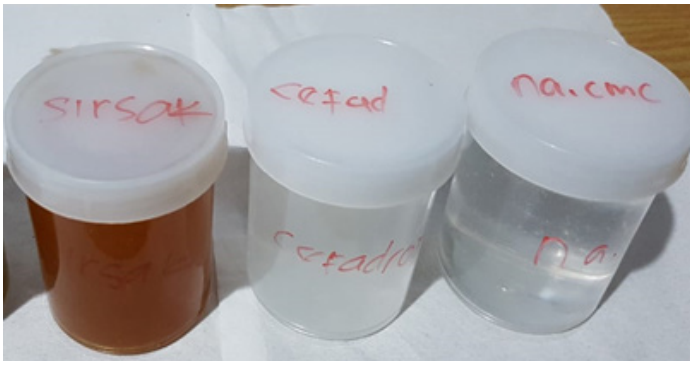
The population in this study was rats of strain *Sprague dawley* with a body weight of 200-250 grams as many as 24 tails. The sample in this study was rats of strain *Sprague dawley* with a weight of 200-250 grams as many as 24 tails. However, random grouping is done to avoid bias because of age. Sampling is done based on trials research guidelines for evaluating the safety and efficacy of herbal medicine according to the standard of WHO (Word Health Organization). Ie there are at least 5 (five) rats of strain *Sprague dawley* in each group and reserve plus 1 (one) each group, so that the number of mice needed is 24 heads divided into 4 groups.

Data analysis

Data is processed and analyzed with the help of a computer. Effect of giving soursop leaf extract, IL-6 cytokine levels are displayed in the form of mean ± (standard deviation) with a confidence interval (95% CI). If the data were normally distributed, a Bivariate Test was used using Paired Samples T test to see differences in IL-6 cytokine levels in the control group, the cefadroxil antibiotic group, the ethanol extract of the soursop leaf group, and the ethanol extract group of soursop leaves combined with the cefadroxil antibiotic. In addition, Test of one way anova was also performed to see differences in IL-6 cytokine levels between groups and continued with post hoc LSD analysis. If the data are not normally distributed then the Kruskal Wallis test is carried out followed by the Mann Whitney test with a significance value of $p \leq 0.05$ at a 95% confidence interval.

The control group was given water with a 0.5% Na.CMC suspension of 100 ml/grBW/day, and a standard feed for 5 days after injection of *S. aureus* bacteria in one of the mammary mice of *Sprague dawley* (2 x10^7 CFU/ml). Cefadroxil antibiotic group was given water, standard feed, and Cefadroxil 45 mg/kgBW/day Na.CMC 0.5% suspension for 5 days after injection of *S. aureus* bacteria in one of the mammae rats of *Sprague dawley* (2 x10^7 CFU/ml). The ethanol extract group of soursop leaves was given water, standard feed, and administration of ethanol

1a



1b

Figure 1a. Ethanol extract of thick liquid soursop leaves ± 250 grams
Figure 1b. Soursop Leaf Extract Suspension, Cefadroxil Antibiotic Suspension, Suspension of Na. CMC

extract of soursop leaf 100 mg/kg/day Na.CMC 0.5% suspension for 5 days after the injection of *S. aureus* bacteria in one of the mammary rats of *Sprague dawley* (2 x10^7 CFU/ml). Ethanol extract group of soursop leaves combined with cefadroxil given water, standard feed, and ethanol extract of soursop leaf extract 100 mg/kg/day Na.CMC 0.5% suspension suspension + administration of Cefadroxil 45 mg /kg/ day Na.CMC 0, 5% for 5 days after injection of *S. aureus* bacteria in one of the mammae rats of Sprague dawley (2 x10^7 CFU/ml), all groups were done orally.

RESULTS AND DISCUSSION

Material preparation

Soursop leaf extraction process in this study produced ethanol extract of soursop leaf (Annona muricata L.) thick liquid as much as ± 250 grams (14.7%) (Figure 1a). The ethanol extract of the thick liquid soursop leaf that has been produced is used to prepare the joint treatment with other ingredients (Figure 1b).

Soursop Leaf Extract Content

Phytochemical testing was conducted qualitatively to determine the content of metabolite compounds contained in soursop leaf extract. Based on the phytochemical test results of soursop leaf ethanol extract showed that soursop leaf ethanol extract contains alkaloids, tannins, saponins, steroids, and flavonoids. Phytochemical test results of compound content on soursop leaves are shown in Table 1.

Research conducted by Londok & Mandey (2014), Soekaryo et al. (2017), Adewole & Ajewole (2009), Rajeswari et al. (2012) and Sugianto et al. (2012) also stated that the ethanol fraction of soursop leaves contained, alkaloids, tannins, saponins, steroids, and flavonoids. Flavonoids and saponins have the ability as antibacterial, antioxidant, anti-inflammatory, and able to prevent cancer (Londok & Mandey, 2014). Flavonoids inhibit bacterial growth (Putri et al., 2017; Solomon-Wisdom, et al 2014).

The content of ethanol extract of soursop leaves which can suppress IL-6 production including flavonoids and saponins which have the ability to be antibacterial, antioxidant, anti-inflammatory, and able to prevent cancer (Londok & Mandey, 2014). Flavonoids can inhibit bacterial gyrase DNA function by damaging the cytoplasmic membrane of bacteria and causing damage to the bacterial cell wall thereby inhibiting bacterial growth (Putri et al., 2017; Stauth, 2007). The results of the identification of the flavonoid group showed soursop leaf extract containing flavonoids, flavones, flavonols, and flavanons (Latifah, 2013). Flavonols (catechins) have antimicrobial properties, strengthen blood vessels, launch urine and inhibit the growth of cancer cells. Tannins have the ability to inhibit the synthesis of chitin and other compounds in polyphenols that interfere with cell permeability by shrinking cell walls or cell membranes so that their growth is inhibited or even dead (Sudira et al., 2014). Saponin is a compound contained in soursop leaf ethanol extract, is antibacterial by working effectively on gram-positive bacteria. The antibacterial mechanism of action of saponins is by increasing cell membrane permeability so that the membrane becomes unstable and results in cell hemolysis (Soetan et al., 2006; Hassan, 2008; Dewi et al., 2015). Steroids have the ability to inhibit bacterial growth (Taleb-Contini et al., 2003). The content of alkaloids in soursop leaf extract has antibacterial ability because it has a quaternary aromatic group that is able to interact with DNA. Alkaloids are also able to disrupt the integrity of the constituent components of peptidoglycan in bacterial cells and cause bacterial death (Cowan,

Table 1. Phytochemical test results of the compound content of soursop leaves (*Annona muricata L.*)

No.	Phytochemical compounds	Stain Viewer	Test results	Description
1	Alkaloids	Dragendorf	There is sediment	+
2	Tannin	FeCl3	Green and sediment formed	+
3	Saponin	Shaken	Foam formed	+
4	Steroid	H2SO4	Red ring formed	+
5	Flavonoids	MgSO4 concentrated HCl	Red orange formed	+

+ (positive) = There were indications of bioactive compounds

1999; Cushnie & Lamb, 2005).

There is the same active compound content in other plants. However, the differences and advantages of soursop leaves because there are more than 200 acetogenin compounds have been identified in soursop plants (Telez, et al., 2016). The content of compounds in the qualitative phytochemical test of soursop leaf extract in this study was obtained alkaloids, tannins, saponins, steroids, and flavonoids which play an important role in inhibiting bacterial growth. So that soursop leaf extract can be an anti-inflammatory herbal therapy. In soursop leaves found acetogenin compounds that are useful in treating various diseases. Acetogenin plays a role in protecting the immune system and preventing deadly infections, compounds that have toxic cytotoxic potential to inhibit the growth of cancer cells (Mardiana & Ratnasari, 2011; Telez at al., 2016).

Differences in IL-6 levels in the 3rd day and in the 6th

Table 2 shows the differences in IL-6 levels in each treatment group of *Sprague Dawley* female rats injected by *S. aureus* on the 3rd day (during treatment) and the 6th day (after treatment). There was no difference in IL-6 cytokine levels in the control group and the cefadroxil antibiotic group. There were differences in IL-6 levels in the soursop leaf ethanol extract group and the soursop leaf ethanol extract group combined with cefadroxil antibiotics.

Table 2: Differences in IL-6 levels in each treatment group of *Sprague Dawley* female rats were injected with *S. aureus* bacteria on the 3rd day (during treatment) and 6th day (after treatment).

Treatment group	IL-6 level (pg/mL)		p value
	3rd Day	6th Day	
	Mean ± SD	Mean ± SD	
Negative Control (n=5)	4,86 ± 3,75	6,97 ± 3,53	0,108[a]
Cefadroxil antibiotics (Cefadroxil 45 mg/kg/day) (n=5)	4,04 ± 2,48	3,76 ± 1,91	0,514[a]
Soursop Leaf Ethanol Extract (Soursop leaf ethanol extract 100 mg /kgBW/day) (n=5)	7,50 ± 1,74	4,51 ± 1,47	*0,037[a]
Soursop leaf ethanol extract group combined with cefadroxil antibiotics (n=5)	7,46 ± 2,66	4,73 ± 1,36	*0.030[a]
p-value	0,143[b]	*0,044[b]	

Description:
[a]Paired Samples T test
[b]*One way Anova*
*p <0.05= there were differences in IL-6 cytokine levels

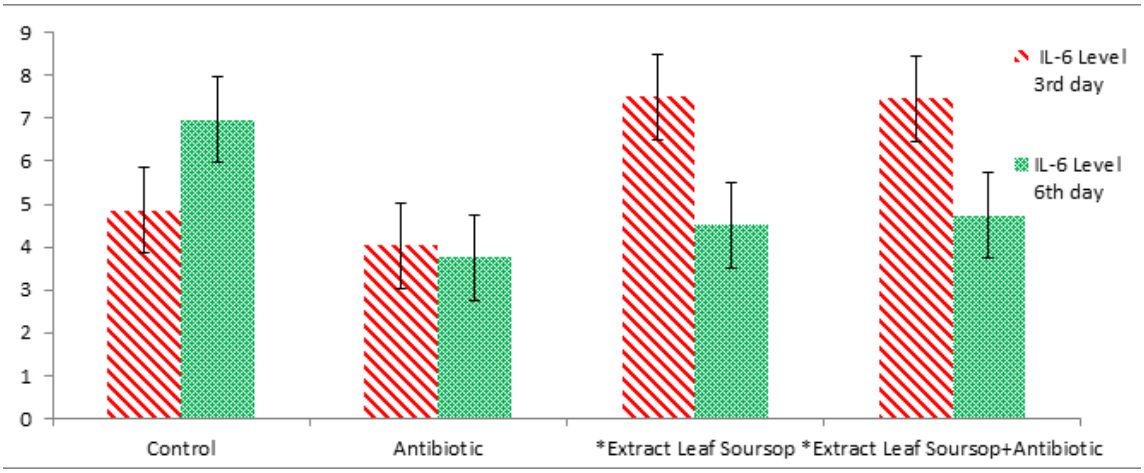


Figure 2. Differences in IL-6 levels in each group of female rats of Sprague dawley on 3rd day and 6th day (* p <0.05)

One way ANOVA test results showed no difference in IL-6 levels during the treatment on the 3rd day between treatment groups. Meanwhile, there were differences in IL-6 levels between treatment groups on the 6th day.

Figure 2 shows the difference in IL-6 levels in each treatment group on the 3rd day and the 6th day. In the control group (only feed + water + Na.CMC), the IL-6 level for the 6th day had an average of 6.97 ± 3.53 pg / mL which was higher than the 3rd day with a mean of 4.86 ± 3 .75 pg / mL. In the cefadroxil antibiotic group, the level of IL-6 on the 6th day had an average of 3.76 ± 1.91 pg / mL which was lower than the 3rd day with an average of 4.04 ± 2.48 pg / mL. In the ethanol extract group of soursop leaves, IL-6 level on the 6th day had an average of 4.51 ± 1.47 pg / ml which was lower than the 3rd day with an average of 7.50 ± 1.74 pg / mL. In the ethanol extract group of soursop leaves combined with cefadroxil antibiotics, IL-6th day 6th had an average of 4.73 ± 1.36 pg / mL which was lower than the 3rd day with a mean of 7.46 ± 2.66 pg / mL.

IL-6 levels on day 3 were still very high in each group as a biomarker that showed inflammation after the induction of S. *aureu*s bacteria. The results of this study are in line with research by Diacci et al. (2017) and Wendy (2014) who showed an increase in IL-6 levels due to the presence of cellular immune responses after the administration of *S. aureus* injection. The activity of proinflammatory cytokines IL-6 and TNF-α will increase, pro-inflammatory cytokines will be synthesized, and systemic inflammation will occur in response to S. *aureus* bacteria. Women with mastitis show an increase in serum cytokines interleukin (IL) 1, 6, and 8, and TNF α. IL-6 levels are increased after the induction of *S. aureus* bacteria. In addition to being used as a biomarker for inflammation, it can also be used as a detection of disease severity. IL-6 levels of pro-inflammatory disease severity are different, including pneumonia for IL-6 levels around 620 pg / mL (sensitivity 71%, specificity 89%), nenonatus sepsis IL-6 31 pg / mL (sensitivity 95 %, specificity 84%), gastric cancer 1.97 pg/mL (sensitivity 81.8%, specificity 66.7%), patients with endometriosis IL-6 2 pg/mL (sensitivity 90%, specificity 67%), IL- 6 in obese and non-obsessed patients an estimated 2 pg/mL, and in patients with active IL-6 psioriasis around 1.1 pg/mL (Arican et al., 2005; Goyal et al., 2012). High levels of IL-6 on the 3rd day after the induction of *S. aureus* bacteria in this study become biomarkers markers of inflammation (pro-inflammatory), as well as a sign of severe infection (inflammation) due to *S. aureus* bacteria (mastitis disease)) (Rahman & Mustari, 2012; Estela, 2016; Friska et al., 2017).

IL-6 levels on the 6th day decreased in the soursop leaf ethanol extract group and the soursop leaf ethanol extract group combined with cefadroxil antibiotics. Soursop leaf extract in rats showed that soursop leaf extract has anti-inflammatory and antibacterial effects at a dose of 0.182 g / kg.

In research on the antibacterial activity of soursop leaf extract using 70% ethanol solvent shows that ethanol extract of soursop leaves with a concentration of 125 mg/mL can inhibit bacterial growth but can also be used 5 concentrations including 150, 125, 100, 70, 50 mg/kg/day (Friska, et al, 2017). Soursop leaf extract concentration in reducing levels of TNF-α and IL-6 showed a decrease in levels of TNF-α and IL-6 found at concentrations of 100 mg/kgBW/day (Estela, et al, 2016). The concentration used in this study

was a concentration of 100 mg/kg/day.

Soursop leaf ethanol extract as a complementary supplement can significantly reduce the production of IL-6 in female rats infected with *S. aureus* bacteria and increase the work of macrophages in phagocytosis because soursop leaf extract has pro-inflammatory and antibacterial properties. The use of cefadroxil antibiotics is as an inhibitor of *S. aureus* bacteria that causes mastitis. This is in accordance with previous studies that antibiotics that can inhibit bacterial mastitis include amoxicillin, gestamin, cephalotin, enrofloxacin, oxytetracycline, amikacin, tetracyclin, chloramphenicol, ciprofloxscin, oxacillin, cefadroxil, gentamycin, lincomycin, fomycloin and frog .

This study proves that the administration of soursop leaf extract (*Annona muricata L.*) and soursop leaf extract which is given together with cefadroxil antibiotics can be used as an effective treatment in mastitis cases. Especially in mastitis caused by *S. aureus* bacteria

If applied to humans, the dose needed per day is 1120 mg, and the dose for dry extract per day is 1400 mg for humans. The dose is obtained from the calculation: (a). Soursop leaf extract dose in rats 100 mg/kg). For rats 200g (200g/1000g) = 0,2, (c). The content of dried soursop leaf extract 80% (ex + filler = 200 g, filler 40 g), (d). The dose of conversion of rats to humans is (56.0), (e). The daily dose of humans is = 100 x 0.2 = 1120 mg, and (f). The dose of dried extract is in humans = = 1400 mg.

Weaknesses in this study are the short treatment time and the researcher did not measure IL-6 levels before induction of *S. aureus* (pre-test) due to the limited number of well elisa kit used. For further research, it is necessary to study the addition of the dose and duration of administration of ethanol extract of soursop leaves to cure mastitis (inflammatory healing) of the breast caused by infection of *S. aureus* bacteria.

CONCLUSION

Soursop leaf extract (*Annona muricata L.*) is effective in decreasing IL-6 levels in mammae rats female strain of *Sprague Dawley* induced by *Staphylococcus aureus* bacteria. Giving soursop leaf extract can be used as a complementary therapy or first aid for mastitis caused by *Staphylococcus aureus* bacteria.

REFERENCES

Adewole, S.O., & Ajewole, J.A.O. 2009. Protective effects of *Annona muricata linn.* (*Annonacaeae*) leaf aqueous stress in serum lipid profiles and oxidative stresss in hepatocytes of Streptozotocin-treated diabeticrat. *African Journal of Traditional, Complementary and Alternative Medicine*, 6 (1): 30–41.

Alasiry, E. 2012. *Buku Indonesia Menyusui*. Retrieved from www.idai.or.id.

Amir L. 2014. ABM Clinical Protocol #4: Mastitis. *Breastfeeding Medicine*, 9(5): 239–243.

Anasari T., & Sumarni. 2014. Faktor-Faktor Yang Mempengaruhi Kejadian Mastitis di RSUD Prof. Dr. Margano Soekarjo Purwokerto. *Jurnal Involusi Kebidanan*, 4(7): 40-52.

Arican O., Aral M., Sasmaz S., & Ciragil P. 2005. Serum Levels of TNF-□, IFNμ, IL-6, IL-8, IL-12, IL-17, and IL-18 in Patients with Active Psoriasis and Correlation with Disease Severity. *Mediators of Inflammation*, 2005(5): 201561. DOI: https://doi.org/10.1155/MI.2005.273

Bauer, I., Gunther, I., Wheeler, T.T., Engelman, S., & Seyfert, H.M. 2015. Extracellular Milieu Grossly Alters Pathogenspecific Immune Response of Mammary Epithelial Cells. *BMC Veterinary Research*, 11: 172.

Chen. 2013. Alpinetin attenuates inflammatory responses by interfering toll-like receptor 4/ nuclear factor kappa B signaling pathway in lipopolysaccharide-induced mastitis in mice. *International Immunipharmacology*, 17(1): 26–32.

Choi, E.J., Kim, H.I., Kim, J.A., Kang, S.H., Park, D.J., Son, S.J., et al. 2015. The Herbal-Derived Honokiol and Magnolol Enhances Immune Response to Infection with Methicillin-Sensitive Staphylococcus Aureus (MSSA) and Methicillin-Resistant *S. aureus* (MRSA). *Applied Microbial and Cell Physiology*, 99: 4387–4396.

Cushnie, T.P.T., & Lamb, A.J. 2005, Antimicrobial activity of avonoids. *Int. J. Antimicrob. Agents*, 26: 343–356.

Cowan, M.M. 1999. Plant products as antimicrobial agents. *Clin. Micobiol. Rev.*, 12(4): 564 – 582.

Diacci, C., Laura, M. D., Bianchini, R., & Pinti, M. 2017. Label-Free Detection of Interleukin-6 Using Electrolyte Gated Organic Field Effect Transistors. *Biointerphases*, 12(5): 05F401

Dewi, Z.Y., Nur, A., & Hertriani, T. 2015. Efek antibakteri dan penghambatan bio lm ekstrak sereh (*Cymbopogon nardus l.*) terhadap bakteri *Streptococcus mutans*. *Majalah Kedokteran Gigi Indonesia*, 1(2): 136–141.

Estela, K.M., Prameshinta, H.F., Dharmana, E., & Kridjamiatun, R.A. 2016. Efektifitas Ekstrak Daun Sirsak (*Annona muricata*) dalam Menurunkan Kadar TNF α dan Meningkatkan Kadar NO Uji Coba pada Mencit Swiss yang Diinokulasi *Plasmodium berghei ANKA*. *Jurnal Kedokteran Brawijaya*, 29(01): 39-42.

Fadel & Anwar. 2014. Antibacterial Efficacy of Variety Plants Against the Resistant *Streptococcus* which Cause Clinical Mastitis in Cows, *AJPRHC*, 5(1): 32-41.

Friska, Tetlana, & Trianna. 2017. Skrining Fitokimia dan Aktivitas Antibakteri Ekstrak Etanol Daun Sirsak (*Annona muricata L.*) pada *Streptococcus mutans ATCC 35668*. *Majalah Kedokteran Gigi Indonesia*, 3(1).

Fursova, K.K., Schchamikova, M.D., Loskutova, I.V., Shepelyakovskaya, A.O., Laman, A.G., Boutanaev, A.M., Sokolov, S.L., Artem'eva, O.A., Nikanova, D.A., Zinovieva, N.A., Brovko, F.A., Kim, Y., & Shin, O.S. 2018. Exotoxin Diversity of Staphylococcus Aureus Isolated from Milk of Cows with Subclinical Mastitis in Central Russia. *Journal of Dairy Science*, 101(5): 4325–4331. DOI: https://doi.org/10.3168/jds.2017-14074.

Goyal, R., Faiz, A., & Singhai, M. 2012. Evaluation of TNF-α and IL-6 levels in obese and non-obese diabetics: Pre- and postinsulin effects. *North American Journal of Medical Sciences*, 4(4): 180-184.

Han, Y., Ripley, B., Jerada, S., Naka, T., & Fujimoto, M. 2016. Interleukin-6 Deficiency Does Not Affect Motor Neuron Disease Caused by Superoxide Dismutase 1 Mutation. *PLoS ONE*, 11(4).

Hassan, SM. 2008. *Antimicrobial activities of saponinrich guar meal extract poultry science*. Doctoral Thesis. Texas: A & M University.

Ishartadiati, K. 2009. *Peranan Tnf, Il-1, dan Il-6 pada Respon Imun Terhadap Protozoa*. Surabaya: FK Universitas Wijaya Kusuma.

Jahanfar, S., Ng, C.J., & Teng, C.L. 2013. Antibiotics for mastitis in breastfeeding women. *Cochrane Database of Systematic Reviews*, 28(2): CD005458.

Kruspe, S., Meyer, C., & Ulrich. H. 2014. Chlorin E6 Conjugated Interleukin-6 Receptor Aptamers Selectively Kill Target Cells Upon Irradiation. *Molecular Therapy Nucleic Acids*, 3(1): e143.

Lai, J.L., & Perg, Y.C. 2017. Indirubin Treatment of Lipopolysaccharide-Induced Mastitis in a Mouse Model and Activity in Mouse Mammary Ephithelial Cells. *Mediators of Immflamation*. 2017: 3082805.

Latifah, W. 2013. *Uji Aktivitas Antioksidan Ekstrak Etanol Daun Sirsak (Annona Muricata L) Gugur.* Master Thesis. Yogyakarta: Universitas Gadjah Mada.

Londok, J.J.M.R., & Mandey, J.S. 2014. Potensi Fitokimia dan Aktivitas Antimikroba Daun Sirsak (*Annona muricata Linn.*) Sebagai Kandidat Bahan Pakan Ayam Pedaging. *Jurnal LPPM Bidang Sains dan Teknologi*, 1(1).

Lutchmedial, M., Ramlal, R., Bridre, N., & Yen, I.C. 2014. Nutritional and Sensory Quality of Stirred Soursop (*Annona Muricata L.*) Yoghurt. *International Journal of Food Sciences and Nutrition*, 55(5): 407– 414.

Mardiana, L., & Ratnasari, J. 2011. *Ramuan dan Khasiat Sirsak*. Jakarta: Penebar Swadaya.

Maramis, R., Kaseke, M., & Tanudjadja, G.N. 2014. Gambaran Histologi Aorta Tikus Wistar dengan Diet Lemak Babi Setelah Pemberian Ekstrak Daun Sirsak (*Annona muricata L.*). *Jurnal E-Biomedik (eBM)*, 2: 430–435.

Mulia, K., Krisanti, E., Maulana, T., & Dianursanti. 2015. Selective Polarity-Guided Extraction and Purification of Acetogenins in *Annona Muricata L*. Leaves. *International Journal of Technology*, 7: 1221–1227.

Oloyede, C. T., Akande F.B., & Oniya O.O. 2015. Moisture Dependent Physical properties of Sour-Sop (*Annona Muricatal.*) Seeds. *CIGR Journal*, 17(2): 185.

Putri, R.A., Sari, N.I, Supartono, & Mursiti, S. 2017. Lotion Ekstrak Daun Sirsak (*Annona muricata L.*) sebagai Antibakteri. *Indonesian Journal of Chemical Science*, 6(3).

Rahman, S., & Mustari. 2012. Uji efek antiinflamasi ekstrak etanol daun sirsak (*Annona muricata Linn.*) terhadap mencit (*Mus musculus*) jantan yang diinduksi dengan karagen. *As-Syifaa Jurnal* Farmasi. 4(1): 7-15.

Rajeswari, V., Gajalakshmi, S., & Vijayalakshmi, S. 2012. Phytochemical and Pharmacological Properties of Annona Muricata: A review. *International Journal of Pharmacy and Pharmaceutical Science*, 4(2): 3-6.

Rosmayanti, K. 2014. Uji Efektivitas Ekstrak Biji Sirsak (*Annona Muricata L*) Sebagai Larvasida pada Larva Aedes Aegypti Instar III/ IV. Undergraduate Thesis. Jakarta: Universitas Islam Negeri Jakarta.

Samantha, K.W., Wennitz, O., Bruckmaier, R.M., & Schwartz, D. 2018. Differential Somatic Cell Count in Milk Before, Dur-

ing, and After Lipopolysaccharide and Lipoteichoic-Acid-Induced Mastitis in Dairy Cows. *Journal of Dairy Science*, 101(6).

Sardi, D. 2009. *Herbal Indonesia Berkhasiat*. Depok: Trubus Swadaya.

Silva, E.M. 2017. High Systemic Il-6 Is Associated with Worse Prognosis in Patients with Non-Small Cell Lung Cancer. *PLoS ONE*, 12 (7).

Soekaryo, E., Setyahadi S., Simanjuntak P. 2017. Identifikasi Senyawa Aktif Fraksi Etanol Daun Sirsak (*Annona Muricata Linn.*) Sebagai Penghambat Siklooksigenase-2. *The 5th Urecol Proceeding*, 862-867

Soetan KO, Oyekunle MA, Aiyelaagbe OO, & Fafunso MA., 2006, Evaluation of the antimicrobial activity of saponins extract of sorghum bicolor l. moench. African J of Biotechnology, 5(23): 2405–2407.

Solomon-Wisdom, G., Ugoh, S., & Mohammed, B. 2014. Phytochemical screening and antimicrobial activities of *Annona muricata (L)* leaf extract. *American Journal Biology Chemistry Pharmaceutical Sciences*, 2(1): 1-7.

Sudira, I. W., Merdana, I., & Wibawa, I. 2011. Uji daya hambat ekstrak daun kedondong (*Lannea Grandis Engl*) terhadap pertumbuhan bakteri Erwinia carotovora. *Buletin Veteriner Udayana*, 3(1), 45-50.

Sugimoto, Y, 2015, Associations between polymorphisms of interleukin-6 and related cytokine genes and serum liver damage markers : A cross-sectional study. *Gene*, 557(2): 158–162.

Sugianto, I.S. 2012. *Uji Fitokimia Ekstrak Pegagan (Centella asiatica) dan Buah Sirsak (Annona muricata L.) Serta Potensinya Sebagai Inhibitor Enzim Xantin Oksidase*. Undergraduate Thesis. Malang: Universitas Negeri Malang

Swarnakar, A. 2014. Literary approach to Annona muticata and its role in cancer- A review. *Int. Journal if Reasearch in Pharmacology & Pharmacotherapeutics*, 3 (4).

Stauth, D. 2007. *Studies Force New View on Biology of Flavonoids*. Oregon: Oregon State University.

Taleb-Contini, S.H., Salvador, M.J., Watanabe, E., Ito, I.Y., & Oliveira, D.C.R. 2003. Antimicrobial activity of flavonoids and steroids isolated from two chromolaena species. *Braz J Pharm Sci*, 39(4): 403–408.

Telez, A.V.C., Gonzalez, E.M., Yahia, E.M., Vazquez, E.N.O. 2016. *Annona muricata*: A Comprehensive Review on Its Traditional Medicinal Uses, Phytochemicals, Pharmacological Activities, Mechanisms of Action and toxicity. *Arabian Journal of Chemistry*,11(5): 662-691.

Wendy, V. I. 2014. Inflammatory Mediators in Mastitis and Lactation Insufficiency. *J Mammary Gland Biol Neoplasia*, 19: 161–167.

Zuhud, A.M. 2011. *Bukti Kedahsyatan Sirsak Menumpas Kanker*. Jakarta: Agro Media Pustaka.

Zou, W., Feng, R., & Yong, Y. 2018. Changes in The Serum Levels of Inflammatory Cytokines in Antidepressant Drug-Naïve Patients with Major Depression. *PLoS ONE*, 13(6): e0197267.