ORIGINAL ARTICLE

Effect of Poguntano leaves extract (*Picria fel-terrae Merr.*) to procalcitonin level in acute bacterial rhinosinusitis model of Wistar mice

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ABSTRACT

Aim Acute rhinosinusitis (AR) is of viral aetiology and only 0.5-2% develop into acute bacterial rhinosinusitis. Herbal therapy is a promising alternative in acute bacterial rhinosinusitis treatment. The aim of this study was to evaluate the effect of ethanol extract of Poguntano leaves (EEPL) to procalcitonin level and the amount of bacteria in acute bacterial rhinosinusitis mice model.

Methods Experimental research with posttest only control group design in 32 Wistar mice that were divided into 4 groups, 3 of which were being inoculated with *Staphylococcus aureus* by inserting a sponge to right nasal cavity of the mice (group K2, K3, and K4); another one was the negative control group (K1). Group K2 was not given any kind of therapy (positive control), group K3 was given 10 mg/kd EEPL for 5 days orally during an induction, and group K4 was given 10 mg/kd EEPL for 5 days orally on the 10th day after induction. Mice in the groups K2 and K3 were sacrificed on the 10th day after induction, while mice in group K4 were sacrificed on the 15th day after induction.

Result A statistically significant decrease in procalcitonin level ($p<0.001$) and amount of bacterial colony ($p<0.001$) was found in four groups.

Conclusion Poguntano leaves extract can lower procalcitonin and amount of bacteria colony, showing an anti-inflammatory and antibacterial effect.

Key words: immunology, inflammation, microbiology, therapeutics, rhinitis

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INTRODUCTION

Acute rhinosinusitis (AR) is an upper respiratory tract infection that is often found in large group of population. In the United States, 30 million cases of AR is reported each year and consumed about 5.8 million USD for treatment each year (1,2). Most AR is caused by viral infection, and thus, antibiotic administration is unnecessary. In reality, it is quite difficult to differentiate an AR of viral etiology to that of bacterial etiology (1).

Rhinosinusitis in adult population is mostly being treated with antibiotic, although the efficacy is still limited or controversial (3). The gold treatment of AR has yet to be established. Antibiotic is not indicated for uncomplicated AR treatment (4). Most AR can resolve without antibiotic administration, but data shows that there are a lot of antibiotic misuse in AR patients (5,6). The difficulty in differentiation AR of bacterial to that of non-bacterial etiology influenced a rise in antibiotic therapy in the last few years (7,8). One of Cochrane reviews stated that antibiotic administration can affect normal flora population and related to antibiotic resistance (1,9). Benefit of antibiotic usage should be carefully evaluated and should always be weighed on its side effects – allergic reaction and development of resistant bacteria – although given for acute bacterial rhinosinusitis. Thus, studies needed to be done to find safe and effective alternative therapy for acute bacterial rhinosinusitis (5).

Procalcitonin (PCT) is one of the pro-inflammatory biomarkers for bacterial infection that is released as a response to bacterial toxin and bacteria-specific pro-inflammatory mediators (10,11). In bacterial infection, PCT level in circulation can increase when in viral infection it stays low (12). In one of the studies, rhinosinusitis is found to have more level of PCT, around 0.8 ± 0.2 ng/mL, a higher amount compared to that of normal population. Antibiotic administration is not given with mean PCT<0.25 ng/mL and it is recommended in cases with PCT>0.25 ng/mL (13).

Herbal medicines have long been used in treating both viral and bacterial AR and there has been an increasing interest in herbal studies, both in the US and in Europe countries (5). Herbal therapy or phytotherapy is a quite promising alternative in AR treatment (14). Several researchers have proven that herbal therapy is beneficial as an additional therapy in rhinosinusitis. This therapy can also lower AR symptoms in adult and paediatric population *in vivo*, showing a quite high tolerance and safety (15).

One of the herbs that is proven to be effective is Poguntano (*Picria fel-terrae Merr*), which leaves are often traditionally used by the people of Tiga Lingga Village of Dairi District in North Sumatera as anti-diabetic therapy (16). There have been a lot of researches done suggesting the benefits of this herb in medical field. Ethanol extract of Poguntano leaves have phytochemistry, which is flavonoid, saponin, tannin, glycoside, and steroid/ triterpenoid (17).

In *in vitro* and *in vivo* studies, steroid/ triterpenoid and flavonoid can inhibit pro-inflammatory cytokines that are secreted by Th1 (TNF-α, IL-2 and IFN-γ) and Th2 (IL-6, IL-4, IL-5, IL-10 and IL-13) cells through limphocytes activation as a response to antigen stimulation, and thus, can lower secretion of acute phase protein, such as procalcitonin (18-20).

Aim of this study was investigate the effect of Poguntano leaves extract (*Picria fel-terrae Merr*.) to procalcitonin level and amount of bacteria colony in acute bacterial rhinosinusitis in Wistar mice model. An evaluation of alternative therapy for acute bacterial rhinosinusitis can hopefully lower the misuse or overuse of antibiotic that have long been known to influence the development of bacterial resistance.

MATERIALS AND METHODS

Study design

This is a true experimental study posttest only control group design. The study on the mice was conducted in the Animal House of Biology Laboratory of Faculty of Mathematics and Natural Sciences of USU (Universitas Sumatera Utara). The preparation of Poguntano leaves extract (EEPL) was conducted in Faculty of Pharmacy of USU. Procalcitonin level was assessed in the Integrated Laboratory (Laboratorium Terpadu) of the School of Medicine of USU. Bacterial colony examination takes place at Microbiology Department of School of Medicine of USU.

This study was approved by the ethics committee of the School of Medicine, Universitas Sumatera Utara.

Methods

Plant materials. Fresh Poguntano leaves were washed and dried at ±40 °C, then grinded until dried powder was obtained. Poguntano leaves extract was produced by maceration of dried powder using ethanol 96%. The powder was soaked for 5 days with periodic stirring. The filtrate was collected, and then evaporated until viscous extract was obtained.

Animals. Thirty-two Wistar mice (*Rattus novergicus,sp*) were used as the sample of this study. According to the Federer's formula (21), the minimum sample size in each group were six of Wistar mice. To anticipate the mice that died during the study, two mice were added to each group. The inclusion criteria were: female mice 8 weeks old, weighed 180 – 200 grams, healthy, and active. The exclusion criteria were: identification of pus within nasal cavity, lowering condition of the mice, or death during the course of the study, and aggressive behavior of the mice that attack other members of its group.

Colonization. All white mice in intervention were injected with 50 mg/kg ketamine hydrochloride intramuscularly for anesthesia (22). The right nasal cavity was then obstructed with sterile sponge to block the ostium of the maxillary sinus. This sponge was first inoculated with 1 mL of *Staphylococcus aureus* bacterial suspension (23). The preparation of bacterial suspension was done in Microbiology Laboratory of USU Hospital Clinic in Medan. *Staphylococcus aureus* ATCC 25924 strain was made in $900x10^6$ cells/mL concentration using McFarland Nephelometer Standard III (24). The right maxillary sinus was dissected in sterile conditions. Secretion swab was taken from right maxillary sinus to evaluate bacteria colony of *Staphylococcus aureus*. If no secretions were found in the maxillary sinus, rinsing with 1 mL of physiological saline solution, and the rinsing fluid was collected for analysis. Secretory samples were inoculated in a mannitol salt agar (MSA) as a culture medium for the *Staphylococcus aureus* bacteria. The MSA disk was incubated at 35 ± 20 °C during 48 hours. Bacterial colonies were counted using colony counter (23).

Samples were divided into four groups: negative control group (K1) that was not inducted by AR and was not given Poguntano leaves extract; positive control group (K2) with AR untreated with Poguntano leaves extract (in this control group, mice were sacrificed on the 10th day after induction); experimental group I (K3) with mice inducted by ABR (acute bacterial rhinosinusitis) and given Poguntano leaves extract 10 mg/kg for 5 days through oral gavage (Poguntano leaves extract was given directly after induction and then sacrificed on the 10th day after induction); and experimental group II (K4) was inducted with ABR and given Poguntano leaves extract 10 mg/kg for 5 days, starting on the 10th day after induction (sacrificed on the 15th day after induction). The mice were grouped through simple randomization. All of the mice were then sacrificed through intra-peritoneal injection of Ketamine hydrochloride (75 mg/kg). Blood was taken from the heart and it was used to assess the PCT level through enzyme-linked immunosorbent assay (ELISA) method using CSB-E13419r Rat Procalcitonin (PCT) Elisa Kit 96 T (CUSABIO, China) was used.

Statistical analysis

The data was presented through mean ± standard deviation. Differences between mean values of PCT were analyzed using Kruskal Wallis test followed by Mann Whitney and t independent posttest method. Differences between means in bacterial colonies were established using ANOVA test analysis of variance followed by Bonferroni's posttest method. A $p<0.05$ was considered statistically significant.

RESULT

The 32 mice in this study was divided into four different groups: negative control group (8 mice), positive control (8 mice), experimental I (8 mice), and experimental II (8 mice). The lowest mean of procalcitonin level was found in group K4 with 191.13 pg/ml (SD=51.75 pg/mL). The highest PCT level found in group K2 (positive control group) with mean 699.38 pg/ml (SD=214.17 pg/mL). Mean level of PCT in group K3 (experimental I) was 338.13 pg/mL (SD=124.19 pg/mL), higher than mean PCT in group K4 (experimental II) (Table 1). A statistically significant difference in mean PCT between four study groups ($p<0.001$) was found. Post Hoc test shows that there was a significant mean

Table 1. Procalcitonin level in the control and treatment group

Group*	Mean (±SD) of procalcitonin (pg/Ml)	p	Post Hoc		
			K2	K3	K4
K1	287.5 (±120.62)	<0.001	0.001	0.422	0.066
K2	699.38 (±214.17)			<0.001	<0.001
K3	338.13 (±124.19)				0.012
K4	191.13 (±51.75)				

*Negative control (K1) that was not inducted by acute rhinitis (AR) and was not given Poguntano leaves extract; positive control (K2) with AR untreated with Poguntano leaves; experimental group I (K3) with mice inducted by AR and given Poguntano leaves extract 10 mg/kg for 5 days directly after induction; experimental group II (K4) was inducted with ABR and given Poguntano leaves extract 10 mg/kg for 5 days, starting on the 10th day after)

between group K1 and K2 (p<0.001), group K2 and K3 (p<0.001), group K2 and K4 (p<0.001). A significant difference is also found in group K3 (experimental I) and group K4 (experimental II) with p=0.012. No significant difference found in group K1 and K3 (p=0.422) and group K1 group K4 (p=0.066) (Figure 1).

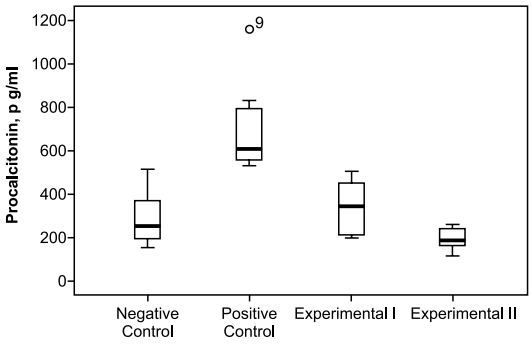

Figure 1. Boxplot graphic of procalcitonin level of four study groups Explanation of the groups as in the Table 1.

In group K1, mean bacteria colony assessed is 34.25 (SD=14.61 colony). The highest amount of colony observed is in group K2 with >300 colony. Mean bacteria colony in group K3 is 148.38 (SD=58.93 colony), more than that of group K4 with 26.25 (SD=21.54 colony) (Table 2).

Table 2. Amount of bacteria colony in the control and treatment group

Group	Mean (SD) amount of bacterial colony colony	p	Post Hoc		
			K2	K3	K4
K1	34.25 (14.61)	<0.001	<0.001	<0.001	1.000
K2	> 300			<0.001	<0.001
K3	148.38 (58.93)				<0.001
K4	26.25 (21.54)				

Explanation of the groups as in the Table 1.

A statistically significant difference in colony amount between the four study groups was found (p<0.001). Using Post Hoc test, there was found a statistically significant difference in mean bacteria colony between group K1 and K2 (p<0.001), group K1 and K3 (p<0.001), group K2 and K3 (p<0.001), group K2 and K4 (p<0.001), and group K3 and K4 (p<0.001). No significant difference in mean amount of bacteria colony found between group K1 and K4 (p=1.000) (Figure 2).

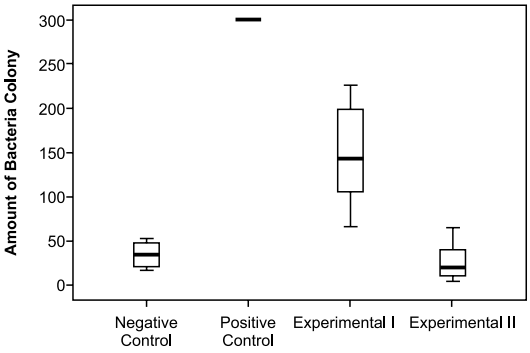

Figure 2. Boxplot graphic of amount of bacteria colony of four study groups Explanation of the groups as in the Table 1.

DISCUSSION

Negative control group (K1) that was not inducted by AR and was not given Poguntano leaves extract; positive control group (K2) with AR untreated with Poguntano leaves extract (in this control group, mice were sacrificed on the 10th day after induction); experimental group I (K3) with mice inducted by ABR and given Poguntano leaves extract 10 mg/kg for 5 days through oral gavage (Poguntano leaves extract was given directly after induction and then sacrificed on the 10th day after induction); and experimental group II (K4) was inducted with ABR and given Poguntano leaves extract 10 mg/kg for 5 days, starting on the 10th day after induction.

The different timings of Poguntano leaves extract between group K3 (with mice inducted by ABR) and K4 (experimental group II inducted with ABR and given Poguntano leaves extract) was due to the observation that group K3 (administration on induction) has not been overgrown by *Staphylococcus aureus* in the mice's maxillary sinus, hence the insufficiency to evaluate the role of Poguntano leaves extract as anti-inflammation and immunomodulator. Group K4 (administration on the 10th day after induction) was expected to be overgrown by *Staphylococcus aureus* in the mice's maxillary sinus, and thus, observation of EEPL effect as anti-bacterial is considered to suffice. This corresponds with the research done

by Cheng et al. (25), Ozcan et al. (26), and Dolci et al. (27) who found bacteria on the 10th day of induction (bacteria-inoculated sponge insertion to nasal cavity), in maxillary sinus that can be confirmed microbiologically, histopathologically, and through CT-scan.

In this study, the highest increase in procalcitonin (PCT) level is observed in a group that was inducted by AR only without the administration of Poguntano (group K2). This is in line with Autio et al. (10) statement that corresponding biomarkers with inflammation reaction, both local and systemic, can show pathophysiology and development of bacteria in AR, where PCT is one of the pro-inflammatory markers that is linked with bacterial infection. An increase in PCT in our study corresponds with a clinical study that documented the increase in PCT level in septic shock patients caused by bacteremia (28). Our results showed significant difference between groups that are only inducted by AR (group K2) with group that is only being administrated with Poguntano extract (group K3 and K4).

Post Hoc test results of presented study showed no significant difference between group K1 and K3, group K1 and K4 suggesting that administration of EEPL can decrease PCT level to normal (group K3) even lower (group K4). A decrease in PCT following Poguntano extract administration in lowering inflammation due to AR induction has indicated in pharmacology studies suggesting that Poguntano leaves extract has anti-inflammatory, anti-pyretic, analgesic, antioxidant, diuretic, anti-diabetic, hepato-protective, and anti-helmintic effect (29). The EEPL contain chemical compounds of glycoside, flavonoid, saponin, tannin, and steroid/triterpenoid (17,30).

Yassine et al. (31) has demonstrated flavonoid role in anti-inflammation process. Flavonoid is shown to inhibit important enzymes, especially prostaglandin and nitric oxide, and also effective in inhibiting arachidonic acid metabolism that mediates prostaglandin biosynthesis. Active substance of flavonoid is considered to be a strong anti-oxidant (31,32).

This research shows that the lowest mean of PCT was found in the group that was treated with Poguntano extract on the 10th day after inducted (group K3) considering that EEPL administration has superior effect in severe infection (bacterial phase). PCT level can increase due to direct induction of peptidoglycan and lipotheichoic acid on bacterial cell wall and direct induction from bacterial enterotoxin, because of indirect induction through pro-inflammatory mediators, such as IL-6 and TNF-α (10,33,34). EEPL administration in bacterial phase (group K4) can lower PCT level more significantly.

Results of our study have shown a significant decrease in amount of colony in experimental II (K4) group (mice was inducted with ABR and given Poguntano leaves extract 10 mg/kg for 5 days, starting on the 10th day after induction) comparing to the negative control group (K1). This corresponds with Mustika study (35) that *Staphylococcus aureus* and *Escherichia coli* growth can be inhibited by EEPL.

Anti-bacterial activity can be caused by the presence of flavonoid and tannin. Flavonoid and tannin are phenol compounds (32,36). Flavonoid has a mechanism as an anti-bacterial due to its ability to form complex with extra-cellular protein and bacteria cell wall. Meanwhile, tannin is able to inactivate microbe adhesion, enzymes, and disturb protein transport on the inner cell lining (35,37,38).

Saponin mechanism of action as an anti-bacterial is through increasing bacteria cell wall permeability and causing protein and enzyme leakage from the cell (29). Steroid also has been reported to have anti-bacterial property, where steroid is linked with membrane lipid and liposome leakage (39). Triterpenoid is also reported to have anti-bacterial effect. This compound disrupts cytoplasm membrane through intracellular leakage, such as potassium that also marks the start of membrane disruption (40).

In conclusion, our study revealed that EEPL administration can lower PCT level in experimental acute bacterial rhinosinusitis animal model, and decrease bacteria colony in maxillary sinus in experimental acute bacterial rhinosinusitis animal model. EEPL can also function as an anti-inflammation, inmmunomodulator, and anti-bacterial due to its ability to lower pro-inflammatory protein, degree of inflammation histopathologically, and amount of bacteria colony in acute bacterial rhinosinusitis Wistar mice model.

The limitation of this study is not to compare the effects of Poguntano leaves extract with standard

therapy in acute rhinosinusitis. Further studies of the phase 1 clinical trials in humans can be conducted to determine whether it may be represent novel therapy for acute rhinosinusitis in humans.

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TRANSPARENCY DECLARATION

Conflicts of interest: None to declare.

REFERENCES

1. Ahovuo-Saloranta A, Rautakorpi UM, Borisenko OV, Liira H, Williams Jr JW, Mäkelä M. Antibiotics for acute maxillary sinusitis in adults. Cochrane Database Syst Rev 2014; CD000243.
2. Sharp AL, Klau MH, Keschner D, Macy E, Tang T, Shen E, Munoz-Plaza C, Kanter M, Silver MA, Gould MK. Low-value care for acute sinusitis encounters : Who's choosing wisely?. Am J Manag Care 2015; 21:479-85.
3. Fokkens WJ, Hoffmans R, Thomas M. Avoid prescribing antibiotics in acute rhinosinusitis. BMJ 2014; 349:g5703.
4. Jund R, Mondigler MA, Stammer H, Stierna P, Bachert C. Herbal drug BNO 1016 is safe and effective in the treatment of acute viral rhinosinusitis. Acta Oto-Laryngologica 2015; 135:50.
5. Bachert C, Schapowal A, Funk P, Kieser M. Treatment of acute rhinosinusitis with the preparation from Pelargonium Sidoides EPs 7630: a randomized, double-blind, placebo-controlled trial. Rhinology 2009; 47:51-8.
6. Schalek P, Hornackova Z, Hahn, A. The relationship of C-reactive protein levels and positive culture with quality of life in acute rhinosinusitis. Patient Prefer Adherence 2015; 9:185-9.
7. Young J, De Sutter A, Merenstein D, Van Essen GA, Kaiser L, Varonen H, Williamson I, Bucher HC. Antibiotics for adults with clinically diagnosed acute rhinosinusitis : a meta-analysis of individual patient data. Lancet 2008; 371:908-14.
8. Gulliford MC, Dregan A, Moore MV, Ashworth M, Van Staa T, McCann G, Charlton J, Yardley L, Little P, McDermott L. Continued high rates of antibiotic prescribing to adults with respiratory tract infection: survey of 568 UK general practices. BMJ Open 2014; 4:e006245.
9. Lemiengre MB, Van Driel ML, Merenstein D, Young, J, De Sutter AI. Antibiotics for clinically diagnosed acute rhinosinusitis in adults. Cochrane Database Syst Rev 2012; 10:CD006089.
10. Autio TJ, Koskenkorva T, Leino TK, Koivunen P, Alho OP. Longitudinal analysis of inflammatory biomarkers during acute rhinosinusitis. The Laryngoscope 2017; 127:E55-61.
11. Schuetz P, Briel M, Mueller B. Clinical outcomes associated with procalcitonin algorithms to guide antibiotic therapy in respiratory tract infections. JAMA 2013; 309:717-8.
12. Kofteridis DP, Samonis G, Karatzanis AD, Fragiadakis GM, Bourolias C A, Maraki S, Papadakis JA, Velegrakis GA. C-reactive protein and serum procalcitonin levels as markers of bacterial upper respiratory tract infections. Am J Infect Dis 2009; 5:282-7.
13. Chauhan P, Sood A, Jain M, Dabla P, Sood S. Serum PCT and CRP levels in upper respiratory tract infections as a marker of infection. AIJCR 2013; 6:1-4.
14. Koch AK, Klose P, Lauche R, Cramer H, Baasch J, Dobos GJ, Langhorst J. A systematic review of phytotherapy for acute rhinosinusitis. Forsch Komplementmed 2016; 23:165-9.
15. Passali D, Cambi J, Passali FM, Bellussi LM. Phytoneering : a new way of therapy for rhinosinusitis. Acta Otorhinolaryngol Ital 2015; 35:1-8.
16. Harfina F, Bahri S, Saragih A. Effect of Poguntano leaves powder (Picria fel-terrae Merr) in patients with diabetes mellitus. J Pharm Pharmacol 2012; 1:112-8.
17. Sibagariang HE. Effect of Poguntano (Picria felterrae (Lour.) Merr.) leaves extract on levels of Superoxide Dismutase (SOD) in patients with type 2 diabetes mellitus. Universitas Sumatera Utara, Medan 2017; MA thesis.
18. Lee S, Kim YJ, Kwon S, Lee Y, Choi SY, Park J, Kwon HJ. Inhibitory effects of flavonoids on TNF-α-induced IL-8 gene expression in HEK 293 cells. BMB Rep 2008; 265-70.
19. Checker R, Sandur SK, Sharma D, Patwardhan RS, Jayakumar S, Kohli V, Sethi G, Aggarwal BB, Sainis KB. Potent anti-inflammatory activity of ursolic acid, a triterpenoid antioxidant, is mediated through suppression of NF-κB, AP-1 and NF-AT. PLoS One 2012; 7:E31318.
20. Chandrasenan P, Neethu MV, Anjumol VM, Anandan V, Selvaraj R. Triterpenoid fraction isolated from Euphorbia tirucalli Linn. amelorates collagen induced arthritis in Wistar rats. J App Pharm Sci 2016; 6:070-5.
21. Ihwah A, Deoranto P, Wijana S, Dewi IA. Comparative study between Federer and Gomez method for number of replication in complete randomized design using simulation: study of Areca Palm (Areca catechu) as organic waste for producing handicraft paper. IOP Science 2018; 131:1-6.
22. Cingi C, Eskiizmir G, Burukoglu D, Erdogmus N, Ural A, Unlu H. The histopathological effect of Thymoquinone on experimentally induced rhinosinusitis in rats. Am J Rhinol Allergy 2011; 25:e268-72.
23. De Campos CAC, Dolci ELL, Da Silva L, Dolci JEL, De Campos CAH, Dolci RLL. Osteitis and mucosal inflammation in a rabbit model of sinusitis. Braz J Otorhinolaryngol 2015; 81:312-20.
24. Yoruk O, Tatar A, Keles ON, Cakir A. The value of Nigella sativa in the treatment of experimentally induced rhinosinusitis. Acta Otorhinolaryngol Ital 2017; 37:32-7.

25. Cheng Y, Wei H, Li Z, Xue F, Jiang M, Chen W, Wang Q. Effects of intranasal corticosteroids in the treatment of experimental acute bacterial maxillary sinusitis in rabbits. ORL J Otorhinolaryngol Relat Spec 2009; 71:57-65.
26. Ozcan KM, Ozcan I, Selcuk A, Akdogan O, Gurgen SG, Deren T, Koparal S, Ozagul C, Dere H. Comparison of histopathological and CT findings in experimental rabbit sinusitis. Indian J Otolaryngol Head Neck Surg 2011; 63:56-9.
27. Dolci ELL, De Campos CAC, Da Silva L, Dolci RLL, Dolci JEL. Evaluation of the ability of an experimental model to induce bacterial rhinosinusitis in rabbits. Braz J Otorhinolaryngol 2014; 80:480-9.
28. Gilbert DN. Procalcitonin as a biomarker in respiratory tract infection. Clin Infect Dis 2011; 52 (Suppl 4):S346-50.
29. Auliafendri N, Rosidah, Yuandani, Suryan, S, Satria D. The immunomodulatory activities of Picria Fel-Terrae Lour herbs towards RAW 264.7 cells. OA-MJMS 2019; 7:24-2.
30. Park M, Cho H. Jung H, Lee H, Hwang KT. Antioxidant and antiinflammatory activities of tannin fraction of the extract from black raspberry seeds compared to grape seeds. J Food Biochem 2013; 38:259-70.
31. Yassine EZ, Dalila B, Latifa EM, Smahan B, Lebtar S, Sanae A, Abdellah F. Phytochemical screening, anti-inflammatory activity and acute toxicity of hydro-ethanolic, flavonoid, tannin and mucilage extracts of Lavandula stoechas L. from Morocco. IJPPR 2016; 8:31-7.
32. Satria D, Silalahi J, Haro G, Ilyas S, Hasibuan PAZ. Antioxidant and antiproliferative activities of an ethylacetate fraction of Picria fel-terrae Lour.Herbs. Asian Pac J Cancer Prev 2017; 18:399-403.
33. Christ-Crain M, Schuetz P, Huber AR, Muller B. Procalcitonin: importance for the diagnosis of bacterial infections. J Lab Med 2008; 32:1-8.
34. Oksuz L, Somer A, Salman N, Erk O, Gurler N. Procalcitonin and C-reactive protein in differentiating to contamination from bacteremia. Braz J Microbiol 2014; 45:1415-21.
35. Mustika N. Making nanoparticles from ethanol extracts of leaves of Pugun Tanoh (Picria fel-terrae Lour.) and anti-bacterial activity test on Staphylococcus aureus and Escherichia coli. Universitas Sumatera Utara, Medan 2018; Undergraduate pharmacy thesis.
36. Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev 1999; 12:564–82.
37. Stefanovic O, Radojevic I, Vasic S, Comic L. Antibacterial activity of naturally occurring compounds from selected plants. In: Bobbalara V, ed. Antimicrobial Agents. Chapter 1. Croatia: Intech Open, 2012. https://www.intechopen.com/books/antimicrobial-agents/antibacterial-activity-of-naturally-occurring-compounds-from-selected-plants (08 October 2019).
38. Sung SH, Kim KH, Jeon BT, Cheong SH, Park JH, Kim DH, Kweon HJ, Moon SH. Antibacterial and antioxidant activities of tannins extracted from agricultural by-products. J Med Plant Res 2012; 6:3072-9.
39. Madduluri S, Rao KB, Sitaram B. In vitro evaluation of antibacterial activity of five indigenous plants extract against five bacterial pathogens of human. Int J Pharm Pharm Sci 2013; 5(Suppl 4):679-84.
40. Leon L, Lopez MR, Moujir L. Antibacterial properties of zeylasterone, a triterpenoid isolated from Maytenus blepharodes, against Staphylococcus aureus. Microbiol Res 2010; 165:617-26.