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Original Article

A Study Comparing Antibacterial Activity of *Ageratum Conyzoides* L. Extract and *Piper Betle* L. Extract in Gel Dosage Forms Against *Staphylococcus Aureus*

Arif Budiman[1,*], Diah Lia Aulifa[2]

ABSTRACT

Background: Microorganisms such as bacteria and viruses often infect humans in their living environments. *Staphylococcus aureus* (SA) are gram-positive bacteria that are widely used in antibacterial activity experiments and cause infection in the body surface of mammals. *Ageratum conyzoides* L. (AC) and *Piper betle* L. (PB)are the natural herbs which have antibacterial activity against SA. **Objective:** This research was aimed to compare the antibacterial activity of AC with PB extracts in gel dosage form against SA. **Methods:** The antibacterial activity of both extracts were determined by disc diffusion method and minimum inhibitory concentration (MIC) were evaluated by the microdilution method. These extracts were formulated into gel dosage form using sodium carboxymethyl cellulose (CMC) with various concentrations and then evaluated for pH, viscosity and antibacterial activity. **Results:** The results show that both AC and PB extracts have antibacterial activity against SA with MIC value of 2 % and 5 %, respectively. Then, the gel containing 4 % sodium CMC showed the best physical stability, either containing AC or PB extract. The gel dosage forms of both extracts did not show any difference in organoleptic properties, pH and viscosity after 28 days storage. The gel dosage forms of AC and PB extracts have antibacterial activity with inhibition zone of 20.3 mm ± 1.3 mm and 15.21 ± 1.3 mm, respectively. **Conclusion:** ,The antibacterial activity of AC extract was higher compared to that of PB extract in the gel dosage form.

Key Words: *Ageratum conyzoides L.* extract, *Piper betle* L. extract, Gel, *Staphylococcus aureus.*

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INTRODUCTION

Microorganisms such as bacteria and viruses often infect humans in their living environments. The antibiotic-resistant microorganisms are on the increase, thereby affecting health-care costs. Many researchers have developed new effective antimicrobial agents that overcome resistance from microorganisms and are cost-effective.[1-3] Interest in natural products or herbs as an alternative medicine has increased in recent years due to the discovery that they reduce microbial resistance in a cost effective way. In recent years. extracts and essential oils from medicinal plants have became a focus of research especially regarding antimicrobial properties.[4]

One of the natural herbs that have antibacterial activity is *Ageratum conyzoides* L (AC). The previous study reported that methanol extract and oil from AC inhibits 20 bacteria and four fungi.[5] The oil from AC has weak activity against *Staphylococcus epidermidis, Staphylococcus aureus* (SA) and *Cladosporium cladosporioides*.[6] AC extract also inhibits the growth of *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Bacillus subtilis.* [7] The presence of alkaloids, flavonoids, saponins, phenols, and tannins was attributed to the antibacterial activity of AC extract.[8]

Piper betle L. (PB) extract is also one of the natural herbs which has potential as an antibacterial agent. A previous article reported that Piper betle L. have antibacterial activity against SA, *Streptococcus pyogenes,* and *Escherichia coli*. The oil from the leaf of PB showed anti-bacterial activity against *Staphylococcus epidermidis, Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli*.[9,10] The previous study reported that Piper betle L. contains fatty acids, hydroxy fatty acid esters and hydroxychavicol that exhibits antibacterial activity. Fatty acids can act as anionic surfactants that have selective against Gram-positive organisms by targeting the structure and function of bacterial cell membranes.[11]

SA are gram-positive bacteria that are widely used in antibacterial activity experiments and cause infection in the body surface of mammals. Furthermore, the structure of SA shows a unique cell envelope structure for Gram-positive organisms which is why it was chosen for the study.[12]

MATERIALS AND METHODS

Materials

Plant material

AC and PB were collected from Research Institute for Medicinal Plants (BALITRO) and were authenticated

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by the School of Life Sciences and Technology, Bandung Institute of Technology (ITB).

Chemicals

Mueller-Hinton Agar (MHA) and Mueller-Hinton Broth were purchased from Sigma Aldrich. All other chemicals used in this study were of technical grade.

Extraction

The AC and PB were extracted using 70 % ethanol by a maceration method for three x 24 hours at ambient temperature. The 70 % ethanol was removed by a rotary evaporator, IKA company, Germany (IKA RV 10) at 60°C to obtain a crude extract of AC and PB.[13,14]

Phytochemical screening extract

The phytochemical screening of AC and PB extract were conducted to determine the presence of secondary metabolites such as flavonoids, alkaloids, tannins, polyphenols, quinones, steroids/triterpenoids, saponins, monoterpenes and sesquiterpenes.[15]

Antibacterial activity of extract

The antibacterial activity of AC and PB extract were conducted using the disc diffusion method. The extracts at various concentrations were dissolved in DMSO 0.01%. Paper discs were soaked in extract solution for 15 minutes and then dried in a laminar air flow for 2 hours. The paper discs containing AC and PB extract, respectively, were placed on the media inoculated SA and then incubated at 37°C for 24 hours.[16]

Gel formulation of AC and PB extract

The gel formulation from AC and PB extracts were made according to the formula showed in Table 1. *Propylene glycol* was mixed with distilled water, and then Sodium carboxymethyl cellulose (CMC) was dispersed into the mixture of *Propylene glycol* and distilled water. AC and PB extract, respectively and other components were dissolved in the distilled water and added into the mixture of *Propylene glycol* and distilled water. The physical stability of the gel formulations from AC and PB extract were determined including organoleptic properties, pH and viscosity.[17]

Antibacterial activity of gel from AC and PB extract

The antibacterial activity of gels from AC and PB extracts were evaluated with the method of disc diffusion. Paper discs were soaked in the solution of extract for 15 minutes and then dried in a laminar air flow for 2 hours. The paper discs containing gels from AC and PB extracts, respectively were placed on MHA media surface inoculated SA and then incubated at 37°C for 24 hours.[18]

Statistical analysis

The result obtained from the experiment were analyzed by the one way analysis of variance (ANOVA) at the level of ($P<0.05$) and all data results were presented as a mean of samples±standard deviation (SD).

RESULTS AND DISCUSSION

Phytochemical screening

Phytochemical screening was conducted to observe the secondary metabolite contained in AC and PB extract. The result showed that AC extract contains alkaloids and flavonoids that have antibacterial activity. Flavonoid constituents such as chalcone, aurone, and flavone have aided the antibacterial activity of the plant.[19]

In addition, PB extract contains sterols, monoterpenes, sesquiterpenes, phenols, flavonoids and tannins base. The Sterols contained in PB extract have great potential as an antibacterial by interacting with the cell walls and membranes of bacteria to change the structure of the ell wall and membrane causing the degradation of bacterial components. Previous study reported that flavonoids and polyphenols contained in the plants have potential as an anti-inflammatory, antiviral, and antioxidant.[20]

Extraction

Maceration method was used in the extraction process to prevent degradation of antibacterial compounds contained in the AC and PB extracts. Ethanol was used as a solvent to optimize the phenol content in the AC and PB extracts. Previous study reported that ethanol extracts have higher phenol levels than water extracts.[21] The previous article reported that using an organic solvent showed more antibacterial activity compared to the extract using water as a solvent. The similarity of polarization between solvent and bioactive compounds contained in the extract can exhibit high antimicrobial activity.[22]

Antibacterial activity

The results of antibacterial activity tests for AC and PB extract can be seen in the Table 2.

Table 2 showed that AC and PB extract have activity against S. aureus. Previous article reported that *Piper betle* L. extract contains fatty acid compounds, hydroxy fatty acid ester and hydroxychavicol which have antibacterial activity. Fatty acids at low pH, acts as antibacterial and antifungal by targeting the structure and function of cell walls and

Table 1: Composition of the gel formulation AC and PB extract.

Materials	Formula (%)					
	I	II	III	IV	V	VI
AC extract	2	2	2	-	-	-
PB extract	-	-	-	5	5	5
Sodium CMC	3	4	5	3	4	5
Propylparaben	0.02	0.02	0.02	0.02	0.02	0.02
Methylparaben	0.18	0.18	0.18	0.18	0.18	0.18
Propylene glycol	2.5	2.5	2.5	2.5	2.5	2.5
Tween 80	2	2	2	2	2	2
Water added	100	100	100	100	100	100

Table 2: The results of antibacterial activity tests for AC and PB extract.

Materials	MIC	Inhibition zone diameter (mm)
Ageratum conyzoides L. extract	2 %	12.4 ± 0.3 mm
Piper betle L. extract.	5 %	10.5 ± 0.8 mm

bacterial membranes. Flavonoid contained in the extract can form complex connections with extracellular and bacterial cell walls.[11] This data almost similar is even better than the previous studies. Tarun et al reported that the range inhibition zone of PB leaves extract with various solvent were 20.5-38.0 mm.[23] While AC leaf extract, durodola reported that 60 fractions of AC leaf extract tested against SA showed antibacterial activity. However, the inhibition zone of antibacterial activity was not reported.[24]

Formulation of gel from AC and PB extract

In this study, CMC is an anionic water-soluble natural polymer derivative, which is widely used as a thickening agent in gel dosage forms because of its viscosity-increasing property.[25] Propylene glycol is a moisturizer and enhancer used to improve drug diffusion through the skin. It also mproves the permeability of compounds contained in the extract in the stratum corneum by changing the solubility parameter of the skin.[26] Methylparaben and propyl paraben were used as preservatives to prevent the degradation of compounds contained in the extract.[27]

Evaluation of the organoleptic properties of the extracts revealed that the viscosity of the gel increases along with the concentration of sodium CMC and carbomer. Gels from AC and PB extracts were easily spreadable, stable in consistency, odor, and color after 28 days storage.

The pH measurement of gel dosage form of AC and PB extract after 28 days storage can be seen in Figure 1.

Based on pH measurements, the pH of gel dosage form of AC and PB extract were in the range of 4.5 – 6.5 which still remained within the acceptable pH for topical dosage forms. The result of statistical analysis showed that there was a significant difference in the pH of gel from AC and PB extract during storage.

The result of viscosity measurements after sterilization can be seen in the following Figure 2.

Based on the measurements, the viscosity of the gel dosage forms of AC and PB extracts increased along with the concentration of sodium CMC. This result is similar with previous data reporting on the ability of polymer to increase the viscosity of gels.[28] The result of statistical analysis showed a significant difference in the viscosity of the gels from AC and PB extracts during storage. Generally, the viscosity of gel used to treat skin disorders are expected around 2000-4000 cPs. In this study, the range of gel viscosity containing either AC leaf extract or PB leaf extract was 2000-5000 cPs. The gel viscosity <4000 cps has advantages such as easy to apply and increase penetration of extract into the skin.[29] The gel containing 4 % sodium CMC showed the best physical stability,

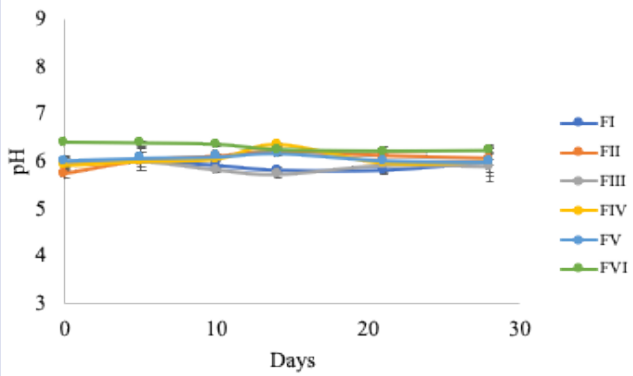

Figure 1: The result of pH measurement of gel from AC and PB extract after 28 days storage (All data results were determined as mean±standard deviation; n=3).

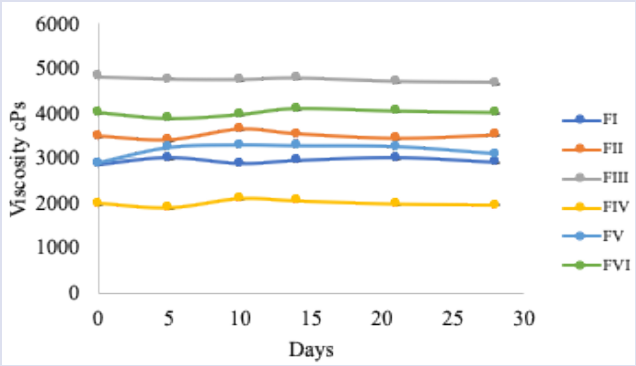

Figure 2: The result of viscosity measurement of gel from AC and PB extract after 28 days storage (All data results were determined as mean±standard deviation; n=3).

Table 3: The result of antibacterial activity test of gel dosage form from AC and PB extract.

Materials	Inhibition zone diameter (mm)
Control	0.0 ± 0.0 mm
Gel without extract	5.21 ± 1.7 mm
Gel of AC extract	20.30 ± 1.3 mm
Gel of PB extract	15.21 ± 1.3 mm

either containing AC or PB extract and subsequently would be used in the antimicrobial tests.

The result of antibacterial activity of gel dosage form can be seen in Table 3.

The results showed that both AC and PB extracts in gel dosage forms have antibacterial activity. An increase in the diameter of the inhibition zone in the gel dosage form was reported to be due to increasing diffusion of antibacterial compounds. The high water content in a gel dosage form can also increase the penetration of antibacterial compounds through the polar peptidoglycan layer.[30]

CONCLUSION

The antibacterial activity of the gel dosage form of AC extract (with inhibition zones of 20.3 mm ± 1.3 mm) was higher compared to that of PB extract (with inhibition zones of 15.21 ± 1.3 mm). The gel dosage forms of AC and PB extracts have antibacterial activity against SA with MIC value of 2 % and 5 %, respectively. The gel containing 4 % sodium CMC showed the best physical stability, either containing AC extract or PB extract. However, the gel dosage forms of both extracts did not show any difference in organoleptic properties, pH and viscosity after 28 days storage.

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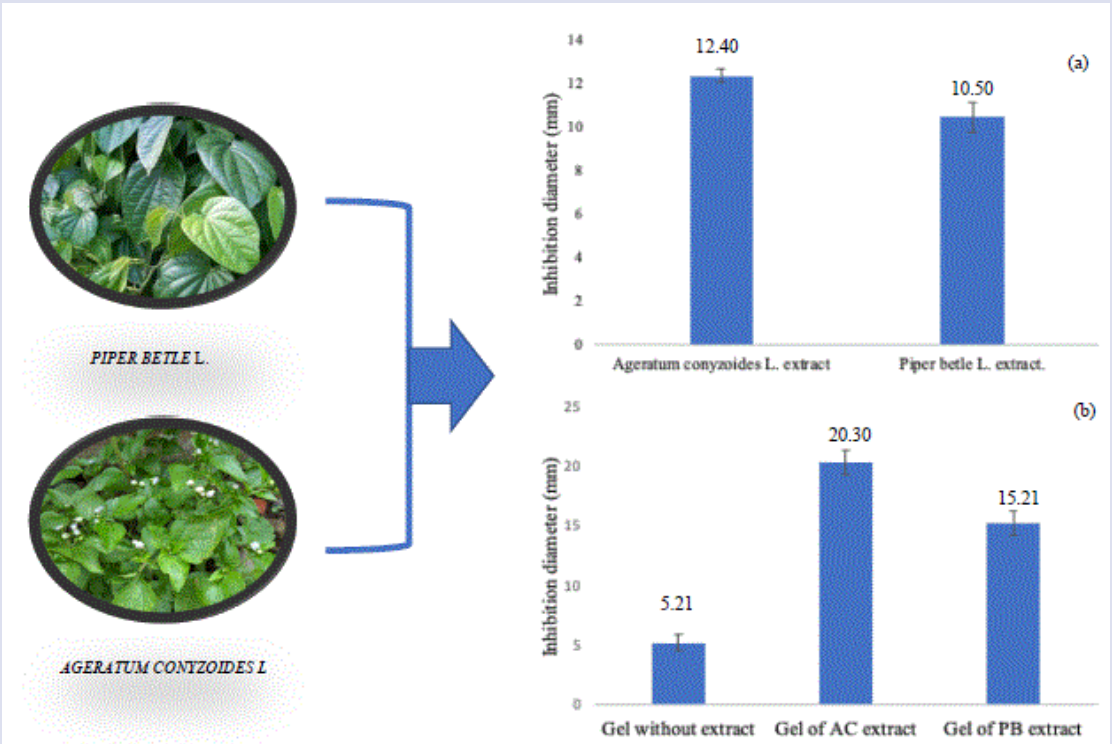
GRAPHICAL ABSTRACT



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