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Comparison of Antimicrobial Activity of Red Betel (*Piper Crocatum* Ruiz & Pav) Leaves Nanoparticle and Powder Ethanolic Extract against Methicillin Resistant *Staphylococcus Aureus*

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Abstract: Objective: Compare the activity of ethanolic extract of red betel (*Piper crocatum* Ruiz & Pav) leaves nanoparticle against MRSA and ethanolic extract of red betel (*Piper crocatum* Ruiz & Pav) leaves powder against MRSA.
Method: Ethanolic extract of red betel (*Piper crocatum* Ruiz & Pav) leaves nanoparticle and ethanolic extract of red betel (*Piper crocatum* Ruiz & Pav) leaves powder was tested against MRSA. Further more, compounds extracted with ethanol were qualitatively analyzed with GCMS.
Results: Ethanolic extract of red betel (*Piper crocatum* Ruiz & Pav) leaves nanoparticle and ethanolic extract of red betel (*Piper crocatum* Ruiz & Pav) leaves powder did not show antibacterial activity in concentration 10 mg/mL. But both of them show antibacterial activity against MRSA in concentration 20 mg/mL. With higher concentration the inhibition activity will also increase, it is observed in the inhibition zone diameter. In all of different concentration, the inhibition zone diameter of ethanolic extract of red betel (*Piper crocatum* Ruiz & Pav) leaves nanoparticle always higher than the ethanolic extract of red betel (*Piper crocatum* Ruiz & Pav) leaves powder.
Conclusion: Ethanolic extract of red betel (*Piper crocatum* Ruiz & Pav) leaves nanoparticle has better activity against MRSA because has more active compound than the ethanolic extract of red betel (*Piper crocatum* Ruiz & Pav) leaves powder.
Keywords: Antimicrobial, Red Betel, *Piper crocatum*, Nanoparticle, Powder, Methicillin Resistant, *Staphylococcus aureus*, MRSA.

Introduction

Staphylococcus aureus (*S. aureus*) is a prominent human pathogen that can cause a diverse range of diseases ranging from relatively minor skin infections to serious and life-threatening infections such as endocarditis, pneumonia, and sepsis. Methicillin resistant *Staphylococcus aureus* (MRSA) strains resistant are resistant to virtually all beta lactam antibiotics [1]. The emergence of MRSA bacterial strains grows up to be a very serious problem in the world of health [2]. Becomes very important to utilize and develop as an antibacterial medicinal plant. Red betel (*Piper crocatum* Ruiz & Pav) leaves (Piperaceae) contains essential oils which make it have antibacterial activity [3].

Betel leaf is popular as an antiseptic and is commonly applied to wounds and lesions for its healing effects. Essential oil extracted from betel leaf may be used as an industrial raw material for manufacturing medicines, perfumes, mouth fresheners, tonics and food additives [4]. Aqueous, methanolic, ethyl acetate and petroleum ether extract of red betel (*Piper crocatum* Ruiz & Pav) leaves has an antibacterial activity against *Streptococcus pyogenes, Staphylococcus aureus, Proteus vulgaris* and *Escherichia coli* [5]. Antibacterial activity of red betel (*Piper crocatum* Ruiz & Pav) leaves methanolic extract against MRSA has been discovered previously [6].

Nanotechnology broadly refers to a field of applied science and technology whose control of the matter in the scales smaller than 1 μm (normally 1-100nm) and its fabrication of a device within the range [7]. It has been widely proposed combining herbal medicine with nanotechnology, because nanostructured systems might be able to potentiate the action, reducing the required dose and side effects, and improving activity. Nanosystems can deliver the active constituent at a sufficient concentration during the entire treatment period, directing it to the desired site of action [8].

The activity comparison between ethanolic extract of red betel (*Piper crocatum* Ruiz & Pav) leaves nanoparticle against MRSA and ethanolic extract of red betel (*Piper crocatum* Ruiz & Pav) leaves powder against MRSA has not been studied yet. In this study also did the comparison between ethanolic extract of red betel (*Piper crocatum* Ruiz & Pav) leaves nanoparticle active compound and ethanolic extract of red betel (*Piper crocatum* Ruiz & Pav) leaves powder.

Materials and Methods

Plant Material

Red betel (*Piper crocatum* Ruiz & Pav) leaves obtained from the market in Pancur Batu Sub District, Deli Serdang District, Medan City, North Sumatra Province, Republic of Indonesia. Sampling was done by purposive without comparing with the same plants from other regions.

Preparation of Plant Material

Red betel (*Piper crocatum* Ruiz & Pav) leaves washed and dried in a drying cabinet at 50°C. Dried red betel (*Piper crocatum* Ruiz & Pav) leaves powdered with a blender until smooth and will be obtained red betel (*Piper crocatum* Ruiz & Pav) leaves powder. To obtained red betel (*Piper crocatum* Ruiz & Pav) leaves nanoparticle used high energy ball mill. Put the balls as a destroyer into the jar (balls with a larger diameter are inserted first then balls with a smaller diameter) and the final sample included. The ratio of the ball and the sample used for milled is 20: 1 (20 gram of the ball and 1 gram of the sample). Jar that contained the ball and sample was sealed and attached jar to the holder. Switched to operate mills and will be obtained red betel (*Piper crocatum* Ruiz & Pav) leaves nanoparticle. The size of the particle was obtained with an electron scanning microscope.

Preparation of Extract

100 grams of sample (red betel (*Piper crocatum* Ruiz & Pav) leaves powder or red betel (*Piper crocatum* Ruiz & Pav) leaves nanoparticle) wrapped in filter paper. Put in a bottle, add 500 mL of 70% ethanol and stirred for 15 minutes. Then soaked for 3 days (stirring frequently), filtered and evaporated the filtrate on a water bath until the viscous extract (ethanolic extract of red betel (*Piper crocatum* Ruiz & Pav) leaves powder or ethanolic extract of red betel (*Piper crocatum* Ruiz & Pav) nanoparticle) obtained.

Gas Chromatography Mass Spectrometry Condition

Column oven temperature 100.0°C with injection temperature 320.00°C and pressure 110.0 kPa. Total flow 109.7 mL/min, column flow 1.47 mL/min, linear velocity 45.1 cm/sec, purge flow 1.0 mL/min and split ratio 73.0. Oven temperature program with temperature gradient. Table 1 shows the gas chromatography mass spectrometry temperature program.

Table 1: Gas chromatography mass spectrometry temperature program

Number	Time	Temperature (°C)	Hold Time (minutes)	Condition
1	0	100	0	Balanced
2	0 → 10	100 → 320	10	Gradient
3	10	320	0	Balanced

Determination of Antimicrobial Activity

The petri dish was sterilized, put 0.1 mL inoculum of MRSA bacteria, inserted about 15 mL of sterile liquid mueller hinton agar medium (45-50°C), homogenized and allowed to solidify at room temperature. Put the reservoir paper containing extract (ethanolic extract of red betel (*Piper crocatum* Ruiz & Pav) leaves powder or ethanolic extract of red betel (*Piper crocatum* Ruiz & Pav) nanoparticle) with various concentrations on the surface of the media, allowed to stand for 15 minutes, then wrapped in a petri dish and incubated at 37°C for 18-24 hours, inhibition zone diameters measured with calipers. Minimum Inhibitory Concentration (MIC) is the lowest concentration of the ethanol extract of red betel leaves is still able to inhibit the growth of bacteria.

Results and Discussion

Both ethanolic extract of red betel (*Piper crocatum* Ruiz & Pav) leaves nanoparticle and ethanolic extract of red betel (*Piper crocatum* Ruiz & Pav) leaves powder did not show antibacterial activity in concentration 10 mg/mL. But both of them show antibacterial activity against MRSA in concentration 20 mg/mL. Figure 1 shows antimicrobial activity of ethanolic extract of red betel (*Piper crocatum* Ruiz & Pav) leaves nanoparticle with various concentrations. Figure 2 shows ethanolic extract of red betel (*Piper crocatum* Ruiz & Pav) leaves powder with various concentrations.

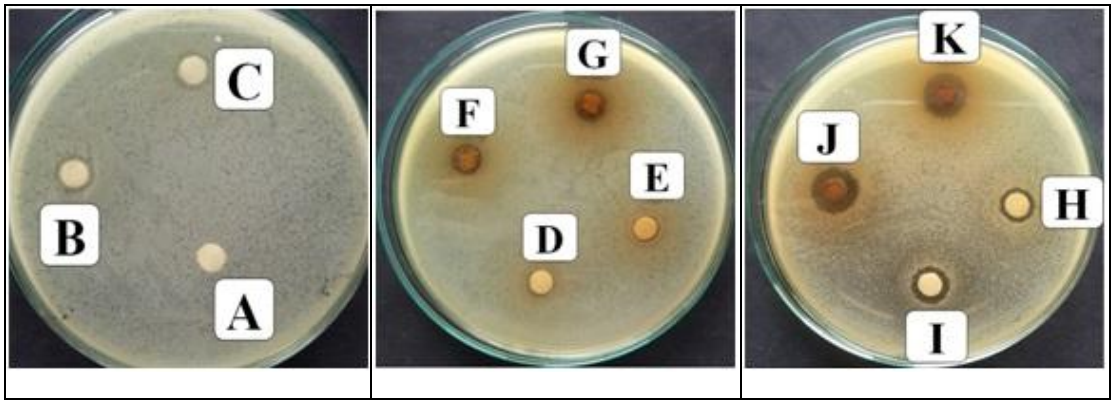

Fig. 1: Antimicrobial activity of ethanolic extract of red betel (*Piper crocatum* Ruiz & Pav) leaves nanoparticle with various concentrations (A=10 mg/mL, B=20 mg/mL, C=30 mg/mL, D=40 mg/mL, E=50 mg/mL, F=75 mg/mL, G=100 mg/mL, H=125 mg/mL, I=150 mg/mL, J=175 mg/mL, K=200 mg/mL)

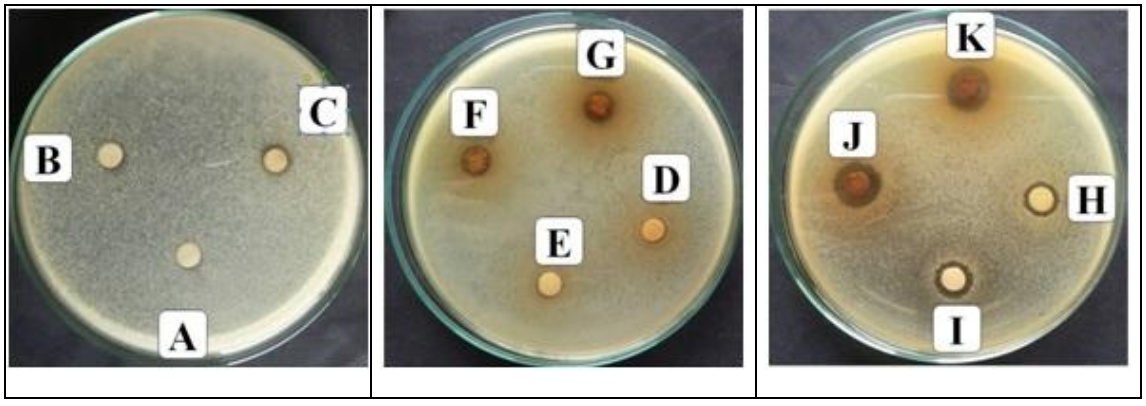

Fig. 2: Antimicrobial activity of ethanolic extract of red betel (*Piper crocatum* Ruiz & Pav) leaves powder with various concentrations (A=10 mg/mL, B=20 mg/mL, C=30 mg/mL, D=40 mg/mL, E=50 mg/mL, F=75 mg/mL, G=100 mg/mL, H=125 mg/mL, I=150 mg/mL, J=175 mg/mL, K=200 mg/mL)

With higher concentration the inhibition activity will increase it is seen from the inhibition zone diameter. Table 2 shows inhibition zone diameters of ethanolic extract of red betel (*Piper crocatum* Ruiz & Pav) leaves nanoparticle and ethanolic extract of red betel (*Piper crocatum* Ruiz & Pav) leaves powder in different concentration.

Table 2: Inhibition zone diameters of of ethanolic extract of red betel (*Piper crocatum* Ruiz & Pav) leaves nanoparticle and ethanolic extract of red betel (*Piper crocatum* Ruiz & Pav) leaves powder in different concentration

Number	Concentration of Extract (mg/mL)	Inhibition Zone Diameter (mm)	
		Nanoparticle	Powder
1	10	-	-
2	20	8.76	6.80
3	30	9.10	6.96
4	40	9.93	7.23
5	50	10.13	7.36
6	75	10.63	7.56
7	100	11.36	8.00
8	125	12.16	8.26
9	150	13.13	8.43
10	125	14.73	9.63
11	200	15.00	10.96

In all of different concentration, the inhibition zone diameter of ethanolic extract of red betel (*Piper crocatum* Ruiz & Pav) leaves nanoparticle always higher than the ethanolic extract of red betel (*Piper crocatum* Ruiz & Pav) leaves powder. Figure 3 shows comparison of the inhibition zone diameter of ethanolic extract of red betel (*Piper crocatum* Ruiz & Pav) leaves nanoparticle and ethanolic extract of red betel (*Piper crocatum* Ruiz & Pav) leaves powder in different concentration.

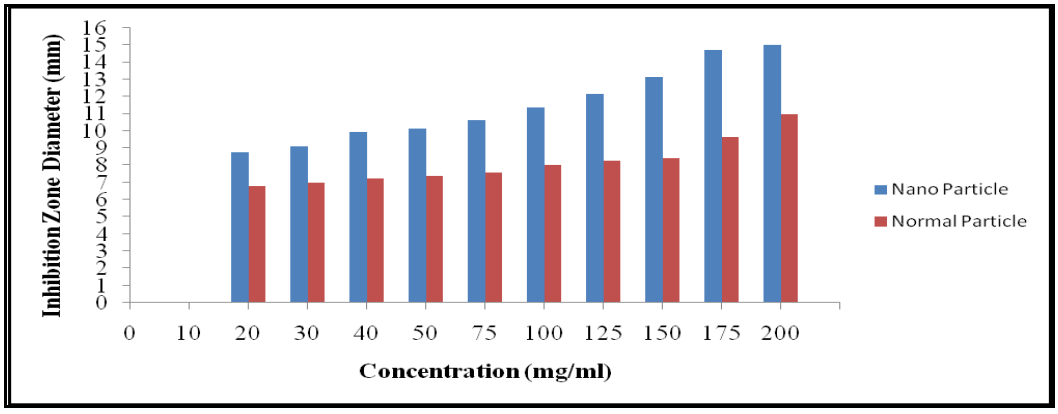

Fig. 3: Comparison of the inhibition zone diameter of ethanolic extract of red betel (*Piper crocatum* Ruiz & Pav) leaves nanoparticle and ethanolic extract of red betel (*Piper crocatum* Ruiz & Pav) leaves powder in different concentration

An ethanolic extract of red betel (*Piper crocatum* Ruiz & Pav) leaves nanoparticle has a better antibacterial activity. This result is due to different particle sizes. The development of nanoparticle in science and technology is a technological advancement that method to resize the ingredients in a smaller form. The shape and size of the particles are two factors that influence the effectiveness of the drug, because the particle size is very influential in the process of dissolution, absorption and distribution of drugs [9]. Figure 4 shows the chromatogram results of gas chromatography mass spectrometry for ethanolic extract of red betel (*Piper crocatum* Ruiz & Pav) leaves nanoparticle. Figure 5 shows the chromatogram results of gas chromatography mass spectrometry for ethanolic extract of red betel (*Piper crocatum* Ruiz & Pav) leaves powder.

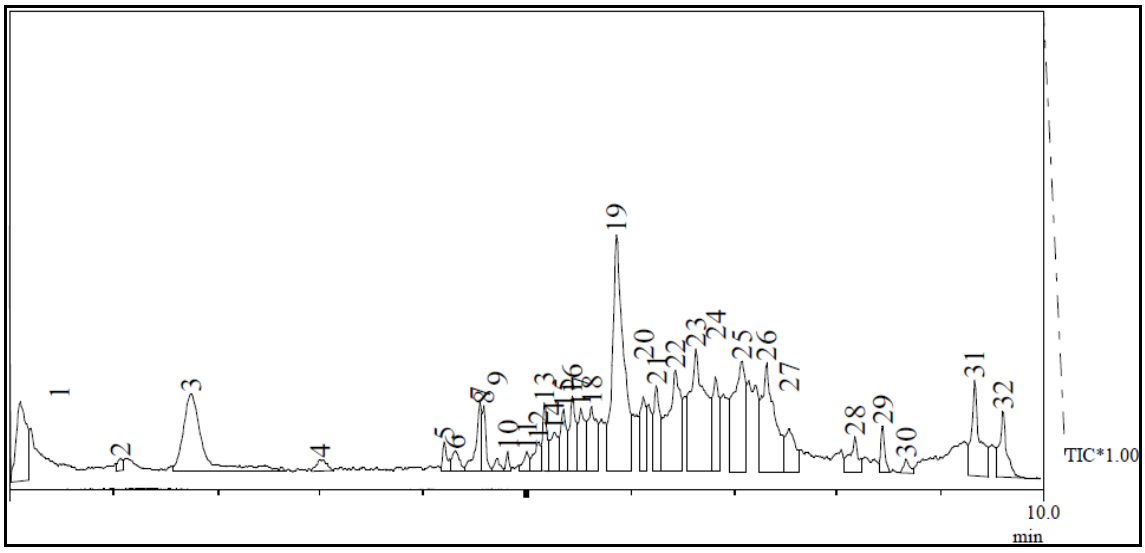

Fig. 4: Chromatogram results of gas chromatography mass spectrometry for ethanolic extract of red betel (*Piper crocatum* Ruiz & Pav) leaves nanoparticle

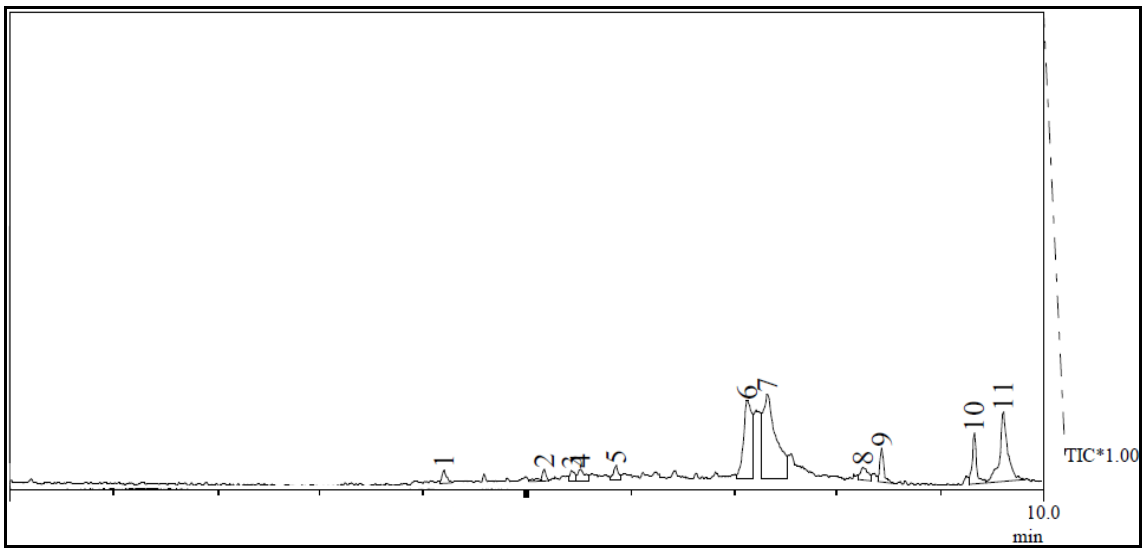

Fig. 5: Chromatogram results of gas chromatography mass spectrometry for ethanolic extract of red betel (*Piper crocatum* Ruiz & Pav) leaves powder

An ethanolic extract of red betel (*Piper crocatum* Ruiz & Pav) leaves nanoparticle has 32 compounds that extracted with ethanol, but an ethanolic extract of red betel (*Piper crocatum* Ruiz & Pav) leaves powder only has 11 compounds that extracted with the ethanol. Because the smaller particle size can improve the solubility of chemical compounds contained in red betel (*Piper crocatum* Ruiz & Pav) leaves [10]. Thus, the surface area of the material to be larger so that the increased solubility of the active substance and a stronger antimicrobial activity [11].

Nanostructured biomaterials, have a unique physicochemical properties such as ultra small and controllable size, large surface area, high reactivity and functionalizable structure. These properties can be applied to facilitate the administration of antimicrobial drugs, thereby overcoming some of the limitations in traditional antimicrobial therapeutics [12].

Rd betel (*Piper crocatum* Ruiz & Pav) leaves contain active compounds are flavonoids, polyphenols, tannins and essential oils [13]. Previous research suggested that the red betel leaf extract can inhibit the growth of Streptococcus mutans. This is presumably because the active compound content of flavonoids, tannins and essential oils [14].

Conclusion

The active compound of ethanolic extract of red betel (*Piper crocatum* Ruiz & Pav) leaves nanoparticle was more than the ethanolic extract of red betel (*Piper crocatum* Ruiz & Pav) leaves powder. The activity of ethanolic extract of red betel (*Piper crocatum* Ruiz & Pav) leaves nanoparticle against MRSA was better than the ethanolic extract of red betel (*Piper crocatum* Ruiz & Pav) leaves powder against MRSA.

Conflict of Interest

We declare that we have no conflict of interest.

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