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Antibacterial activity of mexican sunflower leaf *Tithonia diversifolia* (Hemsl.) A.Gray Aqueous extract against methicillin-resistant *Staphylococcus aureus*

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ABSTRACT

The research of antibacterial activity of Mexican sunflower leaf *Tithonia diversifolia* (Hemsl.) A.Gray aqueous extract against Methicillin-Resistant *Staphylococcus aureus* (MRSA) was carried out. The research aimed to observe the antibacterial activity of Mexican sunflower leaves aqueous extract to inhibit the growth of MRSA with concentrations of 10, 20, 30, 40, and 50%. The extracts were obtained by the maceration method, and the antibacterial activity was tested using the agar well diffusion method. Characterization of Mexican sunflower leaves simplicia were obtained with water level 9%, water-soluble level 21,6%, ethanol-soluble level 10,3%, and total ash level 14,36%. Characterization of Mexican sunflower leaves aqueous extracts were obtained with water level 26,36%, water-soluble level 53,13%, ethanol-soluble level 26,36%, and total ash level 19,98%. Phytochemical screening revealed that aqueous extract of Mexican sunflower leaves contained secondary metabolites of alkaloids, flavonoids, tannins, and saponins. The largest inhibitory zone was shown at a 50% extract concentration with a diameter of 12,40 mm. The aqueous extract of Mexican sunflower leaves was capable to form the inhibition zone on the MRSA growth.

Keywords: Antibacterial, aqueous extract of Mexican sunflower leaves, Methicillin-Resistant *Staphylococcus aureus, Tithonia diversifolia* (Hemsl.) A. Gray

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INTRODUCTION

Staphylococcus aureus is a normal flora in the human body found in the nose and the skin surface. The *S. aureus* might become pathogenic if the cell amount exceeds normal. Nowadays, the infection by *S. aureus* becomes a big challenge in the health field because of the choice of treatment due to its resistance. Resistant bacteria can be caused by the inappropriate administration of antibiotics. One kind of S. aureus resistant to antibiotics is Methicillin-Resistant *Staphylococcus aureus* (MRSA) (Tokajian, 2014).

Research conducted in Indonesia by Kuntaman et al. (2016) at Dr. Soetomo Hospital showed 52 (8%) of 643 patients infected by MRSA; samples were obtained from the patient's throat and nose. The prevalence of MRSA from hospitals in Indonesia is still relatively low compared to other countries. However, the situation must be controlled with the right strategy so that the prevalence will not increase.

One plant that has efficacy and has been traditionally used by people is the Mexican sunflower *Tithonia diversifolia* (Hemsl.) A. Gray. The research by Zurniati (2011) showed the antibacterial activity of *Tithonia diversifolia* leaf aqueous extracts against *Staphylococcus aureus*, which continued to increase from each concentration, which are 25%, 50%, 75%, and 100%, showed that the inhibitory zone of *Tithonia diversifolia* leaf extract against Staphylococcus aureus was 20 mm at 10 mg/mL. Based on that research, *Tithonia diversifolia* leaf has a potential effect as antibacterial, so it has possibilities that also can inhibit the growth of MRSA.

MATERIALS AND METHODS

Sample and Identification of The Plant

The Mexican sunflower plants were collected from Aser-Aser, Central of Aceh Regency. The plant had been identified at Herbarium Bogoriense Botanical Department, The Indonesian Institute of Sciences (LIPI) Bogor.

Preparation of Extracts

Fresh leaves of *Tithonia diversifolia* were harvested and washed with distilled water to remove all the dirty particles. Then the leaves were put on the clean area to air dry for 30 days. After the leaves were well dried, it was mashed using a blender and weighed.

Extraction Procedures

Total of 500 mg powder of Simplicia macerated with distilled water. It was extra using a water solvent (distilled water) with a maceration method that was the ratio of simplicial and the solvent was 1:10. Maceration lasts for 3 days while occasionally stirred, then filtered. The filtrate was subjected to evaporate to remove excess solvent. The crude extract was used for characterization, phytochemical screening, and antimicrobial assay.

CHARACTERIZATION OF SIMPLICIA AND EXTRACT

Determination of water level

A total of 2 g of simplicia powder and extract was put into porcelain that had been heated previously at a temperature of 105ºC for 30 minutes. Then the porcelain with simplicia and extract were placed in the oven with a temperature of 105ºC for 3 hours, then weighed and repeated heating up until the constant weight reached (Depkes RI, 1995).

Determination of the water-soluble level

A total of 5 g simplicia powder and extract was macerated with 100 mL of chloroform for 24 hours and stirred during the first 6 hours, then filtered, 20 mL of the filtrated were put into porcelain, and the filtrated was evaporated using an oven with a temperature of 105ºC. The water-soluble level was calculated in percent (Depkes RI, 1995).

A total of 1 g simplicia powder and extract were put into porcelain, then heated with a temperature of 600°C until it was white. Then cooled in a desiccator and weighed (WHO, 2011).

PHYTOCHEMICAL SCREENING

Test for alkaloid

Total 500 mg of extract was added with 1 mL of HCl 2 N and 9 mL of distilled water; the mixture was heated on a water bath for 2 minutes, then cooled and filtered. The filtrate obtained was transferred 1-3 mL each into three test tubes. The first test tube is added with two drops of Bouchardat reagent; positive alkaloids were characterized by the formation of brown to black color. The second test tube was added with two drops of Mayer reagent; positive alkaloids were represented by white or yellow color. The third test tube was added with two drops of Dragendorff reagent; the formation of orange-yellow color characterized positive alkaloids. Extracts would contain alkaloids if two of these reactions showed positive results (Depkes RI, 1995).

Test for flavonoid

A total of 500 mg extract was dissolved with 1-2 mL methanol. Then the solution was filtered and added with 1-2 mL concentrated HCl; the solution was shaken vigorously and then added 0.1 g of magnesium powder (Mg). If the results showed red-orange to purple-red, the positive sample contains flavonoids (Harborne, 1987).

Test for tannin

A total of 500 mg of extract was added with 10 mL of distilled water for 15 minutes then filtered. The filtrate was diluted with distilled water until it was almost colorless. The filtrate was taken as much as 2 mL and put into a test tube, then added two drops of 10% FeCl3. The extract positive for tannin if it formed a dark blue-black or black-green color (Depkes RI, 1995).

Test for saponin

A total of 10 mL of hot distilled water was added to 500 mg of extract in a test tube. The solution was shaken vigorously and observed for a stable, persistent froth. Positive saponin was characterized by 1-10 cm foam formation and does not disappear when adding one drop of HCl 2 N (Depkes RI, 1995).

Test for steroid/terpenoid

A total of 500 mg extract was added with 10 drops of acetic acid glacial (CH_3COOH) and 2 drops of H_2SO_4. The solution was then allowed to stand for a few minutes. Positive steroids were marked by the formation of blue or green color, while positive triterpenoids were marked in red or purple color (Febrina et al., 2015).

TEST ORGANISMS

The MRSA used as test organisms were obtained from Dr. Zainoel Abidin Hospital, Banda Aceh.

Bacterial Suspension

The bacterial suspension was prepared by taking the bacteria from the bacterial culture using an inoculum needle. The bacteria were added to 5 mL 0.9% NaCl in a test tube; then, the solution was homogenized using a vortex. The suspension was equalized with a 0.5 McFarland standard solution ($1x10^8$ CFU/mL) using a spectrophotometer at a wavelength of 625 nm. The absorbance ranged from 0.08 to 0.1 (WHO, 2003).

Antimicrobial assay

Antibacterial activity testing was carried out by the agar well diffusion method, which was by making a well or hole in *Mueller-Hinton Agar* (MHA) media containing the test bacteria. A total of 25 mL MHA media was poured into a Petri dish and mixed with 1 mL of MRSA bacterial suspension. The Petri dish was shaken until the media and bacteria were mixed. The media was allowed to stand until solidified. There are seven wells or holes in the media; the diameter for each hole is 4 mm. Then, each bottom of the well was dripped slightly with MHA media. Then were dropped 20 µl of an extract with a concentration of 10, 20, 30, 40, 50%, clindamycin 2 µg, and distilled water in each well. Then the Petri disk containing test media was incubated at 37°C for 24 hours. The treatment was carried out in triplicates.

RESULTS AND DISCUSSION

Determination of simplicia and extract

Simplicia and extract water content obtained were9% and 26.36%respectively (Table 1); the result fulfilled the requirement for the water content of the simplicial that is ≤10% and water extract content is <30%. Water content with a high level or even exceeds the requirement might cause enzymatic reactions in cells and trigger the growth of microbes that can reduce the quality of the material (Prasetyo & Inoriah, 2013).

The determination of water-soluble and ethanol was conducted to observe the amount of compound content that can be dissolved in water solvents and ethanol. The level of water-soluble simplicia and extract were 21.6% and 51.13% respectively. The soluble extract of ethanol of simplicia and extracts were 10.3% and 26.36%respectively (Table1). The water-soluble simplicia and extracts are higher than the ethanol-soluble, indicating that the compounds found in the *Tithonia diversifolia* leaves were more polar.

Determination of total ash content was conducted to evaluate the content of inorganic compounds contained in the sample. Total ash content obtained in the simplicia and extracts were 14.36% and 19.98% respectively (Table 1). Total ash content consists of physiological ash from the plant's tissue and non-physiological ash, which can be caused by external factors (WHO, 2011).

Table 1. Characteristic of simplicia and extract of *Tithonia diversifolia* (Hemsl.) A.Gray leaves

Test	Simplicia (%)	Extract (%)
Water level	9	26.36
Water-soluble level	21.6	53.13
Ethanol-soluble level	10.3	26.36
Total ash level	14.36	19.98

Phytochemical screening

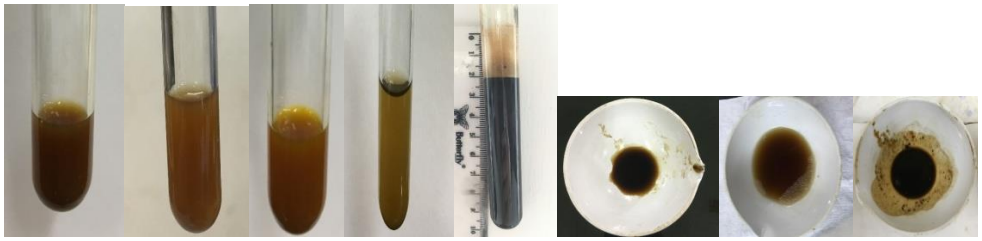
The phytochemical test was carried out to determine the compounds or secondary metabolites contained in the sample. Phytochemical test results showed that the extract of *Tithonia diversifolia* leaves contains secondary metabolites of alkaloids, flavonoids, tannins, and saponins (Table 2). The previous research by Agidigbi & Odeyemi (2014) showed that the leave extracts of *Tithonia diversifolia* possess active components, alkaloids, flavonoids, phenols sesquiterpenes, monoterpenes, and diterpenes as active components.

The difference in the secondary metabolites compound within the same plant might be affected by biological and chemical factors. Biological factors include genetic, location of harvest, time of

harvest, age of the plant, and environmental conditions where the plant grows. Meanwhile, the chemical factors included the extraction method and the selection of solvents used (Depkes RI, 2000).

Table 2. Phytochemical Screening of *Tithonia diversifolia* (Hemsl.) A. Gray leaves extract

Plant constituent	Leaves extract
Alkaloids	Positive
Flavonoids	Positive
Tannins	Positive
Saponins	Positive
Steroid/ triterpenoids	Negative



(a) (b) (c) (d) (e) (f) (g)

Figure 1. Result phytochemical screening test that identifies (a) alkaloid by Bouchardat (b), Mayer (c), Dragendroff reagent; (d) tannins; (e) saponins; (f) flavonoids; (g) steroid/triterpenoids

Antibacterial activity test

Antibacterial activity test was carried out on MRSA using variations in the concentration of *Tithonia diversifolia* leaves extract of 10, 20, 30, 40, and 50%. The positive control used was clindamycin 2 µg. The method of testing antibacterial activity used diffusion wells. The well diffusion method was used because with this method, the extract will diffuse directly to the media so that it will increase the secondary metabolites compounds concentration that will interact with the media compared to the disk diffusion method where the extract will penetrate the disk paper first as a barrier, then will contact with the media (Boateng & Diunase, 2015)

The extract with the largest inhibition zone diameter was at a 50% concentration of 12.40 mm, while the diameter of the smallest inhibition zone was at a 10% concentration of 5.73 mm. The positive control used was 2 µg clindamycin, forming a 31.83 mm inhibition zone (Table 3). The negative control used was distilled water and did not show any inhibitory zones. Clindamycin is an antibacterial that inhibits protein synthesis by binding to the 50s ribosome. The bacteriostatic or bactericidal effect of clindamycin depends on drug concentration, type of organism, and the location of infection. The clindamycin inhibition zone formed (Table 3) shows that MRSA bacteria are still sensitive to clindamycin. *Staphylococcus aureus* bacteria are sensitive to clindamycin if the inhibition zone is >21 mm and resistant if <14 mm (CLSI, 2014).

Previous research conducted by Zurniati (2011) showed that the growth inhibitory zone of *Tithonia diversifolia* leaf extract against *Staphylococcus aureus* was 26.83 mm at 50% extract (Figure 1). The different inhibitory zones results might occur due to differences in the type of the test bacteria and the content of plant secondary metabolites. The MRSA could be resistant against certain

antibacterial compared to *Staphylococcus aureus* so that the MRSA can fight specific antibacterial activity. This is suspected as one of the reasons for inhibition zones produced by *Tithonia diversifolia* leaves extracts against MRSA bacteria smaller than *Staphylococcus aureus*.

Table 3. Antibacterial activity of extract of *Tithonia diversifolia* (Hemsl.) A. Gray leaves against MRSA

Concentration (%)	Zone of Inhibition (mm) ± SD
10	5.73 ± 1.50
20	6.40 ± 0.34
30	8.46 ± 0.45
40	11.10 ± 0.81
50	12.40 ± 1.49
Control +	31.83 ± 0.76
Control -	0 ± 0

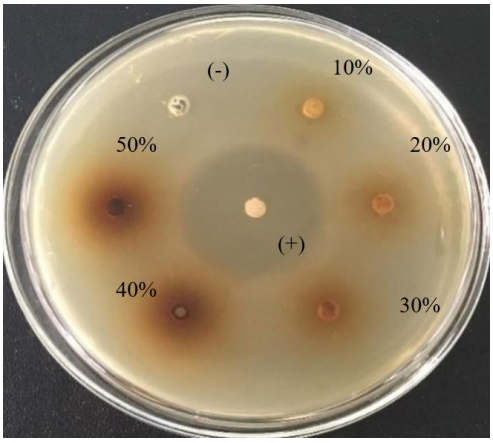


Figure 2. Antibacterial activity of extract of *Tithonia diversifolia* (Hemsl.) A. Gray leaves against MRSA

In this study, secondary metabolite compounds suspected of having an antibacterial role in MRSA are polar. Alkaloid, flavonoid, saponin, and tannin compounds are supposed to play a role in antibacterial activity. Cell walls in Gram-positive bacteria have thick, multi-layered peptidoglycan located outside the cytoplasmic membrane. The peptidoglycan in Gram-positive consists of teichoic acid, which is soluble in water and acts as a positive and negative ion transport to and from the cell. The nature of the water-soluble teichoic acid shows that Gram-positive is more polar. Therefore, polar compounds will enter the cell wall easily then destroy the polar peptidoglycan. On the contrary, Gram-negative bacteria have a more complicated cell wall structure with two membrane layers, namely the outer membrane and the inner membrane consisting of the cytoplasmic membrane. Both membranes are separated by the periplasm space, which also contains a degradative enzyme and as a protein transport. The Gram-negative peptidoglycan layer is thinner, causing its polarity to be lower (Dewi, 2013).

Alkaloids are suspected of having antibacterial activity by disrupting the constituent components of peptidoglycan in bacterial cells so that cells are not formed properly and will cause the cells death. Tannin is a polar secondary metabolite because there are hydroxyl groups and have antibacterial activity by inactivating enzymes and destruction or inactivation of the function of genetic material from bacteria. Flavonoid acts as an antibacterial by forming complex compounds against extracellular proteins that interfere with the integrity of bacterial cell membranes (Karou *et al*., 2005).

Saponin is also suspected of having antibacterial activity by causing leakage of proteins and enzymes in bacterial cells. Saponin will reduce the surface tension of bacterial cell walls and damage membrane permeability. Saponin diffuses through the cell walls and then binds to the cytoplasmic membrane to disrupt and reduce cell membrane stability. This will cause cytoplasm to leak out of the cell resulting in cell death (Madduluri*et al*., 2013).

CONCLUSION

Aqueous extract of *Tithonia diversifolia* leaves exhibited growth inhibitory activity against Methicillin-Resistant *Staphylococcus aureus* (MRSA). It is recommended to test the antibacterial activity of *Tithonia diversifolia* extract against other antibiotic resistant bacteria.

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