Antibacterial and Wound Healing Activity of Ethanolic Extract

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RESEARCH ARTICLE

Antibacterial and Wound Healing Activity of Ethanolic Extract *Melastomamalabathricum* L

Isnaini[1], IkaKustiyah Oktaviyanti[2], Lia Y. Budiarti[3]

[1]Departement of Pharmacology, Faculty of Medicine, Lambung Mangkurat University, Indonesia
[2]Departemen of Patologi Anatomi, Faculty of Medicine, Lambung Mangkurat University, Indonesia
[3]Department of Microbiology and Parasitology, Faculty of Medicine, LambungMangkurat University, Indonesia
*Corresponding Author E-mail: **isnaini@ulm.ac.id**

ABSTRACT:

*Melastomamalabathricum*L are plants that are widely found in South Kalimantan. This plant grows along the riverbanks. Flowers of *M. malabathricum* L have antibacterial activity greater than leaves in *Staphylococcus aureus* bacteria. Every fase of *M. malabathricum* L flowers contents different quercetin and kaempferol. *M. malabathricum* L flower phase consists of bud phase 1 (K1), bud phase 2 (K2), bud phase 3 (K3) and blooming phase (M). Quercetin and kaempferol are also found in *M. malabathricum* L fruit (F). The highest content of quercetin and kaempferol is in the blooming phase. Quercetin and kaempferol have antibacterial and wound healing activity. This research aims to analyze the antibacterial and wound healing activity of ethanolic extracts of *M. malabathricum* L flower (FL) and fruit (FR). The method antibacterial activity is used the liquid dilution method and continued with planting on agar media. The wound healing activity was tested on rat that were smeared with cream for 14 days and examined the histopathology of the skin. Concentration of extract is 1%, 2% dan 4%. The results showed that the skin smeared with FLC (flower cream) 4% had formed adnesia, while the other treatments had not yet formed

KEYWORDS: *Melastomamalabathricum* L, flowers, fruit, antibacterial, wound healing.

INTRODUCTION:

Wound is a condition of damage to the skin caused by tearing of the epithelial layer or deeper due to physical or thermal damage[1]. Wounds are divided into acute wounds and chronic wounds based on their duration[2]. The prevalence of abrasions/bruises is 60.5% and laceration is 22.1% in South Kalimantan. The prevalence of chronic wounds, for example diabetic ulcers, is 66.7% and cancerous wounds is 24.6%[3]. Chronic wounds are not treated immediately can cause amputation. Need immediate treatment of all forms of injuries. One of the plants that has the potential to be developed as a wound healing drug is *Melastomamabathricum* L.

M. malabathricum L. native species of South Kalimantan. This plant grows wild and is considered as a disturbing plant.

M. malabathricum L leaves has several antibacterial activities[4–6], antidiarrheal[7], antioxidants[8,9], gastroprotective[10], wound healing[4,11], antinociceptive[12,13], anticoagulants[9], anti-inflammatory[13,14], antivirals[15], anticancer[8,15].

The antibacterial activity of *M. Malabathricum* L flowers is greater when compared to the ethanolic extract of *M. malabathricum* L leaves in *S. aureus* bacteria. The inhibitory activity of ethanol extract of *M. Malabathricum* L flowers was the same as ampicillin at a concentration of 20%[6]. *M. malabathricum* L flowers and fruits contain flavonoid, specifically quercetin and kaempferol. Flavonoids have antimicrobial activity[16,17]. Each phase of the flower contains different quercetin and kaempferol contents. Bud flower 1 (K1), bud 2 (K2), bud 3 (K3) and bloom (M) flower phases have quercetin content of 19.47µg/g, 17.78µg/g, 31.2µg respectively/g, and 94.32µg/g, while the kaempferol contents were respectively K1, K2, K3 and M, which were 140.99µg/g, 57.28µg/g, 95.32µg/g, and 349.37µg/g[18]. In addition to flowers, the fruit also contains quercetin and kaempferol with 67.78μg/g and 43.52μg/

g respectively. Differences content of quercetin and kaempferol cause differences in antibacterial activity because quercetin and kaempferol have antibacterial activity[19]. Quercetin also have wound healing activity, so this extract have wound healing activity.There are no studies on the antibacterial and wound healing activity of *M. malabathricum* L flowers and fruits.

MATERIAL AND METHOD:

Collection of *M. malabathricum*L. flower and fruit samples *M. malabthricum*L were collected from GunungKupang area, Banjarbaru, South Kalimantan.Flowers are divided into four phases, namely bud 1 (K1), bud 2 (K2), bud 3 (K3), and blooming flowers (L), while fruit (FR) only consists of 1 phase (fig. 1). This plaint was determination in the Laboratory of Basic Sciences, LambungMangkurat University, Banjarbaru, South Kalimantan, Indonesia

Extraction of *M. malabathricum*L. flower and fruit *M. malabathricum*L. was extracted using the maceration method. Pulverized *M. malabathricum*L. extracted with ethanol 96%. Extraction was conducted until the solvent was colorless. The extract was obtained by evaporation using rotary evaporator until thick extract was obtained and the extract was put in the oven until the weight remains.

Turbidity test:

Minimum inhibitory concentration (MIC) was determined using a broth dilution method. Cultures of *Streptococcus aureus* were grown in heart infusion broth (BHI Broth, Oxoid UK) and each culture was adjusted to 3 x 10^8 CFU/ml (1 McFarland standard). Forty percent ethanolic extract *M.malabathricum*L stock solutions were prepared by dissolving 2g of ethanolic extract *M. malabthricum*L in dimethyl sulfoxide (DMSO) 5% and ethanol until 5mL, than the serial dilution until the smallest concentration is obtained which shows the MIC of the ethanolic extract *M. malabathricum*L. One mililitre a *Streptococcus aureus* was put into test tube containing 1mL of various concentration ethanolic extract *M. malabathricum*L in BHI broth. The test tube wereincubated at 37°C for 18–24. A loop full of broth from each test tube was not showing growth; thence they were inoculated into nutrient agar plate and incubated further for 24 h at 37 ºC. Then, the agar plates were examined for growth and turbidity using the una\ided eye[20]. The procedure was repeated three times. The inoculums of the organism without ethanolic extract of *M. malabathricum*L were used as the control. The growth of the control was compared to the growth of the organism exposed to the test material. The MIC is the least concentration of antimicrobial agent that prevents microbial growth[21,22].

Wound Healing Test:

Rats were randomly separated into at least 8 cages for adaptation for 1 week. Each cage contains 5 rats. Rat weight between 200 - 250g. Two groups as a control group, namely negative control (NC), rats were only given cream base, positive control (PC), rats were given povidone iodine solution and three groups were treated as cream treatment group with ethanolic extract of flowers and fruit of *M. malabathricum* L with extract concentration. flower *M. malabathricum* L 1% (FLC1), 2% (FLC2) and 4% (FLC3), while the concentration of fruit extract of *M. malabathricum* L consisted of 1% (FRC1), 2% (FRC2) and 4% (FRC3). Observations were made on the 14th day. During the adaptation period, rats receive food and drink ad libitum.

Before inicision wound, the rats were given anesthetic ketamine injection at a dose of 40mg/kg BW. The rats that had received anesthesia were then initiated on the back with a diameter of 2cm until the skin was detached. Once a day the wound will be smeared with cream or povidone iodine according to the treatment. Treatment is carried out for a maximum of 14 days. Observations were made on day 14 (Modification of Rupina et al., 2016)[23].

Table 1: MIC of ethanolic extract of *M. malabathricum*L in *S. aureus*

Sample	MIC
Bud1	7%
Bud2	8%
Bud3	5%
Bloom	2%
Fruit	2%

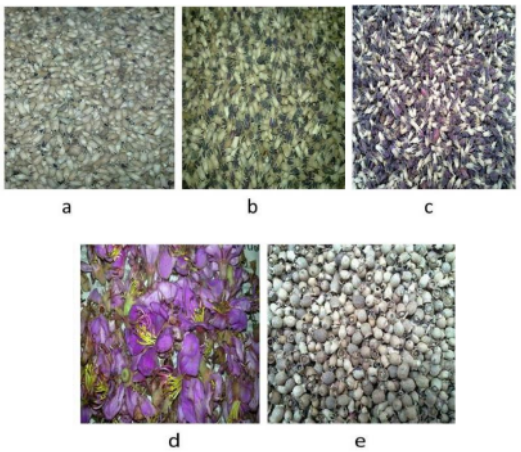
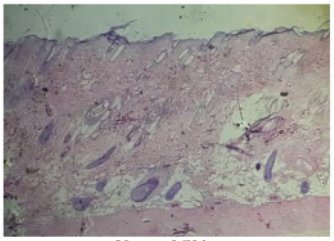
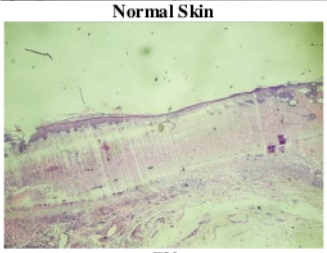


Fig. 1: *M. malabathricum* L in various phase. (a) Bud1, (b) Bud2, (c) Bud3, (d) Bloom and (e) Fruit



Normal Skin



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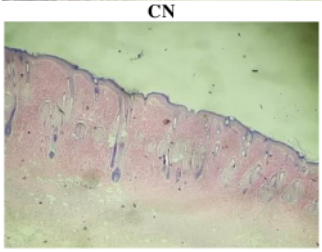

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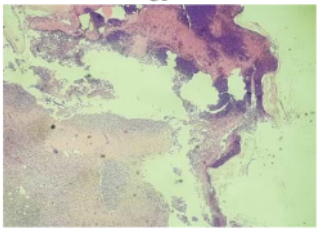
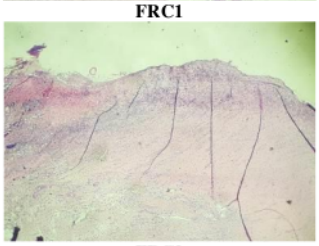
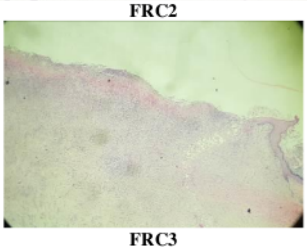


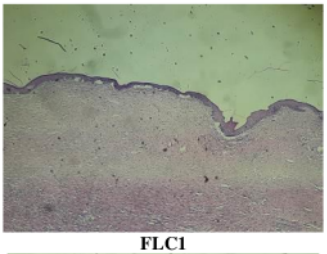
FRC1



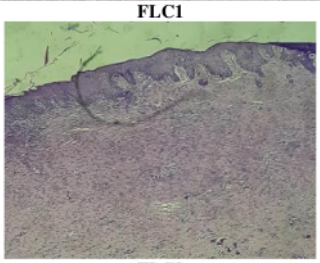
FRC2



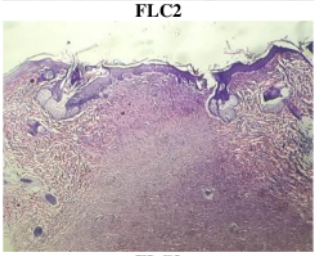
FRC3



FLC1



FLC2



FLC3

Figure 2: Histology skin of rats after treatment cream extract *M. malabathricum*L after 14 days

RESULT AND DISCUSSION:

MIC of extract ethanolic of *M. malabathricum* L:

Ethanolic extract of *M. malabathricum*L phase Bud2 has the highest MIC (8%), and the smallest MIC (2%) is phase Bloom and Fruit. Based on research conducted by Isnaini et al, 2018a and Isnaini*et al.,* 2019, it is known that the smallest quercetin and kaempferol levels are found in the Bud 2, which is 17.78μg/g and 57.28μg/g and the largest quercetin and kaempferol content is phase Bloom, that ccontent of quercetin and kaempferol is 94.32μg/g and 349.37μg/g[6,24], while the content of quercetin and kaempferol in phase F is 67.78μg/g and 43.52μg/g[24]. Phase Fruit has the same activity as Phase Bloom, although the content of quercetin and kaempferol from phase Fruit is smaller compared to phase Bloom. The similarity of these activities is due to the comparison of quercetin content in phase Fruit is greater when compared to the content of kaempferol, whereas in phase bloom, quercetin content is smaller when compared to kaempferol content. Quercetin and kaempferol have antibacterial activity[25]. Quercetin has greater antibacterial activity when compared to kaempferol[19]. Quercetin works by inhibiting the enzyme DNA gyrase and topoisomerase IV, but it also inhibits β

lactamase in MDR strain bacteria. Quercetin can also inhibit the growth of biofilms in *S. mutans* bacteria[26].

Wound Healing Activity:

Wound healing is a repair process that occurs in the skin or other tissues after experiencing trauma[27–29]. Wound healing process is complex and involves anti-inflammatory and anti-bacterial. There are four phases in the wound healing process, namely coagulation, inflammation, proliferation and maturation or remodeling[30–36]. Wound healing aims to achieve tissue integrity and homeostasis[33]. The wound healing process begins with the restoration of damaged tissue, so that it returns to its original state[37]. The wound healing process is very important, so it does not cause physical disabilities and cause scars[38,39].

Wound healing processes that don't take place in a timely and orderly manner will cause chronic wounds. In chronic wounds, wound healing stops in the inflammatory phase. The presence of an inflammatory phase is characterized by high levels of pro-inflammatory cytokines, proteases, senescent cells and persistent infection[40]. Therapeutic interventions are carried out to reduce inflammation and promote tissue regeneration[34,40]. Inflammation occurs due to the stimulation of infectious agents and free radicals[34].The flowers and fruits of *M. malabathricum* L contain quercetin which has activity as an antibacterial and antioxidant[6,41]. In this research, it was found that flower and fruit extracts had the same MIC, which was 2%. Although both have the same antibacterial activity, histological examination shows that the ethanolic extract of *M. malabathricum* L flowers has greater wound healing activity. This can be seen from the histology of rat skin. Histological picture of rat skin when given cream of ethanolic extract of *M. malabathricum* L flowers (FLC3) had better results compared to other treatments, because when administered with *M. malabathricum* L flower extract, adnesia had formed on the skin (fig. 2). M. malabathricum L flower extract contains quercetin[18]. The difference in wound healing activity was due to the ethanolic extract of *M. malabathricum* flower contains more quercetin, compared to fruit[18,24]. Quercetin can increase wound contraction, depending on the dose[42].

Quercetin has strong anti-inflammatory activity. The mechanism of anti-inflammatory action of quercetin is through downregulating IL-1B, IL-6 and IL-8, and upregulating IL-10[33,43,44]. During wound healing, granulation tissue is formed by increasing the expression of IL-10, VEGF and TGF-b1[33,44]. Until now, the mechanism of action of wound healing from the ethanol extract of M. malabathricum L. flowers is still unknown.

CONCLUSION:

The antibacterial activity of the flower and fruit extract of *M. malabathricum* depends on the content of querectin and kaempferol. In extracts containing small quercetin and kaempferol, the antibacterial activity is also small, while in extracts containing large quercetin and kaempferol, the antibacterial activity is also large. The comparison between the content of quercetin and kaempferol also affects the antibacterial activity. Extracts containing more quercetin than kaempferol, the activity will be greater when compared to extracts containing greater kaempferol than quercetin.

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