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Original Article

IDENTIFICATION OF CHEMICAL COMPOUNDS AND ANTIBACTERIAL ACTIVITY OF 96% ETHANOL EXTRACT FROM MORINGA OLEIFERA LAM. LEAVES AGAINST MRSA (METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS)

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ABSTRACT

Objective: Infection with antibiotic-resistant organisms, requiring the selection of the right drug to fight these organisms. *Moringa oleifera* Lam. leaves as natural ingredients have MRSA (Methicillin-Resistant *Staphylococcus aureus*) antibacterial activity. The purpose of this study is to identify the chemical compounds and to determine the antibacterial activity from the 96% ethanol extract of *Moringa oleifera* Lam. leaves against MRSA bacteria.

Methods: The *Moringa oleifera* Lam. leaves were extracted by maceration using 96% (v/v) ethanol. Mass spectrometry was performed on an LC-MS/MS Xevo, G2-XS QTof (Waters MS Technologies) to identified chemical compounds from the extract. The ionization type is ESI. The method of the antibacterial activity test was using agar paper disc diffusion. Antibacterial activity was based on the diameter of the bacterial inhibition zone.

Results: The result of the antibacterial activity test for 96% ethanol extract of *Moringa oleifera* Lam. *leaves* a concentration of 10, 20, and 40 % each has inhibition diameter was 10, 13, and 15.5 mm, and for linezolid as a positive control at 30µg has diameter inhibition was 20 mm, ethanol 96% as a negative control was 0 mm. The 96% ethanol extract of *Moringa oleifera* Lam. *leaves* contains 13-hydroxy-9,11-hexadecadienoic acid, 3-tert-butyl-4-methoxyphenol, bistortaside, daturametelin H, digiprolactone, ephedradine C, kaempferol-3-O-rutinoside,kaempferol-3-O-β-D-glucopyranoside, kaempferol-7-O-α-L rhamnoside, phenyl propionic acid, pyrophaeophorbide A, quercetin, stearidonic acid, stigmastan-3,6-dione.

Conclusion: The 96% ethanol extract of *Moringa oleifera* Lam. leaves contains 14 compounds. The 96% ethanol extract of *Moringa oleifera* Lam. leaves have activity against MRSA bacteria. The antibacterial effect of the extract increased with an increase in its concentration. The extract exerted a greater antibacterial effect on the concentration 40%.

Keywords: *Moringa oleifera* Lam., Antibacterial activity, MRSA (Methicillin-Resistant *Staphylococcus aureus*).

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INTRODUCTION

In generally, diabetic foot ulcer infections are caused by several pathogenic bacteria (polymicrobial). The severity of diabetic foot ulcer infection is determined by the type of pathogen causing it. Pathogenic bacteria that cause osteomyelitis in diabetic foot ulcers are *Staphylococcus aureus*, *Staphylococcus epidermidis*, and gram-negative bacteria [1]. The emergence of *Staphylococcus aureus infection* cases is resistant to Methicillin. This line is known as the Methicillin-Resistant S*taphylococcus aureus* (MRSA) strain [2].

MRSA causes skin and soft tissue infections such as furuncles, cutaneous abscesses, cellulitis [3], and diabetic ulcers. Infection with antibiotic-resistant organisms requires the selection of appropriate drugs to fight these organisms [4]. In 2017, the World Health Organization (WHO) issued MRSA has a high priority for research to find new antibiotics against it, as part of WHO's efforts to address increasing global resistance to antimicrobial drugs [5]. *Moringa oleifera* Lam. leaves are natural ingredient that have antibacterial activity as a more affordable alternative to therapy. The aim of this study were to identification active compounds and determine the potency of 96% ethanol *Moringa oleifera* Lam. leaves extract.

Moringa oleifera Lam. leaves have activities as an analgesic, anticancer, antimicrobial, antihyperlipidemic, antidiabetic, analgesic, hepatoprotective, anticonvulsant, antiulcer [6]. *Moringa oleifera leaves* have antidiabetic activity because they contain quercetin, chlorogenic acid, moringinine [7]. *Moringa oleifera* Lam. *leaves* have hyperglycemic wound healing activities because they contain vicenin-2, an active compound that can accelerate wound healing in hyperglycemic conditions [8]. The antibacterial, antioxidant and wound healing activities with the GC-MS analysis method confirmed there are 17 bioactive compounds [9]. Ethanol extract of *Moringa oleifera* Lam. leaves have MRSA antibacterial activity [10, 11]. It's necessary to find out which compounds have MRSA antibacterial activity.

MATERIALS AND METHODS

Materials

96% Ethanol (Merck, Germany), Methicillin-Resistant Staphylococcus aureus (MRSA), mediaTryptic Soy Agar, Tryptic Soy Broth (TSB) (Merck, Germany), Linezolid 30µg (Liofilchem, Italy). *Moringa oleifera* Lam. leaves from Puri Kelorina, Kunduran, Blora, Centre Jawa 58255. (-7.007617,111.229933).

Preparation of extract

Moringa oleifera Lam. leaves were identified by Herbarium Bogoriense, Research Center for Biology–Indonesian LIPI with the authentic number is 1705/IPH.1.01/If/IX/2019. *Moringa oleifera* Lam. leaves simplicia powder extracted by maceration using 96% (v/v) ethanol. The filtrate is concentrated using a rotary vacuum evaporator. Identification of the chemical compounds contained in the extract using the LC-MS/MS instrument (PerkinElmer, United Kingdom)

Identification of chemical compounds

Mass spectrometry was performed on an LC-MS/MS Xevo, G2-XS QTof (Waters MS Technologies). The ionization type is ESI. The scan range was from 100 to 1200 m/z. The capillary and cone voltage was set at 0.8 kV and 30 kV, respectively, and was used positive electron spray mode. The desolvation gas was set to 1000 L/h at a temperature of 500 °C and the cone gas was set to 50 L/h and the source temperature was set to 120 °C.

The UPLC analysis was performed using a Waters Acquity Ultra Performance LC system. Chromatographic separation was carried out on an ACQUITY UPLC HSS T3 column (100 mm x 2.1 mm, 1.7 µm) at a column temperature of 40 °C. The mobile phase consisted of solvent A (0.1% formic acid in the water, v/v) and solvent B (0,1%

formic acid in acetonitrile), with gradient polarity from 95:0.5 (A: B) to 0.5:95 (A: B). The flow rate was set at 0.3 ml/min. The column and autosampler were maintained at 40 °C and 20 °C, respectively. The injection volume was 1 µl. The data acquisition and processing were performed using UNIFI. The parameter used was retention time (RT) in the range of 1-15 min.

Antibacterial MRSA activity test

The antibacterial activity was carried out by the Kirby-Bauer disc diffusion method using a disc in TSA media. The 96% ethanol extract of *Moringa oleifera* Lam. *leaves* tested against Methicillin-Resistant Staphylococcus aureus (MRSA) with concentrations of 10, 20, and 40%. Linezolid 30µg was used as a positive control, while Ethanol 96% was used for negative control.

Linezolid discs, the paper discs were saturated with ethanol extract of *Moringa oleifera* Lam. *leaves* with a concentration of 10, 20, and 40%, and 96% ethanol was placed on the TSA medium surface where the bacterial suspension was applied. The discs were incubated at 35 °C-37 °C for 18-24 h. The Clear zone around the disc demonstrated bacterial inhibition zone area and was measured in a millimeter (mm) scale.

RESULTS AND DISCUSSION

Identification of chemical compounds

The extract obtained was vicious and blackish-brown in color. The identification results of chemical compounds contained in *Moringa oleifera* Lam. leaf extract using the LC-MS/MS instrument are 13-Hydroxy-9,11-hexadecadienoic acid, 3-Tert-butyl-4-methoxyphenol, bistortaside, daturametelin H, digiprolactone, ephedradine C, kaempferol-3-O-Rutinoside, kaempferol-3-O-β-D-glucopyranoside, kaempferol-7-O-α-L rhamnoside, phenylpropionic acid, Pyrophaeophorbide A, Quercetin, Stearidonic acid, stigmastan-3,6-dione.

Based on searching bistortaside data on PubTator and PubChem, the results found are Bistortaside A. Bistortaside A is a new tannin-related compound [12]. Bistortaside A is gallic acid derivatives (as phenolic acids) [13]. Gallic acid was present in B. kockiana flower could exhibit antibacterial activity towards MRSA. In SEM analysis, bacterial cell membrane was severely disrupted with evident plasmolysis upon treatment. [14]. Kaempferol-3-O-Rutinoside exhibited on wound closure by enhances cell migration involves the induction of filopodia and lamellipodia formation, up-regulation of active Rac1-GTP, increased cellular levels of phosphorylated FAK (Tyr 397) and phosphorylated Akt (Ser 473) [15]. The biological activities of Kaempferol-3-O-β-D-glucopyranoside (Astragalin) are anticancer, anti-inflammatory, antioxidant, neuroprotective, antidiabetic, cardioprotective, antiulcer, and antifibrotic [16]. Kaempferol-7-O-α-L-rhamnoside is a class of flavonoid compounds that has antioxidant activity [17]. Quercetin alone has more active antibacterial activity towards MRSA than Morin (M) and Rutin (R) combination [18]. Stearidonic acid (SDA) is an intermediate fatty acid in the biosynthetic pathway from α-linolenic acid to VLCω-3 PUFAs. Increased SDA consumption can increase red blood cell EPA content [19].

Table 1: Chemical compounds contained in 96% ethanol extract of *Moringa oleifera* Lam. leaves

Component name	Formula	Observed M/Z	Neutral mass (DA)	Observed RT (min)
Bistortaside	$C_{22}H_{24}O_{14}$	535.1087	512.11661	6.59
Daturametelin H	$C_{34}H_{46}O_9$	621.3078	598.31418	10.18
Kaempferol-3-O-rutinoside	$C_{27}H_{30}O_{15}$	595.1668	594.15847	4.62
Kaempferol-3-O-β-D-glucopyranoside	$C_{21}H_{20}O_{11}$	449.1076	448.10056	6.23
Kaempferol-7-O-α-L-rhamnoside	$C_{21}H_{20}O_{10}$	433.1126	432.10565	5.66
Phenylpropionic acid	$C_9H_{11}NO_2$	166.0859	165.07898	2.70
Quercetin	$C_{15}H_{10}O_7$	303.0494	302.04265	4.71
Stearidonic acid	$C_{18}H_{28}O_2$	277.2161	276.20893	9.82
Stigmastan-3,6-dione	$C_{29}H_{48}O_2$	429.3728	428.36543	11.18
Digiprolactone	$C_{11}H_{16}O_3$	197.1169	196.10994	5.54
3-Tert-butyl-4-methoxyphenol	$C_{11}H_{16}O_2$	181.1218	180.11503	8.03
Ephedradine C	$C_{30}H_{40}N_4O_5$	537.3036	536.29987	9.30
Phenylpropionic acid	$C_9H_{11}NO_2$	166.0857	165.07898	2.14
Pyrophaeophorbide A	$C_{33}H_{34}N_4O_3$	535.2713	534.26309	9.53

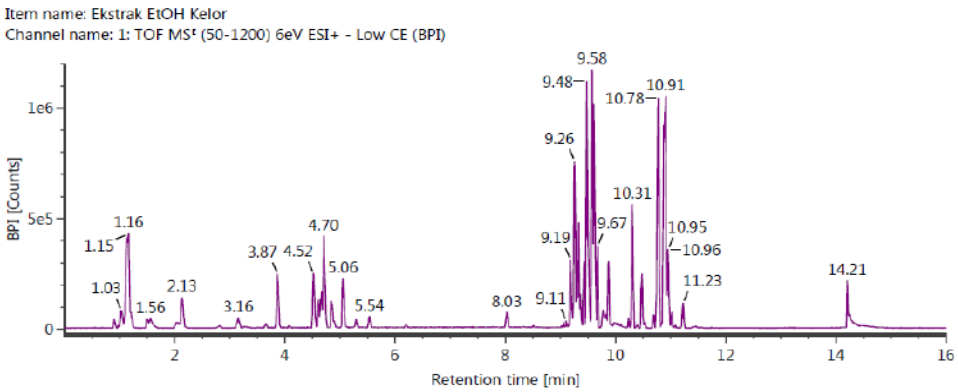

Fig. 1: Chromatogram of 96% ethanol extract of *Moringa oleifera* Lam. Leaves

Antibacterial MRSA activity test

The antibacterial activity test was performed using the agar diffusion method with linezolid 30µg as positive control and 96% ethanol as the negative control. Linezolid 30µg was chosen as a positive control because of its bacteriostatic activity that inhibits the initiation of protein synthesis at the ribosome. Unlike vancomycin, linezolid achieves high levels in the epithelial lining fluid of the lungs, making it a promising candidate for the treatment of MRSA [21]. The results of the antibacterial activity test were summarized in table 2 and fig. 2.

Table 2: Zone of inhibition of antibacterial MRSA activity

Sample	Zone of inhibition (mm)
Ethanol extract of *Moringa oleifera* Lam. Leaves 10%	10+0.0
Ethanol extract of *Moringa oleifera* Lam. Leaves 20%	13+0.0
Ethanol extract of *Moringa oleifera* Lam. Leaves 40%	15.5+0.7
Positive control (linezolid 30µg)	20+0.0
Negative control (ethanol 96%)	0+0.0

Notes: The data was given in mean+SD; n=2, The result of the antibacterial activity test showed that ethanol 96% extract of *Moringa oleifera* Lam. leaves has antibacterial activity from 10%.

Fig. 2: Antibacterial activity test for 96% ethanol extract from Moringa oleifera Lam. leaves.

Note
10% : Ethanol extract of *Moringa oleifera* Lam. leaves 10%
20% : Ethanol extract of *Moringa oleifera* Lam. leaves 20%
40% : Ethanol extract of *Moringa oleifera* Lam. leaves 40%
K+ : Positive Control (Linezolid 30 µg)
K- : Negative Control (Ethanol (96%)

The zone of inhibition of antibacterial MRSA activity in this research lower than Kalugendo E *et al.* (2019), who had showed 30 mm inhibition zone of inhibition against MRSA from *M. oleifera* seed extract [22]. Compared than research by Singh K *et al.* (2014) the zone of inhibition of antibacterial MRSA activity in this research more superior [23]. In this research, the highest zones of inhibition were lower than positive control linezolid 30µg than known as the first oxazolidinones of antibacterial drugs [24]. It has inhibitory activity against a broad range of Gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA) [25].

The present research was conducted to obtain a preliminary study on the anti-MRSA activity of *Moringa oleifera* Lam. leaves. The extract of 40% powder from fresh leaf (dissolved in ethanol 96%) has greater anti-MRSA activity than the 10% and 20% extracts. The more high *Moringa oleifera* Lam. leaves concentration, the more powerfull as anti-MRSA agent therapeutic.

CONCLUSION

The 96% ethanol extract of *Moringa oleifer*a Lam. leaves contains 13-hydroxy-9,11-hexadecadienoic acid, 3-tert-butyl-4-methoxyphenol , bistortaside, daturametelin H, digiprolactone, ephedradine C, kaempferol-3-O-rutinoside, kaempferol-3-O-β-D-glucopyranoside, kaempferol-7-O-α-L rhamnoside, phenylpropionic acid, pyrophaeophorbide A, quercetin, stearidonic acid, and stigmastan-3,6-dione.

The 96% ethanol extract of *Moringa oleifera* Lam. leaves have activity against MRSA bacteria with a concentration of 10, 20, dan 40%. According to the results of this study, the 96% ethanol extract of *Moringa oleifera* Lam. leaves can be added as a natural antibacterial constituent. The use of this 96% ethanol extract of *Moringa oleifera* Lam. leaves in traditional medicine suggests that they represent an economic and safe alternative to treat infectious diseases.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

No conflicts of interest with respect to the research, authorship, and/or publication of this article.

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