Isolation of Antibacterial Compound from the Leaves of *Mikania Micrantha* Kunth

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ABSTRACT

Aim: The purpose of this research was to analyze secondary metabolites from the leaves of the *M. micrantha* was potentials as antibacterials.
Methods: Research methods started with the collection of the sample, extraction with ethanol 70%, isolation, test of antibacterial activity, and characterization of antibacterial compounds using spectrophotometry UV and FT-IR.
Results: The screening of ethanol extracts leaves of phytochemicals *M. micrantha* contain alkaloid, polyphenols, flavonoids, saponins, and steroids. Testing of antibacterial activity of extracts, indicating the fraction of ethyl acetate (Mm-II), subfraction (Mm-II-D), isolate (Mm-II-D4) showed the greatest activity against the bacteria *Staphylococcus aureus* and *Escherichia coli*. The separation of compound was done using liquid-liquid extraction, column chromatography and preparative thin layer chromatography. Purification of isolate performed with two-dimensional thin layer chromatography. Characterization of the active antibacterial isolate (Mm-II-D4) was showed UV spectra indicate a maximum wavelength at 234 nm, and FTIR analysis indicated the existence of C=C aromatics, C=O, and –OH.
Conclusion: The allenged compound of antibacterial active isolate from leaves of *Mikania micrantha* was benzoic acid.

Keywords: *Mikania micrantha*, antibacterial, *Staphylococcus aureus, Escherichia coli*

INTRODUCTION

The infection diseases is still a problem in the public health issues. Where infection can be caused by bacteria, fungi, viruses, or parasites. The bacteria is one of the factors the causes of infections that often occur [1]. This is the underlying source of new antibacterial agents.

Mikania micrantha H.B.K is a plant that is utilized by the community as traditional medicine. Communities of Suku Anak Dalam in the area of the National Park of Bukit Dua Belas Jambi, has been utilizing *Mikania micrantha* leaves to treat wounds. Beside it, this plant use to medicine and pesticide [2]. In the area of Fiji, the plant has traditionally been used to stop bleeding, while Bangladesh is used as an antiseptic [3].

This plant has a diverse bioactivities include antitumor, antifungal, cytotoxic, analgesic, antimicrobial, antioxidant, antiviral [3–5]. Secondary metabolites are reported in *M. micrantha* among others of the phenolic substances, flavonoids, alkaloids, terpenoids and its derivatives [2,6]. In preliminary research to new antibacterial agents from natural product, reported from extract which extracted from dried leaves of *M. micrantha* against the *E. coli*, *K. pneumoniae*, *P. vulgaris* and *S. dysenteriae* in *in-vitro* condition. From the results, showed the extract of *M. micrantha* having a good antibacterial activity, addition ethyl acetate extracts of this plant

exhibited antibacterial [7,8]. Beside it, research about investigations of antibacterial from fractions of *M. micrantha* had been reported [9]. Isolation of pure compounds is recommended to identity the secondary metabolites responsible for antibacterial. The purpose of this research was to analyze secondary metabolites from the leaves of the *M. micrantha* was potentials as antibacterials.

MATERIALS AND METHODS

Materials

Leaf material of *M. micrantha* was collected from Muara Sabak, Tanjung Jabung Regency East, province of Jambi, Indonesia. Identified in the laboratory identification and determination of its biodiversity, Biological Science and Technology School (SITH), Bandung Institute of Technology, no identification 4337/II.CO2.2/PL/2017.

Bacterial Strain

One Gram-positive bacterial strain, *S. aureus*, and one Gram-negative strains *E.coli* were used during this study. Strains were obtained from the Microbiology Laboratory, Stikes Harapan Ibu Jambi. Strains were regularly subcultured using agar medium for bacterial strain at 37^0C.

Extraction and Isolation

Samples were macerated with 70% ethanol for one week, and filtered through Whatman No.1 filter paper. The filtrate was evaporated at 50^0C until a semi-concentrated of crude extract was obtained. The crude extract was analized by analytical thin layer chromatography on TLC silica gel 60 F_{254}, and elution was`optimized with n-hexane:ethyl acetate (1:1).

Phytochemical analysis

Qualitative phytochemical analysis of crude extract was carried out to identity secondary metabolites of *M. micrantha* leaves include alkaloids, flavonoids, polyphenols, tannins, saponins, steroids, terpenoids, monoterpenoid, seskuiterpenoid, and quinones.

Fraction preparation

The crude extract of *M. micrantha* leaves was subjected to partitioned by using liquid-liquid extraction methods. Each fractions (n-hexane fraction (Mm-1), ethyl acetate fraction (Mm-II), n-butanol fraction (Mm-III), and water fraction (Mm-IV)) was evaporated and tested to antibacterial activities [10]**.** Potential fractions were subjected to column chromatography yielding 4 subfraction ((Mm-II-A), (Mm-II-B), (Mm-II-C), (Mm-II-D)). The active antibacterial activity was separated with preparative thin layer chromatography Silica GF_{254} no 1.07730 as stationary phase, and n-hexane : ethyl acetate (1:1) as mobile phase ((Mm-II-D1), (Mm-II-D2), (Mm-II-D3), (Mm-II-D4)). Purification of isolate was performed with two-dimensional thin layer chromatography.

Characterization of the purified active component

UV-VIS and FTIR Spectroscopic analysis

Three mg of the purified isolate with antibacterial activity were characterized by using spectrophotometer UV and FTIR. UV-Visible spectrophotometric analysis of *M. micrantha* leaves using a UV-Visible spectrophotometer (Varian Cary 100 Conc UV) to known the maximum wavelength and absorbance of isolate. Fourier Transform Infrared (FTIR) was used to characterization of functional groups in the purified active compound [11,12].

RESULTS AND DISCUSSION

Phytochemicals Identified

The identified phytochemicals constituents of the *M. micrantha* leaves were in the table 1. From the table 1, presence of alkaloid, flavonoid, polyphenol, saponin, and steroid in ethanol extract. In methanolic extract of *M. micrantha* revealed the presence of alkaloids, flavonoids, reducing sugars, saponins, phenolic compounds, tannins, amino acids and proteins [3]. Meanwhile phytochemical study from methanolic extract of whole plants parts of *M. micrantha*, there were presences of tannin, phenol, flavonoid, glycoside, cardiac glycoside, carotenoid, and saponin [13]. These variation of secondary metabolites from *M. micrantha* extract, occur because of habitat differences of the plants which affected production of secondary plant metabolite[13]. The presence of tannins, polyphenols, alkaloids, saponins, and triterpenoids that responsible for antibacterial [9].

Table 1. Phytochemicals identified from *M. micrantha*

Chemical classification	Ethanol extract	n-hexane fraction	Ethyl acetate fraction	n-butanol fraction	Water fraction
Alkaloid (Mayer)	+	-	+	-	-
Alkaloid (Dragendorff)	+	-	+	-	-
Flavonoid	+	-	+	-	-
Polyphenol	+	-	+	+	+
Tannin	-	-	+	-	-
Saponin	+	-	-	+	-
Steroid	+	+	-	-	-
Monoterpen and sesquiterpen	-	-	-	-	-
Quinone	-	-	-	-	-

(+) indicates presence of constituents; (-) indicates absence of constituents

The antibacterial activity of ethanol extracts of *M. micrantha* with its concentration of 5%, 10%, and 15% against the bacteria *S. aureus* belongs in the strong category, and *E. coli* belongs in the middle category. Antibacterial activity of extract can be seen in Figure 1.

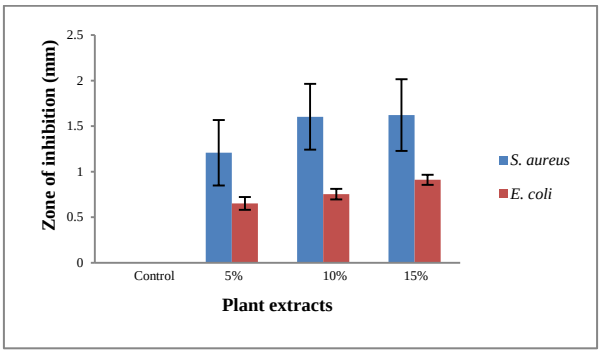

Figure 1. Antibacterial activity of ethanol extracts of *M. micrantha*

Our results showed that at 15% was the highest antibacterial activity against *S. aureus* and *E. coli*. Antibacterial activity of all fractions, ethyl acetate fraction (Mm-II) was performed highest antibacterial activity against *S. aureus*. This active fraction was eluted with n-hexane:ethyl acetate, produced four subfraction (Mm-II-A), (Mm-II-B), (Mm-II-C), (Mm-II-D). Sub fraction of (Mm-II-D) showed the highest antibacterial activity.

To test antibacterial activity from four subfraction, in preliminary doing separated with preparative thin layer chromatography. The result showed four isolates (Mm-II-D1), (Mm-II-D2), (Mm-II-D3), (Mm-II-D4), can be seen in Figure 2.

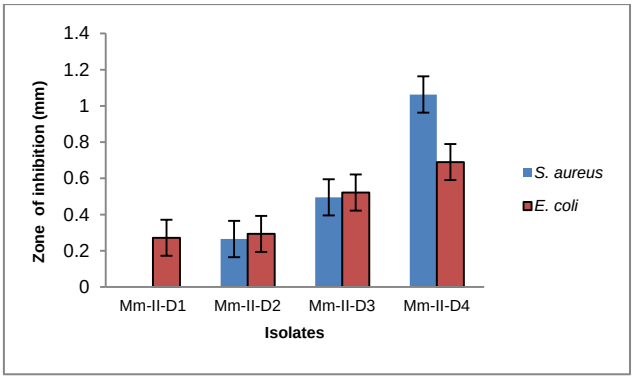

Figure 2. Antibacterial activity of isolate Mm-II-D of *M. micrantha*

From figure 2, isolate of Mm-II-D4 showed highest antibacterial activity against *S. aureus* and *E. coli*. *M. micrantha* has been reported contained sesquiterpene lactones, flavonoids, phenolic compounds and diterpenes that mostly responsible for antibacterial activities [14].

Characterization of purified active component

Absorption spectra of active subfractions were compared with the absorption spectrum of known compounds. Purity of one active isolates Mm-II-D4 was further analyzed by UV-Vis Spectrum (Table 2, Figure 3) and FTIR spectrum (Table 3, Figure 4).

Table 2. UV-Vis Spectrum Peak values of isolates Mm-II-D4 of *M. micrantha*

No	Wavelength (nm)	Absorbance
1	234.00	0.580
2	233.00	0.694
3	213.00	0.569

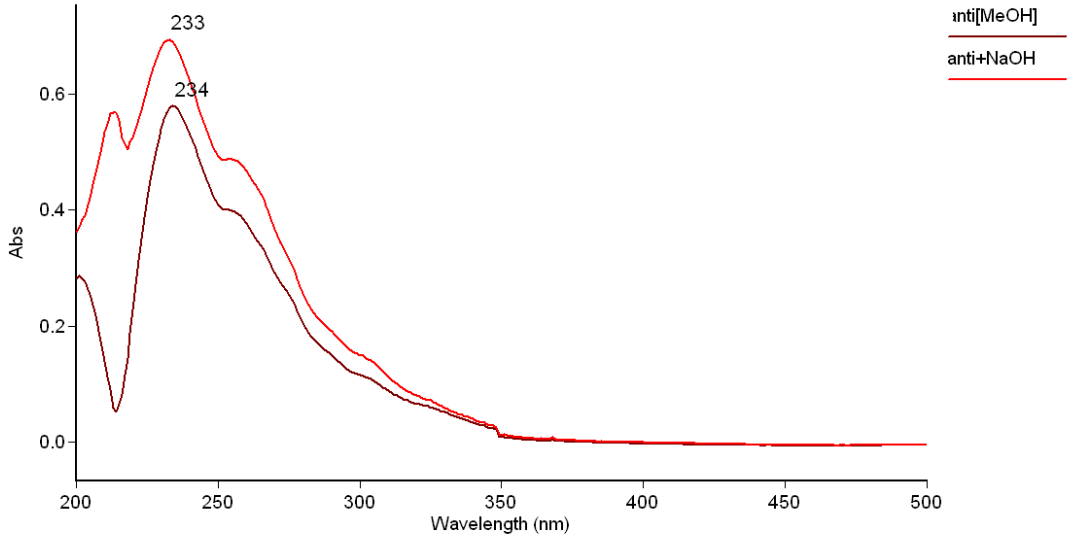

Figure 3. UV-Vis Spectrum of isolates Mm-II-D4 of *M. micrantha*

Purified active isolates were characterized by comparing absorption spectra with standard (benzoic acid)**.** Literature studies have been conducted on various compounds has been reported from ***M. micrantha,*** particularly parts of the leaves. Secondary metabolites are reported from the leaves of this plant, among others deoxymikanolide, mikanolide, scandenolide, dihydroscandenolide, dihydromikanolide, and m-methoxy benzoic acid [5]. The wavelength of the UV spectrum of benzoic acid on this study of the simultaneous determination of benzoic acid with UV spectroscopy of 230 nm[15,16].

Table 3. FTIR Peak values and functional groups of isolates Mm-II-D4 of *M. micrantha*

FTIR Peak values of isolates Mm-II-D4 (cm^{-1})	FTIR Peak Benzoic Acid Literatures (Khan, 2016)	Functional Group
1122,56	-	C-O-R
1458.18	1496	
1600.92	1622	
1726.29	1714	C=O
3427.51	3443.12	OH

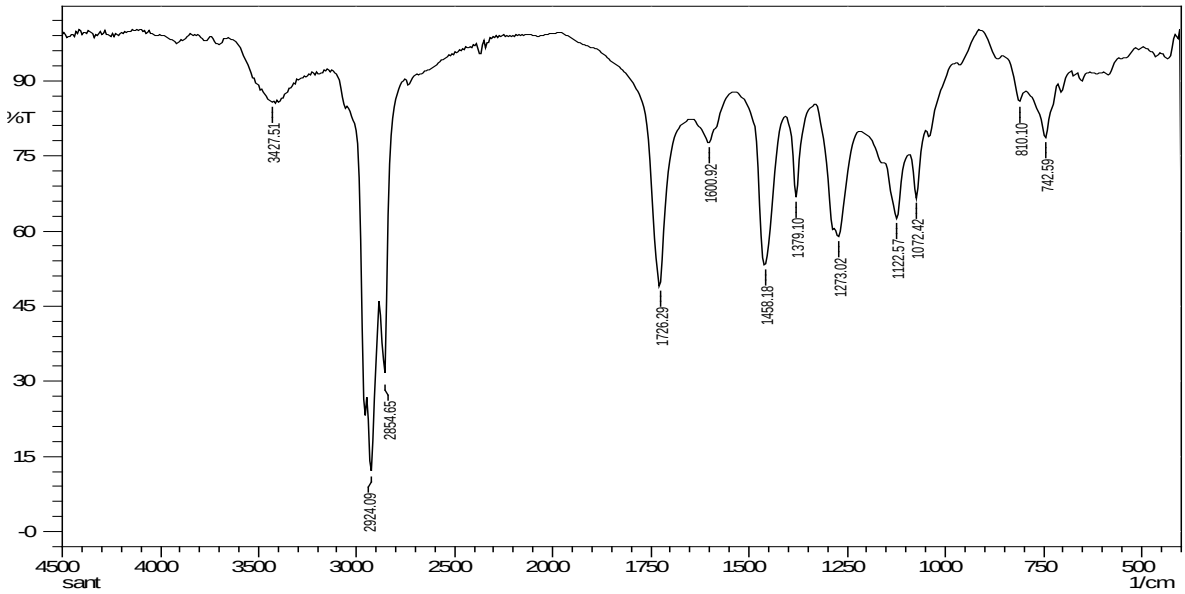

Figure 4. FT-IR spectrum of isolates Mm-II-D4 of *M. micrantha*

Addition about characterized of antibacterial compounds from isolates Mm-II-D4 of *M. micrantha* used FT-IR spectrum. The results of the comparison with other IR spectrum (Table 3), this is confirmed by research separation of benzoic acid from industrial waste streams, which similiarly with FT-IR spectrum of isolates [17].

Based on isolate wavelength showed similiarly UV spectrum with benzoic acid, and from FT-IR spectrum also similiarly with FT-IR benzoic acid, so our assumed benzoic acid as antibacterial from isolate of *M. micrantha* . For next investigation need NMR spectrum to completely characterization.

CONCLUSION

Based on the results of the spectrum, the allenged compound of antibacterial active isolate from leaves of *Mikania micrantha* was benzoic acid.

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