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Bioautography and FTIR Analysis of Ethanol Fraction Morel Berry Root (*Physalis angulata* L.) Against *Staphylococcus epidermidis* and *Pseudomonas aeruginosa*

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ABSTRACT

Morel berry root contains flavonoids, tannins, and alkaloids that have an antibacterial characteristics. This study aims to determine the antibacterial activity of the morel berry root ethanol fraction against *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* and the most effective active compounds to inhibit such activity. Extractions of morel berry root used the maceration method with ethanol 70% of and then continued by fractionation using ethanol, chloroform, and n-hexane. Test of the compound fraction was performed using Thin Layer Chromatography (TLC) and characterization with FTIR Spectrophotometer. The results of the antibacterial activity with inhibitory zone diameter were at a 100% concentration of 18.69 mm in *S. epidermidis* while *P. aeruginosa* of 20.00 mm. Bioautographic results for ethanol fraction indicate an inhibited zone with an Rf value of 0.72 in the *S. epidermidis* and *P. aeruginosa* which is thought to be a flavonoid compound. Characterization with FTIR spectrophotometer functional groups O-H phenol, C = C aromatic, C-H aromatic, C-O alcohol, and C-H aliphatic in ethanol fraction with Rf 0.72.
Keywords: Ciplukan; Thin Layer Chromatography; Antibacteria

INTRODUCTION

Infection up to now is still a major problem for the health of the Indonesian people. Treatment of infectious diseases usually uses antibiotics. But the use of this antibiotic sometimes gives unwanted side effects to the body (Andr *et al*, 2022). This situation shows the need for research to develop new antibacterial drugs derived from plants.

Morel berry (*Physalis angulata* L.) is a plant that only grows wild in gardens, moorings, curbs, bushes, light forests, and forest edges. Empirically by the community, this plant is often used as a medicine for diabetes by brewing. This plant can be used as an anticoagulant, anti-inflammatory, analgesic, antiseptic, diuretic, antibacterial, and antiviral drug (Akbar and Ernah, 2018). Morel berry plants of various active compounds such as saponins, flavonoids, polyphenols, alkaloids, physalin B, physalin D, physalin F, protein, and chlorogenic citric acid, with angulation A, palmitic acid, acetate acid, protein, vitamin C, tannins, malic acid (Luthfiyanti *et al*, 2021).

The ethanol extract of morel berry root had been shown to have antibacterial activity against *P. aeruginosa* and not on *S. epidermidis* bacteria. Ethanol extract of morel berry root at a concentration of 20%, 40%, 60%, 80%, and 100% can inhibit *P. aeruginosa* with inhibition zone diameters of 10.01 mm, 11.01 mm, 11.86 mm, 12.27 mm, and 15.08 mm respectively. With inhibitory power at 14% MIC and MBC at 20%. Whereas for *S. epidermidis* morel berry root extract has no antibacterial activity (Viogenta *et al*, 2017). Based on the phytochemical test results from morel berry root extract, it is known to contain flavonoid compounds, tannins, saponins, and alkaloids which function as antibacterial (Viogenta *et al*, 2017). In morel berry root extract, the compound content is still mixed between polar, semipolar, and nonpolar properties so that compounds that do not have the potential to be antibacterial are also extracted (Viogenta *et al*, 2017).

This study aims to detect spots on the TLC chromatogram and functional groups that have antibacterial activity of the ethanol fraction of morel berry roots against *S. epidermidis* and *P. aeruginosa.* Bioautography is a simple method used to show antibacterial activity by combining the use of the Thin Layer Chromatography (TLC) technique which aims to detect chromatogram spots from TLC results and see active compounds that are effective in inhibiting bacteria (Jesionek *et al*, 2013). Identifying the functional groups of active compounds that have the potential as antibacterial can use the Fourier Transform Infrared (FTIR) Spectrophotometer technique (Utami *et al,* 2016).

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METHODOLOGY

Materials

The materials used in this study included morel berry root (*Physalis angulata* L.), *Staphylococcus epidermidis, Pseudomonas aeruginosa, Nutrient Agar* (NA), *Nutrient Broth* (NB), 125 µg/µL amikacin, aquadest (H_2O), ethanol 70% (C_2H_6O), ethanol pa, chloroform pa ($CHCl_3$), n-Hexane pa ($CH_3(CH_2)_4CH_3$), methanol, $FeCl_3$, ammonia (NH_3), Liebermann Burchard, and Bourchadat reagents.

Methods

Simplicia Processing Process

The morel berry root was taken from Kemiling District, Bandar Lampung City, Lampung Province. The morel berry plant was determined at the Biology Laboratory of the University of Lampung to ensure that the plant taken was morel berry. The morel berry root was taken by digging and then cleaned of impurities attached to running water, then the root was weighed around 3000 g, then continued by drying indirectly (covered with black cloth) under the sun to dry, and the last that simplicia weighed again to produces 500 g.

Extraction Process

Simplicia morel berry root as much as 500 g macerated with 70% ethanol. Solvents were replaced once every 24 hours for 6 days. Then the extract was evaporated using a rotary evaporator until a 250 ml liquid extract was obtained.

Fractionation Process

A liquid extract of 100 ml morel berry root was inserted into a separating funnel then 100 ml of n-hexane was added, then shaken until there was a separation between the ethanol fraction and the n-hexane fraction and repetition until getting the pure solvent. The n-hexane fraction was separated while the ethanol fraction was fractionated again with 100 ml chloroform solvent and then shaken up to obtain the ethanol fraction and chloroform fraction and repetition until getting the pure solvent. The ethanol fraction was evaporated with a rotary evaporator.

Antibacterial Activity Test

Each bacterial suspension (100 µL) was suspended in a sterile petri dish and then added NA medium which had not been compacted, homogenized, and then allowed to solidify. Some holes were made in the media using a blue tip. Then the ethanol fraction test solution was inserted into these holes using a micropipette. All petri dishes were incubated for 24 hours at 37 ºC. Furthermore, observations and measurements of the inhibitory zone formed around the diffusion were carried out using a caliper. The fraction that produced the inhibition zone diameter was then tested with a concentration of 20%, 40%, 60%, 80%, and 100%, with amikacin as a positive control and aquadest as a negative control with the same method namely the diffusion method. Antibacterial power is categorized according to Davis and Stout (1971) where the diameter of the inhibitory zone <5 mm is classified as weak, 5-10 mm is classified as moderate, 10-20 mm is classified as strong and> 20 mm is classified as very strong.

Thin Chromatography Testing (TLC)

The stationary phase to be used was the silica gel plate G60F254 which was activated first by heating in an oven at a temperature of 100 ºC for 1 hour. The mobile phase used for ethanol fraction was chloroform: methanol: water (2: 5: 3). The samples that will be used were ethanol fraction was bottle 5 × on each TLC plate using capillary pipes, left for a few minutes to dry and then put in a chamber that was saturated with the eluting liquid. Let it elute until the chromatogram plate boundary had been determined. The plate is removed from the chamber, then the stain that appears to be detected with uv 254 nm, 366 nm.

The Bouchard at spray reagent for detecting alkaloids would show a brown color, ammonia spray for flavonoids shows a brownish yellow, green, brown, or pink, $FeCl_3$ for tannins would produce strong green, red, purple, blue, or black colors, and Liebermann-Burchard for saponins produces purple (Ekawati *et al*, 2017). Furthermore, Rf measurements of the chromatogram of the results of TLC were carried out.

Testing by TLC-Bioautography

The test to find out the most active fraction was carried out by TLC method using methanol: water (2:5:3) as the mobile phase and silica gel G60F254 as the stationary phase. After elution, spots on the TLC plate were then observed under visible light and UV light with wavelengths of 254 and 366 nm. Furthermore, the chromatogram plate was contacted with the surface of NA media which had been inoculated by *S. epidermidis or P. aeruginosa* for 3 hours. The plate was removed and the culture was incubated at 37 °C for 24 hours then observed the zone of resistance formed then calculate the value of the Rf (Isnaeni *et al,* 2018). Rf with the same value or close to the Rf value in TLC is thought to be the most effective active compound as an antibacterial.

Identification of Group Functions of Active Compounds with FTIR Spectrophotometers

Identification of functional groups was carried out by scraping the plate as a result of TLC-bioautography which was thought to contain the active compound as an antibacterial from the ethanol fraction of the morel berry root. Then it was identified using the FTIR (*Agilent / Cary* 630) spectrophotometer.

RESULT AND DISCUSSION

Based on the results of the determination in Lampung University, Morel berry has the species name *Physalis angulata* L. from the Solanaceae family. The results obtained in the antibacterial test were positive control (amikacin), and ethanol fraction have inhibitory zone diameters while the negative control (aquadest) haven't inhibitory zone diameters. This can indicate that the antibacterial compounds of the morel berry root fraction were extracted in polar ethanol solvents (Figure 1).

The antibacterial activity with concentrations of 20%, 40%, 60%, 80%, 100%, amikacin as a positive control, and distilled water as a negative control which was then tested against *S. epidermidis* and *P aeruginosa*. The results of the antibacterial test showed that the ethanol fraction of morel berry root could inhibit the growth of gram-positive *S. epidermidis* bacteria and gram-negative *P. aeruginosa* bacteria. This is evidenced by the formation of clear zones around the diffusion. The ethanol fraction of morel berry root has an inhibitory effect on *S. epidermidis* for all concentrations of 20%, 40%, 60%, 80%, and 100% (v / v), with the formation of inhibition zones around the well with an average diameter were of 7.71 mm, 8.79 mm, 10.40 mm, 12.79 mm and 18.69 mm, while the amikacin control inhibition zone diameter was 29.91 mm, and aquades control, was 0 mm.

The ethanol fraction of morel berry root has inhibition on *P. aeruginosa* for all concentrations of 20%, 40%, 60%, 80%, and 100% (v / v), with the formation of inhibition zones around diffusion with an average diameter of 10.08 mm, 11.76 mm, 14.11 mm, 17.36 mm and 20.00 mm while the amikacin control inhibition zone diameter was 30.27 mm, and aquades control was 0 mm. This increase in concentration affects the working power of antibacterial substances against bacterial growth. This is caused by levels of active compounds that are contained in high concentrations that are higher than in low concentrations (Table I).

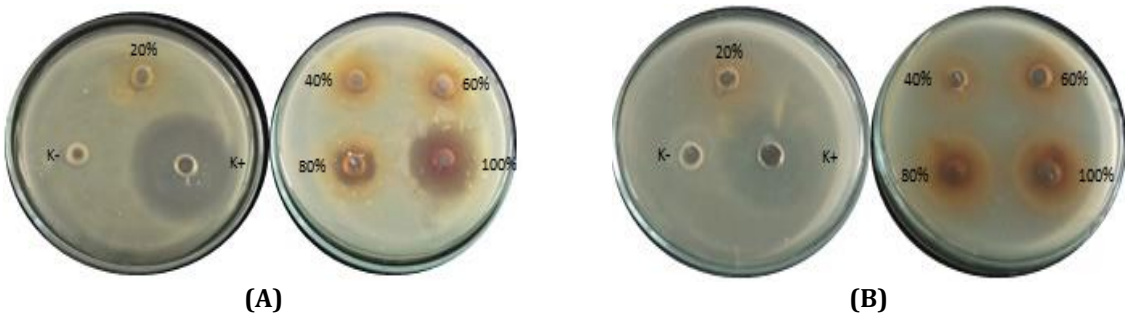
The results of the Kruskal Wallis test showed p-value (Asymp.Sig.) = 0.00 (p 5 0.05), meaning that the ethanol fraction of morel berry root had a significant effect on the inhibition zone of *S. epidermidis* and *P. aeruginosa* (Table I). The results of the Mann Whitney U test on the average diameter of the inhibition zone of *S. epidermidis* and *P. aeruginosa* in the negative control (aquadest) and the ethanol fraction had different antibacterial activity for other treatments. The positive control (amikacin) was significantly different from all ethanol fractions concentration. It can be concluded that the ethanol fraction of morel berry roots has not been able to replace the antibacterial activity of amikacin antibiotics. Amikacin is an aminoglycoside antibiotic that works by inhibiting protein synthesis on the bacterial ribosome. The antibiotics used were pure amikacin active compound with a concentration of 125 mg/mL.

Against *S. epidermidis* and *P. aeruginosa* (Table I), the antibacterial activity of the ethanol fraction of morel berry root with a concentration of 20% was the same as that of 40% but different for other concentrations. At a concentration of 40% ethanol fraction and 60% ethanol concentration, it has the same antibacterial activity but is different from other concentrations. The 60% ethanol fraction and 80% ethanol fraction had the same antibacterial activity but were different for other treatments. The concentration of the 100% ethanol fraction was significantly different from the treatment of the ethanol fraction at concentrations of 20%, 40%, 60%, and 80%. Therefore, the antibacterial activity of the ethanol fraction of morel berry roots can be concluded that there is a very strong positive correlation where the higher the concentration of the ethanol fraction, the larger the inhibition zone that appears.

The TLC analysis aims to determine the compound content of each ethanol fraction. The mobile phase used for ethanol fraction, chloroform: methanol: water (2: 5: 3), was chosen because it was adjusted to the solubility properties of the analyzed compounds, which are polar (Anggreany *et al*, 2020).

Furthermore, the spots are detected with a spray reagent, namely ammonia for flavonoid compounds, and will show a brownish-yellow, orange or red color. $FeCl_3$ will produce green, red, purple, blue, or black colors if it contains tannins. Bouchardat reagent for alkaloids will produce a brown color and Liebermann-Burchard will produce purple color for saponins (Parbuntari *et al*, 2018). The results of cross-sectional spots and color on TLC of the ethanol fraction of morel berry roots can be seen in Figure 2.

The cross-sectional results of thin layer chromatography on the ethanol fraction of morel



(A) **(B)**

Figure 1. Antibacterial Activity of Ethanol Fraction Morel Berry Root Needs With Concentration of 20%, 40%, 60%, 80%, 100%, (-) = Aquades and (+) = Amikacin. A. *S. epidermidis* and B. *P. aeruginosa*

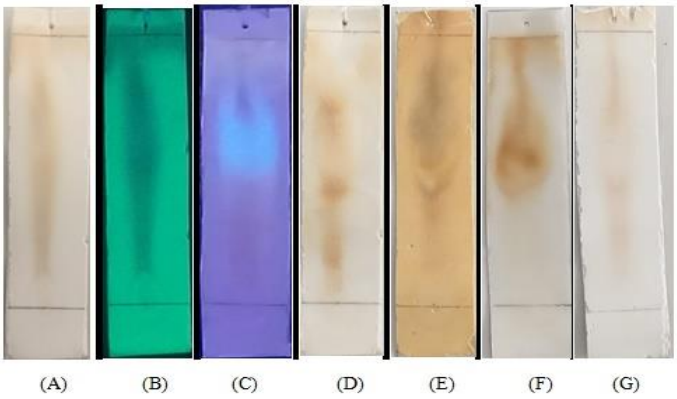


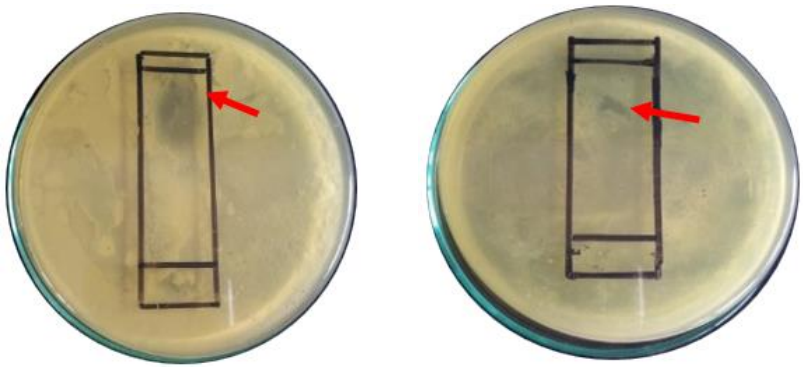
Figure 2. Results of TLC Test Ethanol Fractination of Morel Berry Root. A: Visible rays, B: UV 254, C: UV 366, D: Ammonia, E: FeCl3, F: Bouchardat Reagent and G: Liebermann-Burchard.

Table I. An Inhibitory Zone Diameterof Ethanol Fraction of Morel Berry root against *S. epidermidis* and *P. aeruginosa*

Treatment	Inhibition zone (mm)	
	S. epidermidis	***P. aeruginosa***
E -	0^{a}	0^{a}
E +	29.91^{b}	30.27^{b}
E. 20%	7.71^{c}	10.08^{c}
E. 40%	8.79^{cd}	11.76^{cd}
E. 60%	10.40^{de}	14.11^{de}
E. 80%	12.79^{e}	17.36^{e}
E. 100%	18.69^{f}	20.00^{f}

berry roots showed positive results containing flavonoids after being sprayed with ammonia reagent which was indicated by the formation of a brownish-yellow color on the plate. From the results of the ammonia spray test, it can be stated that it contains flavonoid compounds with Rf 0.32, 0.54, and 0.72. Rf 0.72 of ethanol fraction of morel berry root fraction, has the same Rf as flavonoids of the flavanone group. So that the flavonoids in the ethanol fraction of morel berry roots are thought to be from the flavanone group. Identification of flavonoids in Alchemilla species shows the presence of flavonoid compounds that have the same Rf value as flavonoids with Rf of 0.72 and 0.44 respectively (Kaya et al, 2012).

The results of thin layer chromatography spots on the ethanol fraction of morel berry roots showed positive results containing tannins after being sprayed with $FeCl_3$ which was indicated by the formation of black color on the plate. The formation of a black color after being added with $FeCl_3$ is because tannins will form complex



(A) (B)

Figure 3. Bioautography of Morel Berry Root Ethanol Fraction with Rf 0.72 A. *S. epidermidis*; B. *P. aeruginosa*

Tabel II. TLC Test Results of the Ethanol Fraction of Morel berry roots with a development distance of 5 cm

Compound	Detection	Positive Results	Result Research	Statement	Rf
Flavonoid	Ammonia	Brownish-yellow.	Brownish-yellow	**+**	**0,32; 0,54; 0,72**
Tannins	$FeCl_3$	green, red, purple, blue or black	Black	**+**	**0,58; 0,64; 0,82**
Alkaloid	Bouchardat Reagents	Brown	Brown	**+**	**0,48; 0,64; 0,74; 0,94**
Saponins	Lieberman Burchat	Purple	No change	**-**	

compounds with Fe^{3+} ions (Ergina and Pursitasari, 2014). The most common Rf value for tannins is 0.55 and the ethanol fraction of ceplukan roots produces Rf values of 0.58, 0.64, and 0.8239.

The results of thin layer chromatography spots on the ethanol fraction of morel berry roots showed positive results containing alkaloids after being sprayed with Bouchardat reagent which was marked by the formation of brown color on the plate. In the Bouchardat test, metal ions K^+ will form coordinate covalent bonds with nitrogen in the alkaloids to form a precipitated potassium-alkaloid complex (Ergina and Pursitasari, 2014). The Rf value of the most common alkaloids is 0.07 – 0.62 and the ethanol fraction of morel berry roots produce Rf values, respectively, for the Rf of ethanol fraction of morel berry roots of 0.48, 0.64, 0.74, and 0.94. One of the spots from the ethanol fraction with Rf 0.48 for the ethanol fraction was following the Rf in general so it can be said that the ethanol fraction contains compounds alkaloids (Valiyeva and Garaev, 2019). Thin layer chromatography spots on the ethanol fraction of ceplukan roots showed negative results containing saponins after spraying with Liebermann-Burchard because there was no purple color on the plate.

The purpose of the bioautography test was to determine which compounds in the TLC chromatogram had antibacterial activity. Active spots are indicated by the formation of a clear zone. The Rf value of the clear zone was calculated and matched with the Rf value of the chromatogram plate. The results of the TLC-Bioautography test of the ethanol fraction on both test bacteria *S. epidermidis* and *P. aeruginosa* showed one active spot with an Rf value of 0.72 and had an inhibition zone diameter on the agar diffusion activity test.

From the results of the TLC test, spots on the ethanol fraction Rf 0.72 are flavonoid compounds, so the active compounds that have the most role as an antibacterial in the ethanol fraction of morel berry roots are flavonoid compounds. The inhibition zone formed is caused by the presence of active compounds from the chromatogram spots that diffuse into the media and cause inhibition of

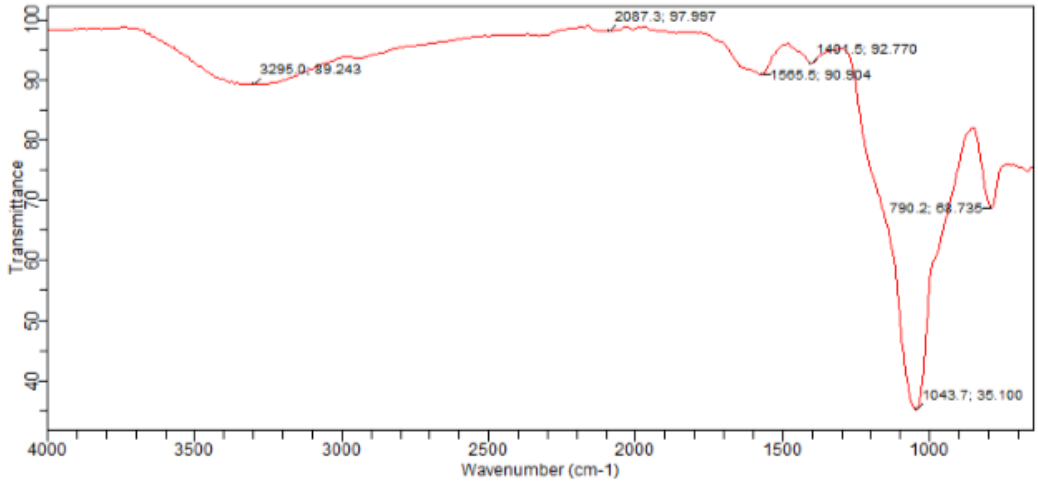


Figure 4. Results of FTIR Spectrum Ethanol Fraction of Morel Berry Root

bacterial growth at the diffusion site of the active compounds (Souto *et al*, 2021).

The results of FTIR analysis on the ethanol fraction of morel berry root of Rf 0.72 in the TLC plate showed the presence of functional groups OH phenol (3295 cm^{-1}), C = C aromatic (1565.5 cm^{-1}), CH aromatic (790.2 cm^{-1}), CO alcohol (1043.7 cm^{-1}) and CH aliphatic (1401.5 cm^{-1}), where the functional groups belong to flavonoids and include flavonoid flavones containing OH groups on C3, C3 'and C4'. Other studies on the isolation and identification of flavonoid compounds in Sembukan leaves (*Paederia foetida* L) explained that the functional groups O-H, C = C aromatic, C-H aromatic, C-O alcohol, and C-H aliphatic were included in flavonoid compounds (Ekawati *et al,* 2017).

CONCLUSION

Ethanol fraction morel berry root has antibacterial activity against *S. epidermidis* and *P. aeruginosa* bacteria and has proven to contain flavonoids, tannins, and alkaloids. The active compounds which are most effective in inhibiting bacteria in the morel berry root ethanol fraction are flavonoids for the flavonone group with Rf 0.72. The active compound functional group based on the FTIR spectrophotometer characterization in the ethanol fraction at Rf 0.72 shows the presence of OH phenol groups, C = C aromatic, CH aromatic, CO alcohol, and CH aliphatic. Based on the research that has been done, further research is recommended to purify the active compound using the column chromatography method, conducting further research on the isolation of flavonoid compounds which are used as inhibitors of the growth of *S. epidermidis* and *P. aeruginosa*, and preparations of fractions ethanol morel berry root as antibiotics.

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