

ORIGINAL ARTICLE

Antibacterial Activity of Ethanolic Extract of Morel Berry (*Physalis angulata* L.) towards *Staphylococcus aureus*

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ABSTRACT

Introduction: Infectious disease is still a major health problem in Indonesia. One of its bacteriological pathogens is *Staphylococcus aureus*. Morel berry (*Physalis angulata* L.) is a traditional herb that can be utilized as an antimicrobial agent because of its chemical substances such as flavanoid, alkaloid, tannins, and polyphenol. **Purpose:** The aim from this study is to determine the antibacterial activity of ethanolic extract of morel berry (*Physalis angulata* L.). **Methods:** This study used the diffusion method and concentration of 50%, 70%, and 90%. The obtained data were analyzed by One-Way ANOVA. Phytochemical screening disclosed the presence of alkaloids, polyphenol, flavonoids, and tannins in ethanolic extracts. Sole extract of morel berry (*Physalis angulata* L.) at a concentration of 90% showed the largest inhibition zone as much as 17 mm to *Staphylococcus aureus* ATCC 25923. **Conclusion:** The ethanol extracts prove to be potentially effective as natural alternative preventive to fight against *Staphylococcus aureus* ATCC 25923.

Keywords: Antibacterial activity, Morel berry (*Physalis angulata* L.), *Staphylococcus aureus*.

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INTRODUCTION

Staphylococcus aureus is a serious human pathogen known to cause numerous bacterial infections at the level of the bloodstream, lower respiratory tract, and skin and soft tissue (1). *Staphylococcus aureus* infections cause high mortality and the resistance to almost all currently used antibiotics (2). Penicillin was initially highly effective for the treatment of *Staphylococcus aureus* infections. However, the widespread use of penicillin led to the emergence of penicillin-resistant *Staphylococcus aureus* (PRSA)(3). With the release of beta-lactamase-resistant penicillins such as methicillin and oxacillin in the 1970s, methicillin-resistant *Staphylococcus aureus* (MRSA) emerged and became an important cause of infectious diseases acquired in hospitals(1). Due to the presence of this situation, it is important to prevent MRSA. The treatment of MRSA infections continued to be complex due to the fact that they have developed resistance.

Various herbal medicines in current use are not effective to completely cure bacterial infections.

Several newer classes of antibiotics have proven to have activity against MRSA, including linezolid of the oxazolidinone class and daptomycin of the lipopeptide class (4). The shortage and limited affordability of pharmaceutical drugs to treat MRSA infections are still considered as the main hindrance. From that standpoint, this is an area of concern that highly calls extensive and collaborative researches to find novel germicidal drugs to replace the old antibiotics. Several studies have shown that extracts from plant species may be active against multi-drug resistant bacteria, including MRSA (1).

Indonesia has many plants that can be used as natural antibiotics one of them is *Physalis angulata* L. It is a plant that belongs to the genus *Physalis* of the family Solanaceae. It is known by different names, including morel berry, camapu, mullaca, winter cherry, etc. In Indonesia, it is known as Ciplukan. Its biological properties include, antimycobacterial, anticancerous, antitumoral, anticoagulant, immunostimulant, etc (5).

MATERIALS AND METHODS

Sample Preparation

Morel berry (*Physalis angulata* L.) were rigorously collected in Pontianak, Indonesia. Morel berry fruits were chosen depending on their solidity, absence of

mechanical injury, and observable rot. Following that, the fruits were dried in an oven at 60°C and eventually blended into powder with the help of a porcelain mortar and pestle.

The extraction was aseptically performed in a Pharmaceutical laboratory. Morel berry powder weighed as much as 100 grams was put in a brown bottle filled with 1000 ml of 70% ethanol solvent, then soaked for 5 days. The extract obtained was then filtered and the filtrate was evaporated with a vacuum rotary evaporator at 40°C, after which the filtrate was oven at 40°C to obtain a thick or concentrated extract.

Phytochemical Screening

Qualitative analysis tests were performed for various phytoconstituents such as flavonoids (Shinoda test), tannins (Ferric chloride test), alkaloids (Wagner test), and phenolic compounds were examined by diluting the extract with distilled water up to 5 mL and added with 3 drops of 5% ferric chloride solution. The availability of alkaloids in the extract was confirmed by a yellowish-white precipitate after adding a Mayer solution. The flavonoid in the extract was confirmed by adding samples with ethanol then heated, filtered, shaken, and then added with magnesium powder and dripped with HCL. The test result would bear a red color. Phenols in the extracts were confirmed by the change in colors to dark green. Tanin was tested by The test material is mixed with distilled water until it is soaked and heated for 3 to 5 minutes. Then add 2 drops of FeCl₃ 1%. Blackish green color changes indicate the presence of tannins.

Antibacterial Assay of The Extracts

This study used bacteria from human pathogens isolated from clinical specimens. Pure cultures of *Staphylococcus aureus* ATCC 25923 used to assess the antibacterial properties were obtained from bacteriology laboratory Politeknik Kesehatan Kemenkes Pontianak. A single colony of each test bacteria was diluted in 9 mL of peptone water and acclimatized to give the equal concentration of bacterial cells of 10⁶ colony-forming unit/mL (6).

Antibacterial activity of the extracts was determined by agar well diffusion method (7). A cotton swab was used to swab 25 microliters of diluted bacteria and a Pasteur pipette was used to create holes in the plate. Twenty milligrams of each extract (20 mg/mL) was impregnated in created wells and 20 mg of Vancomycin (20 mg/mL) was used as control. The plates were then incubated in an upright position at 37°C for 24 hours. All tests were done in duplicate and the antibacterial potential was recorded as mean by estimating the inhibition zones with a vernier calliper (6,7).

RESULTS

Phytochemical Analysis

Table I shows that in the intervention group most of the responPhytochemical screening confirmed the presence of alkaloids, tannins, flavonoids, polyphenol compounds in ethanolic extracts, recorded in Table I.

Antibacterial Activity

The findings of the antimicrobial assay revealed that vancomycin had more potential compared to ethanolic extracts. The extracts were able to inhibit

Table I : The results of the chemical tests of extracts *Physalis angulata* L. fruits.

Extracts	Flavonoid	Alkaloids	Tannins	Polyphenol
Ethanolic	+	+	+	+

Tables II. The inhibited diameter of extract Morel berry (*Physalis angulata* L.) against *Staphylococcus aureus* ATCC 25923 in diffusion method (Mean ± SD).

Sample	Concentration	Zone of inhibition (mm)
Morel berry	50 %	11.333±0.577
Morel berry	70 %	14.333±0.577
Morel berry	90 %	17.000±0.000
Control (+) Vancomycin	50 %	15.666±0.557
	70 %	18.000±0.000
	90 %	18.777±0.557
Control (-) DMSO 3%	50 %	0
	70 %	0
	90 %	0

Staphylococcus aureus ATCC 25923 the largest inhibition zone as much as 17 mm. (Table II). Antibacterial activity of ethanolic extract of Morel berry (*Physalis angulata* L.) was performed using the diffusion method. A stock solution was prepared in three concentrations of 50%, 70%, and 90% respectively. DMSO 3% was used as a solvent and negative control. Vancomycin 15µg was used as a positive control. Antibacterial activity test was performed using a diffusion test for 24 hours at 37°C. Antibacterial activity of each extract was marked by a transparent zone around the disc and its size was measured and stated in millimeter (mm) (Table II).

DISCUSSION

The findings presented in Table I disclosed the existence of tannins, saponins, alkaloids, and flavonoids in the ethanolic extract which have antibacterial activities. Alkaloids acted as an antibacterial agent by disrupting constituent of peptidoglycan in bacterial cells, leading to the disorganization of the cell wall and its death. Flavonoids acted as an antibacterial agent by forming a complex compound with extracellular protein which impaired the integrity of bacterial cell membranes. Its mechanism of action was by denaturing bacterial cell protein and alter cell membrane (8). Flavonoids acted as an antibacterial agent by hampering the formation of DNA and RNA. Flavonoid caused the disruption of bacterial cell wall permeability, microsome, and lysosome as the results of the interaction between flavonoid and bacterial DNA (9).

Tannins acted as an antibacterial agent by shrinking the bacterial cell wall of its membrane, leading to impaired cell permeability. This made the cell unable to undergo its normal activity, causing the delay of its growth or even worse, its death. Tannin exerted its antibacterial activity by precipitating protein, similar to the mechanism showed by a phenolic compound. Tannin interacted with the cell membrane, inactivated its enzyme, and inactivated the function of genetic materials (10).

Polyphenol can link with and disable some bacterial enzymes essential for bacterial cell wall synthesis, action for simple phenols are attributed to the interaction with sulfhydryl groups that are present in microbial enzymes which leads to the enzyme inhibitions or through the interactions with a non-specific chain of amino acids (11).

In the present investigation, antibiotics exhibited high inhibitory activity than the prepared plant extracts as shown in Table II. Antibiotics are more effective than plant extracts probably because they

are in refined states and naturally purified while plant extracts are still in crude states (12).

CONCLUSION

Extracts of *Physalis angulata* L. have potential bioactive substances such as flavanoid, alkaloid, tannins, and polyphenol. From that perspective, it contains antimicrobial agents that could be contemplated to develop the effectual treatment modalities for fighting pathogens that are resistant to typical antibiotics.

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REFERENCES

1. Yu H, Liu M, Liu Y, Qin L, Jin M, Wang Z. Antimicrobial Activity and Mechanism of Action of *Dracocephalum moldavica* L. Extracts Against Clinical Isolates of *Staphylococcus aureus*. *Front Microbiol.* 2019;10:1249.
2. Lan L, Hao C, Yanyan L, Shuangli X, Maomao W, Zhou L, et al. Synergistic effect of Linezolid With Fosfomycin Against *Staphylococcus Aureus* in Vitro and in an Experimental *Galleria Mellonella* Model. *J Microbiol Immunol Infect.* 2018.
3. Chen CJ, Huang YC. New epidemiology of *Staphylococcus aureus* infection in Asia. *Clin Microbiol Infect.* 2014;20:605–623.
4. Kali A. Antibiotics and bioactive natural products in treatment of methicillin resistant *Staphylococcus aureus*: a brief review. *Pharmacogn Rev.* 2015;9:29–34.
5. Nostro A, Germano M, D'Angelo V, Marino A, Cannattelli M. Extraction methods and bioautography for evaluation of medicinal plants for antimicrobial activity. *Lett Appl Microbiol.* 2000;30(5):379–84.
6. Osho A, Adetunji T, Fayemi S, Moronkola D. Antimicrobial activity of essential oils of *Physalis angulata* L. *African J Tradit Complement Altern Med.* 2010;7(4):303–6.
7. National Committee for Clinical Laboratory Standards (NCCLS). M02-A12 Performance Standards for Antimicrobial Disc Susceptibility Tests; Approved Standard. 12th Edition. Villanova: NCCLS. 2015.
8. Juliantina FR. Manfaatsirihmerah (piper crocatum) sebagai agen anti Bacteria terhadap bakteri gram

- positif dan gram negatif. JKKI – J Kedokt dan Kesehat Indones. 2008.
9. Cushnie TPT, Lamb AJ m. Antimicrobial Activity of Flavonoids. *Int J Antimicrob Agents*. 2005;26:343–56.
 10. Ajizah A. Sensitivitas Salmonella Typhimurium terhadap Ekstrak Daun Psidium Guajava L. *Bioscientiae*. 2004;1(1):8-31.
 11. Omar A, Aldulaimi. General Overview of Phenolics from Plant to Laboratory, Good Antibacterials or Not. *Pharmacogn Rev*. 2017;11(22):123–127.
 12. Doughari J, Pukuma M, De N. Antibacterial effects of *Balanites aegyptiaca* L. Drel. and *Moringa oleifera* Lam. on *Salmonella typhi*. *Afr J Biotechnol*. 2007;6(19):2212–5.