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Antibacterial activity of *Piper betle* L. (Piperaceae) and its products

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ABSTRACT

Introduction: Piper betle L. is a common species in many countries and is used in the production of natural products mainly for its antibacterial activity. Objective: To investigate the antibacterial ability of 96%, 70%, 50% ethanol extracts, essential oils, and products from P. betle. Research methods: agar disc diffusion and dilution methods to determine MIC (minimum inhibitory concentration). Results: The extracts and essential oils of P. betle have good effects on 2 strains of bacteria E.coli and S.aureus, in which the extract with the best MIC is 96% ethanol extract (0.312 mg/mL and 0.078 mg/mL for E. coli and S. aureus, respectively). The MICs of essential oils were 0.5% and 0.0312% for E. coli and S. aureus, respectively, while products from P. betle were only active against S. aureus but had no effect on E.coli strains. Conclusion: Extracts, essential oils, and preparations from P.betle have antibacterial activity against two strains of bacteria, E.coli and S.aureus. Research should be directed to other strains of bacteria for more comprehensive evaluation.

Keywords: *Piper betle, antibacterial, essential oil*

1. INTRODUCTION

Piper betle L. originated in Central and East Malaysia and was cultivated as early as 2,500 years ago, before spreading to Madagascar and East Africa. Today, *P. betle* is widely grown across tropical Asian countries, including India, Sri Lanka, Vietnam, and others. In these regions, large-scale *P. betle* plantations cater to industrial demands for products such as toothpaste, shampoo, personal care items, herbal supplements, and herbal beverages [1 - 4]. Historically, *P. betle* was commonly chewed in combination with lime betle, hard fruit, areca nuts, and gambier as a traditional mouth refresher [4]. These chewing formulations have been known to strengthen teeth and promote oral freshness [4 - 5]. Beyond oral applications, *P. betle* paste has also been traditionally used for hair cleansing and intimate hygiene. The bioactive constituents of *P. betle* include tannins, flavonoids (such as quercetin), eugenol, hydroxychavicol, and chavibetol, which contribute to its pharmacological properties [5 - 8]. *P. betle* has been reported to exhibit antimicrobial, antifungal, antioxidant, antidiabetic, and anticancer activities [3, 9, 10]. Thus, this study aims to evaluate the antibacterial activity of essential oils, extracts, and formulations derived from *P. betle* through in vitro analysis.

2. MATERIALS AND METHODS

2.1. Materials

The materials: *Piper betle* L. leaves from VAN XUAN CO., LTD. Chloroform, Ethanol, Mueller-Hinton Agar, DMSO*, Tween 80 (Merck), Tryptic Soy Aga (TSA), Escherichia coli ATCC 25922, Staphylococcus aureus* ATCC 29213. LIDA. VX herbal cream and Van Xuan Huong herbal mouthwash was provided by Van Xuan Co., Ltd.

2.2. Method

2.2.1. Extraction

500g of *Piper betle* leaves were dried in the oven at 50°C. For extraction, the dried leaves were percolated with 96%, 70%, and 50% ethanol at room temperature. Ethanol was then removed by using a rotary evaporator with a vacuum pressure (120 mbar) at 50°C to obtain a crude extract.

Fresh leaves of *P. betle* were used steam distillation technique to extract essential oils. Before the first steam distillation, the *P. betle* leaves are cut into small pieces to facilitate the distillation and

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insulate the essential oils inside the *P. betle* leaves. The essential oil extraction efficiency was determined by the formula:

$$\%Efficiency = \frac{m_{essential\ oils}}{m_{leaves\ P.\ betle}} x\ 100\%$$

2.2.2. Antibacterial activity of essential oils from P. betle

Test strain activation medium: Tryptic Soy Agar (TSA).

Test medium: Mueller - Hinton Agar (MHA).

Sample dilution solvent: DMSO, Tween 80 (Merck).

Test environment conditions: Room temperature.

The minimum inhibitory concentration (MIC) of the test substances was determined using the agar dilution method.

The essential oils were dissolved in DMSO containing 0.02% Tween 80. When used, the solution was directly mixed with the test medium to achieve a series of decreasing concentrations, with each subsequent concentration being half of the previous one. The final concentrations in the test medium (% wt/wt) were as follows: 1%, 0.5%, 0.25%, 0.125%, 0.0625%, 0.0312%, and 0.0156%.

2.2.3. Antibacterial activity of extracts and products from P. betle

The antibacterial and antifungal properties of the test substances were determined using the agar diffusion method.

Each extract (0.05 g) was dissolved in DMSO to obtain a test concentration of 50 mg/mL. Then, 100 µl of the solution was introduced into a 6 mm diameter well on an agar plate to assess antibacterial activity.

Table 1. Classification of bacterial growth inhibitory responses based on the diameter of the clear zone [12]

Diameter of clear zone (mm)	Response to bacterial growth inhibition
> 20	Strong
16 - 20	Moderate
10 - 15	Weak
< 10	None

The MIC of the test substances was determined using the agar dilution method.

Each extract (0.1 g) was dissolved in DMSO. When used, the solution was directly diluted in the test medium to achieve a series of decreasing concentrations, with each subsequent concentration being half of the previous one. The final concentrations in the test medium (mg/ml) were: 10; 5; 2.5; 1.25; 0.625; 0.312; 0.156; 0.078; 0.039, and 0.00195.

The antibacterial activity was evaluated against *Escherichia coli* (*E. coli*) and Staphylococcus aureus (*S. aureus*) due to their prevalence and their representation of Gram-negative and Gram-positive bacteria, respectively. The tests were conducted in triplicate, and the data were processed using Prism 10 software.

3. RESULTS

3.1. Extraction

The maceration method protects the compounds in the *P. betle* from degradation, especially potentially antibacterial compounds. The solvent used for the maceration process is ethanol because it is the solvent that can dissolve polar and nonpolar compounds, and is safe for topical applications. *Piper betle* essential oil is extracted by steam distillation method and the essential oil extraction efficiency is 0.2%.

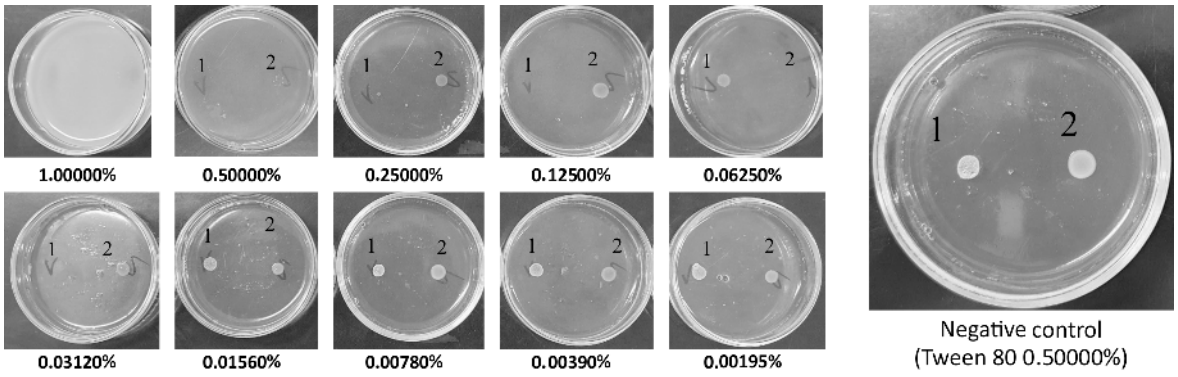

Figure 1. Results MIC of essential oil *P. betle* L.
1: *Staphylococcus aureus* ATCC 29213; 2: *Escherichia coli* ATCC 25922

MIC of essential oil *P. betle* on *Staphylococcus aureus* ATCC 29213 is 0.0312%, MIC of essential oil *P. betle* on *Escherichia coli* ATCC 25922 is 0.5 %.

3.3. Antibacterial Activity Test on extracts and products from *P. betle*

Qualification of antibacterial properties of test substances

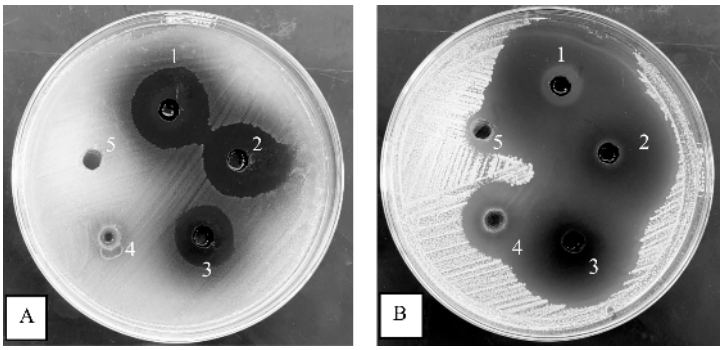

Figure 2. Qualitative of antibacterial properties of *P. betle* extracts

A: Diffusion results on Escherichia coli ATCC 25922
B: Diffusion results on Staphylococcus aureus ATCC 29213
1: 96% ethanol extract of P. betle; 2: 70% ethanol extract of P. betle; 3: 50% ethanol extract of P. betle; 4: VX herbal cream; 5: Van Xuan Huong herbal mouthwash

Table 2. Results of antibacterial circle diameter of *P. betle* extracts (mm)

	Escherichia coli	***Staphylococcus aureus***
96% ethanol extract of *P. betle*	12.02 ± 0.02	21.00 ± 1.09
70% ethanol extract of *P. betle*	12.17 ± 0.93	23.03 ± 0.53
50% ethanol extract of *P. betle*	8.93 ± 1.32	20.87 ± 0.72
VX herbal cream	-	11.08 ± 1.14
Van Xuan Huong herbal mouthwash	-	8.21 ± 0.34

Determine the MIC of the test substance

The antibacterial ability of Van Xuan Huong herbal mouthwash is weak, so MIC determination of this sample was not performed.

Table 3. MIC of *P. betle* L.

	Escherichia coli	***Staphylococcus aureus***
96% ethanol extract of *P. betle*	0.312 mg/mL	0.078 mg/mL
70% ethanol extract of *P. betle*	1.250 mg/mL	0.312 mg/mL
50% ethanol extract of *P. betle*	0.625 mg/mL	0.165 mg/mL
VX herbal cream	> 10.000 mg/mL	5.000 mg/mL

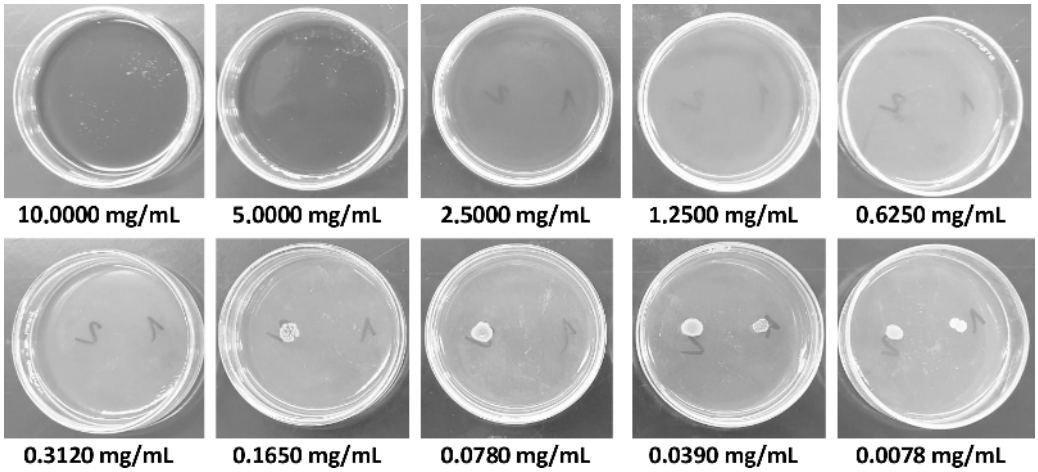

Figure 3. MIC of 96% ethanol extract of *P. betle*

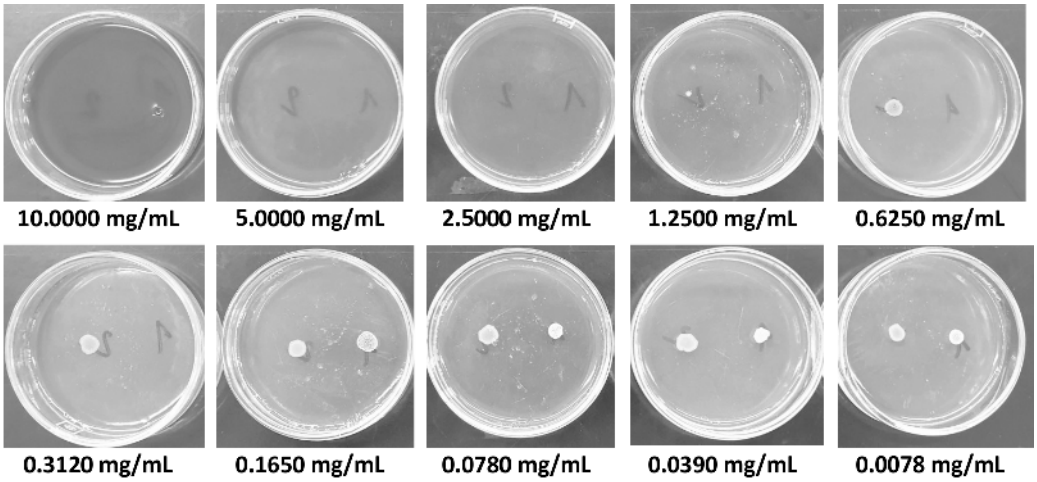

Figure 4. MIC of 70% ethanol extract of *P. betle*

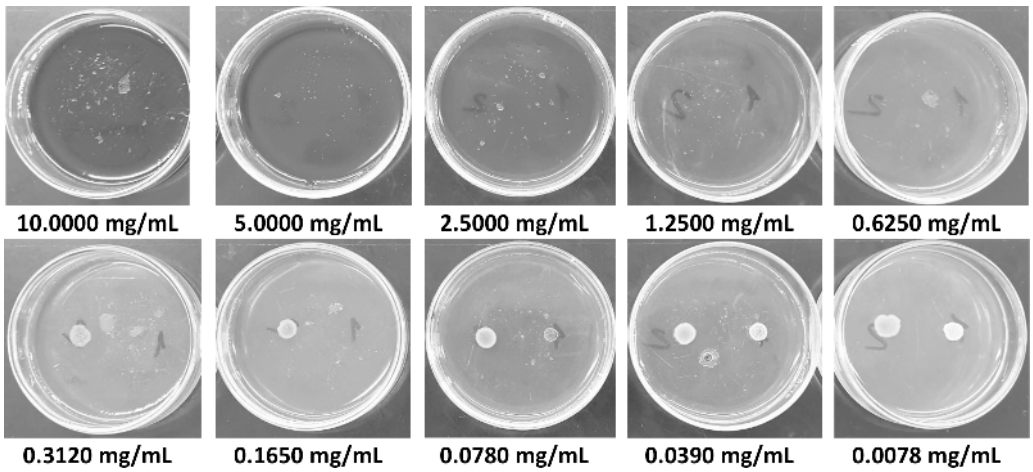

Figure 5. MIC 50% ethanol extract of *P. betle*

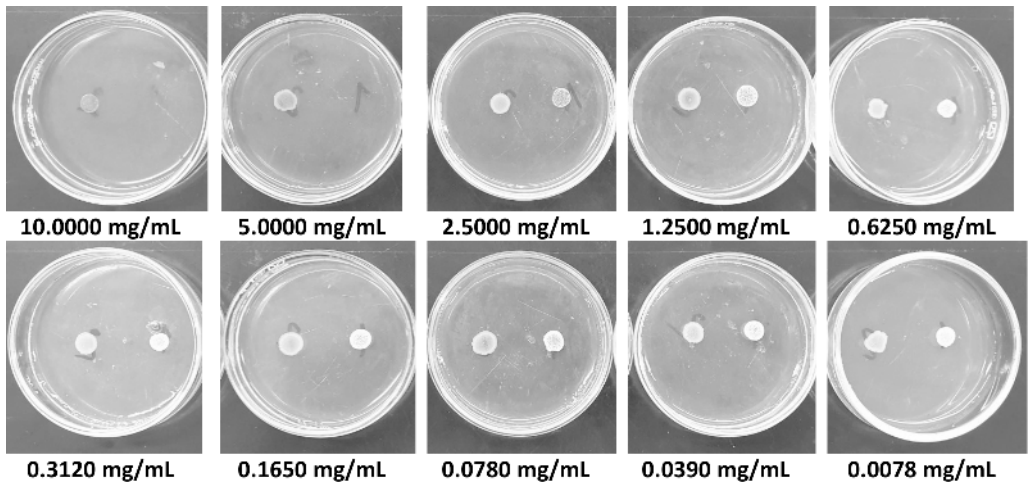

Figure 6. MIC of VX herbal cream

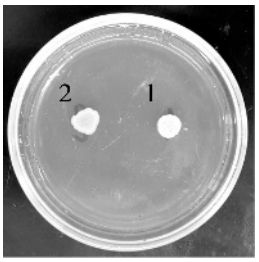

Figure 7. Negative control (DMSO)
1. Staphylococcus aureus ATCC 29213, 2. Escherichia coli ATCC 25922

4. DISCUSSION

Extracts and essential oils from *Piper betle* exhibited antibacterial effects, with the 96% ethanol extract and essential oil showing strong activity against Staphylococcus aureus and Escherichia coli. Previous studies have reported similar antibacterial properties of ethanol extracts [11, 12].

Furthermore, ethanol extracts have been found to be active against various bacterial strains, including *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Serratia marcescens*, *Methicillin-resistant Staphylococcus aureus* (MRSA), *Vancomycin-resistant Enterococcus* (VRE), *Enterobacter faecalis, Lactobacillus fermentum, Lactobacillus salivarius, Streptococcus sobrinus*, and *Streptococcus mutans* [13].

Products derived from *P. betle*, such as VX herbal cream and Van Xuan Huong herbal mouthwash, exhibited moderate to weak antibacterial activity against S. aureus but showed no effect on *E. coli*. The limited efficacy against these bacterial strains may be attributed to their intended application on different body parts.

In future studies, the research team plans to investigate the antibacterial activity of these products against a broader range of bacterial strains to further evaluate their potential applications.

5. CONCLUSION

The antibacterial results showed that the essential oil of *P. betle* had strong activity on 2 strains of *S. aureus* and *E. coli* at MIC of 0.0312% and 0.5000%. In addition, extracts and products from *P. betle* all had antibacterial activity, in which the 96% ethanol extract had the highest activity among the extracts on 2 strains of *S. aureus* and *E. coli* at MIC of 0.078 mg/mL and 0.312 mg/mL. Products from *P. betle* had antibacterial activity on *S. aureus* but not on *E. coli*. *P. betle* products may be potent against other bacterial strains, so research is planned to test antibacterial and antifungal activity on other strains.

REFERENCES

[1] Voon, W.W.Y; Ghali A.N., Rukayadi Y., Meor H.A.S. “Application of betel leaves (*Piper betle* L.) extract for preservation of homemade chili bo”. *Int Food Res J*. 21(6):2399-403, 2014.

[2] Ravindran P.N., Pillai G.S. and Nirmal B.K., *Handbook of Herbs and Spices* vol. 2, ed K V Peter, Cambridge: Woodhead, pp 53–103, 2004

[3] Madhumita M., Guha P., Nag A.. “Bio-actives of betel leaf (*Piper betle* L.): A comprehensive review on extraction, isolation, characterization, and biological activity”. *Phytother Res*. 34(10):2609-2627, 2020.

[4] Hoque M.M. et al, “Antibacterial Activity of Ethanol Extract of Betel Leaf (*Piper betle* L.) Against Some Food Borne Pathogens”, *Bangla. J. Microbiol.*, vol. 28, no. 2, pp. 58–63, Sep. 2012

[5] Mohanto S., Shreyasi D., and Surabhi M. "*Piper betel* Linn.; a brief study." *Int J Curr Med Pharm Res* 3.2, 1290-1296, 2017.

[6] Vandana D., Shalini T., “ Review study on potential activity of *Piper betle*”, *Journal of Pharmacognosy and Phytochemistry* 3(4): 93-98, 2014.

[7] Patil R.S., Harale P.M., Shivangekar K.V., Kumbhar P.P., Desai R.R.. “Phytochemical potential and in vitro antimicrobial activity of *Piper betle* Linn. leaf extracts”. *J Chem Pharm Res*. 7:1095-101, 2015.

[8] Pradhan D., Suri D.K.A., Pradhan D.D.K and Biswasroy P.. “Golden Heart of the Nature: *Piper betle* L.”, *Journal of Pharmacognosy and Phytochemistry,* Vol. 1 No. 6, 147-167, 2013.

[9] Thamaraikani and Kulandhaivel M.. “Purification of Hydroxychavicol from *Piper betle* Linn and Evaluation of Antimicrobial Activity against Some Food Poison Causing Bacteria”, *Journal Of Pure And Applied Microbiology,* Vol. 11(4), p. 1883-1889, 2017.

[10] Durani L.W., et al. “*Piper betle* L. modulates senescence-associated genes expression in replicative senescent human diploid fibroblasts”. *BioMed Res. Int.* May 17:2017.

[11] Ermawati F., “Antimicrobial activity analysis of *Piper betle* Linn leaves extract from Nganjuk, Sidoarjo and Batu against *Escherichia coli*, *Salmonella sp.*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*”, *Journal of Physics: Conference Series*, vol. 1951, no. 1, p. 012004, 2021, doi: 10.1088/1742-6596/1951/1/012004.

[12] Azahar N.I., Mokhtarand M.N., Arifin M.A., “*Piper betle*: a review on its bioactive compounds,

pharmacological properties, and extraction process", *IOP Conference Series: Materials Science and Engineering*, vol. 991, no. 1, p. 012044, 2020, doi: 10.1088/1757-899x/991/1/012044.

[13] Nayaka N.M.D.M.W., Sasadara M.M.V., Sanjaya D.A., et al. "*Piper betle* (L): Recent Review of Antibacterial and Antifungal Properties, Safety Profiles, and Commercial Applications." *Molecules*. 26(8):2321, 2021.

Hoạt tính kháng khuẩn của cây Trầu không (*Piper betle* L. Piperaceae) và các chế phẩm từ dược liệu

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TÓM TẮT

Tổng quan: Piper betle L. là một loài phổ biến ở nhiều quốc gia và được sử dụng vào việc sản xuất các sản phẩm từ tự nhiên chủ yếu là hoạt tính kháng khuẩn của loài này. Mục tiêu: Khảo sát khả năng kháng khuẩn của các cao chiết ethanol 96%, 70%, 50%, tinh dầu và các sản phẩm từ P. betle. Phương pháp nghiên cứu: phương pháp khuếch tán đĩa thạch và phương pháp pha loãng trong thạch để xác định MIC (nồng độ ức chế tối thiểu). Kết quả: Các cao chiết và tinh dầu của P. betle có tác dụng tốt trên 2 chủng vi khuẩn E.coli và S. aureus, trong đó cao chiết có MIC tốt nhất là cao ethanol 96% (0.312 mg/mL và 0.078 mg/mL tương ứng E.coli và S. aureus). MIC của tinh dầu là 0.5% và 0.0312% tương ứng E.coli và S. aureus), còn các sản phẩm từ P. betle chỉ có hoạt tính trên S. aureus nhưng có không có hiệu quả trên chủng E. coli. Kết luận: Các cao chiết, tinh dầu và các chế phẩm từ Trầu không có hoạt tính kháng khuẩn trên hai chủng vi khuẩn E.coli và S.aureus. Nghiên cứu định hướng các chủng vi khuẩn khác để đánh giá toàn diện hơn.

Từ khoá: *Piper betle, kháng khuẩn, tinh dầu*

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