Original Article

Antimicrobial Activity of Alkaloid from Roots of *Vetiveria zizanoides* (L.) Nash ex Small

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ABSTRACT

Objective: This study aimed to isolate active compounds from the root of *Vetiveria zizanoides* (L.) Nash ex Small cultivar Surat Thani and test for antimicrobial activity. **Method:** Crude methanolic extract of the root of *V. zizanoides* (L.) Nash ex Small root cultivar Surat Thani was screened for antimicrobial activity by agar diffusion method. Crude methanolic extract was purified by column chromatography and further purified by preparative thin layer chromatography (PTLC) twice by using 2% dichloromethane in ethyl acetate as the first mobile phase. The residue was developed in the second PTLC which used 2% ethyl acetate in dichloromethane as the mobile phase. Structured elucidation was performed by UV, IR, NMR and MS and was also confirmed by ^{1}H-^{1}H COSY and ^{1}H-^{13}C HMBC techniques. **Result:** Five pure compounds were isolated. Four of these pure compounds showed antifungal activity against *Trichophyton mentagrophytes*. The most active compound, vetiverin, which was an alkaloid showed minimum inhibitory concentration (MIC) of 1,628 μg/ml. The structure of vetiverin was elucidated successfully. **Conclusion:** Root of *V. zizanoides* (L.) Nash ex Small cultivar Surat Thani showed antimicrobial activity especially vetiverin. However, antimicrobial activity of *V. zizanoides* was more likely a result of the additive or synergistic effect of several compounds.

Keyword: Antimicrobial activity, *Vetiveria zizanoides*

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Introduction

Vetiver grass *(Vetiveria spp.)* has been widely used not only for water conservation and riverbank stability but also for medical purposes. The genus *Vetiveria* (Gramineae) has a total of 26 species in the world, two of which are found in Thailand. The two vetiver grass in Thailand, “Yah fake don” – *Vetiveria nemioralis* (Balan.) Holtt. Camus and “yah fake hawm” – *V.zizanioides* (L.) Roberty, are characterized by distinguished flower morphology and anatomy of their roots and leaves. The term *zizanioides* means riverside. *V. zizanoioides* (L.) Roberty can grow in swamps and can endure 45 days in flood, but can also grow on hills or mountains and resist drought for several months. Vetiver grass had rhizome buds which are used to propagate and easy to control. Germination by seeds is not common. This grass does not tend to become a noxious weed. Pruning techniques are applied to promote root and leaf growth and to retard and stunt the flowers that can cause outbreeding and mutations. Furthermore, this grass can grow vigorously on any kind of soil and local climate.

Yah fake hawm roots have volatile oil which is hot and spicy. Thai herbalists use vetiver grass to prepare medicine for various purposes including heart tonic, digestive tract cleaning, bloat relief, exhaustion relief and urine purity. Kindra and Satayanaraya claimed that vetiver oil from *Vetiveria spp.* showed antimicrobial activity.[1] Since such antimicrobial activity and related compound have not been studied, this investigation aimed to report on antimicrobial activity of crude extract, partially purified extract and pure compounds from root of *V. zizanioides* cultivar Surat Thani, and on elucidation of active chemical constituents from the cultivar.

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Materials and Methods

Plant material and preparation of extracts

V. zizanoides (L.) Nash ex Small cultivar Surat Thani were collected from the north of Thailand. These plants were identified in the herbarium of the Pharmaceutical Science Department, Chiang Mai University in which voucher specimens were deposited. All cultivars of *V. zizanoides* roots were dried at 40 - 60 ºC, powdered and passed through sieve No. 60. Two hundred grams of each were macerated with 1 litre of methanol for 1 day. They were filtered and macerated twice. The filtrate was evaporated under vacuum.

Equipments

UV spectrum was recorded by JASCO. IR spectrum was recorded by JASCO FT/IR-5000. Nuclear Magnetic Resonance (NMR) spectra were recorded by Bruker FT NMR 500 MHz. GC-MS spectrum was recorded by Shimatzu.

Antimicrobial activity screening of crude extract

Crude methanolic root extract of *V. zizanoides* (L.) Nash ex Small cultivar Surat Thani was screened for antimicrobial activity by agar diffusion method.[2,3] It was screened against *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Klebsiella pneumoniae* ATCC 67120. For antifungal activity, it was tested against *Candida albicans, Aspergillus flavus, Trichophyton mentagrophytes* and *Microsporum gypsum* by agar well diffusion method at 1 - 10% w/v concentrations.

Purification of crude extract

Crude methanolic extract was purified by column chromatography. Silicagel 60 (35 - 70 mesh) was used as the adsorbent and the column was eluted with 2% dichloromethane in ethyl acetate. A total of 12 fractions, 20 ml each, were collected. Each fraction was found to produce the same spot in thin layer chromatography. As a result, all fractions were combined and evaporated under vacuum.

This partially purified extract was further purified with preparative thin layer chromatography (PTLC) twice. Silica gel 60 GF 254 was used as adsorbent, with the thickness of 1 mm. PTLC was developed first with 2% dichloromethane in ethyl acetate. The residue from first PTLC was separated and purified further with the second PTLC which was developed with 2% ethyl acetate in dichloromethane. Five pure compounds were obtained from the second PTLC.

Determination for antimicrobial activity

Antimicrobial activity of five pure compounds isolated from root of V. zizanoides (L.) Nash ex Small cultivar Surat Thani against all pathogenic bacteria and fungi as previously described were tested by agar diffusion method. Any pure compounds with the highest antimicrobial activity was selected for further determination of minimum inhibitory concentration (MIC) by agar dilution method.[2,3] Partially purified extract was also determined for MIC for comparing with that of pure compounds.

Chemical structure elucidation

Based on microbial assay, any pure compounds with antimicrobial activity were further studied for chemical structure spectra. Structures were elucidated by UV, IR, NMR, MS, and IR techniques, and confirmed by 1H-1H Cosy and 1H-13C HMBC techniques.

Results and Discussion

Antimicrobial activity

Crude root extract of *V. zizanoides* (L.) Nash ex Small cultivar Surat Thani showed antimicrobial activity against four pathogenic bacteria and four pathogenic fungi at 1 - 10% w/v concentration (Table 1). Since crude extract showed the highest antifungal activity against *T. mentagrophytes* at a wide range of crude extract concentrations, antimicrobial activity of the five pure compounds was tested against only this pathogenic fungi. While four of five pure compounds showed antifungal activity against *T. mentagrophytes*, the second compound (vetiverin) showed the highest activity (Table 2). MIC of partially purified extract against *T. mentagrophytes* was 78 μg/ml which was much lower than that of the second pure compound (1,628 μg/ml) (Table 3).

Table 1 Antimicrobial activity screening of crude methanolic root extract of *V. zizanoides* (L.) Nash ex Small cultivar Surat Thani by agar diffusion method.

Conc. of crude extracts	Diameter of inhibition zone* (mm.) (from 2 replicates)			
	Pathogenic bacteria			
	S. aureus ATCC 25923	*E. coli* ATCC 25922	*P. aeruginosa* ATCC 27853	*K. pneumoniae* ATCC 67120
Control (MeOH)	0,0	0,0	0,0	0,0
Crude extract 1% w/v	0,0	0,0	0,0	0,0
Crude extract 5% w/v	12,12	0,0	0,0	0,0
Crude extract 10% w/v	16,17	11,11	10,10	10,10
	Pathogenic fungi			
	C. ablicans	*A. flavus*	*T. mentagrophytes*	*M. gypsum*
Control (PEG 200)	0,0	0,0	0,0	0,0
Crude extract 1% w/v	0,0	0,0	15,15	12,12
Crude extract 5% w/v	0,0	0,0	20,20	20,21
Crude extract 10% w/v	11,12	12,12	30,30	25,30

* Diameter of the cup was 8.0 mm.

Table 2 Antifungal activity of five pure compounds isolated from root of *V. zizanoides* (L.) Nash ex Small cultivar Surat Thani against *T. mentagrophytes* by agar diffusion method.

Types of pure compound	Diameter of inhibition zone* (mm.) (from 2 replicates)		
	1	2	Average
Control (PEG 200)	0	0	0
1st compound, 1.7% w/v	35	37	36
2nd compound, 1.16% w/v	40	42	41
3rd compound, 1.47% w/v	25	25	25
4th compound, 1.45% w/v	45	45	45
5th compound, 1.23% w/v	0	0	0

* Diameter of the cup was 8.0 mm.

Table 3 MIC of partially purified extract and second pure compound from root of *V. zizanoides* (L.) Nash ex Small cultivar Surat Thani against *T. mentagrophytes* by agar dilution method.

Types of test compound	MIC (μg/ml)
Partially purified extract[1]	78
Second pure compound[2]	1,628

[1] Two-fold dilutions with a conc. range of 19 – 10,230 μg/ml, from 2 replicates.

[2] The two-fold dilutions with a conc. range of 407 - 417,000 μg/ml.

Chemical structure elucidation

Among four compounds with active antimicrobial activity, the second compound (vetiverin) was found to have adequate amount to be studied for structure spectra and was further undergone for such study. According to structure spectra, the second compound (vetiverin) was an alkaloid (Figure 1). All structure study results are as follows.

UV Spectrum

The alkaloid (vetiverin) in methanol showed 2 peaks at 235 and 280 nm.

IR Spectrum

NH- stretching of imine at ν = 3450 cm^{-1}.

CH- stretching of alkyl growp at ν = 2950 cm^{-1}.

C=C stretching of R1 CH = CH R2 at ν = 1650 cm^{-1}.

^{1}H NMR Spectra

The structure of vetiverin based on 1HNMR is detailed as follow; aromatic proton, δ = 7.3 ppm 3H of methyl group coupled with 1H of olefinic group which was the mixture of trans and cis-isomer, position 1 and 2 (δ = 1.25 ppm, d, J = 6.29 Hz, δ = 4.6 ppm, qd, J = 16.48 Hz) position 2 and 3 (δ = 4.6 ppm, qd, J = 16.48 Hz, δ = 4.8 ppm, m, J = 14 Hz).

^{13}C NMR

Carbon of methyl group (position 1) was shown at δ = 29.2 ppm. Carbon of imino group (position 4) was indicated at δ = 158 ppm. Carbon of phenyl ring were shown at δ = 106 ppm. Two groups of olefinic carbon (position 2 and 3) were shown at δ = 124 and 126 ppm.

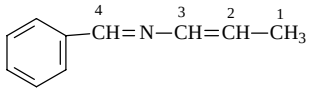
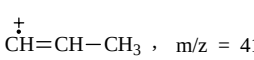
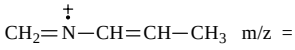


Figure 1 Structure of isolated alkaloid

Mass spectrum

This alkaloid showed fragment ion at:





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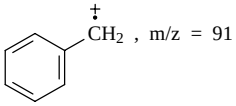

Conclusion

Root of *V. zizanoides* (L.) Nash ex Small cultivar Surat Thani showed antimicrobial activity. Four pure compounds from PTLC showed antifungal activity against *T. mentagrophytes.* Second compound, vetiverin from second PTLC showed MIC against *T. mentagrophytes* at 1,628 μg/ml and its structure was alkaloid. Since MIC of partially purified extract against *T. mentagrophytes* was only 78 μg/ml, antimicrobial activity of *V. zizanoides* was more likely a result of the additive or synergistic effect of several compounds.

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