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Pharmacognostic Identification and Antimicrobial Activity Evaluation of *Vetiveria Zizanioides* (L.) Nash. ex Small Root

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ABSTRACT

The study of Vetiveria zizanioides (L.) Nash. ex Small root from six different cultivars namely : Surat Thani, Phimai, Wiang Chai, Pang Bong, Rachaburi and Indonesia revealed a very minor difference in shape and size. The name of the cultivar was derived from the area in which it was first cultivated in Thailand and the samples we investigated came from Doi Tung Palace. Determination of antimicrobial activity revealed that crude methanolic extracts of 6 cultivars of V. zizanioides root showed antifungal activity against Trichophyton mentagrophytes at 1% W/V. Some cultivars showed antibacterial activity against Staphylococcus aureus ATCC 25923, Escherichia coli ATCC 25922 and Pseudomonas aeruginosa ATCC 278533 at 10% W/V. The most active cultivar was PangBong. We purified crude methanolic extract of cv. SuratThani by column and preparative thin layer chromatography. Five components were collected and analysed and four of them showed antifungal activity against T. mentagrophytes by using the agar diffusion method. The minimum inhibitory concentration (MIC) of the purified combined column chromatographic fractions against T. mentagrophytes, as determined by the agar dilution method, was 78 μg/mL, and MIC of one from four components was 1,628 μg/mL. In addition to the pharmacognostic identification and antimicrobial activity evaluation of these six cultivars, the crude methanolic extracts may be able to cure dermatophytic infection that is associated with some types of skin disorder.

Key words : V*etiveria zizanioides* (L.) Nash. ex Small, Pharmacognostic identification, Antimicrobial activity

INTRODUCTION

There are 11 species of Genus *Vetiveria* in the world but only 2 species are found in Thailand: *Vetiveria nemoralis* A. Camus and *V. zizanioides* (L.) Nash. ex Small which can grow in all types of soil and climate. It can conserve moisture, nitrogen and toxic substances and can prevent soil erosion. Kindra and Satayanaraya (1978) claimed that vetiver oil from *Vetiveria spp*. had antimicrobial activity. Present investigations report on pharmacognostic identification and antimicrobial activity of *V. zizanioides* root against some human pathogens.

MATERIALS AND METHODS

Plant material and preparation of extracts

V. zizanioides (L.) Nash. ex Small has 6 cultivars namely : Surat Thani, Phimai, Viang-Chai, Pang-Bong, Rachaburi and Indonesia, and they 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 are deposited. All cultivars of *V. zizanioides* roots were dried at 40–60°C, powdered and passed through a sieve no.60. These powdered drugs were examined under microscope for pharmacognostic identification, and 200 g of each were macerated with 1 litre of methanol for 1 day. They were filtered and macerated twice more. The filtrate was evaporated under vacuum and the % yield of the extracts determined.

Determination of antimicrobial activity of crude methanolic extracts.

All crude methanolic extracts were screened for antibacterial activity against *Staphyllococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 and for antifungal activity against weak pathogenic fungus-*Tricrophyton mentagrophytes.* In the latter experiment, the extracts were tested with two more potent pathogenic fungi, *Candida albicans* and *Aspergillus flavus*.

Antimicrobial activity of purified combined column chromatographic fractions of *V. zizanioides* (L.) Nash. ex Small cv. Surat Thani root.

Nine hundred grams of root of cultivars *zizanioides* (L.) Nash. ex Small cv. Surat Thani were macerated with 7 liters of methanol for 1 day and filtered. Maceration was repeated twice with methanol 2.5 liters each time. The filtrate was evaporated under vacuum. Crude methanolic extract was further purified by column chromatography. Silica gel 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 sound to produce the same spot in thin layer chromatography. As a result, all fractions were combined and evaporated under vacuum. The purified fractions were tested for antibacterial activity against S. aureus ATCC 25923, E. coli ATCC 25922, *P. aeurginosa* ATCC 27853 and for antifungal activity against *C. albicans, A. flavus, T. mentagrophytes* and *Microsporum gypsum* by agar well diffusion method. (Lenette, 1980; Washington, 1981).

Determination of minimum inhibitory concentration (MIC) of *V. zizanioides* (L.) Nash. ex Small cv. Surat Thani root against *T. mentagrophytes.*

MIC of the purified column chromatographic fractions against *T. mentagrophytes* was determined by the agar dilution method (Lenette, 1980; Washington, 1981). Column chromatographic fractions were further purified with preparative thin layer chromatography (PTLC) twice. Silica gel 60 GF 254 was used as the adsorbent, with a thickness of the adsorbent 1 mm. PTLC was developed first with 2% dichloromethane in ethyl acetate. The residue from the first PTLC was separated and purified further with the second PTLC developed with 2% ethyl acetate in dichloromethane.

RESULTS

Identification of *V. zizanioides* (L.) Nash. ex Small

Botanical identification

A densely-tufted perennial grass. Rootstock branching with spongy aromatic roots. Culms start up to over 4 ft. tall, glabrous. Leaf-sheaths compressed, blades stiffish narrowly linear, acute, 30–90 cm. long, 4–10 mm. wide, erect, rigid, firm or somewhat spongy, usually glabrous, rarely more or less hairy downwards on the face, pale green, midrib slender, lateral

nerves close, 6 or more on each side, margin spinoushy rough. Panicle oblong, up to over 30 cm. long, very narrow, rachis stout, smooth, whorls 6–10 with up to 20 rays; branches oblique to suberect. Racemes up to 5 rarely 7.5 cm. long very slender; joints about as long as the sessile spikelets, pedicels similar but shorter. Sessile spikelet, dorsally compressed awned; callus small, shorthy bearded. Imvolucral glumes equal, thinly chartaceous to membranous; lower 2-keeled, with narrow sharply inflexed margins; upper boat-shaped, 3-newed, acutely keeled. Lower floral glume hyaline, upper a hyaline linear stipe. Palea 0 or very minute; lodicules 2, minute glabrous. Stamen 3. Stigmas exerted laterally usually low down, longer than the styles. Pollen grain oblong, obtuse dorsally slightly compressed (Bailey, 1949).

Pharmacognostic identification

Investigation of internal composition of vetiver roots revealed isolated and aggregated starch grains both inside and outside of parenchyma cells. Also found were oil granules and calcium oxalate crystals scattered inside and outside the cells. Large vessels, fibre cells, stone cells and sclereid cells were also present. Single hair cells (trichomes), whose identity are slightly different among 6 cultivars, were also found. Theses cells of *Vetiveria zizanioides* (L.) Nash. ex Small have specific identity and size. The details of each strain are shown in complete report reference.

Antimicrobial activity

V. zizanioides (L.) Nash. ex Small cv. Pang-Bong gave the highest yield after methanol extract which was 16.42%. Crude methanolic extracts of *V. zizanioides* (L.) Nash. ex Small of all 6 cultivars showed antibacterial activities against S. aureus ATCC 25923, E. coli ATCC 25922 and *P. aeruginosa* ATCC 27853 at 10% W/V concentration (Table 1) and antifungal activity against *T. mentagrophytes* at as low as 1% W/V concentration (Table 2). Although Pang Bong cultivar showed the greatest overall antimicrobial activity, Surat Thani cultivar was selected for further investigation because large quantity of this sample was available throughout the study. Further test of crude extract of cv. Surat Thani against 3 more fungi revealed that at least 10% W/V concentration is required for inhibition of all fungi. Less concentration failed to inhibit growth of *C. albicans* and *A. flavus* (Table 3).

Purified combined column chromatographic fractions of *V. zizanioides* (L.) Nash. ex Small cv. Surat Thani root showed less activity only against *S. aureus* ATCC 25923 (Table 4) and MIC of these fractions against *T. mentagrophytes* was 78 μg/mL. Five components were produced as a result of the second PTLC. Four of these showed antifungal activity against *T. mentagrophytes*. The second component showed the highest activity (Table 5). MIC of the second component was 1,628 μg/mL.

Table 1. Antibacterial activity of crude methanolic extracts of 6 cultivars of *V. zizanioides* (L.) Nash. ex Small by agar diffusion method.

Descriptions	% w/v	Average inhibition zone (m.m.)		
		S. aureus **ATCC 25923**	***E. coli*** **ATCC 25922**	***P. aeruginosa*** **ATCC 27853**
1. Control (Methanol)	-	0	0	0
2. Surat Thani extract	1	0	0	0
	5	12.3	0	0
	10	13.6	0	0
3. Indonesia extract	1	10	0	12
	5	11.6	0	12
	10	12.6	11	12
4. Phimai extract	1	0	0	0
	5	11	0	11.6
	10	12.3	12.3	12
5. Wiang Chai extract	1	0	0	0
	5	10.3	11	11
	10	11.6	12	11.6
6. Pang Bong extract	1	0	0	0
	5	13	0	11.6
	10	13	12	12
7. Rachaburi extract	1	0	0	0
	5	0	0	11
	10	11	11	11.3

Table 2. Antifungal activity against T. mentagrophytes of crude methanolic extracts of 6 cultivars of V. zizanioides (L.) Nash. ex Small by agar diffusion method.

Descriptions	%w/v	Average inhibition zone (m.m.)
1. Control (PEG 200)	-	0
2. Surat Thani : Crude methanolic extract	1	40
	5	49
	10	53
3. Indonesia : Crude methanolic extract	1	40
	5	49
	10	53
4. Phimai : Crude methanolic extract	1	26
	5	36
	10	50
5. Wiang Chai : Crude methanolic extract	1	25
	5	48
	10	50
6. Pang Bong : Crude methanolic extract	1	50
	5	56
	10	62
7. Rachaburi : Crude methanolic extract	1	29
	5	48
	10	50

Table 3. Antifungal activity of crude methanolic extracts of *V. zizanioides* (L.) Nash. ex Small cv. Surat Thani root by agar well diffusion method.

Descriptions	% w/v	Average inhibition zone (m.m.)			
		C. albicans	***A. flavus***	***T. mentagrophytes***	***M. gypsum***
Control (PEG 200)	-	0	0	0	0
Crude extract	1	0	0	15	12
	5	0	0	20.8	19.3
	10	11.5	12.6	29.2	22.7

Table 4. Antibacterial activity of purified combined column chromatographic fractions of *V. zizanioides* (L.) Nash. ex Small cv. Surat Thani root by agar diffusion method.

Descriptions	Aerage inhibition zone (m.m.)		
	S. aureus **ATCC 25923**	***E. coli*** **ATCC 25922**	***P. aeruginosa*** **ATCC 27853**
Control (MeOH)	0	0	0
St[d] Ampicillin Sodium 50 μg/mL	43	20	0
0.5% Purified combined chromatographic fractions	11	0	0

Table 5. Antifungal activity against T. mentagrophytes of pure 5 components from PTLC by agar well diffusion method.

Descriptions	% w/v	Inhibition zone (m.m.)		
		Plate 1	**Plate 2**	**Average**
Control (PEG 200)	-	0,0,0	0,0,0	0,0
1st component	1.7	25,40,37	37,35,35	36.5
2nd component*	1.16	34,40,40	37,42,40	39
3rd component	1.47	25,25,25	22,22,24	23.8
4th component	1.45	45,45,46	42,48,45	45
5th component	1.23	0,0,0	0,0,0	0

* Highest antifungal activity against *T. mentagrophytes* (determined from the lowest concentration and highest inhibition zone).

DISCUSSION AND CONCLUSION

From pharmacognostic identification, there was minor difference among 6 cultivars of *V. zizanioides* (L.) Nash. ex Small, probably because they were collected from the same area.

We did not investigate antibacterial activity of Surat Thani cultivar further because purified combined chromatographic fractions showed less activity (Table 4). It is likely that antibacterial activity of the crude methanolic extract was the result of the combined or synergistic effect of several components.

MIC of the purified combined column chromatographic fractions was found to be 78 μg/mL. and MIC of the second component (from four components) was found to be 1,628 μg/mL. MIC of purified combined column chromatographic fractions was lower than that of the second component because it was the result of addition or synergistic effect of many compounds in purified combined column chromatographic fractions.

The active constituents need to be isolated and elucidated. The results from this investigation provide preliminary data for future development of this plant product for antifungal and antibacterial treatment.

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