

Antimicrobial Activity of *Gelonium multiflorum*, A. Juss, Leaves

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ABSTRACT

Phytochemical analysis of the leaves of Gelonium multiflorum from Euphorbiaceae family resulted in the isolation and yield product of many compounds. Various solvent (hexane, dichloromethane and methanol) extracts of leaves were tested against 3 human pathogenic bacteria and 2 opportunistic fungi by the agar-well diffusion method. As a result of the low solubility of substances, therefore, the agar dilution method was employed instead. Solvent extract from dichloromethane expressed antifungal activity against Trichophyton mentagrophytes and Trichophyton rubrum with the minimum inhibitory concentration (MIC) at 3200 µg/ml. By column chromatography technique, purification of this extract yielded in three fractions, two of these had antifungal property and contained many constituents.

Key words: *Gelonium multiflorum* A. Juss., Antimicrobial activity

INTRODUCTION

Gelonium multiflorum A. Juss is a plant of the Euphorbiaceae family, native to the tropical forests of eastern Asia. It was used in traditional medicine to treat cancer, ringworm and venereal diseases (Phooutpong, 1987 ; Sunthorntummo, 1987). Some phytochemical components can be applied in many arrays of medicine, *e.g.* anti-cancer (Rosenblum et al., 1992), anti-parasitic (Surolia and Misquith, 1996) and anti-viral (Foa-Tomasi et al., 1982) effects by the plant proteins such as gelonin, a ribosome-inactivating protein (RIP) (Stirpe et al., 1980). MAP 30 and GAP 31 from *G. multiflorum* had anti-herpes simplex activity (Bourinbaiar et al., 1996). Although there are many studies of phytochemical molecules, there is little information about antifungal and antibacterial activities of *G. multiflorum*.

This study examined antibacterial and antifungal activities of *G. multiflorum*. At the beginning, the leaves of *G. multiflorum* were extracted by polarity order with hexane, dichloromethane and methanol. The selected fractions from previous step were subsequently isolated by column chromatography. Agar diffusion method

was performed to examine antimicrobial activity of this plant. Finally, thin layer chromatography was performed to demonstrate the components in the selected fractions.

MATERIALS AND METHODS

Plant material and preparation of extracts

Gelonium multiflorum A. Juss specimens were collected from Chom Tong forest, Chiang Mai in 2002. A voucher specimen (Maxwell 007285) is kept in the Chiang Mai University Pharmacy Herbarium. The leaves were dried at 40°C and then powdered. Fifty grams of powder were macerated with hexane of 350 ml. Each replicate was macerated for a day, filtered and repeated twice. The filtrate was evaporated in a vacuum and the percentage of yield was 3.93. The powder residue was macerated further with dichloromethane of 250 ml and methanol of 250 ml, respectively, using the same method as hexane extract. The percent harvest from dichloromethane extract was 3.33 and methanol extract was 5.44.

Determination of antimicrobial activity of hexane, dichloromethane and methanol crude extract by agar diffusion and agar dilution method

Hexane, dichloromethane and methanol crude extracts were screened for the antimicrobial activity against *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Candida albicans* ATCC 90028 and *Aspergillus flavus* at 0.1%, 0.5% and 1% concentrations by the agar diffusion method. Three crude extracts were further examined for the antifungal activity by the agar dilution method against *Trichophyton mentagrophytes*, *Trichophyton rubrum* and *Microsporium gypsum* (Lenette, 1980 ; Washington, 1981).

Purification of dichloromethane crude extract

Dichloromethane crude extract was purified by column chromatography. Silica gel 60 (35-70 mesh) was used as an adsorbent and the column was eluted with 3% of methanol in dichloromethane of 200 ml and further 10% methanol in dichloromethane of 200 ml. A total of 15 fractions, 20 ml each, were collected.

The total fractions were separated into 2 parts by a thin layer chromatogram. First part was fractions 2 to 3 and another was fractions 6 to 8.

Determination of antifungal activity of purified column chromatographic fractions by the agar diffusion method

The agar diffusion method was set up to determine the antifungal activity against *T. mentagrophytes* of purified fractions 2-3 and fractions 6-8 (Lenette, 1980; Washington 1981).

Determination of the components in purified column chromatographic fractions by thin layer chromatography

Thin layer chromatography determination of fractions 6-8 with 2 developing solvents was done. Developing solvent 1 was 1:1 mixture of dichloromethane and

methanol. Developing solvent 2 was 1:1 mixture of ethyl acetate and dichloromethane.

From thin layer chromatograms, it demonstrated that fractions 6 and 7 consisted of three spots and fraction 8 consisted of 6 spots.

RESULTS

As demonstrated in Tables 1-5, three crude extracts did not reveal the activity against the pathogenic bacteria (*S. aureus* ATCC 25923, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853) and fungi (*C. albicans* ATCC 90028, *A. flavus*) by the agar diffusion method. The crude extract of dichloromethane showed the antifungal activity against *T. mentagrophytes* and *T. rubrum* except for *M. gypsum* by the agar dilution method and it gave the MIC at 3200 µg/ml (Tables 6-8). However, crude extracts of hexane and methanol did not show antifungal activity against three dermatophytic fungi. From Table 9, it suggests that MIC of standard cotrimazole against *T. mentagrophytes*, *T. rubrum* and *M. gypsum* was 1.25 µg/ml, 10 µg/ml and 20 µg/ml, respectively. The purified fractions from column chromatography exhibited the narrow inhibition zone against *T. mentagrophytes* by the agar diffusion method. These fractions consisted of many spots as examined by thin layer chromatograms (Table 10).

Table 1. The anti bacterial activity of hexane crude extract by agar diffusion method.

Descriptions	Inhibition zone (mm.)		
	<i>S. aureus</i> ATCC 25923	<i>E. coli</i> ATCC 25922	<i>P. aeruginosa</i> ATCC 27853
Hexane extract:			
Standard 10.5 µg ^a	39, 39	14, 14	0, 0
	37, 37	12, 12	0, 0
Sample 0.1%	0, 0	0, 0	0, 0
	0, 0	0, 0	0, 0
Standard 10.5 µg	39, 39	14, 14	0, 0
	41, 42	12, 12	0, 0
Sample 0.5%	0, 0	0, 0	0, 0
	0, 0	0, 0	0, 0
Standard 10.5 µg	39, 37	12, 12	0, 0
	36, 37	14, 14	0, 0
Sample 1%	0, 0	0, 0	0, 0
	0, 0	0, 0	0, 0
Control ^b	0, 0, 0, 0	0, 0, 0, 0	0, 0, 0, 0

^a Ampicillin sodium 50 µg/ml, 0.21 ml. (concentration 10.5 µg/well) was used as standard. Diameter of agar well was 8.0 mm.

^b Control was PEG 200 of 5 ml and DMSO of 0.2 ml.

Table 2. The antibacterial activity of dichloromethane crude extract by agar diffusion method.

Descriptions	Inhibition zone (mm.)		
	<i>S. aureus</i> ATCC 25923	<i>E. coli</i> ATCC 25922	<i>P. aeruginosa</i> ATCC 27853
Dichloromethane extract: Standard 10.5 µg ^a	37, 37	12, 13	0, 0
	39, 37	14, 14	0, 0
Sample 0.1%	0, 0	0, 0	0, 0
	0, 0	0, 0	0, 0
Standard 10.5 µg	37, 39	12, 13	0, 0
	38, 37	14, 14	0, 0
Sample 0.5%	0, 0	0, 0	0, 0
	0, 0	0, 0	0, 0
Standard 10.5 µg	40, 40	12, 13	0, 0
	38, 37	14, 14	0, 0
Sample 1%	0, 0	0, 0	0, 0
	0, 0	0, 0	0, 0
Control ^b	0, 0, 0, 0	0, 0, 0, 0	0, 0, 0, 0

^a Ampicillin sodium 50 µg/ml, 0.21 ml. (concentration 10.5 µg/well) was used as standard. Diameter of agar well was 8.0 mm.

^b Control was PEG 200 of 5 ml and DMSO of 0.2 ml.

Table 3. The antibacterial activity of methanol crude extract by agar diffusion method.

Descriptions	Inhibition zone (mm.)		
	<i>S. aureus</i> ATCC 25923	<i>E. coli</i> ATCC 25922	<i>P. aeruginosa</i> ATCC 27853
Dichloromethane extract: Standard 10.5 µg ^a	39, 40	12, 12	0, 0
	37, 36	12, 13	0, 0
Sample 0.1%	0, 0	0, 0	0, 0
	0, 0	0, 0	0, 0
Standard 10.5 µg	39, 41	12, 13	0, 0
	37, 37	12, 13	0, 0
Sample 0.5%	0, 0	0, 0	0, 0
	0, 0	0, 0	0, 0
Standard 10.5 µg	39, 40	12, 13	0, 0
	38, 39	12, 12	0, 0

Table 3. The antibacterial activity of methanol crude extract by agar diffusion method. (Continue)

Descriptions	Inhibition zone (mm.)		
	<i>S. aureus</i> ATCC 25923	<i>E. coli</i> ATCC 25922	<i>P. aeruginosa</i> ATCC 27853
Sample 1%	0, 0	0, 0	0, 0
	0, 0	0, 0	0, 0
Control ^b	0, 0, 0, 0	0, 0, 0, 0	0, 0, 0, 0

^a Ampicillin sodium 50 µg/ml, 0.21 ml. (concentration 10.5 µg/well) was used as standard. Diameter of agar well was 8.0 mm.

^b Control was PEG 200 of 5 ml and DMSO of 0.2 ml.

Table 4. The antifungal activity against *C. albicans* ATCC 90028 of three crude extracts of *G. multiflorum* leaves by agar diffusion method.

Descriptions	Inhibition zone (mm.)	
	Plate I	Plate II
Standard ^c	30, 30, 31, 31	29,30,34,30
Hexane:		
0.1% extract	0, 0, 0, 0	0, 0, 0, 0
0.5% extract	0, 0, 0, 0	0, 0, 0, 0
1% extract	0, 0, 0, 0	0, 0, 0, 0
Dichloromethane:		
0.1% extract	0, 0, 0, 0	0, 0, 0, 0
0.5% extract	0, 0, 0, 0	0, 0, 0, 0
1% extract	0, 0, 0, 0	0, 0, 0, 0
Methanol:		
0.1% extract	0, 0, 0, 0	0, 0, 0, 0
0.5% extract	0, 0, 0, 0	0, 0, 0, 0
1% extract	0, 0, 0, 0	0, 0, 0, 0
Control ^d	0, 0, 0, 0	0, 0, 0, 0

^c Cotrimazole 0.001%, 0.21 ml (concentration 2.1 µg/well) was used as standard. Diameter of agar well was 8.0 mm.

^d Control was PEG 200 of 5 ml.

Table 5. The antifungal activity against *A. flavus* of three crude extracts of *G. multiflorum* leaves by agar diffusion method.

Descriptions	Inhibition zone (mm.)	
	Plate I	Plate II
Standard ^c	30, 30, 28, 28	30,30,33,30
Hexane:		
0.1% extract	0, 0, 0, 0	0, 0, 0, 0
0.5% extract	0, 0, 0, 0	0, 0, 0, 0
1% extract	0, 0, 0, 0	0, 0, 0, 0

Table 5. The antifungal activity against *A. flavus* of three crude extracts of *G. multiflorum* leaves by agar diffusion method. (Continue)

Descriptions	Inhibition zone (mm.)	
	Plate I	Plate II
Standard ^c	30, 30, 28, 28	30,30,33,30
Dichloromethane:		
0.1% extract	0, 0, 0, 0	0, 0, 0, 0
0.5% extract	0, 0, 0, 0	0, 0, 0, 0
1% extract	0, 0, 0, 0	0, 0, 0, 0
Methanol extract:		
0.1% extract	0, 0, 0, 0	0, 0, 0, 0
0.5% extract	0, 0, 0, 0	0, 0, 0, 0
1% extract	0, 0, 0, 0	0, 0, 0, 0
Control ^d	0, 0, 0, 0	0, 0, 0, 0

^c Cotrimazole 0.001%, 0.21 ml (concentration 2.1 µg/well) was used as standard.
Diameter of agar well was 8.0 mm.

^d Control was PEG 200 of 5 ml.

Table 6. Determination of MIC of hexane crude extract against dermatophytic fungi.

Concentration of hexane crude extract (µg/ml)	Growth of dermatophytic fungi		
	<i>T. mentagrophytes</i>	<i>T. rubrum</i>	<i>M. gypsum</i>
Positive control (before)	+	+	+
Solvent control (before)	+	+	+
3200	+	+	+
1600	+	+	+
800	+	+	+
400	+	+	+
200	+	+	+
100	+	+	+
20	+	+	+
25	+	+	+
Positive control (after)	+	+	+
Solvent control (after)	+	+	+

+ indicates the growth of fungi.

Table 7. Determination of MIC of dichloromethane crude extract against dermatophytic fungi.

Concentration of dichloro- methane crude extract (µg/ml)	Growth of dermatophytic fungi		
	<i>T. mentagrophytes</i>	<i>T. rubrum</i>	<i>M. gypsum</i>
Positive control (before)	+	+	+
Solvent control (before)	+	+	+
3200	-	-	+
1600	+	+	+
800	+	+	+
400	+	+	+
200	+	+	+
100	+	+	+
20	+	+	+
25	+	+	+
Positive control (after)	+	+	+
Solvent control (after)	+	+	+

+ indicates the growth of fungi.

- indicates no growth of fungi.

Table 8. Determination of MIC of methanol crude extract against dermatophytic fungi.

Concentration of methanol crude extract (µg/ml)	Growth of dermatophytic fungi		
	<i>T. mentagrophytes</i>	<i>T. rubrum</i>	<i>M. gypsum</i>
Positive control (before)	+	+	+
Solvent control (before)	+	+	+
3200	+	+	+
1600	+	+	+
800	+	+	+
400	+	+	+
200	+	+	+
100	+	+	+
20	+	+	+
25	+	+	+
Positive control (after)	+	+	+
Solvent control (after)	+	+	+

+ indicates the growth of fungi.

Table 9. Determination of MIC of standard cotrimazole against dermatophytic fungi.

Concentration of standard cotrimazole ($\mu\text{g/ml}$)	Growth of dermatophytic fungi		
	<i>T. mentagrophytes</i>	<i>T. rubrum</i>	<i>M. gypsum</i>
Positive control (before)	+	+	+
Solvent control (before)	+	+	+
20	-	-	-
10	-	-	+
5	-	+	+
2.5	-	+	+
1.25	-	+	+
Positive control (after)	+	+	+
Solvent control (after)	+	+	+

+ indicates the growth of fungi.

- indicates no growth of fungi.

Table 10. The antifungal activity against *T. mentagrophytes* of purified column chromatographic fractions by agar diffusion method.

Descriptions	Inhibition zone (mm.)		
	Plate I	Plate II	Plate III
Fractions 2 and 3	0, 0, 0, 0	0, 0, 0, 0	0, 0, 0, 0
Fractions 6 and 7	20, 21, 24, 20	20, 20, 18, 20	22, 23, 20, 20
Fraction 8	16, 15, 17, 16	20, 20, 17, 17	21, 21, 20, 20
Control ^e	0, 0, 0, 0	0, 0, 0, 0	0, 0, 0, 0

^e was the absolute ethanol.

Diameter of agar well was 8.0 mm.

DISCUSSION AND CONCLUSION

Three crude extracts in these experiments revealed no antimicrobial activity against pathogenic bacteria and fungi, tested by the agar diffusion technique, due to the solubility problem. This problem can be solved by using the agar dilution method instead.

From this preliminary study, it was concluded that crude extract of dichloromethane from *G. multiflorum* leaves showed the low antifungal activity as reflected by high MIC and narrow inhibition zone and this activity was originated from the action of many components. Although we cannot use this extract as antifungal treatment in AIDS patients as we expected in the purpose of the study, the weak antifungal activity of *G. multiflorum* may explain and support the usage of the purpose of the study this crude preparation in traditional medicine.

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