Antimicrobial Activity of *Cassia alata* Linn. Leaves (Caesalpinioideae)

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ABSTRACT

35% ethanolic extract from Cassia alata Linn. leaves showed antifungal activity against Trigrophyton mentagrophyte, Trigrophyton rubum and Microsporum gypsum. The activity was determined by agar diffusion method. Further purification of 35% ethanolic extract was done by dissolving in methanol and the undissolved methanolic material could dissolve in water. This methanolic extract showed antifungal activity against T.mentagrophyte while aqueous extract showed no activity. Isolation and purification of methanolic extract by column chromatography and preparative layer chromatography showed no principal component but consisted of many minor components. These minor components showed antifungal activity against T.mentagrophyte Minimum inhibitory concentration (MIC) of methanolic extract against T.mentagrophyte as determined by agar dilution method was found to be 15 mg/ml. It was also found that 35% ethanolic extract showed less activity against Staphyllococcus aureus ATCC 25923 at 10% W/V concentration.

The result from this investigation suggest that ethanolic extract from Cassia alata Linn. leaves can be used for the treatment of ringworm and it is desirable to introduce people to use this plant as in the traditional use.

Key words: *Cassia alata* Linn. Leaves, Antimicrobial activity

INTRODUCTION

Cassia alata Linn. is a shrub in family caesalpinioideae. Its traditional use of the leaves is for the treatment of ringworm, diuretics and laxative. The tree is also used as an anthelmintic for earthworm (Pongbunrod, 1984). Active constituents of this plant are anthraquinones and flavonoids (Ultasit et al., 1987).

Palanochamy and Nagarajan (1990) found that 85% ethanolic extract of *C.alata* could treat dermatophyte such as *T.mentagrophyte, T.rubum* and *M.gypsum* at 20% W/V concentration. Crockette et al., (1992) found that water extract of *C.alata* could inhibit the growth of *Escherichia coli* and *Candida albicans*. MIC and minimum bactericidal concentration (MBC) against *E.coli* were 1.6 mg/ml and 60 mg/ml., respectively. MIC and minimum fungicidal concentration (MFC) against C.albicans were 0.39 mg/ml and 60 mg/ml., respectively. The objective

of this investigation was to extract and isolate active constituents in the leaves of *C.alata*, Linn.

MATERIALS AND METHODS

Plant material

C.alata Linn. specimen was collected from Amphur Muang, Chiang Mai Province, in 1995. A voucher specimen No.009569 was kept in Chiang Mai University Pharmacy Herbarium.

Extraction

Extraction by polarity

Extracted *C.alata* Linn. leaves by hexane for 1 day, filtered, evaporated filtrate and macerated again twice. Dichloromethane and methanol were macerated in the same manner after hexane extraction and determined antifungal activity by agar diffusion method. It was observed that there was fungal growth in hexane and dichloromethane extracts but methanolic extract could inhibit *T.mentagrophyte*, so fungal contamination of these dried leaves was determined. It was found that they were contaminated with *Aspergillus niger* and *Rhizopus spp*., so 35% ethanol was used as the solvent for extracting the leaves and fungal contamination disappeared.

Extraction with 35% ethanol

35% ethanol was used to macerate *C. alata* Linn. dried leaves. They were macerated for 1 day, filtered, evaporated the filtrate and macerated again twice. Antifungal and antibacterial activity of ethanolic extract was determined against *T.mentagrophyte, T.rubrum, M.gypsum, E. coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *S.aureus* ATCC 25923 for 3 lots. It was found that 35% ethanolic extract could inhibit fungal growth at 50% W/V and could inhibit *S.aureus* at 10% W/V concentration.

Partial purification

Ethanolic extract was dissolved with methanol and undissolved material was dissolved with water. For simplicity *T.mentagrophyte* was used as the fungal micro-organism test. It was found that 5% W/V of methanolic extract could inhibit fungal growth but aqueous extract did not show antifungal activity. MIC of methanolic extract against *T.mentagrophyte* was 15 mg/ml. Methanolic extract was purified by column and preparative layer chromatography. Six fractions were isolated and each fraction showed antifungal activity but they were of small amount and their Rf-values were near each other, so isolation of pure compounds from these fractions was not done.

RESULTS

Ethanolic extracts showed antifungal activity by using agar diffusion method (Lenette, 1980; Washington, 1981). (Table 1).

Table 1. Antifungal activity of ethanolic extract.

Descriptions	Diameter of clear zone (mm.)		
	T.mentagrophyte	***T.rubum***	***M.gypsum***
1. Distilled water	0	0	0
2. 35% ethanolic solvent	0	0	0
3. Ethanolic extract No.1	31	31	31
4. Ethanolic extract No.2	31	35	31
5. Ethanolic extract No.3	35	40	35

Ethanolic extract showed antibacterial activity against only *S.aureus* ATCC 25923. (Lenette, 1980; Washington, 1981). (Table 2).

Table 2. Antibacterial activity of ethanolic extract.

Descriptions	Diameter of clear zone (mm.)								
	S.aureus ATCC 25923			***E.coli*** ATCC 25922			***P.aeruginosa*** ATCC 27853		
	1	2	Average	1	2	Average	1	2	Average
Distilled water	0	0	0	0	0	0	0	0	0
1% Ethanolic extract	0	0	0	0	0	0	0	0	0
5% Ethanolic extract	0	0	0	0	0	0	0	0	0
10% Ethanolic extract	20	20	20	0	0	0	0	0	0

Methanolic extract showed antifungal activity at 5% W/V and aqueous extract showed no activity (Lenette, 1980; Washington, 1981). (Table 3).

Table 3. Antifungal activity of methanolic and aqueous extract against *T.mentagrophyte.*

Descriptions	Diameter of clear zone (mm.)		
	1	**2**	**Average**
1. Distilled water	0	0	0
2. PEG 200	0	0	0
3. 5% Aqueous extract	0	0	0
4. 10% Aqueous extract	0	0	0
5. 5% Methanolic extract	30	30	30
6. 10% Methanolic extract	40	35	37

MIC of methanolic extract against *T.mentagrophyte* by agar dilution method was 15 mg/ml (Lenette 1980; Washington 1981). (Table 4).

Table 4. MIC of methanolic extract against *T.mentagrophyte* by agar dilution method.

Concentration of methanolic extract (mg/ml)	Results	
	1	2
Positive control before experiment	+	+
Solvent control before experiment	+	+
30	-	-
15	-	-
7.5	+	+
3.7	+	+
1.8	+	+
Solvent control after experiment	+	+
Positive control after experiment	+	+

Six fractions were obtained from purification steps (column and preparative layer chromatography) and they showed antifungal activity by agar diffusion method against *T.mentagrophyte* (Lenette, 1980; Washington, 1981). (Table 5).

Table 5. Antifungal activity of purified fractions from methanolic extract by agar diffusion method against *T.mentagrophyte.*

Descriptions	Diameter of clear zone (mm.)		
	1	2	Average
PEG 200	0	0	0
Fraction 1.1	40	40	40
Fraction 1.2	30	30	30
Fraction 2.1	30	30	30
Fraction 3-5	20	20	20

DISCUSSION AND CONCLUSION

C.alata Linn. dried leaves were contaminated with *A.niger* and *R.spp*. Fungal contaminations were treated by extracting these dried leaves with 35% ethanol. Methanol was used to dissolve dried 35% ethanolic extract and purified by column and preparative layer chromatography. Six fractions were obtained and they showed antifungal activity against *T.mentagrophyte* but they were of small amounts and their Rf-values were near each other. It could cost a big budget to isolate them, therefore, the isolation of pure components was not carried out. From this investigation, 35% ethanolic extract can be used for treating ringworm.

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