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Original Article

Antimicrobial activity of *Desmos chinensis* leaf and *Maclura cochinchinensis* wood extracts

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Abstract

The antimicrobial activity of crude extracts of *Desmos chinensis* leaves and *Maclura cochinchinensis* wood were tested against human pathogens, including bacteria, yeast and dermatophytic fungi, using the agar disc diffusion and agar dilution methods. The crude chloroform extract of *D. chinensis* was active against *Staphylococcus aureus, Staphylococcus epidermidis* and *Bacillus subtilis* with MIC values ranging from 500-1000 µg/ml. The crude hexane and chloroform extracts of *D. chinensis* exhibited the strongest activity against all dermatophytes tested (*Trichophyton rubrum*, *Trichophyton mentagrophytes* and *Microsporum gypseum*), with MIC values ranging from 31.25-62.50 µg/ml. However, methanol and water extracts of *D. chinensis* showed no activities against all of the microorganisms tested.

The crude chloroform extract of *M. cochinchinensis* exhibited strong antibacterial activity against *S. aureus*, *S. epidermidis* and *B. subtilis* with MIC value ranging from 125-250 µg/ml. and showed inhibition against all the dermatophytes, with MIC of 250 µg/ml. Hexane extract of *M. cochinchinensis* was not active against all microorganisms. None of the crude extracts were active against *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Shigella sonnei* and *Candida albicans*. These findings indicated that *D. chinensis* and *M. cochinchinensis* had antibacterial activity against Gram-positive bacteia and anti-dermatophytic activity.

Keywords: *Desmos chinensis*, *Maclura cochinchinensis*, Antibacterial activity, Antifungal activity, Antimicrobial activity

1. Introduction

Desmos chinensis Lour. (Annonaceae) has been used as a folk medicine for treatment of malaria (Kakeya *et al*., 1993), parturition and vertigo (Rahman *et al*., 2003). In Thailand it is used traditionally to treat pyretic and dysentery (Bunyapraphatsara *et al*., 2000). The leaf chloroform extract of this plant contains flavonoids such as flavones, chalcone, flavanones, 2'4'dihydroxy-3'-(2,6-dihydroxybenzyl)-6'-methoxychalcone, uvaretin and isouvaretin (Rahman *et al*., 2003). This plant exhibited antibacterial activity (Qais *et al*., 1996), cytotoxicity (Nakanishi *et al*., 1965) and tyrosine kinase enzyme inhibitory properties (Kakeya *et al*., 1993).

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Maclura cochinchinensis Corner. (Moraceae) has been reported to possess antipeptic ulcers (Manandhar, 1995), antipyretic (Mokkhasmit *et al*., 1971; Lin *et al*, 1999), antihepatitis (Lin *et al*., 1990), antihistamine and antispasmodic (Mokkhasmit *et al*., 1971), antiviral (Bunyapraphatsara *et al*., 2000; Yoosook *et al*., 2000), antifungal (Shirata and Takahashi, 1982), antimycobacterial (Chang *et al*., 1977) and anti-inflammatory activities (Lin *et al*., 1999). The wood extract of this plant contain campesterol steroid, resveratrol stilbene, stilbene, 2-3'-4-5'-tetrahydroxy:stilbene, stigmasterol steroid, phenols sterol and/or triterpene (Kanjanapee and Natori., 1966), morin (Bunyapraphatsara *et al*., 2000), prenylated benzophenones and prenylated xanthones (Hou *et al*., 2001).

The present study aimed to investigate the crude extracts of *D. chinensis* and *M.cochinchinensis* for *in vitro* antimicrobial activity.

2. Material and Methods

2.1 Plant materials

Fresh leaves of *Desmos chinensis* (Figure 1) were collected from Hat Yai, Songkhla Province, Thailand. The dried woods of *Maclura cochinchinensis* (Figure 2) were purchased from a Thai herbal drugstore in Hat Yai, Songkhla Province, Thailand. The plants were identified at the Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University. A voucher specimen No. SKP 011-04-03-01 for *D. chinensis* and No. SKP 117-13-03-01 for *M. cochinchinensis* were deposited at the Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Songkhla, Thailand.

2.2 Preparation of plant extracts

Plant extracts were prepared according to the procedure of Subhadhirasakul and Pechpongs (2005). The dried leaves of *D. chinensis* and wood of *M. cochinchinensis* (1 kg each) were ground and macerated with n-hexane (3 l) at room temperature for seven days. The macerate of each plant was filtered through Whatman No.1 filter paper and concentrated with a rotary vacuum evaporator to dryness at reduced pressure. The marc was re-macerated with n-hexane (3 l) three times, filtered and concentrated. The dried masses were combined to give the n-hexane extract. The marc was dried in a hood and then macerated with chloroform and methanol using the same procedure as described above to give the chloroform and methanol extract, respectively. For water extraction, dried final marc was boiled for 30 minutes in distilled water (3 l) and the macerate was removed the powder from this aqueous extract by filtration through cotton cloth and Whatman No.1 filter paper, respectively. The filtrate was gently heated until the volume was reduced to about 500 ml and then freeze-dried. Each crude extract was weighed, kept in an air tight bottle and stored in the refrigerator at 4°C until further use.

Figure 1. *Desmos chinensis* Lour.

Figure 2. *Maclura cochinchinensis* Corner.

2.3 Antimicrobial activity assay

2.3.1 Microorganisms

The tested organisms used for biological evaluation consisted of bacteria and fungi. Four Gram-positive bacteria: *Staphylococcus aureus* ATCC 25923*, Staphylococcus epidermidis* TISTR 518*, Bacillus subtilis* and *Enterococcus faecalis* TISTR 459, and four Gram-negative bacteria: *Escherichia coli* ATCC 25922*, Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhi*, and *Shigella sonnei* were provided by the Department of Pathology, Faculty of Medicine, Prince of Songkhla University, or in some cases purchased from the Thailand Institute of Scientific and Technological Research (TISTR). Fungi including one yeast: *Candida albicans* TISTR 5779 and five dermatophytes: two *Trichophyton rubrum*: two *Trichophyton mentagrophytes and* one *Microsporum gypseum* were provided by the Department of Microbiology, Faculty of Science, Prince of Songkhla University, and the Institute of Dermatology, Department of Medical Services, Ministry of Public Health, Bangkok Thailand.

2. 3.2 Media

Media used in this study were Mueller Hinton agar (MHA), Mueller Hinton broth (MHB), (Difco, Becton Dickinson and Company, Spark USA) for bacteria, and Sabouraud Dextrose agar (SDA), Sabouraud Dextrose broth (SDB), (Difco, Becton Dickinson and Company, Spark, USA) for the fungi.

2.3.3 Reference antimicrobial drugs

Tetracycline (30 µg/disc) and Norfloxacin (10 µg/disc) (Oxoid, Oxoid Limited, England), Tetracycline (Fluka, Fluka Chemie GmbH, Switzerland), Amphotericin B (Sigma, Sigma-Aldrich Chemie GmbH, Germany) and Ketoconazole (Sigma, Sigma-Aldrich Chemie GmbH, Germany) were used as control antimicrobial drugs.

2.3.4 Preliminary susceptibility test

The disc diffusion method (Lorian, 1996) was used to

screen for antimicrobial activity of extracts of *D. chinensis* and *M. cochinchinensis*. The bacterial were grown on Mueller Hinton agar (MHA) overnight at 37°C for 24 h, the yeast was grown on Sabouraud dextrose agar (SDA) at 37°C for 48 h, and dermatophytic fungi were grown on SDA at 30° C for 10 days. Sterile discs (6 mm) were impregnated with 10 µl of reconstituted crude extracts (concentration 200 mg/ml in dimethyl sulfoxide, DMSO) and placed on the surface of bacterium inoculated MHA for bacteria and fungus inoculated SDA for fungi. Control discs contained 10 µl DMSO and water. Standard antimicrobial drugs composed of tetracycline (30 µg/disc) for the Gram-positive bacteria, norfloxacin (10 µg/disc) for the Gram-negative bacteria, amphotericin B (25 µg/disc) for the yeast and ketoconazole (25 µg/disc) for dermatophytic fungi. Each extract was tested in triplicate. Tested agar plates were incubated at 37°C for 24 h and 48 h for bacteria and yeast, respectively. Dermatophytes were incubated at 30°C for 4 days. Inhibition zones were recorded as the diameter of growth-free zones, included the diameter of the disc, in mm at the end of the incubation period.

2.3.5 Determination of minimum inhibitory concentration (MIC)

The agar microdilution method (Lorian, 1996 and CLSI, 2002) was used to determine the minimum inhibitory concentration (MIC) of the crude extracts of plants which produced an inhibition zone in the preliminary screening. Tetracycline and ketoconazole were used as reference standards for bacteria and dermatophytes, respectively. The bacterial were grown on MHA overnight at 37°C for 24 h, and dermatophytic fungi were grown on SDA at 30°C for 10 days. The inocula containing of 10^3 -10^4 cfu of each microorganism were spotted on agar supplemented with an extract or antimicrobial agent at concentrations ranging from 3.9-2000 µg/ml for crude extracts, 0.12-64 µg/ml for antimicrobial agent. A number of wells were reserved in each plate for sterility control (no inoculum added), inoculum viability (no extract added) and the DMSO control. Each extract was tested in triplicate. Tested agar plates were incubated at 37°C for 24 h for bacteria and at 30°C for 7 days for dermatophytic

Table. 1. Antimicrobial activity of crude extracts of the leaves of *D. chinensis* , wood of *M. cochinchinensis* and antimicrobial agents

Microorganisms	Diameter[a] of inhibition zone (mm)									
	D. chinensis				*M. cochinchinensis*				microbial agent	
	H	C	M	W	H	C	M	W		
Gram positive bacteria										
Staphylococcus aureus ATCC 25923	7.7	11.2	-	-	-	12.1	12.8	8.5	Te	26.5
Staphylococcus epidermidis TISTR 518	9.7	10.5	-	-	-	13.9	17.3	11.4	Te	34.6
Enterococcus faecalis TISTR 459	-	-	-	-	-	-	-	-	Te	29.4
Bacillus subtilis	7.4	10.2	-	-	-	9.9	13.4	9.7	Te	19.2
Gram negative bacteria										
Escherichia coli ATCC 25922	-	-	-	-	-	-	-	-	Nor	38.0
Pseudomonas aeruginosa ATCC 27853	-	-	-	-	-	-	-	-	Nor	32.8
Salmonella typhi	-	-	-	-	-	-	-	-	Nor	23.6
Shigella sonnei	-	-	-	-	-	-	-	-	Nor	34.3
Fungi-yeast										
Candida albicans TISTR 5779	-	-	-	-	-	-	-	-	Am	17.9
Fungi-dermatophytes										
Trichophyton rubrum 1	12.6	11.2	-	-	-	15.5	-	-	Ke	31.9
Trichophyton rubrum 2	11.7	13.3	-	-	-	15.3	11.6	-	Ke	36.5
Trichophyton mentagrophytes 1	12.8	12.1	-	-	-	15.6	12.0	-	Ke	27.0
Trichophyton mentagrophytes 2	11.7	10.8	-	-	-	11.2	9.4	-	Ke	27.1
Microsporum gypseum 1	12.5	14.4	-	-	-	9.2	-	-	Ke	10.3

Key : (-) = No inhibition zone, Concentration of extracts = 2 mg/disc. H = hexane extract, C = chloroform extract, M = methanol extract, W = water extract

Te = Tetracycline 30 µg/disc, Nor = Norfloxacin 10 µg/disc, Am = Amphotericin B 25 µg/disc, Ke = Ketoconazole 25 µg/disc

[a] Includes diameter of disc (6mm)

Table 2. Antimicrobial activity of crude extracts of the leaves of *D. chinensis*, wood of *M. cochinchinensis* and microbial agents expressed as minimum inhibitory concentrations (MIC)

Microorganisms	MIC (μg/ml)					MIC (μg/ml)	
	D. chinensis		*M. cochinchinensis*			microbial agent	
	H	C	C	M	W	Te	Ke
Staphylococcus aureus ATCC 25923	2000	1000	250	1000	2000	1.0	N.T.
Staphylococcus epidermidis TISTR 518	500	500	250	500	1000	0.25	N.T.
Bacillus subtilis	2000	1000	125	1000	1000	2.0	N.T.
Trichophyton rubrum 1	31.25	31.25	250	N.T.	N.T.	N.T.	0.5
Trichophyton rubrum 2	62.50	62.50	250	1000	N.T.	N.T.	1.0
Trichophyton mentagrophytes 1	31.25	62.50	250	1000	N.T.	N.T.	8.0
Trichophyton mentagrophytes 2	31.25	31.25	250	1000	N.T.	N.T.	8.0
Microsporum gypseum 1	62.50	62.50	250	N.T.	N.T.	N.T.	16.0

Key : N.T.= Not tested, H = hexane extract, C = chloroform extract, M = methanol extract, W = water extract
Te = Tetracycline, Ke = Ketoconazole

fungi. MIC was defined as the lowest concentration of the extract at which no visible growth was observed after incubation.

3. Results and Discussion

The percentage yield of extracts from *D. chinensis* is as follows: hexane (4.14), chloroform (8.47), methanol (6.24) and water (4.80); and from *M. cochinchinensis*: hexane (0.14), chloroform (0.23), methanol (14.20) and water (0.90).

D. chinensis and *M. cochinchinensis* extracts were tested for antimicrobial activity against Gram-positive bacteria, Gram-negative bacteria and fungi. The results of antimicrobial activity of extracts are shown in Table 1 and Table 2.

The results revealed that the hexane and chloroform extracts of *D. chinensis* exhibited strong antibacterial activity against three of the four Gram-positive bacterial species tested, *S. aureus S. epidermidis* and *B. subtilis* with an inhibition zone ranged from 7.4-11.2 mm and with MIC values ranging of 500-2000 μg/ml. The hexane and chloroform extracts of *D. chinensis* were active against all dermatophytes. Their inhibition zones ranged from 10.8-14.4 mm and with MIC values ranging from of 31.25-62.50 μg/ml. However, methanol and water extracts of *D. chinensis* showed no activity against any of the microorganisms tested. *D. chinensis* ethanol leaf extract has been reported to show antibacterial activity against *S. aureus, Bacillus cereus*, *B. subtilis*, *Salmonella typhi* A, *Shigella boydii, Shigella shiga* and *S. sonnei* at concentration of 400 μg/disc but did not inhibit *E. faecalis* at the same concentration (Qais, *et al*., 1996.).

The crude chloroform and methanol extracts *M. cochinchinensis* exhibited strong antibacterial activity against three of the four Gram-positive bacterial species tested, *S. aureus*, *S. epidermidis* and *B. subtilis*. Their inhibition zones ranged from 9.9-17.3 mm and MIC values ranging between 125-1000 μg/ml. The crude chloroform extract of *M. cochinchinesis* showed appreciable antifungal activity with an inhibition zone ranged from 9.2-15.6 mm and MIC values of 250 μg/ml against all the tested dermatophytes, whereas the crude methanol extract possessed moderate effects (MIC = 1000 μg/ml). The hexane extract of *M. cochinchinensis* showed no activity against any of the microorganisms tested. In another study, the *M. cochinchinensis* root extract possessed antimycobacterial activity against *Mycobacterium smegmatis* (Chang *et al*., 1977) and antifungal activity against plant pathogens (Shirata and Takahashi, 1982).

However, none of *D. chinensis* and *M. cochinchinensis* extracts were active against all Gram-negative bacteria, one Gram-positive bacterium (*E. faecalis*) and yeast (*C. albicans*). It has been shown that most antibacterial medicinal plants attack Gram-positive bacteria while few are active against Gram-negative bacteria (Herrera *et al*., 1996; Meng *et al*., 2000).

4. Conclusion

The results obtained from this study indicated that the chloroform extracts of *D. chinensis* and *M. cochinchinensis* showed potential antimicrobial activity. It is suggested that the extracts of these plants may be useful for the treatment of the diseases caused by Gram-positive bacteria and dermatophytic fungi.

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