African Journal of Microbiology Research Vol. 6(19), pp. 4132-4137, 23 May, 2012
Available online at http://www.academicjournals.org/AJMR
DOI: 10.5897/AJMR11.1374
ISSN 1996-0808 ©2012 Academic Journals

Full Length Research Paper

In vitro antimicrobial activities of 26 Yunnan plant extracts against multi-drug resistant pathogens

Cui-Xian Yang [1,2], Guo-Ying Zuo[1]*, Jun Han[2], Gen-Chun Wang[1] and Zhong-Qi Bian[3]

[1]Research Center for Natural Medicines, CPLA Kunming General Hospital, Kunming 650032, China.
[2]Yunnan Traditional Chinese Medical University, Kunming 650500, China.
[3]Center for Infectious Diseases, CPLA Kunming General Hospital, Kunming 650032, China.

Accepted 15 February, 2012

***In vitro* activities of 80% ethanol extracts from 26 plants native to South-Eastern Yunnan, China, were evaluated against clinical multi-drug resistant (MDR) pathogens. The extracts were initially screened by the agar hole diffusion test. Then the minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) or minimum fungicidal concentration (MFCs) were determined through serial dilution with a standard broth micro-dilution method. Of the 26 extracts, 9 showed different potencies against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans*. The most active plants against both *S. aureus* and methicillin-resistant *S. aureus* (MRSA) were *Rhodoleia parvipetala* Tong, *Diplopanax stachyanthus* Hand.-Mazz and *Sarcosperma kachinense* Exell var. *simondii* Lam.et Royen, and their MIC/MBCs were 512, 512, 256-512 mg/L, respectively. *Sladenia celastrifolia* Kurz extract showed strong activity against *P. aeruginosa* and *C. albicans*, and their MICs were 512-2048 and 2048 mg/L, together with no activities of MBC or MFC up to the concentrations of 2048 mg/L. All these plants showed weak inhibition against *E. coli*.**

Key words: Plants extracts, antimicrobial activity, MRSA, MIC.

INTRODUCTION

With the widespread use of antibiotics and other antimicrobial agents, more and more of the clinical multidrug-resistant (MDR) pathogens appeared, and the degree of resistance has become increasingly serious(Güneş et al., 2012). At the same time, because of the difficulty in developing chemical synthetic drugs and because of their side-effects, scientists are making more efforts to search for new drugs from plant resources to combat MDR microbial infections (Tan and Vanitha, 2004; Zuo et al., 2005; Wang et al., 2006; Zhang et al., 2006; Sibanda and Okoh, 2007; Dong et al., 2008; Ameril et al., 2011). The importance of Chinese herbal medicines has been increasingly recognized. Our group has been studying the antimicrobial activity of the Chinese herbal medicines against clinical MDR strains, especially methicillin-resistant *Staphylococcus aureus* (MRSA) (Zuo et al., 2008a, b; Meng et al., 2009). To expand the screening scope, we turned to find out new plant extracts with anti-MDR microbial activities. In the present study, we screened the antimicrobial activities of 80% ethanol extracts from 26 southeastern Yunnan plants against *S.*

*Corresponding author. E-mail: zuoguoying@263.net. Tel: +86-871-4774941. Fax: +86-871-5414186.

Abbreviations: ATCC, American Type Culture Collection**; CFU,** colony forming unit**; CLSI,** Clinical Laboratory Standards Institute**; DMSO,** dimethyl sulfoxide**; IPRA,** imipenem-resistant *pseudomonas aeruginosa*; **IZD,** inhibition zone diameters**; KIB,** Kunming Institute of Botany**; MBC,** minimum bactericidal concentration**; MFC,** minimum fungicidal concentration**; MH-A,** Standard Mueller–Hinton agar; **MH-B,** Standard Mueller–Hinton broth**; MIC,** minimum inhibitory concentration; **MDR,** multidrug-resistant**; MRSA,** Methicillin-resistant *Staphylococcus aureus*; **MSSA,** Methicillin-sensitive *Staphylococcus aureus*; **NCCLS,** National Committee for Clinical Laboratory Standards**; S-A,** Sabouraud agar**; S-B,** Sabouraud broth.

aureus, *E. coli*, *P. aeruginosa* and *C. Albicans* and the related MDR pathogens.

MATERIALS AND METHODS

Bacterial strains

The strains of *S. aureus* (ATCC25923), *E. coli* (ATCC25922), *P. aeruginosa* (ATCC27853), *C. albicans* (ATCCY0109) and antibiotic susceptibility disks were provided by the National Institute of Control of Pharmaceutical and Biological products (NICPBP, Beijing, China). Clinical Imipenem-resistant strains of *P. aeruginosa* (IRPA120, IRPA132) and Fluconazole-resistant strains of *C. albicans* (FRCA842, FRC885) and MRSA strains (MRSA008, MRSA098, MRSA128, MRSA144, MRSA167, MRSA321) were isolated and characterized from the infectious sputum samples of critically ill patients in CPLA Kunming General Hospital (CLSI 2006; CLSI 2007; Kloos *et al*. 1999; Zuo et al., 2008a).

Plant materials

The selected plants were collected in Jinping, located in southeastern Yunnan, at altitudes of 1000m-2400m in June 2010. They were identified at the Botany Department, Kunming Institute of Botany (KIB), the Chinese Academy of Sciences. The voucher specimens are preserved at the herbarium of KIB (Shui et al., 2003).

Media used

Standard Mueller–Hinton agar and broth (MHA and MHB), and Sabouraud agar and broth (SA and SB) (Tianhe Microbial Agents Co., Hangzhou, China) were used as the bacterial and fungal culture media, respectively.

Extract preparation

An amount of air-dried and ground plant materials (25 g, each) was macerated with 80% ethanol for one week, filtered and the mare was further macerated twice with the same solvent overnight and filtered after being sonicated for 30 min. The filtrate was combined and the solvent was evaporated at 40℃ in vacuum to afford each of the plant extract.

Susceptibility test

The ethanol extracts of the 26 plants were initially subjected to susceptibility test according to the agar diffusion method on MHA (for the bacterium) or SA (for the fungi) plates. The samples (50 mg/ml in dimethylsulfoxide) were pipetted into the 6 mm (diameter) holes of agar punched in the agar plates, plating with inoculums of 1.5×10^8 CFU/ml for bacteria and 5×10^5 CFU/ml for fungi in advance. The plates were incubated at 35℃ for 24 h and measur ed and recorded the inhibition zone diameters (IZDs). The samples with IZDs larger than 12 mm against standard *S. aureus*, *E. coli*, *P. aeruginosa* or *C. albicans* were further subjected to assay of their minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) or minimum fungicidal concentration (MFCs) (Vandenbossche, et al., 2002; Zuo, et al, 2005).

The MICs and MBCs or MFCs were determined by standardized broth microdilution techniques with starting inoculums of 5×10^5 CFU/mL for bacteria and 2.5×10^3 CFU/mL for fungi and incubated at 35℃ for 24h according to CLSI guidelines (NCCLS, 19 99; NCCLS M27-A, 2002; CLSI, 2006, 2007). They were determined in duplicate, with concentrations ranging up to 2048 mg/L. For the MBCs or MFCs assays, 0.1ml aliquots from drug dilution wells with visual growth inhibition were plated onto MHA or SA media. The lowest drug concentration that yielded three or fewer microorganism colonies was recorded as the MBC or MFC (Radojević et al., 2011).

RESULTS AND DISCUSSION

The IZDs of ethanol extracts (50 mg/ml) from 26 plant samples were tested (Table 1), of which 11 extracts with IZDs ≥12 mm and belonging to 8 different plant families were subjected to the determination of the MICs and MBCs against different standard and MDR resistant pathogens. The values are in the ranges of 256->2048 mg/L against both MRSA and MSSA strains (Table 2), and 512->2048 mg/L against *C. albicans* and IRPA (Table 3), respectively.

The present study aimed at the antibacterial and antifungal activities of southeastern Yunnan plants. Based on the screening results, we found that some of the plant extracts had strong inhibition against gram-positive strains, especially *S. aureus*, and the inhibition was weak against gram-negative bacteria and fungi strains. Several plant samples showed a broad spectrum of antimicrobial activities. It is interesting that these plants also showed, to some extent, inhibition against MDR strains which were isolated from the clinical infectious samples. As the constitution of the plant extracts was complex, it is encouraging to perform detailed tracking of their more active compounds (Zuo et al., 2008b).

The antibacterial activity of *R. parvipetala* was strong against the MRSA and MSSA strains. But so far to the best of our knowledge, the chemical components and pharmacological activities of it had not been reported. We are beginning further study on the active compounds. There were no chemical and pharmacological reports about *S. bicolor and C. leucantha,* but the literature (Li et al., 2005) pointed out that the main components of Orchidaceae were terpenoids and phenanthrenes which suggested that they may be the antimicrobial composition in the two plants. *D. stachyanthus* was the specific plant in China. Previous literature (Yan et al., 2004) showed that it contained oleanolic acid, ursolic acid, quercetin, ellagic acid and so on. And there was no report about the pharmacological especially antimicrobial activities. Our experiments showed that it had strong inhibitory effect against both *S. aureus* and MRSA, which is worthy of further investigation. We study the antimicrobial activities of *S. celastrifolia* and find that it had a wide spectrum effects on both gram-positive and gram-negative bacteria, also acting on fungi. It had been reported (He et al., 2010) that *S. celastrifolia* contained flavanols and terpenoids, and its leaves and root bark have insecticidal activity. We could infer that maybe the insecticidal components were the antimicrobial composition.

Table 1. The inhibition zone diameters of 26 plants of *in vitro* antimicrobial activity (mm).

No.	Plant species	Specimen No.	SA[a]	MA1	MA2	EC	PA	CA
1	*Allomorphia baviensis* Guillaum.(Melastomataceae)	KUN 317	12	8	11	10	10	8
2	*Alpinia blepharocalyx* K. Schunm.(Zingiberaceae)	IBSC 2454	10	0	9	9	10	8
3	*Blumea balsamifera* (Linn.) DC.(Compositae)	KUN 21	8	7	0	0	10	0
4	*Brassaiopsis fatsioides* Harms(Araliaceae)	KUN 115	0	0	0	10	7	0
5	*Breynia fruticosa* Hook.f.(Euphorbiaceae)	KUN 1451	10	8	8	7	11	8
6	*Cayratia japonica* (Thunb.) Gagnep. Var. *Japonica* (Vitaceae)	PE 2777	10	9	10	10	9	10
7	*Celastrus orbiculatus* Thunb. (Celastraceae)	KUN 110	9	10	9	11	11	7
8	*Coelogyne leucantha* W. W. Smith (Orchidaceae)	KUN 12	15	8	13	7	9	9
9	*Diplopanax stachyanthus* Hand.-Mazz (Cornaceae)	KUM 4026	15	13	15	11	12	8
10	*Embelia undulata* (Wall.) Mez (Myrsinaceae)	YFS 226	8	8	8	11	9	7
11	*Hydrangea yunnanensis* Rehd. (Hydrangeaceae)	KUN 580565	11	0	9	8	8	11
12	*Illicium majus* Hook.f.et Thoms. (Illiciaceae)	YUKU 83	11	0	8	11	0	0
13	*Luculia pinciana* Hook.var. *Pinciana* (Rubiaceae)	TOYA 16013	10	7	7	11	9	8
14	*Manglietia forrestii* W.W.Smith ex Dandy (Magnoliaceae)	US 11988	11	0	8	0	6	0
15	*Meliosma squimulata* Hance (Sabiaceae)	KUN 2411	13	9	11	10	10	10
16	*Michelia balansae* (A. DC.) Dandy (Magnoliaceae)	SWFC 1056	7	0	0	10	0	0
17	*Oenanthe didlsii* de Boiss. (Umbelliferae)	KUN 3047	12	7	9	12	7	0
18	*Pilea martini* Hand.-Mazz (Urticaceae)	KUN 271	0	0	0	11	0	0

Table 1 Cont:

19	*Polygala fallax* Hemsl. (Polygalaceae)	YCP 85197	0	0	0	10	0	9
20	*Rhodoleia parvipetala* Tong (Hamamelidaceae)	KUN 185	15	12	16	12	12	9
21	*Sarcosperma kachinense* Exell var. *simondii* Lam.et Royen (Sapotaceae)	KUN 42	14	12	11	12	12	10
22	*Sladenia celastrifolia* Kurz (Sladeniaceae)	YCP 85250	13	10	10	10	13	15
23	*Sunipia bicolor* Lindl. (Orchidaceae)	KUN 4937	14	7	12	10	0	10
24	*Tupistra wattii* C.B.Clarke (Liliaceae)	KUN 580634	11	8	7	9	8	0
25	*Turpinia Montana* Kurz (Staphyleaceae)	KUN 82620	11	7	10	8	11	7
26	*Wrightia coccinea* (Loddiges) Sims (Apocynaceae)	NY 13574	0	0	9	9	0	10

[a]SA: *Staphylococcus aureus* (ATCC25923); MA1: MRSA008; MA2: MRSA128; EC: *Escherichia coli* (ATCC25922); PA: *Pseudomonas aeruginosa* (ATCC27853); CA: *Candida albicans* (ATCCY0109). All samples were tested in triplicate.

Table 2. The MIC and MBC of nine plant extracts against MSSA and MRSA strains (mg/L).

Plant species		ATCC25923	MRSA008	MRSA098	MRSA128	MRSA144	MRSA167	MRSA321
S. kachinense	MIC	256	512	512	1024	512	512	512
	MBC	2048	2048	2048	n.a.[a]	n.a.	2048	n.a.
C. leucantha	MIC	512	1024	1024	1024	1024	1024	1024
	MBC	1024	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
R. parvipetala	MIC	512	1024	512	512	512	512	512
	MBC	1024	n.a.	n.a.	2048	2048	n.a.	n.a.
D. stachyanthus	MIC	512	512	512	1024	512	512	512
	MBC	2048	2048	n.a.	n.a.	2048	n.a.	n.a.
S. bicolor	MIC	512	1024	1024	1024	152	1024	512
	MBC	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
A. baviensis	MIC	1024	2048	1024	1024	1024	1024	1024
	MBC	n.a	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
O. didlsii	MIC	1024	2048	2048	2048	2048	2048	2048
	MBC	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

Table 2 Cont:

S.celastrifolia	MIC	1024	2048	1024	1024	1024	1024	1024
	MBC	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
M. squimulata	MIC	2048	2048	2048	1024	2048	2048	1024
	MBC	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

[a]n.a.: not active at the concentration up to 2048 mg/L; All samples were tested in triplicate.

Table 3. The activities of *S. celastrifolia* extract against *P. aeruginosa* and *C. albicans* strains (mg/L).

Strains		MIC	MBC or MFC
PA	ATCC 27853	2048	n.a.[c]
	IPRA 120[a]	1024	n.a.
	IPRA 132	512	2048
CA	ATCC Y0109	2048	n.a.
	FR 842[b]	2048	n.a.
	FR 885	2048	n.a.

[a]IPRA120, IPRA132: Imipenem-resistant *Pseudomonas aeruginosa*; [b]FR 842, FR 885: Fluconazole-resistant *Candida albicans*; [c]n.a.: not active at the concentration up to 2048 mg/L; All samples were tested in triplicate.

Conclusion

From this study we can conclude that *R. parvipetala*, *D. stachyanthus* and *S. kachinense* were the most active anti-MRSA plants, and *S. celastrifolia* were more active against *P. aeruginosa*, *C. albicans*, and their resistant strains. Most of these species are perennial plants widely distributed in Jinping and other districts in southeastern Yunnan. As little was known about their phytochemical constitution and antimicrobial activity prior to our investigation, we are making further investigations of the antimicrobial components in these plants.

ACKNOWLEDGEMENTS

This research was financially supported by a grant from the National Natural Science Foundation of China (NSFC 81073126, NSFC 30972629). The specimens were identified at the Botany Department, Kunming Institute of Botany (KIB), the Chinese Academy of Sciences.

REFERENCES

Ameril A, Vaidya JG, Deokule SS (2011). *In vitro* evaluation of anti-staphylococcal activity of *Ganoderma lucidum*, *Ganoderma praelongum* and *Ganoderma resinaceum* from Pune, India. Afr. J. Microbiol. Res. 5(3): 328-333.

Clinical Laboratory Standards Institute (2006). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically—seventh edition, Approved standard M7-A7. Wayne, PA, CLSI.

Clinical Laboratory Standards Institute (2007). Performance standards for antimicrobial susceptibility testing—17th informational supplement. Approved standard M100-S17. Wayne, PA, CLSI.

Dong Y, Wang XY, Zhou H, Zhou J (2008). A study on bacteriostatic action of Chinese herbal medicine on MRSA clinical strain. Chin. Nursing Res., 22(4): 863-865.

Güneş H, Çetin ES, Kaya S, Arıdoğan BC (2012). Investigation of reduced glycopeptid susceptibility among methicillin resistant staphylococci. Afr. J. Microbiol. Res. 6(2): 453-459.

He R, Qi RP, Yang W (2010). Studies on the chemical constituents of *Sladenia Celastrifolia* Lurz. J. Yunnan Univ. Nationalities, 19(2): 102-104.

Kloos WK, Bannerman TL (1999). *Staphylococcus* and *Micrococcus*. In Murray et al. (eds) Manual of clinical microbiology, 7th ed. ASM Press, Washington, DC, pp. 264-282.

Li S, Wang CN, Guo SX (2005). Advances in studies on chemical components and pharmacology of epiphytic type medicinal plants in the Orohid Family. China J. Chin. Mater. Med., 30(19): 1489-1496.

Meng FY, Zuo GY, Hao XY, Wang GC, Xiao HT, Zhang JQ, Xu GL (2009). Antifungal activity of the benzocphenanthridine alkaloids from *Chelidonium majus* Linn against resistant clinical yeast isolates. J. Ethnopharmacol., 125(3): 494-496.

National Committee for Clinical Laboratory Standards (2002). Minutes US NCCLS antifungal susceptibility subcommittee meeting on interpretive breakpoints. NCCLS, Wayne, PA.

National Committee for Clinical Laboratory Standards (1999). Methods for determining bactericidal activity antimicrobial agents. Approved guidelines M26-A. NCCLS, Wayne, PA.

Radojević ID, Stanković MS, Stefanović OD, Topuzović MD, Čomić LR, Ostojić AM (2011). Anti-Aspergillus properties of different extracts from selected plants. Afr. J. Microbiol. Res., 5 (23): 3986-3990.

Shui YM, Chen WH, Li ZY, Huang SH, Zhang KP (2003). Seed plants of Honghe region in Se Yunnan, China. Yunnan Science and Technology Press, Kunming, China.

Sibanda T, Okoh AI (2007). The challenges of overcoming antibiotic resistance: Plant extracts as potential sources of antimicrobial and Mresistance modifying agents. Afr. J. Biotech. 6(25): 2886-2896.

Tan BKH, Vanitha J (2004). Immunomodulatory and antimicrobial effects of some traditional Chinese medicinal herbs: A review. Curr. Med. Chem., 11: 1423-1430.

Vandenbossche I, Vaneechoutte M, Vandevenne M, Baere TD, Verschraegen G (2002). Susceptibility testing of fluconazole by the NCCLS broth macrodilution method, E-test and disk diffusion for application in the routine laboratory. J. Clin. Microbiol., 40: 918-921.

Wang H, Meng C, Shi XA (2006). Screening for antibacterial Chinese herbal medicines and extraction and preliminary identification of their effective ingredients. China Pharmaceut., 15(20): 15-16.

Yan XH, Guo YW (2004). Studies on chemical constituents from leaves of *Diplopanax stachyathus*. Chin. Tradit. Herbal Drugs, 35(2): 125-127.

Zhang XZ, Guo AJ, Li YL (2006). Antimicrobial Activities of Traditional Chinese Materia Medica Extracts. Chin. J. Nosocomiol., 16(5): 563-565.

Zuo GY, Han J, Yu W (2005). Antimicrobial activities screening of 47 traditional Chinese herbal medicine extracts. China Pharm., 12(10): 798-799.

Zuo GY, Wang GC, Zhao YB, Xu GL, Hao XY, Han J, Zhao Q (2008a). Screening of Chinese medicinal plants for inhibition against clinical isolates of methicillin-resistant S*taphylococcus aureus* (MRSA). J. Ethnopharmacol., 120(2): 287-290.

Zuo GY, Meng FY, Hao XY, Zhang YL, Wang GC, Xu GL (2008b). Antibacterial alkaloids from *Chelidonium majus* Linn (Papaveraceae) against clinical isolates of methicillin-resistant *Staphylococcus aureus*. J. Pharm. Pharmaceut. Sci., 11(4): 90-94.