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Full Length Research Paper

Biological activities of extracts from a naturally wild kiwifruit, *Actinidia macrosperma*

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***Actinidia macrosperma* C. F. Liang is a medicinal plant in China and has been well known for its attraction to cats and activities against leprosy and cancers. In this paper, the petroleum ether, chloroform, ethyl acetate, *n*-hexane and aqueous successive extracts from the stems of *A. macrosperma* were screened for antibacterial and antifungal activities *in vitro* for the first time, using the disc diffusion method, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) / minimum fungicidal concentration (MFC). General toxicity of these extracts has also been assessed by brine shrimp lethality assay. From the results, the chloroform extract exhibited the most significant antimicrobial (MIC in the range of 60 to 500 µg/mL, MBC in the range of 150 to 1000 µg/mL, MFC in the range of 170 to 600 µg/mL) and cytotoxic activities (LC_{50} = 16.82 µg/mL at 24 h).**

Key words: *Actinidia macrosperma*; antimicrobial activity, cytotoxicity, brine shrimp.

INTRODUCTION

Actinidia macrosperma C. F. Liang, endemic to China, is a kind of naturally wild kiwifruit. It is commonly known as 'Ginseng for Cat' (Jiangsu New Medicine College, 1994), due to its attractant effect on cats by giving off a specific odor and then cats preferred to eat its fresh leaves or twigs to excite themselves and cure wounds (Lai and Zhang, 2002). The plant grows in shady places, such as thickets, forest margins, moist ditches, or sides of low mountains at an altitude below 800 m (Qiu, 1993). *A. macrosperma* has its reputation in folk treatment on various ailments in East China, e.g. leprosy, abscess, rheumatism, arthritis inflammation, jaundice and abnormal leucorrhea (Jiangsu New Medicine College, 1994; Lai and Zhang, 2002; Jiang et al., 2003). As previously reported, the roots and stems of this plant have also been claimed to have effectiveness in some local cancer treatments (Yao and Wang, 1989; Wan et al., 2004). Previously, isolated constituents included β-carotene from fruits (McGhie and Ainge, 2002), inorganic elements and amino acids from stems (Feng et al., 2004; Lu et al., 2004).

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Because of the enormous demand, the wild resource of this species has decreased rapidly and even become exhausted, based on our field and market investigation and folk inquiry. Great attention should be paid to its effective protection and reasonable development. Therefore, in recent years, we have conducted a series of research projects focusing on the chemistry and tissue culture (Jiang and Li, 2003; Feng et al., 2004; Lu et al., 2004, 2007a, 2007b; Zhao et al., 2006). This article was a follow-up of our previous review. And it was a necessary first step to investigate *in vitro* antimicrobial and cytotoxicity potential of *A. macrosperma*. Because the current botanical products used unextracted material, different extraction methods were used as a means of improving activity.

MATERIALS AND METHODS

The material studied was collected from hilly areas of Fuyang County, Zhejiang Province, China in August 2009. A specimen was identified and kept in Zhejiang University Herbarium (ZJUH), China (Voucher Number: HZU-A2009086).

Powdered stems (300 g) from the air-dried material were extracted successively with petroleum ether (pet-ether), chloroform ($CHCl_3$), ethyl acetate (EtOAc), *n*-hexane and water. The solvents were concentrated under vacuum at 40 to 50°C by a rotary flash

Table 1. Antibacter and antifungal activity of extracts from *A. macrosperma* stems.

Test samples [b]	Zone of inhibition diameter (mm) [a]						
	Gram-positive bacterium		Gram-negative bacterium		Fungi		
	S. aureus	***B. subtilis***	***E. coli***	***P. aeruginosa***	***C. albicans***	***A. fumigatus***	***M. canis***
Pet-ether extract	8	7	7	–	–	–	–
$CHCl_3$ extract	11	14	15	8	13	10	11
EtOAc extract	9	12	13	–	9	–	–
n-Hexane extract	11	10	9	7	9	–	7
Aqueous extract	–	–	–	–	–	–	–
Kanamycin	23	22	25	18	nt	nt	nt
Clotrimazole	nt	nt	nt	nt	22	21	18

S. aureus: *Staphylococcus aureus*; *B. subtilis*: *Bacillus subtilis*; *E. coli*: *Escherichia coli*; *P. aeruginosa*: *Pseudomonas aeruginosa*; *C. albicans*: *Candida albicans*; *A. fumigatus*: *Aspergillus fumigatus*; *M. canis*: *Microsperum canis*. –: no activity; nt: not tested. Solvent blanks showed no inhibition. [a] Values are the average of triplicate; Inhibition zones including the diameter of the paper disc (6 mm). [b] Crude extracts are tested at concentration of 200 µg/mL; Kanamycin (30 µg/mL) and Clotrimazole (20 µg/mL) are used as positive controls.

evaporator. The weight of the solid residue was recorded and taken as yield of crude extract (yields: 2.99, 0.13, 1.02, 1.84 and 7.81%, respectively). On phytochemical screening (Xu and Chen, 1983), ethyl acetate, *n*-hexane and aqueous extracts gave positive tests for saponins, but only the chloroform extract was positive for alkaloids. A portion of each dry extract was tested for antimicrobial and brine shrimp cytotoxic activity.

Antimicrobial activities were tested by disc diffusion technique (Bauer et al., 1966; Gnanamanickam and Smith, 1980). The extracts, which showed activities were then subjected to the determination of minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC)/minimum fungicidal concentration (MFC) (Irobi and Daramala, 1994; Zgoda and Porter, 2001). Cytotoxicity activity was determined by the brine shrimp (*Artemia salina*) lethality bioassay (Meyer et al., 1982). For the antimicrobial assay, the test samples were freshly dissolved at a concentration of 200 µg/ml in 5% dimethyl sulphoxide (DMSO) separately. Bacterial or fungal concentration of 5×10^5 cfu/mL was used. For the cytotoxicity assay, each test was carried out at three concentrations (the aqueous fraction in distilled water, the other fractions in 5% DMSO), including positive control.

Used microorganisms, listed in Table 1, are collected as pure cultures from the Department of Food Science and Nutrition, Zhejiang University. Laboratory hatched nauplii *A. salina* (obtained commercially from Bo Hai, China) were tested for 24 h with the mortalities of shrimps at each concentration of the extract, and LC_{50} values were determined by probit analysis using a Finney computer program.

RESULTS

The results of antimicrobial activity are shown in Tables 1. The MIC and MBC/MFC values are shown in Table 2. The results of cytotoxic activity are list in Table 3.

Except for the aqueous extract, the other four extracts exhibited varying degrees of inhibitory effects and a clear selectivity towards the studied bacterial strains except *Pseudomonas aeruginosa*, which is naturally resistant to many antibacterial agents (Walker and Edwards, 1999). However, only the chloroform extract inhibit the growth of three fungi used in the study. The data demonstrated a mild antifungal activity in ethyl acetate extract against *Candida albicans,* no inhibition on *Aspergillus fumigates* and *Microsperum canis*. Extract of *n*-hexane showed a mild inhibition against *C. albicans*, appreciable inhibition against *M. canis*, and no inhibition on *A. fumigatus*. Both aqueous extract and petroleum ether extract had no antifungal effect against the tested microorganisms. In the brine shrimp lethality bioassay, the petroleum ether and aqueous extracts were inactive all the same, whereas chloroform, ethyl acetate, *n*-hexane extracts of stems showed a cytotoxic activity even if it was found to be less active than podophyllotoxin, a well known cytotoxic lignin.

In conclusion, among the tested extracts of *A. macrosperma*, the chloroform extract showed more potent cytotoxic activity, interestingly, while the most significant antimicrobial activity was also found in chloroform extract. Aqueous extract showed neither antimicrobial nor cytotoxic activity.

DISCUSSION

A number of studies have shown multiple actions

Table 2. Minimum inhibitory concentration (MIC) (µg/mL) and minimum bactericidal concentration (MBC)/minimum fungicidal concentration (MFC) (µg/mL) of *A. macrosperma* stem extracts.

Test samples	*S. aureus*		*B. subtilis*		*E. coli*		*P. aeruginosa*		*C. albicans*		*A. fumigatus*		*M. canis*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MFC	MIC	MFC	MIC	MFC
Pet-ether extract	550	900	600	>1000	600	1000	nt	nt	nt	nt	nt	nt	nt	nt
$CHCl_3$ extract	210	480	95	180	60	150	500	1000	170	170	350	600	220	250
EtOAc extract	400	840	150	330	100	120	nt	nt	480	480	nt	nt	nt	nt
n-Hexane extract	220	500	260	500	420	450	700	>1000	500	500	nt	nt	>1000	>1000
Kanamycin	30	30	30	30	30	30	10	15	nt	nt	nt	nt	nt	nt
Clotrimazole	nt	nt	nt	nt	nt	nt	nt	nt	3	6	2	3	1	2

S. aureus: *Staphylococcus aureus*; *B. subtilis*: *Bacillus subtilis*; *E. coli*: *Escherichia coli*; *P. aeruginosa*: *Pseudomonas aeruginosa*; *C. albicans*: *Candida albicans*; *A. fumigatus*: *Aspergillus fumigatus*; *M. canis*: *Microsperum canis*. nt: not tested.

Table 3. Cytotoxicity of extracts from *A. macrosperma* stems on brine shrimp[a].

Test samples	Concentration (µg/mL)	LC_{50}-24h (µg/mL)
Pet-ether extract	1000:100:10	>1000
$CHCl_3$ extract	500:50:5	16.82
EtOAc extract	1000:100:10	662.73
n-Hexane extract	1000:100:10	933.28
Aqueous extract	1000:100:10	>1000
Podophyllotoxin[b]	1:2:4	2.76

[a]Results are the mean of three repeats (10 nauplii per concentration plus control in one measurement; dead nauplii were counted); both 5% DMSO and distilled water (solvent blanks) show 100% surviving. [b] Positive control.

of *A. macrosperma* on immune system responses. Several clinical trials on patients receiving chemotherapy or radiotherapy have found that this plant significantly improved appetite, alleviate weakness, anorexia, vomiting, spontaneous or night sweat and pain, increases weight and stabilizes white blood cell counts, NK cells, IL-2 and CD4/CD8 ratio (Zhou, 2000). Others have shown that *A. macrosperma* could improve the quality of life of the subjects by enhancing physical function and healthy transition (respondents' amount of change in their health in general over a 1-year period) without any adverse side effects in gastric, esophageal, liver and lung cancers (Lai and Zhang, 2002).

To our knowledge, this study is the first report of the antimicrobial and cytotoxic properties of *A. macrosperma*. Results presented here may provide a support to some traditional uses of this plant, and is therefore a potential source of biological ingredients for the food and pharmaceutical industry. Based on our continual study of *A. macrosperma*, certainly, further chemical and pharmacological investigations should be recommended.

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