Antimicrobial Activity of 11 Insects Extracts Against Multi Drug Resistant (MDR) Strains of Bacteria and Fungus

Antimicrobial Activity of 11 Insects Extracts Against Multi Drug Resistant (MDR) Strains of Bacteria and Fungus

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Abstract. Objective: 11 kinds of insects, which are traditional Chinese medicine (TCM), were detected by antimicrobial experiments to find new natural antibacterial extractives. Method: The body or shell of insects were extracted by two stage extraction solvent. Firstly, the body were impregnated two times with dichloromethane solvent, and then, soaked with 70% methanol and extracted two times. Disk diffusion test and Broth Dilution Assay were used to test MIC (MIC-minimum inhibitory concentration) value of these extractions. Multi Drug Resistant (MDR) Strains of Bacteria and Fungus were used as detected strains in the test. Result: Of all eleven species of insects, extractives of the shell of *Cryptotympanapustulata Fabricius* and the body of *Mole cricket* have significant antibacterial activity on *Staphylococcus aureus* and *Mycobacterium tuberculosis*; the different organic solvents components of *C. Fabricius* and *M.cricket* also have inhibit effects on *S.aureus* and *M.tuberculosis*; The MIC of the ethyl acetate fraction of *M.cricket* on *M.tuberculosis* was even reached to 0.01 g/mL. Moreover, the effect of water extractives from *C. Fabricius* on *M.tuberculosis* was 0.01 g/mL eighter. Discussion: The discovery of new antimicrobial medicine is always an urgent and successive need in the field of Medicine. Insects is a new field for the discovery of antibiotics.

1. Introduction

As we all know, the emergence of drug-resistant bacterial strains has caused the infection treatment more difficult and complicated. The discovery of new antimicrobial medicine is always an urgent and successive need in the field of Medicine [1]. Traditional antibiotics have been unable to meet the current needs of treatment; the research and development of new antibiotics cannot keep up with the rate of infection caused by pathogenic microbes. Therefore, more and more scientists are paying attention to the research and development of new antibacterial drugs [2, 3]. Insects are an important part of the Chinese traditional medicine (TCM), such as *Cryptotympanapustulata Fabricius*, the shells of the *C. Fabricius* is a kind of common TCM, which has the efficacy of dispersing wind and relieving heat, clearing the lung, clearing out the rash, relieving the antichloristic and clearing the liver, relieving the antitussive, relieving asthma, breathing the spasmodic and retreating the eyes. It is commonly used in the treatment of wind and fever, cough and sore throat. Pain, epilepsy in children, convulsions, convulsions, rubella, tetanus and other diseases [4, 5]. Most insects are also used as part of Chinese medicine prescription, and have special function of agaist infection diseases based on Compendium of Materia Medica (Table 1). However, few scientists have studied the anti-infection effect of insects deeply.

In recent years, the antibacterial and anti-inflammatory effects of insects were notified by some

natural chemists [6-8]. For example, in 2012, Sun Long studied the activity of antimicrobial peptides from two species of *Coleoptera* insects, and found that their antibacterial peptides had certain antibacterial activity [9]. In 2009, Li Xiu-hua studied the antibacterial activity of the metabolites of nematode bacteria, and found a metabolite of bacteriostatic to gray mould [10].

In the study, 11 kinds of insects were extracted by the two stage solvent extraction method. And the antibacterial activity of the insect extracts were detected by Broth Dilution Assay. Five kinds of Multi Drug Resistant (MDR) Strains of Bacteria and Fungus were used as experimental strains. The effective components were successively separated by different extraction methods, and the antibacterial activity of the extracts from various extraction methods was studied in vitro to provide basic datas for understanding the chemical composition and the special function of agaist infection diseases of Chinese medicine prescription.

2. Method and materials

2.1. Matereial

The dry shell or bodies of 11 insects, such as *Pillbug armadillidium*; *Cryptotympanapustulata Fabricius*; *Mole cricket*; *Musca domestica larvae; Scolopendra subspinipes; Statilia maculate*; *Eupolyphaga sinensis; Bombycid male; Silkworm larva; Tabanus*; *Coridius chinensis* (Table-1)*;* were bought from Tianji Tang in Zhangshu, Jiangxi province, China ppraiser, Jiangxi University of traditional Chinese medicine, Zhang Puzhao).

Multi Drug Resistant (MDR) Strains: *Monilia albican* ATCC10231; *Staphylococcus aureus* ATCC6538; *Enterococcus faecalis* ATCC29212; *Escherichia coli* ATCC8739; *Mycobacterium tuberculosis, H37RA.* All strains were bought from ATCC (American type culture collection).

Mueller-Hinton broth and agar were bought from Shanghai Ruichubio Reagent Co., Ltd.

2.2. Methods

2.2.1. Preparation of crude extractives. The crude extractives of insects were extracted by two stage solvent extraction method [11-13]. Insects were lyophilisation, crushed and dipped with dichloromethane for 2 times, each time for 5 days, and the leach solution was recovered to recover the insect body. The fat soluble components of insects were obtained after solvent extraction. The above recovered insects were dried to constant weight, then soaked in 2 times with 70% methanol for 3 days. After solvent extraction, insect extract II was obtained. 11 extracts were labelled and placed in 4^{o}C. for preservation.

2.2.2. Organic solvents extracts from crude extracts. The crude extracts powder of C fabricius and M crickets were extracted by percolation at room temperature with different organic solvents, respectively; those solvent were evaporated to dryness under low pressure [1]. 7 kinds of re-extracted drug components were got: No.1 is butanol parts of C fabricius, No.2 is butanol parts of M crickets, No.3 is ethyl acetate parts of M crickets, No.4 is acetate parts of C fabricius, No.5 is petroleum ether parts of M crickets, No.6 is water parts of C fabricius, No.7 is water part of M crickets. All the powders were dissolved in DMSO, Label separately, store in -20^{o}C.

2.2.3. The recovery of experimental strains. The cryopreservation strains were inoculated on the corresponding plate by three line method and cultured in the corresponding incubator. Among them, C albicans should be placed at 28^{o}C, and other strains were cultured at 37^{o}C. 24 h later, strains were kept in 4^{o}C.

2.2.4. Disk diffusion test. Disk diffusion test is a test of the antibiotic sensitivity of bacteria. According to Kirby–Bauer test method [14]**.** Fistly, bacteria strains were inoculated on the whole plate, each plate was attached with 6 discs containing specific antibiotics, each disc were added 10 μg extractives of

different insects (one of them was added nothing as a blank control). Then, the zone diameter of inhibition were tested after 24 hours culture at the corresponding temperature to observe the growth rate. However, M tuberculosis is growing slower than other strains, and its observation was generally two days).

2.2.5. Broth Dilution Assay. Broth Dilution Assay were used to test the susceptibility of bacteria. To antibiotics. Here, it were used to test MIC of the organic solvents extracts of insects [14, 15]. 6 concentration gradients were tested, each gradients were inoculated with S aureus and M tuberculosis respectively. Cultured at 37°C. and shake for 24 h. And repeated twice. All those experiments were carried out in laboratories with biosecurity level II. DMSO were included in every experiment as control.

Table 1. Data of the insects investigated

Latin binomial	Family	Common name	Part used	Popular use in TCM[a]
Pillbugs armadillidium	woodlice	Pillbugs/Shufuchong	Dry whole body	Relieving asthma, diuresis, detoxification, pain relief, sedation
Cryptotympanapustulata Fabricius	Cicadidae	Chantui	Slough of Cicadidae	Dispersing wind heat, promoting pharynx and opening the rash, activating rash, relieving wind to stop spasms,
Mole cricket	Gryllotalpida	short winged *mole cricket*	Dry whole body	Clear eyesight, cure blindness, and sore wounds, etc
Musca domestica larvae	Oriental Latrine	Maggot	Dry whole body	Treat malnutrition, abdominal distention and malnutrition
Scolopendra subspinipes	centipede	Chinese red-head,	Dry whole body	Wind and spasm, dredging collaterals and relieving pain, attacking toxin and dispersing stagnation
Statilia maculata	Mantis	Ootheca mantidis	Dry whole body	Semen, premature ejaculation, enuresis, etc
Eupolyphaga sinensis	Wingless	Tubiechong	Dry whole body	Activating blood circulation to dissipate blood stasis, promoting menstruation and relieving pain
Bombycid	Bombycidae	silkworm moth/Xiongchane	Dry whole body of male	Stop bleeding, blood and warm water, treat storm, gold sores, chilblain, soup fire sores, and eliminate scar, etc
Bombycidae	Bombycidae	Silkworm larva/Baijiangchan	Dry whole body of larva	Dispelling wind and spasms and dissipating phlegm and resolving stagnation
Tabanus	Oestridae	botflies/Gadfly insect/Munching	Dry whole body	Cure the disease, accumulate, reduce blood stasis in the abdomen, stagnation of blood stasis, and attack blood stasis
Coridius chinensis	*Chinensis*	Aspongopus chinensis	Dry whole body	Spleen deficiency, chest and abdominal congestion, etc

A Based on Compendium of Materia Medica

3. Results

3.1. Disk diffusion test of insects extractives

Among the 11 crude extracts, only extractive of *C fabricius* and *M crickets* extracts have inhibit effects to *S aureus* and *M tuberculosis* (Table 2).

Table 2. The antibacterial results of insect extractives/ mm

Insects	Antimicrobial spectrum (zone diameter of inhibition) /mm				
	Staphylococcus aureus ATCC6538	*Monilia albican* ATCC10231	*Enterococcus faecalis* ATCC29212	*Escherichia coli* ATCC8739	*Mycobacterium tuberculosis H37RA*
Pillbugs armadillidium	-	-	-	-	-
Cryptotympanapustulata Fabricius	13	-	-	-	16
Mole cricket	-	-	9	-	10
Musca domestica larvae	-	-	-	-	-
Scolopendra subspinipes	-	-	-	-	-
Statilia maculate	-	-	8*	-	-
Eupolyphaga sinensis	-	-	-	-	-
Bombycidae	-	-	-	-	-
Bombycidae	-	-	-	-	-
Tabanus	-	-	-	-	-
Coridius chinensis	-	-	-	-	-
DMSO	-	-	-	-	-

Note: * indicates that the phenomenon of bacteriostatic is not obvious.

3.2. Disk diffusion test of organic solvents extracts

All 7 organic solvents components of *C fabricius* and *M cricket* were ineffective to *Enterococcus faecalis* ATCC29212; the components of No 1,3,4,6 show inhibit effects on *S aureus* and *M tuberculosis* (Table 3).

3.3. Broth Dilution Assay analyse the effective parts of insects

Four parts of *M cricket* and *C fabricius* have obvious inhibit effect on *S aureus* and *M tuberculosis*. The MIC of No.3, the ethyl acetate parts of *M cricket*, is 0.1 g. mL^{-1} and 0.01 g.m L^{-1} on *S aureus* and *M tuberculosis separately*; however, the MIC of No.4, ethyl acetate parts of *C fabricius*, is 0.01 g. mL^{-1} on *S aureus.* Moreover, No 6, the effect of water extract parts from *C fabricius*, is also 0.01 g.mL^{-1} on *M tuberculosis*. All those four components have strong inhibit effect on *S aureus* and M tuberculosis (Table 4).

Table 3. The antimicrobial tests of organic solvents extracts of Cryptotympanapustulata Fabricius and mole cricket

Bacterial strains	Antimicrobial spectrum (diameter of bacteriostatic) /mm							
	1	2	3	4	5	6	7	DMSO
Staphylococcus aureus ATCC6538	15	0	9	7	0	9	0	0
Enterococcus faecalis ATCC29212	0	0	0	0	0	0	0	0
Mycobacterium tuberculosi H37RA	20	0	7.5	12*	0	5*	0	0

Note: * indicates that the phenomenon of bacteriostatic is not obvious.

No.1 is butanol parts of *Cryptotympanapustulata Fabricius*, No.2 is butanol parts of *Mole crickets*, No.3 is ethyl acetate parts of *Mole crickets*, No.4 is acetate parts of *Cryptotympanapustulata Fabricius*, No.5 is petroleum ether parts of *Mole crickets*, No.6 is water parts of *Cryptotympanapustulata Fabricius*, No.7 is water part of *Mole crickets*.

Table 4. The MIC results of organic parts of insects /g•mL-1

No.	Bacterial strains	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
1	*Staphylococcus aureus* ATCC6538	-	-	+	+	+	+
	Mycobacterium tuberculosis H37RA	-	+	+	+	+	+
3	*Staphylococcus aureus* ATCC6538	-	+	+	+	+	+
	Mycobacterium tuberculosis H37RA	-	-	+	+	+	+
4	*Staphylococcus aureus* ATCC6538	-	-	-	-	+	+
	Mycobacterium tuberculosis H37RA	-	+	+	+	+	+
6	*Staphylococcus aureus* ATCC6538	-	+	+	+	+	+
	Mycobacterium tuberculosis H37RA	-	-	+	+	+	+

Note: "-" no bacteria growing; "+" bacteria growing.

No.1 is butanol parts of *Cryptotympanapustulata Fabricius*, No.2 is butanol parts of *Mole crickets*, No.3 is ethyl acetate parts of *Mole crickets*, No.4 is acetate parts of *Cryptotympanapustulata Fabricius*, No.5 is petroleum ether parts of *Mole crickets*, No.6 is water parts of *Periostracum cicada*, No.7 is water part of *Mole crickets*.

4. Conclusion

Mycobacterium tuberculosis (Mtb), the pathogen of tuberculosis, is a broad-spectrum antibiotics tolerance bacteria strains [16, 17]. Staphylococcus aureus ATCC6538 is a strain of Methicillin-resistant *Staphylococcus aureus* (MRSA), which can cause different kind of infections in human [18-20]**.** It is a life-threatening pathogen to patients, because it has resistance to almost all the commonly used antibiotics. *S aureus* and *M tuberculosis* are both the Multi Drug Resistant (MDR) Strains.

In our study, the Bacteriostatic of butanol parts of *M crickets* have inhibit effect on *S aureus* and *M tuberculosis*. The ethyl acetate parts of *M crickets* also have strong inhibit effect on that two kinds strains; However, the other insect's extracts had no inhibit effect on the those bacteria strains and *M albican* ATCC10231. It can be found that the chemical components of n-butanol part of *C fabricius* have strong antibacterial activity to *S aureus* and *T bacilli*; The MIC of ethyl acetate extracts of *C fabricius* was $1 * 10^{-2}$ g mL^{-1}, which shows that ethyl acetate extracts of *C fabricius* contains some materials have effects on *S aureus*. These results indicate that insect extracts possess antibiotic activity and are potential natural antibiotics and have certain development prospects.

C falbricias is a kind of TCM, and usually used as part of TCM prescription to cure tuberculosis [21]; such as Inner Xiao Sheng Xuan Decoction; it is also commonly used in the treatment of wind and fever, cough and sore throat. Pain, epilepsy in children, convulsions, convulsions, rubella, tetanus and other diseases [4, 5]. In our study, we found that the shell of *C falbricias* have inhibit effect on *S aureus* and *M tuberculosis.* Which can partly explain the function of *C falbricias* in TCM.

In this paper, the research on the antibacterial activity of insect extracts is limited to the insects that have been purchased. However, the insect resources of our country are extremely rich and the number is very large, which provides a new research resource for the further exploration of the antibacterial active substances in the body of medicinal insects, pests and pathogens. On the basis of this experiment, according to the established experimental method, the research object of the insect source will be extended to provide new ideas for us to find new natural antibiotics or antibiotic precursor compounds.

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