Acaricidal activities of traditional Chinese medicine against the house dust mite, *Dermatophagoides farinae*

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SUMMARY

Objective. This paper assessed the potential of traditional Chinese medicine (TCM) preparations for using as environmentally acceptable and alternative commercial acaricides. **Methods.** 22 kinds of TCM, which contained abundant essential oils and showed insecticidal effects, were collected. Samples extracted with petroleum ether, ethyl acetate and methanol were tested against house dust mites *Dermatophagoides farinae* and their toxicity assessed. **Results.** The results showed that 3 TCM of *Cinnamonum cassia, Eugenia caryophyllata* and *Pogostemon cablin* have higher activity, and the parallel tests showed that the petroleum ether extract had higher activities (0·0046 mg/cm^2, 0·005 mg/cm^2 and 0·006 mg/cm^2 respectively, 24 h, LD_{50}) than the extracts of ethyl acetate and methanol. The acaricidal activity of the ethyl acetate extracts from *C. cassia*, *P. cablin* and *Asarum sieboldii* (0·00144 mg/cm^2, 0·00347 mg/cm^2 and 0·05521 mg/cm^2 respectively, 24 h, LD_{50}) were almost comparable to that of benzyl benzoate and dibutyl phthalate. However, the methanolic extracts of were less effective. **Conclusions.** This study shows the use of extracts with petroleum ether of *C. cassia*, *P. cablin* and *E. caryophyllata* as eco-friendly biodegradable agents for the control of the house dust mite.

Key words: natural acaricides, traditional Chinese medicine, extracts, house dust mite, *Dermatophagoides farinae*, mode of action.

INTRODUCTION

Allergic diseases such as asthma, allergic rhinitis and atopic dermatitis are the most popular diseases. Among the kinds of inhalant allergens inside homes in humid geographical areas, the most important are dust mites, especially the American house dust mite, *D. farinae* (Hughes), and the European house dust mite, *D. pteronyssinus* (Trouessart) (Arlian, 1989). Changes in living environments such as a rise in the number of apartment households with centrally installed heating, space heating, tighter windows and fitted carpets have improved conditions for mite growth.

Control of house dust mite is currently achieved by 3 different approaches: implementation of exceptional cleaning standards (Adilah *et al*. 1997; Nishioka *et al*. 1998), reduction of humidity levels (Bischoff *et al*. 1998) and reduction of allergens by effective acaricides (Mitchell *et al*. 1985; Green *et al*. 1989) such as γ-benzene hexachloride (γ-BHC), pirimiphos-methyl, benzyl benzoate, N,N-diethyl-*m*-toluamide (DEET), and dibutyl phthalate (Pollart *et al*. 1987). The latter option must only be employed after careful consideration because acaricides could kill the house dust mite quickly and inhibit the breeding of them effectively. Although good control was obtained by these synthetic acaricides, their repeated use has sometimes resulted in the development of resistance (van Bronswijk and Sinha, 1971), has undesirable effects on non-target organisms, and has fostered environmental and human health concerns (Pollart *et al*. 1987). These problems have highlighted the need for the development of safer and more efficient alternatives in controlling mites in the indoor environment.

Plant products have been suggested as a promising alternative to chemical acaridices for mite control (Kim *et al*. 2003; Rezk and Gadelhak, 2004; Rembold, 2005). In China, there are abundant plant materials designated as traditional Chinese medicine (TCM), which is compiled of different components of the same or dissimilar species of plant. The flowers, seeds, leaves, bark and roots etc. are prepared and purified by a chemical or physical process to provide the different TCM products, which exert different effects to cure disease. Table 1 lists 22 kinds of TCM having the characteristic of abundant essential oils or insecticidal or antibacterial effects. These 22 samples were selected to screen for activity against the house dust mite. This investigation could lead to the development of new classes of potentially

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Table 1. Crude materials collected from traditional Chinese medicine

Sample	Latin name	Chinese name	Part
1	*Cinnamomum cassia* Presl	Rou-Gui	Cortex
2	*Pogostemon cablin* (Blanco) Benth	Guang-Huo-Xiang	Acrial part
3	*Asarum sieboldii* Miq.	Xi-Xin	Root
4	*Mentha haplocalyx* Briq.	Bo-He	Acrial part
5	*Eugenia caryophyllata* Thunb	Ding-Xiang	Bud
6	*Foeniculum vulgare* Mill.	Xiao-Hui-Xiang	Fruit
7	*Zanthoxylum bungeanum* Maxim.	Hua-Jiao	Pericarp
8	*Areca catechu* L.	Bing-Lang	Seed
9	*Camellia sinensis* (L.) O. Ktze	Cha-Ye	Leafage
10	*Agrimonia pilosa* Ledeb.	Xian-He-Cao	Acrial part
11	*Citrus reticulata* Blanco	Chen-Pi	Pericarp
12	*Cnidium monnieri* (L.) Cuss.	She-Chuang-Zi	Fruit
13	*Coptis chinensis* Franch	Huang-Lian	Rhizome
14	*Coriandrum sativum* L.	Hu-Sui	Whole herba
15	*Hydnocarpus anrhelminiica* Pierre	Da-Feng-Zi	Seed
16	*Melia toosendan* Sieb. Et Zucc.	Chuan-Lian-Zi	Fruit
17	*Phellodendron chinense* Schneid.	Huang-Bo	Cortex
18	*Quisqualis indica* L.	Shi-Jun-Zi	Fruit
19	*Rosa rugosa* Thunb.	Mei-Gui-Hua	Bud
20	*Sophora flavescens* Ait	Ku-Shen	Root
21	*Stemona sessilifolia* (Miq.) Miq.	Bai-Bu	Rhizome
22	*Veratum nigrum* Linn.	Li-Lu	Root

safer acaricides for application inside homes and other hiding places. Comparative data have demonstrated that *Eugenia caryophyllata* (5), *Narcissus pseudonarcissus*, *Chenopodium ambrosioides*, *Rosa rugosa* (19) all of which contained caraway oils, have high activity against *D. pteronyssinus* (Saad *et al.* 2006). *Eugenia caryophyllata* (5, Clove) bud oil-derived methyleugenol was much more effective than benzyl benzoate and N,N-diethyl-m-toluamide (DEET) against *D. pteronyssinus* and *D. farinae* adults (Kim *et al.* 2003). Acaricidal activities of components from *Foeniculum vulgare* (10) fruit oil against *D. farinae* and *D. pteronyssinus* were much more effective than benzyl benzoate (Lee, 2004). Materials derived from the rhizome of *Cnidium officinate* (12) were also shown to have higher acaricidal activity than DEET, but equitoxic to benzyl benzoate (Kwon and Ahn, 2002*a*). Therefore, this paper describes a laboratory study that assessed the potential of TCM preparations for use as commercial acaricides. Acaricidal activities of petroleum ether extracts, ethyl acetate extracts and methanolic extracts from 22 TCM towards the adult house dust mite, *D. farinae*, were examined using direct contact and vapour-phase toxicity bioassay and compared with those of 2 current chemical acaricides of benzyl benzoate and dibutyl phthalate.

MATERIALS AND METHODS

Chemicals

The two chemical acaridides used in this study were benzyl benzoate (98%) and dibutyl phthalate (98%) purchased from China National Medicines Corporation Ltd. All other chemicals were of reagent grade and commercially available.

Stock culture of house dust mites

A stock culture of the house dust mite, *Dermatophagoides farinae*, was maintained in our laboratory for 5 years without exposure to any known acaricide. Mites were reared on a finely ground mixture of dust, dried yeast and bean cake (1 : 1 : 0·5 by weight) in complete darkness. Stock jars were kept in an incubator at an average temperature of (25 ± 2 °C) and relative humidity of (80 ± 5%).

TCM and sample preparation

Twenty-two TCM were used in this study (Table 1). They were purchased from Shenzhen Accordance Pharmaceutical Co., Ltd, China. Each sample (100 g) was pulverized and extracted with 400 ml of petroleum ether by soxhlet's extraction and filtered. The filtrate was dried by anhydrous Na_2SO_4 and concentrated to dryness by rotary evaporation at 50 °C. Then, filter residues were extracted successively with 500 ml of ethyl acetate twice and 300 ml of methanol twice at room temperature for 3 days and filtered. The respectively combined filtrate was also dried by anhydrous Na_2SO_4 and concentrated to dryness by rotary evaporation at 50 °C.

Contact toxicity bioassay

A filter paper contact toxicity bioassay (Lee, 2004) was used to evaluate the toxicity of petroleum ether extracts, ethyl acetate extracts and methanolic extracts from 22 TCM and 2 chemical insecticides currently used against adult *D. farinae*. In a preliminary experiment, 0·00953 mg/cm² was found to be an appropriate starting dose for a primary screening of the acaricidal activity of these extracts. Eight concentrations (100, 50, 25, 12·5, 6·25, 3·12, 1·56, 0·78 mg/ml) of each extract in petroleum ether were used. Amounts (1·21951, 0·60976, 0·30488, 0·15244, 0·07622, 0·03811, 0·01905, 0·00953 mg/cm² or other doses) of each test material were applied to Whatman filter papers (5·6 cm diameter). The 2 acaricides tested were also dissolved in petroleum ether. Control filter papers received an equal volume of petroleum ether. After drying in a fume hood (20°C) for 2 min, each treated filter paper was separately placed on the bottom of a Petri dish (5·5 cm diameter × 1·2 cm). Groups of 55–65 adult mites were introduced into Petri dishes containing a piece of cotton (5 mm × 5 mm) impregnated with 2 ml of distilled water. Each Petri dish was then covered with a lid and kept in an incubator at an average temperature of (25 ± 2 °C) and relative humidity of (80 ± 5%). Mortalities were determined at 24 h (or 0·5, 1, 1·5, 2, 2·5 h) post-treatment under a binocular microscope (20 ×). Adults were considered to be dead if the legs did not move when they were prodded with a fine pin (Kwon and Ahn, 2002*b*). All treatments were replicated 3 times.

Vapour-phase toxicity bioassay

Experiments were conducted to determine whether the lethal activity of these extracts against adult *D. farinae* was attributed to contact or vapour-phase toxicity (Kim *et al.* 2004). One selected dose above which mortality of the extract was up to 100% in the preliminary fumigant bioassay of each preparation in petroleum ether was used. The amount of each selected test material was applied to Whatman filter papers (5·6 cm diameter). And each treated filter paper was separately attached to the top of a lid. When mites were introduced into the bottom of a Petri dish, each dish was covered with a lid with a treated filter paper tightly fitted (a closed container, method A) to investigate the potential vapour-phase toxicity of the extracts used, or 95% of the Petri dish was covered with the lid lightly fitted (an open container, method B). Mortalities were determined at 24 h post-treatment. Other operations were performed as described above.

Data analysis

Lethal activity was classified as follows: strong, mortality > 80%; moderate, mortality 80–50%; weak, mortality 50–20%; little or no activity, mortality < 20%. The mortality percentages were determined and transformed to arcsine square root values for analysis of variance (ANOVA). The Bonferroni multiple comparison method was used to test for significant differences among the test TCM preparations. A paired *t*-test was used to test for significant differences between two treatment methods. Means (± S.E.) of untransformed data are reported. The LD_{50} values were calculated by probit analysis. Acaricidal activity was considered to be significantly different when 95% confidence limit levels failed to overlap.

RESULTS

Contact toxicity with treated filter paper

Toxic effects of the petroleum ether extracts, ethyl acetate extracts and methanolic extracts of 22 TCM on adult *D. farinae* were bioassayed by direct contact. Lethal activity varied according to medicine species, extracted components and exposure dose tested. At the dose of 1·219 mg/cm², the results in Table 2 show that the petroleum extracts of samples 1–7 exhibited 100% mortality. Among the 22 petroleum ether extracts, *C. cassia* (1), P. *cablin* (2) and *E. caryophyllata* (5) preparations exhibited the highest lethal activities against *D. farinae* adults. At the dose of 2·65 mg/cm², the ethyl acetate preparations of samples 1–4 and 10 exhibited strong (Table 3). Samples 1 and 2 demonstrated 100% lethal activity when they were tested at 0·13 mg/cm².

In a comparative listing, although 7 methanolic extracts exhibited strong lethal activity at the dose of 3·98 mg/cm², the other 15 methanolic preparations showed little or no activity (Table 4). Methanolic extracts were less effective than petroleum ether extracts and ethyl acetate extracts against *D. farinae* adults by mortality values. There was no mortality in the solvent-treated controls.

The acaricidal activity of the petroleum ether extracts, ethyl acetate extracts and methanolic extracts was compared with that of 2 acaricides currently used. As judged by 24 h LD_{50} values, *C. cassia* (1) extract was the most toxic material (0·0046 mg/cm²) among the 22 petroleum ether extracts (Table 5). Ethyl acetate extract from *C. cassia* (1) also was the most toxic material (0·00144 mg/cm²) (Table 6). And the acaricidal activity of many other petroleum ether and ethyl acetate extracts was higher than that of 2 currently used acaricides benzyl benzoate and dibutyl phthalate. By contrast, methanolic extracts from the 22 TCM exhibited much lower acaricidal activity. Even the most toxic material (0·16537 mg/cm²), *P. cablin* (2), was less effective than benzyl benzoate and dibutyl phthalate (see Table 7).

In addition, the acaricidal activity of the 5 selected petroleum ether extracts and the 3 ethyl acetate

Table 2. Lethal activity of petroleum ether extracts from 22 TCM towards adult *D. farinae* using the filter paper contact toxicity bioassay, exposed for 24 h

Sample[a]	Mortality (mean ± S.E. (%))[b] No. of mites	1·219[c]	No. of mites	0·304[c]	No. of mites	0·019[c]
C. cassia	189	100 ± 0·0	181	100 ± 0·0	188	100 ± 0·0
P. cablin	180	100 ± 0·0	183	100 ± 0·0	184	100 ± 0·0
A. sieboldii	183	100 ± 0·0	184	100 ± 0·0	190	3·4 ± 1·6
M. haplocalyx	182	100 ± 0·0	186	66 ± 9·7	186	7·5 ± 2·2
E. caryophyllata	183	100 ± 0·0	189	100 ± 0·0	177	100 ± 0·0
F. vulgare	195	100 ± 0·0	182	37 ± 3·8	185	20·2 ± 2·8
Z. bungeanum	185	100 ± 0·0	176	26 ± 2·5	179	13·3 ± 2·4
A. pilosa	188	61 ± 4·1	185	23 ± 4·0	183	3·3 ± 1·6
C. monnieri	187	29 ± 3·8	189	19·3 ± 3·9	178	0·0
C. sativum	190	22 ± 1·5	179	14·8 ± 5·2	186	10·8 ± 2·9
H. anrhelminiica	185	41 ± 7·5	182	25 ± 2·8	187	4·8 ± 1·4
R. rugosa	189	23·1 ± 4·0	185	14·3 ± 3·6	180	0·0
S. flavescens	179	40 ± 3·3	186	18·9 ± 3·1	182	0·0
S. sessilifolia	184	25·4 ± 2·7	182	20 ± 6·0	186	0·0

[a] TCM preparations causing ⩾20% mortality at 1·219 mg/cm^2 are presented.
[b] Means within a column followed by the same letter are not significantly different ($P = 0·05$, Bonferroni's test).
[c] Dose (mg/cm^2).

Table 3. Lethal activity of ethyl acetate extracts from 22 TCM towards adult *D. farinae* using the filter paper contact toxicity bioassay, exposed for 24 h

Sample[a]	Mortality (mean ± S.E. (%))[b] No. of mites	2·65[c]	No. of mites	1·33[c]	No. of mites	0·13[c]
C. cassia	189	100 ± 0·0	182	100 ± 0·0	188	100 ± 0·0
P. cablin	179	100 ± 0·0	183	100 ± 0·0	183	100 ± 0·0
A. sieboldii	182	100 ± 0·0	185	100 ± 0·0	188	9·9 ± 3·5
M. haplocalyx	179	100 ± 0·0	180	100 ± 0·0	185	7·9 ± 1·7
E. caryophyllata[d]	185	< 20%	181	0	182	0
F. vulgare	191	68·0 ± 6·2	187	31·2 ± 4·8	186	6·2 ± 1·9
Z. bungeanum	188	60·0 ± 5·8	179	12·8 ± 7·5	182	3·2 ± 1·4
C. sinensis	189	58·8 ± 8·4	180	10·2 ± 8·3	192	0·0
A. pilosa	187	100 ± 0·0	184	97·6 ± 2·7	185	11·3 ± 2·8
C. reticulata	190	63·4 ± 9·6	179	17·4 ± 8·3	181	1·6 ± 1·5
C. monnieri	186	93·2 ± 9·1	190	45·6 ± 8·0	178	5·3 ± 1·7
C. sativum	190	68·1 ± 9·4	179	14·3 ± 9·5	182	2·4 ± 1·0
H. anrhelminiica	179	67·2 ± 9·1	177	28·1 ± 7·0	181	3·3 ± 1·5
M. toosendan	182	66·8 ± 4·7	189	39·1 ± 2·0	184	0·0
Q. indica	190	100 ± 0·0	188	99·6 ± 0·7	188	6·3 ± 1·7
R. rugosa	182	27·0 ± 6·9	186	0·0	178	0·0
S. flavescens	178	79·7 ± 7·9	182	9·1 ± 5·8	183	1·1 ± 0·9
S. sessilifolia	186	79·8 ± 7·9	182	39·0 ± 7·0	181	4·1 ± 2·6

[a] TCM preparations causing ⩾20% mortality at 2·65 mg/cm^2 are presented.
[b] Means within a column followed by the same letter are not significantly different ($P = 0·05$, Bonferroni's test).
[c] Dose (mg/cm^2).
[d] Ethyl acetate extract from *E. caryophyllata* exhibited very low solubility in petroleum ether.

extracts was compared with that of 2 currently used acaricides by direct contact 0·5, 1, 1·5, 2 and 2·5 h after treatment. It was shown that petroleum ether extracts from *C. cassia* (1), *P. cablin* (2) and *A. sieboldii* (3) worked faster than other preparations (Table 8). The mortality of benzyl benzoate was comparable with that of petroleum ether extracts. There was no mortality recorded in the controls.

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Table 4. Lethal activity of methanolic extracts from 22 TCM towards adult *D. farinae* using the filter paper contact toxicity bioassay, exposed for 24 h

Sample[a]	Mortality (mean ± S.E. (%))[b]					
	No. of mites	3·98[c]	No. of mites	0·66[c]	No. of mites	0·13[c]
C. cassia	190	<20%	181	0	182	0
P. cablin	181	100±0·0	184	97·7±2·5	182	8·5±3·2
A. sieboldii	180	100±0·0	186	35·6±7·9	189	11·8±1·0
M. haplocalyx	179	100±0·0	189	17·5±4·8	185	3·3±1·6
E. caryophyllata	188	100±0·0	190	82·3±6·3	179	4·3±3·4
F. vulgare	187	<20%	187	0	192	0
Z. bungeanum	186	<20%	182	0	188	0
A. pilosa	181	<20%	179	0	186	0
C. monnieri	188	<20%	184	0	191	0
C. sativum	185	<20%	187	0	182	0
H. anrhelminiica	185	97·1±2·7	179	18·6±4·7	184	3·2±1·6
Q. indica	188	99·4±1·0	189	14·9±6·0	192	12·7±3·2
R. rugosa	184	<20%	190	0	184	0
S. flavescens	186	<20%	186	0	185	0
S. sessilifolia	185	100±0·0	191	63·4±4·2	186	12·2±4·6

[a] TCM preparations causing ⩾20% mortality at 3·98 mg/cm² are presented.
[b] Means within a column followed by the same letter are not significantly different ($P=0·05$, Bonferroni's test).
[c] Dose (mg/cm²).

Table 5. Toxicity of petroleum ether extracts and 2 currently used pesticides towards adult *D. farinae* using the filter paper contact toxicity bioassay, exposed for 24 h

Sample[a]	No. of mites	Slope (±S.E.)	LD_{50} (mg/cm²)	95% CL
C. cassia	671	5·27±0·26	0·0046	0·0039–0·0054
P. cablin	672	3·28±0·16	0·006	0·0056–0·0066
A. sieboldii	640	6·43±0·45	0·037	0·0331–0·0427
M. haplocalyx	674	4·60±0·31	0·226	0·1825–0·2685
E. caryophyllata	636	4·36±0·22	0·005	0·0044–0·0055
F. vulgare	668	5·51±0·35	0·358	0·3284–0·3901
Z. bungeanum	597	5·90±0·42	0·399	0·3759–0·4221
A. pilosa	631	2·13±0·11	0·826	0·7528–0·9075
C. monnieri	655		>2·0	
C. sativum	643		>2·0	
H. anrhelminiica	626	1·35±0·16	1·723	1·4706–2·0432
R. rugosa	670		>2·0	
S. flavescens	628	2·63±0·26	1·696	1·3576–1·9599
S. sessilifolia	661		>2·0	
Benzyl benzoate	621	3·56±0·21	0·00931	0·0074–0·0122
Dibutyl phthalate	612	4·55±0·31	0·05852	0·0523–0·0652

[a] TCM preparations causing ⩽2·0 mg/cm² 24 h LD_{50} are presented.

Route of acaricidal action

The fumigant activity of these selected extracts which showed strong acaricidal activity in the contact toxicity test was investigated using a vapour-phase toxicity bioassay in 2 formats. After 24 h of exposure to the different extracts at different doses, there was a significant difference in lethal activity of these extracts between exposure in a closed container (method A), which resulted in 100% mortality, and exposure in an open container (method B), which resulted a mortality range from 0 to 11·05% against *D. farinae* adults (Table 9). *C cassia* (1), *P. cablin* (2) and *A. sieboldii* (3) ethyl acetate preparations exhibited slightly weak lethal activity compared with their petroleum ether extracts in method A. This may be the result of the different volatilities of petroleum ether extracts and ethyl acetate extracts from the same TCM. The fumigant activity of these 8 extracts was comparable with that of benzyl

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Table 6. Toxicity of ethyl acetate extracts and 2 currently used pesticides towards adult *D. farinae* using the filter paper contact toxicity bioassay, exposed for 24 h

Sample[a]	No. of mites	Slope (±S.E.)	LD_{50} (mg/cm^2)	95% CL
C. cassia	701	4·62±0·22	0·00144	0·00120–0·00170
P. cablin	725	6·58±0·34	0·00347	0·00322–0·00371
A. sieboldii	716	2·71±0·16	0·05521	0·04204–0·07715
M. haplocalyx	721	2·43±0·14	0·13080	0·11414–0·14822
E. caryophyllata[b]	649		>2·0	
F. vulgare	745	2·81±0·15	1·78940	1·53632–2·06169
Z. bungeanum	712		>2·0	
A. catechu	649	3·39±0·21	1·32324	1·05595–1·60793
C. sinensis	702		>2·0	
A. pilosa	689		>2·0	
C. reticulata	651	4·11±0·27	1·95673	1·68892–2·42728
C. monnieri	688	5·71±0·47	1·25707	0·98706–1·43694
C. sativum	687	4·44±0·24	1·97780	1·78269–2·21388
H. anrhelminiica	674	4·88±0·28	1·83425	1·63661–2·02555
M. toosendan	753	2·84±0·22	1·90666	1·68255–2·14592
Q. indica	758	3·56±0·23	0·41726	0·34347–0·48240
S. flavescens	771	5·51±0·28	1·76171	1·44385–2·11330
R. rugosa	726		>2·0	
S. sessilifolia	736	3·64±0·29	1·48390	1·36991–1·58589
V. nigrum	697	2·78±0·31	1·07185	0·68541–1·32782
Benzyl benzoate	626	3·56±0·21	0·00931	0·0074–0·0122
Dibutyl phthalate	615	4·55±0·31	0·05852	0·0523–0·0652

[a] TCM preparations causing ⩽2·0 mg/cm^2 24 h LD_{50} are presented.
[b] Ethyl acetate extract from *C. sinensis* exhibited very low solubility in petroleum ether.

Table 7. Toxicity of methanol extracts and 2 currently used pesticides towards adult *D. farinae* using the filter paper contact toxicity bioassay, exposed for 24 h

Sample[a]	No. of mites	Slope (±S.E.)	LD_{50} (mg/cm^2)	95% CL
C. cassia	623		>2·0	
P. cablin	639	3·97±0·24	0·16537	0·09594–0·23337
A. sieboldii	587	5·05±0·28	0·99121	0·69807–1·32269
M. haplocalyx	592	3·76±0·19	1·02286	0·87044–1·18817
E. caryophyllata	575	9·48±0·56	0·41358	0·39654–0·43004
F. vulgare	582		>2·0	
Z. bungeanum	595		>2·0	
A. pilosa	571		>2·0	
C. monnieri	589		>2·0	
C. sativum	596		>2·0	
H. anrhelminiica	563	2·77±0·19	1·25805	1·00905–1·55886
Q. indica	614	4·20±0·23	0·77252	0·67246–0·88279
R. rugosa	612		>2·0	
S. flavescens	602		>2·0	
S. sessilifolia	582	3·33±0·21	0·4280	0·39548–0·46392
Benzyl benzoate	620	3·56±0·21	0·00931	0·0074–0·0122
Dibutyl phthalate	618	4·55±0·31	0·05852	0·0523–0·0652

[a] TCM preparations causing ⩽2·0 mg/cm^2 24 h LD_{50} are presented.

benzoate in method A but slightly less effective in method B. There was no mortality in the dibutyl phthalate control exposures in both method A and in method B.

DISCUSSION

TCM components are potential products for house dust mite control because many of them are selective

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Table 8. Lethal activity of 5 selected petroleum ether extracts, 3 ethyl acetate extracts and 2 currently used acaricides towards adult *D. farinae* using the filter paper contact toxicity bioassay at different times

Sample	No. of mites	Dose[d] mg/cm²	Mortality (mean ± S.E. (%))				
			0·5 h	1 h	1·5 h	2 h	2·5 h
C. cassia[a]	945	0·0122	23·28 ± 9·72[e]	87·67 ± 2·75[e]	92·39 ± 4·14[e]	100 ± 0·00	100 ± 0·00
C. cassia[b]	935	0·0122	2·36 ± 2·04[e]	6·39 ± 5·71[e]	24·98 ± 8·82[e]	53·96 ± 5·55[e]	87·78 ± 10·77[e]
P. cablin[a]	937	0·01951	20·62 ± 6·13[e]	87·87 ± 6·18[e]	97·29 ± 2·43[e]	100 ± 0·00	100 ± 0·00
P. cablin[b]	944	0·00853	5·41 ± 1·14[e]	25·05 ± 7·40[e]	56·53 ± 5·3[e]	76·03 ± 10·73[e]	88·54 ± 10·97[e]
A. sieboldii[a]	931	0·04265	63·04 ± 5·86[e]	84·92 ± 8·42[e]	96·19 ± 3·75[e]	100 ± 0·00	100 ± 0·00
A. sieboldii[b]	946	0·12185	7·60 ± 2·06[e]	20·65 ± 5·26[e]	37·07 ± 10·79[e]	47·20 ± 5·32[e]	84·06 ± 10·95[e]
M. haplocalyx[a]	939	0·31682	17·17 ± 6·30[e]	34·64 ± 6·51[e]	57·57 ± 10·00[e]	82·5 ± 7·22[e]	97·27 ± 0·97[e]
E. caryophyllata[a]	942	0·00976	46·68 ± 6·20[e]	56·61 ± 9·26[e]	83·92 ± 5·34[e]	97·00 ± 3·46[e]	100 ± 0·00
Benzyl benzoate[c]	939	0·03656	14·84 ± 3·74[e]	70·06 ± 1·27[e]	85·43 ± 5·79[e]	90·79 ± 4·80[e]	94·59 ± 4·82[e]
Dibutyl phthalate[c]	931	0·14143	0·00	3·83 ± 1·63[e]	5·07 ± 1·42[e]	7·52 ± 1·10[e]	13·24 ± 2·11[e]

[a] Petroleum ether extracts.
[b] Ethyl acetate extracts.
[c] Currently used acaricides.
[d] Extracts holding 100% mortality at this and at higher than this dose in 24 h in contact bioassay.
[e] Significant at $P < 0{\cdot}05$, according to a paired *t*-test.

Table 9. Fumigant activity of 5 selected petroleum ether extracts, 3 ethyl acetate extracts and 2 currently used acaricides towards adult *D. farinae* using the vapour-phase toxicity bioassay in 2 formats, exposed for 24 h, at different doses

Sample	No. of mites	Dose[d] (mg/cm²)	Method[e]	Mortality (mean ± S.E. (%))
C. cassia[a]	184	0·00609	A	100 ± 0·00
	194		B	10·2 ± 1·45[f]
C. cassia[b]	178	0·00975	A	100 ± 0·00
	181		B	8·58 ± 1·97[f]
P. cablin[a]	191	0·01584	A	100 ± 0·00
	180		B	10·2 ± 1·45[f]
P. cablin[b]	174	0·01828	A	100 ± 0·00
	182		B	4·48 ± 2·44[f]
A. sieboldii[a]	189	0·04265	A	100 ± 0·00
	175		B	4·68 ± 4·05[f]
A. sieboldii[b]	185	0·14622	A	100 ± 0·00
	189		B	2·34 ± 1·01[f]
M. haplocalyx[a]	179	0·13403	A	100 ± 0·00
	171		B	0·00
E. caryophyllata[a]	181	0·0122	A	100 ± 0·00
	178		B	11·05 ± 2·41[f]
Benzyl benzoate[c]	196	0·093	A	100 ± 0·00
	187		B	42·15 ± 5·41[f]
Dibutyl phthalate[c]	182	0·14143	A	0·00
	189		B	0·00

[a] Petroleum ether extracts.
[b] Ethyl acetate extracts.
[c] Currently used acaricides.
[d] Extracts holding 100% mortality at this and at higher than this dose in fumigant bioassay.
[e] A, vapour in closed container; B, vapour in open container.
[f] Significant at $P < 0{\cdot}05$, according to a paired *t*-test.

for pests, and have no or little harmful effects on non-target organisms and the environment. Some extracts from the medicines or corresponding medicinal plants are known to possess acaricidal activity against house dust mites (Kwon and Ahn, 2002*a*; Kim *et al.* 2003; Rezk and Gadelhak, 2004; Rembold, 2005; Saad *et al.* 2006) and even other acarid parasites such as the tick *Ixodes ricinus* (Garboui *et al.* 2007; Pålsson *et al.* 2008). Most of the reported naturally occurring acaricidal components

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are essential oils. Little work has been done with respect to the acaricidal activity of other components from the medicine or corresponding medicinal plants.

Although it has been reported that susceptibility to some plant essential oils such as almond bitter oil, caraway oil, and perilla oil was greater in *D. farinae* adults than in *D. pteronyssinus* adults (Watanabe *et al.* 1989; Furuno *et al.* 1994), *D. farinae* adults are also found to be more tolerant to the wood oils of *Thuga heteropylla* Sarg. and *Cryptomeria japonica* D. Don than *D. pteronyssinus* adults (Miyazaki *et al.* 1989). So, the acaricidal activity of 3 kinds of extracts from 22 TCM was tested against *D. farinae* adults using direct contact and vapour-phase toxicity bioassays in the present study.

Lethal activity of petroleum ether extracts from the 22 TCM was almost comparable with that of ethyl acetate extracts but much stronger than that of methanolic extracts. Petroleum ether extracts 3 and 4 showed stronger toxic activity than benzyl benzoate and dibutyl phthalate, respectively, as judged by 24 h LD_{50} values, and ethyl acetate extracts 2 and 3 showed stronger toxic activity than benzyl benzoate and dibutyl phthalate respectively. The toxic activity of all of the methanolic extracts was less effective than that of the 2 currently used acaricides. As a whole, *C. cassia* (1), *P. cablin* (2), *A. sieboldii* (3), *M. haplocalyx* (4) and *E. caryophyllata* (5) preparations showed stronger lethal activity than the other medicines.

There was a significant difference in lethal activity of the different extracts from the same medicine. For example, acaricidal activities of the ethyl acetate extracts from *V. nigrum* (22), *Fructus cnidii* (12) and *A. catechu* (8) were strong (1·07185, 1·25707 and 1·32324 mg/cm^2 respectively), as judged by 24 h LD_{50}, but the petroleum ether and methanolic extracts from them had little or no lethal activity against house dust mite adults.

Potencies also varied according to exposure times and, as a whole, petroleum ether extracts worked faster than ethyl acetate extracts. Mortalities of *C. cassia* (1), *P. cablin* (2) and *A. sieboldii* (3) were higher than 80% after 1 h of treatment by direct contact, but at the same exposure time, ethyl acetate extracts from them only caused 6·39%, 25·05% and 20·65% mortality respectively. Furthermore, the acaricidal activity of these extracts was dose dependent.

Volatile essential oils from the medicines or corresponding medicinal plants, such as clove bud oil (Kim *et al.* 2003) and *Cnidium* rhizome preparation (Kwon and Ahn, 2002*a*) exhibit fumigant activity against adult *D. farinae*. In this paper, the fumigant activity of extracts was also tested using a vapour-phase toxicity bioassay. These 5 petroleum ether preparations and 3 ethyl acetate preparations were much more effective against *D. farinae* adults in closed containers than in open containers. These results indicate that the mode of delivery of these preparations was largely due to action in the vapor phase: they may be toxic when they penetrate the dust mite body via the respiratory system. However, the exact acaricidal mode of action remains unknown.

In conclusion, petroleum ether extracts from the bark of *C. cassia* (1), *E. caryophyllata* (5) and *P. cablin* (2), ethyl acetate extracts from *C. cassia* (1), *P. cablin* (2) and *A. sieboldii* (3), and methanolic extracts from *P. cablin* (2), *E. caryophyllata* (5) and *S. sessilifolia* (21) emerged as potential TCM-derived agents that provide promising results against the house dust mite, *D. farinae*, in our comprehensive screening. These studies suggest that some TCM preparations described herein may be useful as potentially effective, environmentally acceptable, inexpensive, simple and alternative acaricides. Further research is, however, necessary on the safety issues of these TCM on human health and insecticidal constituents of these extracts.

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