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Evaluation of benzaldehyde derivatives from *Morinda officinalis* as anti-mite agents with dual function as acaricide and mite indicator

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Severe fever with thrombocytopenia syndrome (SFTS) is an emerging infectious disease caused by SFTS virus with 12–30% fatality rate. Despite severity of the disease, any medication or treatment for SFTS has not developed yet. One approach to prevent SFTS spreading is to control the arthropod vector carrying SFTS virus. We report that 2-methylbenzaldehyde analogues from *M. officinalis* have a dual function as acaricide against *Dermatophagoides* spp. and *Haemaphysalis longicornis* and indicator (color change) against *Dermatophagoides* spp. Based on the LD₅₀ values, 2,4,5-trimethylbenzaldehyde (0.21, 0.19, and 0.68 µg/cm³) had the highest fumigant activity against *D. farinae*, *D. pteronyssinus*, and *H. longicornis*, followed by 2,3-dimethylbenzaldehyde (0.46, 0.44, and 0.79 µg/cm³), 2,4-dimethylbenzaldehyde (0.66, 0.59, and 0.95 µg/cm³), 2,5-dimethylbenzaldehyde (0.65, 0.68, and 0.88 µg/cm³), 2-methylbenzaldehyde (0.95, 0.87, and 1.28 µg/cm³), 3-methylbenzaldehyde (0.99, 0.93, and 1.38 µg/cm³), 4-methylbenzaldehyde (1.17, 1.15, and 3.67 µg/cm³), and *M. officinalis* oil (7.05, 7.00, and 19.70 µg/cm³). Furthermore, color alteration of *Dermatophagoides* spp. was shown to be induced, from colorless to dark brown, by the treatment of 2,3-dihydroxybenzaldehyde. These finding indicated that 2-methylbenzaldehyde analogues could be developed as functional agent associated with the arthropod vector of SFTS virus and allergen.

Appearance of new infectious diseases creates serious health threat to the world. Ebola and Severe fever with thrombocytopenia syndrome are the typical example of new infectious diseases, which were recently discovered in West Africa and Northeast Asia¹. The SFTS is characterized by fever, leukocytopenia, respiratory problems, gastrointestinal symptoms, and thrombocytopenia¹. The etiological agent of SFTS is SFTS virus, a novel bunyavirus, which is carried by a tick, *Haemaphysalis longicornis*. Although the prevalence of SFTS is rare, the disease is a serious disease in which fatality rate range from 12 to 30%². However, there has not been developed any medication or treatment for SFTS yet. More seriously, the population of *H. longicornis* is increasing because of global warming², suggesting that SFTS could threaten the world near future.

House dust mites, such as *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*, play directly as a strong allergen to cause a number of allergic diseases, of which atopic dermatitis and allergic rhinitis are the most prevalent diseases¹. Although changes in housing environments with local heating, repeated ventilation, and limited use of fitted carpets are a solution to control acari, which are the subclass of ticks and mites, the control of acari by improvement of house conditions has a definite limitation³. Therefore, there is urgent demand for development of an agent controlling acari effectively to prevent allergic diseases and diseases spread by acari including SFTS.

Traditional control methods have been performed using many approaches, such as the following: (1) implementation of exceptional cleaning standards; (2) improvement of habitat of mites and ticks; and (3) use of synthetic acaricides, such as avermectin, γ-benzene hexachloride, chlopyrifos-methyl, DEET, fenitrothion, pirimiphos-methyl, etc^{4–6}. In spite of the outstanding effects of these synthetic acaricides against mites and ticks, their repeated treatment has resulted in the occurrence of resistance, environmental impact, and side effects in non-target organisms⁷. For example, Van Leeuwen et al.⁸ had reported that avermectin in mites acts on glutamate-gated chloride channels and γ-aminobutyric acid (GABA). Resistance of avermectin causes an increase of

excretion and decreases of absorption with conjugated compound⁸. In light of these problems, there is a need for the development of alternatives for the control of mites and ticks, such as more efficient and safer chemicals of natural origin⁹.

Plants have been found as prospective alternatives to a number of chemicals because of the abundance of materials designated as traditional herbal medicine^{10–11}. *Morinda officinalis* How (family Rubiaceae), cultivated in subtropical and tropical regions, has been used in traditional herbal medicines and nutrient supplements for more than 2,000 years^{12–13}. To date, various biological activities of *M. officinalis* roots have been reported, including antiosteoporosis¹⁴, antifatigue¹⁵, antioxidant¹⁶, hypoglycemic¹⁷, and antidepressant agents¹⁷. In addition, various chemicals have been found in *M. officinalis* roots, including anthraquinone, carbohydrate, iridoid glucoside, iridoid lactone, rotungenic, and β -sitosterol¹⁷. These findings indicated that *M. officinalis* roots should have important pharmacological properties^{14–18}. In this regard, the potential ability of *M. officinalis* roots to act as a natural acaricide against mites and ticks was assessed in the present study.

Results and Discussion

Essential oil was extracted from *M. officinalis* roots with a yield of 0.03%. To determine the acaricidal potential, the acaricidal activity of *M. officinalis* oil was evaluated using the contact and the fumigant bioassays against *Dermatophagoides* spp. and *H. longicornis*. Compared with the LD₅₀ values of DEET, the acaricidal activity of *M. officinalis* oil (7.05, 7.00, and 19.70 $\mu\text{g}/\text{cm}^3$) proved to be more toxic than that of DEET (36.50, 34.23, and 52.03 $\mu\text{g}/\text{cm}^3$) against *D. farinae*, *D. pteronyssinus*, and *H. longicornis* in the fumigant assay, respectively (Table 1). In case of the contact bioassay, the acaricidal effect of *M. officinalis* oil (5.57, 5.00, and 15.48 $\mu\text{g}/\text{cm}^2$) was also more potent than that of DEET (19.64, 14.12, and 47.77 $\mu\text{g}/\text{cm}^2$) against *D. farinae*, *D. pteronyssinus*, and *H. longicornis*, respectively (Table 1). The negative treatment, injection of acetone alone, did not result in the death of dust mites and ticks in the fumigant and the contact bioassays. The acaricidal potential of *M. officinalis* oil depends on the species of mites, chemicals, and biological conditions¹⁹. Thus, *M. officinalis* oil has the potential ability as a natural acaricide against *Dermatophagoides* spp. and *H. longicornis*.

The *M. officinalis* oil was analyzed by GC–MS, and the retention index, retention time, and mass spectra of each compound were compared to those reported in the literature²⁰. A list of the constituents from *M. officinalis* oil is provided in Table 2. The relative composition (%) of the constituents of *M. officinalis* oil was 1-allyl-4-methoxybenzene (2.42%), 1,2-benzenedicarboxylic acid (4.54%), γ -butyrolactone (2.74%), hexadecanoic acid (6.47%), hexanoic acid (2.42%), 2-methylanthraquinone (8.00%), 2-

methylbenzaldehyde (31.97%), 8-methylundecene (2.94%), myristaldehyde (6.53%), 1,3,12-nonadecatriene (2.63%), nonanoic acid (4.27%), 9,17-octadecadienal (2.09%), paeonol (11.26%), and γ -stearolactone (2.20%). The constituents of *M. officinalis* oil were grouped as acids (1,2-benzenedicarboxylic acid, hexadecanoic acid, hexanoic acid, and nonanoic acid), aldehydes (2-methylbenzaldehyde and myristaldehyde), alkenes (8-methylundecene, 1,3,12-nonadecatriene, and 9,17-octadecadienal), benzenes (1-allyl-4-methoxybenzene), lactones (γ -butyrolactone and γ -stearolactone), phenols (paeonol), and quinones (2-methylanthraquinone). According to Yong-tao (2009)²¹, the volatile components of *M. officinalis* oil were borneol, diisobutyl phthalate, linoleic acid, 3-methylbenzaldehyde, and oleic acid²². In the present and previous studies, the constituents of *M. officinalis* oil were influenced by the environmental conditions, including the handling method, harvest time, intraspecific variability, storage period, and the experimental conditions, which included the method of extraction and the parts of the plant extracted²⁰.

To isolate the active constituent of *M. officinalis* oil, various chromatographic analyses were conducted using column chromatography and liquid chromatography. The MO322 fraction was finally isolated from *M. officinalis* oil, and the structure was identified by spectroscopic analyses including ¹H-NMR, ¹³C-NMR, and DEPT-NMR spectra. The isolated MO322 fraction was characterized as 2-methylbenzaldehyde (Figure 1) (C₈H₈O); EI-MS (70 eV) m/z M⁺ 120.06; ¹H-NMR (CDCl₃, 400 MHz) δ 2.48 (s, 3H), 7.26–7.44 (d, J = 7.2 Hz, 1H), 7.45–7.59 (t, J = 5.6 Hz, 1H), 7.61–7.75 (t, J = 5.6 Hz, 1H), 7.77–7.90 (d, J = 5.2 Hz, 1H), and 10.36 (s, 1H); ¹³C-NMR and DEPT-NMR (CDCl₃, 100 MHz) δ 18.6 (CH₃), 191.0 (CH), 126.2 (CH), 131.9 (CH), 131.9 (CH), 134.4 (C), 134.4 (CH), 139.6 (C). The findings of 2-methylbenzaldehyde were compared with those of a previous study²³. The acaricidal activity of 2-methylbenzaldehyde isolated from *M. officinalis* oil was evaluated using the contact and the fumigant bioassays against *Dermatophagoides* spp. and *H. longicornis* and compared with that of DEET. The LD₅₀ values of 2-methylbenzaldehyde in the fumigant bioassay were 0.95, 0.87, and 1.28 $\mu\text{g}/\text{cm}^3$ against *D. farinae*, *D. pteronyssinus*, and *H. longicornis*, respectively (Table 3). In the contact bioassay, the LD₅₀ values of 2-methylbenzaldehyde were observed to be 0.51, 0.47, and 0.94 $\mu\text{g}/\text{cm}^2$ against *D. farinae*, *D. pteronyssinus*, and *H. longicornis*, respectively (Table 4). In comparison with the LD₅₀ values of DEET in the fumigant bioassay, 2-methylbenzaldehyde was approximately 38.58, 39.30, and 40.58 times more effective than DEET (36.50, 34.23, and 52.03 $\mu\text{g}/\text{cm}^3$) against *D. farinae*, *D. pteronyssinus*, and *H. longicornis* (Table 3). In the contact bioassay, it was about 38.48, 30.04, and 50.60 times more toxic than DEET (19.64, 14.12, and 47.77 $\mu\text{g}/\text{cm}^2$) against *D. farinae*, *D. pteronyssinus*, and *H. longicornis* (Table 4). In this regard, the acaricidal activity of 2-methylbenzaldehyde was much more potent than that of DEET, a

Table 1 | Acaricidal activities of *M. officinalis* oil and acaricide against *Dermatophagoides* spp. and *H. longicornis*¹

Samples	Bioassay	Species	LD ₅₀ $\pm$ SE	95% CL	RT ²
<i>M. officinalis</i> oil	Fumigant ($\mu\text{g}/\text{cm}^3$)	<i>D. farinae</i>	7.05 $\pm$ 0.11	6.84–7.24	5.18
		<i>D. pteronyssinus</i>	7.00 $\pm$ 0.04	6.87–7.13	4.89
		<i>H. longicornis</i>	19.70 $\pm$ 0.11	19.63–19.77	2.64
	Contact ($\mu\text{g}/\text{cm}^2$)	<i>D. farinae</i>	5.57 $\pm$ 0.04	5.50–5.64	3.53
		<i>D. pteronyssinus</i>	5.00 $\pm$ 0.06	4.95–5.06	2.82
		<i>H. longicornis</i>	15.48 $\pm$ 0.12	15.42–15.55	3.09
DEET	Fumigant ($\mu\text{g}/\text{cm}^3$)	<i>D. farinae</i>	36.50 $\pm$ 0.12	36.44–36.55	1.00
		<i>D. pteronyssinus</i>	34.23 $\pm$ 0.03	34.18–34.29	1.00
		<i>H. longicornis</i>	52.03 $\pm$ 0.07	51.99–52.07	1.00
	Contact ($\mu\text{g}/\text{cm}^2$)	<i>D. farinae</i>	19.64 $\pm$ 0.05	19.63–19.66	1.00
		<i>D. pteronyssinus</i>	14.12 $\pm$ 0.16	14.11–14.13	1.00
		<i>H. longicornis</i>	47.77 $\pm$ 0.04	47.72–47.82	1.00

¹Exposed for 24 h.

²RT, Relative toxicity = LD₅₀ value of DEET/LD₅₀ value of each compound.

Table 2 | Analysis of various constituents from *M. officinalis* oil identified by GC–MS

Retention Time	Constituents	Retention Index ¹		
		DB–5	HP–Innowax	Relative composition (%)
5.56	Hexanoic acid	974	1005	2.42
8.94	2-Methylbenzaldehyde	1095	1128	31.97
10.70	Nonanoic acid	1272	1301	4.27
11.13	1-Allyl-4-methoxybenzene	1190	1224	2.42
11.97	8-Methylundecene	1140	1168	2.94
12.27	γ -Butyrolactone	1284	1313	2.74
13.48	Paeonol	1439	1472	11.26
16.81	Myristaldehyde	1601	1633	6.53
18.81	9,17-Octadecadienal	1997	2035	2.09
19.51	Hexadecanoic acid	1968	1999	6.47
20.90	1,3,12-Nonadecatriene	1916	1948	2.63
21.06	γ -Stearolactone	2178	2214	2.20
21.36	2-Methylanthraquinone	2069	2101	8.00
25.00	1,2-Benzenedicarboxylic acid	2248	2284	4.54

¹Retention Index, Kovats index of retention.

commercial acaricide, against *D. farinae*, *D. pteronyssinus*, and *H. longicornis*.

To establish the structural relationships between 2-methylbenzaldehyde analogues and acaricidal activities against *Dermatophagoides* spp. and *H. longicornis*, 2,3-dihydroxybenzaldehyde, 2,5-dihydroxybenzaldehyde, 2,4-dihydroxybenzaldehyde, 2,3-dimethylbenzaldehyde, 2,4-dimethylbenzaldehyde, 2,5-dimethylbenzaldehyde, 2-hydroxybenzaldehyde, 3-hydroxybenzaldehyde, 4-hydroxybenzaldehyde, 3-methylbenzaldehyde, 4-methylbenzaldehyde, 2,4,5-trihydroxybenzaldehyde, and 2,4,5-trimethylbenzaldehyde were selected as 2-methylbenzaldehyde analogues for testing (Figure 1). The acaricidal activities of 2-methylbenzaldehyde analogues were evaluated using the contact and the fumigant bioassays against *D. farinae*, *D. pteronyssinus*, and *H. longicornis*. In the fumigant bioassay (Table 3), the acaricidal activities of 2,4,5-trimethylbenzaldehyde (0.21, 0.19, and 0.68 $\mu\text{g}/\text{cm}^3$) were approximately 178.02, 178.30, and 76.07 times more toxic than those of DEET (36.50, 34.23, and 52.03 $\mu\text{g}/\text{cm}^3$) against *D. farinae*, *D. pteronyssinus*, and *H. longicornis*, followed by 2,3-dimethylbenzaldehyde (0.46, 0.44, and 0.79 $\mu\text{g}/\text{cm}^3$), 2,4-dimethylbenzaldehyde (0.66, 0.59, and 0.95 $\mu\text{g}/\text{cm}^3$), 2,5-dimethylbenzaldehyde (0.65, 0.68, and 0.88 $\mu\text{g}/\text{cm}^3$), 2-methylbenzaldehyde (0.95, 0.87, and 1.28 $\mu\text{g}/\text{cm}^3$), 3-methylbenzaldehyde (0.99, 0.93, and 1.38 $\mu\text{g}/\text{cm}^3$), and 4-methylbenzaldehyde (1.17, 1.15, and 3.67 $\mu\text{g}/\text{cm}^3$). In the contact bioassay (Table 4), the acaricidal effects of 2,4,5-trimethylbenzaldehyde (0.12, 0.19, and 0.46 $\mu\text{g}/\text{cm}^2$) were about 170.99, 73.53, and 103.61 times more active than those of DEET (19.64, 14.12, and 47.77 $\mu\text{g}/\text{cm}^2$) against *D.*

farinae, *D. pteronyssinus*, and *H. longicornis*, followed by 2,3-dimethylbenzaldehyde (0.25, 0.24, and 0.56 $\mu\text{g}/\text{cm}^2$), 2,4-dimethylbenzaldehyde (0.32, 0.32, and 0.62 $\mu\text{g}/\text{cm}^2$), 2,5-dimethylbenzaldehyde (0.35, 0.36, and 0.74 $\mu\text{g}/\text{cm}^2$), 2-methylbenzaldehyde (0.51, 0.47, and 0.94 $\mu\text{g}/\text{cm}^2$), 3-methylbenzaldehyde (0.53, 0.50, and 1.02 $\mu\text{g}/\text{cm}^2$), and 4-methylbenzaldehyde (0.63, 0.61, and 1.84 $\mu\text{g}/\text{cm}^2$). In contrast, 2,3-dihydroxybenzaldehyde, 2,4-dihydroxybenzaldehyde, 2,5-dihydroxybenzaldehyde, 2-hydroxybenzaldehyde, 3-hydroxybenzaldehyde, 4-hydroxybenzaldehyde, and 2,4,5-trihydroxybenzaldehyde did not show the acaricidal activity against *D. farinae*, *D. pteronyssinus*, or *H. longicornis* in the contact and the fumigant bioassays. Comparison of the LD₅₀ values from the contact and the fumigant bioassays against *D. farinae*, *D. pteronyssinus*, and *H. longicornis* revealed that the acaricidal activities of the structural analogues containing a methyl (CH₃) functional group (2,3-dimethylbenzaldehyde, 2,4-dimethylbenzaldehyde, 2,5-dimethylbenzaldehyde, 2-methylbenzaldehyde, 3-methylbenzaldehyde, 4-methylbenzaldehyde, and 2,4,5-trimethylbenzaldehyde) were more potent than those of structural analogues containing a hydroxyl (OH) functional group (2,3-dihydroxybenzaldehyde, 2,4-dihydroxybenzaldehyde, 2,5-dihydroxybenzaldehyde, 2-hydroxybenzaldehyde, 3-hydroxybenzaldehyde, 4-hydroxybenzaldehyde, 2,4,5-trihydroxybenzaldehyde). In particular, the number of methyl functional groups influenced the acaricidal activity against *D. farinae*, *D. pteronyssinus*, and *H. longicornis*. These observations are similar with the results in the study by Oh et al.¹⁸ in which 2'-methylacetophenone, 3'-methylacetophenone, and 4'-methylacetophenone were

Figure 1 | Structure–activity relationships of 2-methylbenzaldehyde analogues. (a) 2-Methylbenzaldehyde; (b) 3-Methylbenzaldehyde; (c) 4-Methylbenzaldehyde; (d) 2-Hydroxybenzaldehyde; (e) 3-Hydroxybenzaldehyde; (f) 4-Hydroxybenzaldehyde; (g) 2,3-Dimethylbenzaldehyde; (h) 2,4-Dimethylbenzaldehyde; (i) 2,5-Dimethylbenzaldehyde; (j) 2,3-Dihydroxybenzaldehyde; (k) 2,4-Dihydroxybenzaldehyde; (l) 2,5-Dihydroxybenzaldehyde; (m) 2,4,5-Trimethylbenzaldehyde; (n) 2,4,5-Trihydroxybenzaldehyde.

Table 3 | Acaricidal activities of 2-methylbenzaldehyde analogues and commercial acaricide against *Dermatophagoides* spp. and *H. longicornis*, using a fumigant bioassay¹

Compounds	Species	LD ₅₀ ± SE (μg/cm ³)	95% CL	RT ²
2-Methylbenzaldehyde	<i>D. farinae</i>	0.95 ± 0.04	0.89–1.01	38.58
	<i>D. pteronyssinus</i>	0.87 ± 0.02	0.84–0.90	39.30
	<i>H. longicornis</i>	1.28 ± 0.12	1.19–1.38	40.58
3-Methylbenzaldehyde	<i>D. farinae</i>	0.99 ± 0.06	0.91–1.07	36.86
	<i>D. pteronyssinus</i>	0.93 ± 0.02	0.90–0.97	36.69
	<i>H. longicornis</i>	1.38 ± 0.15	1.33–1.43	37.62
4-Methylbenzaldehyde	<i>D. farinae</i>	1.17 ± 0.07	1.07–1.27	31.19
	<i>D. pteronyssinus</i>	1.15 ± 0.02	1.12–1.18	29.79
	<i>H. longicornis</i>	3.67 ± 0.05	3.62–3.71	14.19
2-Hydroxybenzaldehyde	<i>D. farinae</i>	1.32 ± 0.14	1.12–1.52	27.63
	<i>D. pteronyssinus</i>	1.27 ± 0.07	1.17–1.37	26.98
	<i>H. longicornis</i>	— ³	—	—
3-Hydroxybenzaldehyde	<i>D. farinae</i>	—	—	—
	<i>D. pteronyssinus</i>	—	—	—
	<i>H. longicornis</i>	—	—	—
4-Hydroxybenzaldehyde	<i>D. farinae</i>	—	—	—
	<i>D. pteronyssinus</i>	—	—	—
	<i>H. longicornis</i>	—	—	—
2,3-Dimethylbenzaldehyde	<i>D. farinae</i>	0.46 ± 0.02	0.45–0.48	78.99
	<i>D. pteronyssinus</i>	0.44 ± 0.02	0.41–0.47	77.80
	<i>H. longicornis</i>	0.79 ± 0.15	0.73–0.85	65.78
2,4-Dimethylbenzaldehyde	<i>D. farinae</i>	0.66 ± 0.08	0.56–0.76	55.30
	<i>D. pteronyssinus</i>	0.59 ± 0.03	0.55–0.63	58.02
	<i>H. longicornis</i>	0.95 ± 0.05	0.87–1.02	55.00
2,5-Dimethylbenzaldehyde	<i>D. farinae</i>	0.65 ± 0.05	0.58–0.71	56.58
	<i>D. pteronyssinus</i>	0.68 ± 0.10	0.54–0.82	50.34
	<i>H. longicornis</i>	0.88 ± 0.12	0.83–0.93	58.92
2,3-Dihydroxybenzaldehyde	<i>D. farinae</i>	7.53 ± 0.30	7.11–7.95	4.85
	<i>D. pteronyssinus</i>	6.69 ± 0.59	5.87–7.51	5.12
	<i>H. longicornis</i>	—	—	—
2,4-Dihydroxybenzaldehyde	<i>D. farinae</i>	—	—	—
	<i>D. pteronyssinus</i>	—	—	—
	<i>H. longicornis</i>	—	—	—
2,5-Dihydroxybenzaldehyde	<i>D. farinae</i>	—	—	—
	<i>D. pteronyssinus</i>	—	—	—
	<i>H. longicornis</i>	—	—	—
2,4,5-Trimethylbenzaldehyde	<i>D. farinae</i>	0.21 ± 0.02	0.18–0.23	178.02
	<i>D. pteronyssinus</i>	0.19 ± 0.01	0.18–0.20	178.30
	<i>H. longicornis</i>	0.68 ± 0.09	0.64–0.73	76.07
2,4,5-Trihydroxybenzaldehyde	<i>D. farinae</i>	—	—	—
	<i>D. pteronyssinus</i>	—	—	—
	<i>H. longicornis</i>	—	—	—
DEET	<i>D. farinae</i>	36.50 ± 0.12	36.44–36.55	1.00
	<i>D. pteronyssinus</i>	34.23 ± 0.03	34.18–34.29	1.00
	<i>H. longicornis</i>	52.03 ± 0.06	51.99–52.07	1.00

¹Exposed for 24 h.

²RT, Relative toxicity = LD₅₀ value of DEET/LD₅₀ value of each compound.

³—, no activity.

observed to have potent activities while 2',4'-dihydroxyacetophenone and 2',6'-dihydroxyacetophenone possessed no activities against *D. farinae* and *D. pteronyssinus*. According to Lee and Lee²², the acaricidal effects of 2-methyl-1,4-naphthoquinone, which has a methyl functional group added onto 1,4-naphthoquinone, were more toxic against *D. farinae* and *D. pteronyssinus* than those of 2-hydroxy-1,4-naphthoquinone, containing a hydroxyl functional group on 1,4-naphthoquinone.

Color alterations of *Dermatophagoides* spp. were observed through an optical microscope when before and after treatment with *M. officinalis* oil and 2-methylbenzaldehyde structural analogues. In particular, *Dermatophagoides* spp. treated with *M. officinalis* oil and 2,3-dihydroxybenzaldehyde presented with skin discoloration to a dark brown color in the body, while the untreated mites were colorless (Figure 2). The color change of the mites treated with *M. officinalis* oil and 2,3-dihydroxybenzaldehyde allowed *D. farinae* and *D.*

pteronyssinus to be distinguish with the naked eye. According to a previous study, complete elimination is actually impossible due to residual mite excrement and dead mites²⁴. In addition, a vicious cycle of the treatments of the commercial acaricides was continued. These problems have emphasized the demand for the development of new technologies to control the allergens caused by *Dermatophagoides* spp. In this regard, the mite indicator in this study is valuable because of the color alteration of *Dermatophagoides* spp. induced by *M. officinalis* and 2,3-dihydroxybenzaldehyde. For these reasons, a new concept for natural acaricides was found in the present study, involving both the acaricidal activity and the mite indicator against *Dermatophagoides* spp. The discoloration is likely to be related to the benzene ring metabolisms of the defense mode of action in plants^{22,25}. A similar reaction was previously observed in the whole body of dust mites treated with quinone²². Lee and Lee reported that polyphenol oxidase catalyzed two reactions: hydroxylation and

Table 4 | Acaricidal activities of 2-methylbenzaldehyde analogues and commercial acaricide against *Dermatophagoides* spp. and *H. longicornis*, using a contact bioassay¹

Compounds	Species	LD ₅₀ ± SE (μg/cm ²)	95% CL	RT ²
2-Methylbenzaldehyde	<i>D. farinae</i>	0.51 ± 0.02	0.48–0.54	38.48
	<i>D. pteronyssinus</i>	0.47 ± 0.01	0.45–0.49	30.04
	<i>H. longicornis</i>	0.94 ± 0.12	0.92–0.97	50.60
3-Methylbenzaldehyde	<i>D. farinae</i>	0.53 ± 0.03	0.49–0.57	37.03
	<i>D. pteronyssinus</i>	0.50 ± 0.01	0.49–0.52	28.07
	<i>H. longicornis</i>	1.02 ± 0.14	0.96–1.09	46.69
4-Methylbenzaldehyde	<i>D. farinae</i>	0.63 ± 0.04	0.58–0.68	31.13
	<i>D. pteronyssinus</i>	0.61 ± 0.01	0.60–0.62	23.26
	<i>H. longicornis</i>	1.84 ± 0.06	1.80–1.89	25.90
2-Hydroxybenzaldehyde	<i>D. farinae</i>	0.70 ± 0.06	0.62–0.78	28.13
	<i>D. pteronyssinus</i>	0.71 ± 0.08	0.61–0.82	19.80
	<i>H. longicornis</i>	— ³	—	—
3-Hydroxybenzaldehyde	<i>D. farinae</i>	—	—	—
	<i>D. pteronyssinus</i>	—	—	—
	<i>H. longicornis</i>	—	—	—
4-Hydroxybenzaldehyde	<i>D. farinae</i>	—	—	—
	<i>D. pteronyssinus</i>	—	—	—
	<i>H. longicornis</i>	—	—	—
2,3-Dimethylbenzaldehyde	<i>D. farinae</i>	0.25 ± 0.01	0.24–0.26	78.03
	<i>D. pteronyssinus</i>	0.24 ± 0.01	0.22–0.26	59.07
	<i>H. longicornis</i>	0.56 ± 0.15	0.50–0.63	84.69
2,4-Dimethylbenzaldehyde	<i>D. farinae</i>	0.32 ± 0.01	0.30–0.34	61.07
	<i>D. pteronyssinus</i>	0.32 ± 0.01	0.30–0.34	44.26
	<i>H. longicornis</i>	0.62 ± 0.09	0.57–0.67	76.79
2,5-Dimethylbenzaldehyde	<i>D. farinae</i>	0.35 ± 0.02	0.31–0.38	56.83
	<i>D. pteronyssinus</i>	0.36 ± 0.05	0.30–0.43	39.00
	<i>H. longicornis</i>	0.74 ± 0.05	0.69–0.80	64.22
2,3-Dihydroxybenzaldehyde	<i>D. farinae</i>	3.79 ± 0.10	3.65–3.93	5.19
	<i>D. pteronyssinus</i>	3.45 ± 0.09	3.33–3.57	4.09
	<i>H. longicornis</i>	—	—	—
2,4-Dihydroxybenzaldehyde	<i>D. farinae</i>	—	—	—
	<i>D. pteronyssinus</i>	—	—	—
	<i>H. longicornis</i>	—	—	—
2,5-Dihydroxybenzaldehyde	<i>D. farinae</i>	—	—	—
	<i>D. pteronyssinus</i>	—	—	—
	<i>H. longicornis</i>	—	—	—
2,4,5-Trimethylbenzaldehyde	<i>D. farinae</i>	0.12 ± 0.01	0.10–0.13	170.99
	<i>D. pteronyssinus</i>	0.19 ± 0.02	0.18–0.20	73.53
	<i>H. longicornis</i>	0.46 ± 0.07	0.43–0.50	103.61
2,4,5-Trihydroxybenzaldehyde	<i>D. farinae</i>	—	—	—
	<i>D. pteronyssinus</i>	—	—	—
	<i>H. longicornis</i>	—	—	—
DEET	<i>D. farinae</i>	19.64 ± 0.05	19.63–19.66	1.00
	<i>D. pteronyssinus</i>	14.12 ± 0.16	14.11–14.13	1.00
	<i>H. longicornis</i>	47.77 ± 0.04	47.72–47.82	1.00

¹Exposed for 24 h.

²RT, Relative toxicity = LD₅₀ value of DEET/LD₅₀ value of each compound.

³—, no activity.

oxidation in the benzene ring metabolisms²¹. In addition, disease resistance in both insects and mites occurred due to the existence of polyphenol oxidase²⁶.

In the present study, acaricidal effects of *M. officinalis* oil and 2-methylbenzaldehyde structural analogues were found against *Dermatophagoides* spp. and *H. longicornis*. In particular, 2,4,5-trimethylbenzaldehyde had potent activity against *Dermatophagoides* spp. and *H. longicornis*. From a point of view, *M. officinalis* oil and 2-methylbenzaldehyde are the most promising because of the high sensitivity against *D. farinae*, *D. pteronyssinus*, and *H. longicornis*. Interestingly, skin color alteration of *Dermatophagoides* spp. was exhibited, changing from colorless to dark brown through the treatment with 2,3-dihydroxybenzaldehyde. In this regard, 2,3-dihydroxybenzaldehyde could be very useful to observe and remove the allergens.

In conclusion, this work showed that 2-methylbenzaldehyde analogues from *M. officinalis* oil have a dual function as acaricide and

acar indicator, meaning that these compounds could be developed as acaricidal agents. Especially, we believe that 2,3-dihydroxybenzaldehyde have a strong potential as an ideal acaricidal agent to control *Dermatophagoides* spp. and *H. longicornis* which are the vector of SFTS virus and allergen. In the registration process, considering the fact that *Morinda officinalis* is a very cheap plant which can be easily cultivated, the cost would not be a barrier for the commercial development of 2-methylbenzaldehyde isolated from *Morinda officinalis*.

Methods

Compounds. 2,3-Dihydroxybenzaldehyde (97%, Cat No. 189839), 2,4-dihydroxybenzaldehyde (98%, Cat No. 168637), 2,5-dihydroxybenzaldehyde (98%, Cat No. D108200) 2,3-dimethylbenzaldehyde (97%, Cat No. 515353), 2,4-dimethylbenzaldehyde (90%, Cat No. 151041), 2,5-dimethylbenzaldehyde (99%, Cat No. 151068), 2-hydroxybenzaldehyde (98%, Cat No. W300403), 3-hydroxybenzaldehyde (97%, Cat No. H19808), 4-hydroxybenzaldehyde (97%, Cat No. W398403), 3-methylbenzaldehyde (97%, Cat No. T35505), 4-

Figure 2 | Mite indicators against *D. farinae* and *D. pteronyssinus*. (a) untreated mites, (b) mites, treated with 2,3-dihydroxybenzaldehyde.

methylbenzaldehyde (97%, Cat No. T35602), 2,4,5-trihydroxybenzaldehyde (99%, Cat No. 526452), and 2,4,5-trimethylbenzaldehyde (99%, Cat No. 493864) were provided from Aldrich (St. Louis, MO, USA). DEET (95%, Cat No. 32570) was obtained from Fluka (Buchs, Switzerland).

Essential oil from *M. officinalis*. Dried roots of *M. officinalis* (4 kg) were obtained from a market (Jeonju city, Republic of Korea) and extracted using the steam distillation extraction method²⁷. The essential oil (1.2 g, yield 0.03%) of *M. officinalis* roots was concentrated by an evaporator (model name: NAJ-100, EYELA, Tokyo, Japan) at 27°C and stored at 4°C to prevent loss of the volatile constituents.

Gas chromatography–mass spectrometer. The volatile components of *M. officinalis* oil were analyzed by GC–MS (6890 and 5973 series, Agilent, USA) and were separated using DB–5 and HP–Innowax capillary columns (0.25 mm i.d. × 3,000 cm L. × 0.25 µm thickness)⁷. The initial temperature of the GC column was 50°C, which was gradually increased up to 210°C. The ion source temperature was 230°C. Helium gas as the carrier gas was flowed at a rate of 0.85 mL/min⁷. Effluents from GC column were introduced into the mass spectrometer. Mass spectra (*m/z*) were obtained in electron ionization (70 eV) set to scan a range of 50–600 amu for 2 seconds. The volatile components of *M. officinalis* oil were identified by retention index, retention time, and mass spectra, and were confirmed by comparison to the published mass spectra data²⁸. The relative composition (%) of volatile components was calculated by the standard of the internal amount.

Isolation and identification. Silica gel (70–230 mesh) was purchased from Merck (Rahway, NJ, USA). *M. officinalis* oil (12 g) was loaded onto a silica gel column (8 cm i.d. × 70 cm L.) and eluted gradually using a mixed organic solvent (hexane:ethyl acetate, gradient, v/v). The eluted fractions were separated by thin-layer chromatography and a UV lamp (at 264 nm) to obtain eight fractions (MO1–MO8). The acaricidal activities of the eight fractions were evaluated using the fumigant bioassay against *Dermatophagoides* spp. and *H. longicornis* at a concentration of 59.0 µg/cm². Fraction MO3 fraction (1.98 g, yield 16.5%) was found to possess potent activity against *Dermatophagoides* spp. and *H. longicornis*, and was chromatographed on a silica gel column using a mixed organic solvent (ethyl acetate : hexane = 1 : 9, v/v) to further separate into five fractions (MO31–MO35). Fraction MO32 (887 mg, yield 44.8%) was identified as the toxic fraction. Next, preparative high performance liquid chromatography (model name: LC-908, JAI Co., Ltd, Tokyo, Japan) was performed. Fraction MO32 was separated into three fractions (MO321–MO323) using a GS column (model name: GS 310, 2 cm i.d. × 50 cm L.) with methanol solvent as the mobile phase at a flow rate of 4.8 mL/min. Finally, the active compound (MO322, 130 mg, yield 14.7%) was isolated. The structure of the compound in MO322 fraction was identified by spectroscopic analyses.

The chemical structure of MO322 fraction isolated from *M. officinalis* oil was determined using nuclear magnetic resonance (NMR). To confirm the number of carbons (C) and protons (H), ¹³C-, ¹H-, and DEPT–NMR (model name: JNM-ECA600 spectrometer, JEOL Ltd., Tokyo, Japan) were implemented at 400 and 100 MHz. The MO322 was melted in CDCl₃, and tetramethylsilane was used as the internal standard. In addition, the molecular weight of the MO322 fraction was investigated using EI–MS (model name: JEOL GSX 400 mass spectrometer, JEOL Ltd., Tokyo, Japan) spectra.

Target. The rearing method of *D. farinae* and *D. pteronyssinus* modified by Yang and Lee was utilized³. The mites were reared without exposure to any synthetic acaricides. The feed consisted of fry powder and dried yeast (Korea special feed meal Co. Ltd., Jeonju, Republic of Korea) given in a rearing case (15 × 12 × 6 cm) at 25°C and 75% relative humidity (RH) in a dark area. The protein content of the fry powder was maintained above 49%. For experiments, the *Dermatophagoides* spp. mites were

placed in a petri dish (9 cm i.d. × 1.5 cm L.). *H. longicornis* ticks were collected around Jeonju River and were identified morphologically and genetically. However, the *H. longicornis* ticks were not maintained in our laboratory.

Acaricidal activity. The acaricidal effects of the oil, 2-methylbenzaldehyde, and its analogues were evaluated using the contact and the fumigant bioassays against *Dermatophagoides* spp. and *H. longicornis* through the method modified by Yang and Lee³. The experimental concentrations in the study were set to scan a range of 60.0–0.10 µg/cm². Each sample was dissolved in 50 µL acetone and then applied to filter paper (5.5 cm i.d. × 25 µm thickness, Whatman, UK). Acetone alone was applied as the negative control, and DEET was designated as the positive control. The residual solvent of each filter paper was dried under the fume hood for 14 min. In the contact bioassay, each piece was placed on the bottom of a petri dish (6 cm i.d. × 1.5 cm L.). In the case of the fumigant bioassay, a piece of filter paper was put in the lid of a petri dish (6 cm i.d. × 1.5 cm L.) and a thin cotton fabric was inserted to prevent contact of the inoculated mites with the applied filter paper. Each twenty experimental mites (*Dermatophagoides* spp. and *H. longicornis*), which included a mixture of males and females, were inoculated in each petri dish, and the lids were sealed. All treatments were repeated four times and incubated for 24 h at 25°C in the dark. Mortality was measured by the number of dead ticks and mites, which did not move when prodded with a pin, under an optical microscope (20×, Olympus, Japan).

Color alteration of mites. Color alterations to *D. farinae* and *D. pteronyssinus* exposed to 2-methylbenzaldehyde analogues were evaluated using a light microscope (100×), as described by Lee et al²⁵. Color alteration of the mites before and after treatment was compared.

Statistics. Mortalities in all the treatments were measured under an optical microscope (20×, Olympus, Japan). *D. farinae* and *D. pteronyssinus* were regarded to be dead when they did not move when touched with a pin. The LD₅₀ values were determined by probit analysis²⁹. Relative toxicity (RT) was calculated by the ratio of the commercial acaricide LD₅₀ value to the LD₅₀ value observed for each compound.

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Author contributions

J.-Y.Y. designed and carried out the experiments, prepared most of the data, and wrote the paper; M.-G.K. designed and carried out the experiments, prepared most of the data, and wrote the paper; J.-H.P. carried out the experiments for the bioassay and assisted in paper-writing; S.-T.H. wrote the paper; H.-S.L. proposed the key idea of this paper, designed the experiments, carried out color alteration by introduction of functional radicals, managed the research process, and wrote the paper.

Additional information

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