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Acaricidal Effects of Quinone and Its Congeners and Color Alteration of *Dermatophagoides* spp. with Quinone

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Abstract Acaricidal activity of the active constituent derived from *Pyrus ussuriensis* fruits against *Dermatophagoides farinae* and *D. pteronyssinus* was examined and compared with that of the commercial benzyl benzoate. The LD_{50} value of the ethyl acetate fraction obtained from the aqueous extract of *P. ussuriensis* fruits was 9.51 and 8.59 $\mu g/cm^3$ against *D. farinae* and *D. pteronyssinus*, respectively. The active constituent was identified as quinone by spectroscopic analyses. On the basis of LD_{50} values with quinone and its congeners, the compound most toxic against *D. farinae* was quinone (1.19 $\mu g/cm^3$), followed by quinaldine (1.46), benzyl benzoate (9.32), 4-quinolinol (86.55), quinine (89.16), and 2-quinolinol (91.13). Against *D. pteronyssinus*, these were quinone (1.02 $\mu g/cm^3$), followed by quinaldine (1.29), benzyl benzoate (8.54), 4-quinolinol (78.63), quinine (82.33), and 2-quinolinol (86.24). These results indicate that the acaricidal activity of the aqueous extracts can be mostly attributed to quinone. Quinone was about 7.8 and 8.4 times more toxic than benzyl benzoate against *D. farinae* and *D. pteronyssinus*. Additionally, quinaldine was about 6.4 and 6.6 times more toxic than benzyl benzoate against *D. farinae* and *D. pteronyssinus*, respectively. Furthermore, the skin color of the dust mites was changed from colorless-transparent to dark brown-black by the treatment of quinone. These results indicate that quinone can be very useful as potential control agents, lead compounds, or the indicator of house dust mites.

Keywords: Acaricide, *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, *Pyrus ussuriensis*, quinone, dust mite indicator

Changes in a living environment 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 the growth of dust mites [19]. The most important pyroglyphid mites are *Dermatophagoides pteronyssinus* (Trouessart) and *Dermatophagoides farinae* (Hughes), for the following three reasons: [19] Their cosmopolitan occurrence and abundance; [2] They are a major source of multiple potent allergens; [25] Their causal association with sudden infant death syndrome [2, 5, 19, 25]. Toward the development of diagnostics and a therapeutic vaccine, important dust mite allergens have been explored and are now classified as major house dust mite antigens [5, 7, 25]. Control of these mite populations has been principally through the use of chemicals such as benzyl benzoate and *N,N*-diethyl-*m*-toluamide [19]. Although effective, their repeated use has sometimes resulted in the widespread development of resistance, had undesirable effects on non-target organisms, and fostered environmental and human health concerns [6, 12, 19, 25]. These problems have highlighted the need for the development of new strategies for selective control of house dust mites.

Plant extracts or their constituents may provide an alternative to currently used mite-control agents against house dust mites [13, 15, 22]. Since many of them are largely free from adverse effects and have excellent biological actions [17], they could lead to the development of new classes of possibly safer mite-control agents [10, 12, 13, 16]. *Pyrus ussuriensis*, more commonly known as Chinese pear, is naturally inhabited in southeast Siberia, east China, and Korea [1]. In China, the pear has been considered not only a fruit but also a herbal medicine with antitussive, antiinflammatory, and diuretic effects [23]. Additionally, chemical studies have revealed that the pear contains various phenolic compounds such as chlorogenic acid, rutin, tannins, procyanidins, and arbutin [21, 23]. Phenolic compounds have recently aroused considerable interest because of their broad pharmacological activity as antioxidants or coloring factors in the fruit [9, 11, 13, 23, 26]. Although several reports have indicated that the stems and leaves of pear contain quinonic compounds as antimicrobial substances,

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other functions of quinonic compounds derived from the pear have not been investigated in detail. From this point of view, we evaluated the color alteration and acaricidal effect of the active component isolated from *P. ussuriensis* fruits against the house dust mites.

Chemicals and Plant Material

Benzyl benzoate was purchased from Aldrich (Milwaukee, WI, U.S.A.). Quinone, quinine, quinidine, quinaldine, 2-quinolinol, and 4-quinolinol were supplied by Sigma (St. Louis, MO, U.S.A.). All other chemicals were of reagent grade. The methanol and water used for the HPLC mobile phase were of HPLC grade, and those used for other purposes were of analytical grade. The fruits of *Pyrus ussuriensis* Max. (Family Rosaceae) were collected during fall, 2005, in Chonbuk Province, Korea.

Isolation and Identification

The fruits (10 kg) of *P. ussuriensis* were homogenized with a grinder after washing, and then extracted once with an ethanol-water (7:3, v/v) solvent system for 5 h in a heating mentle at 80°C. Aqueous extract was applied to filtration through filter paper (Toyo filter paper No. 2, Toyo Roshi, Japan) *in vacuo.* The filtrate was concentrated *in vacuo* at 50°C using a rotary vacuum evaporator (Model: N-3NW, EYELA, Japan) to yield about 4.2%. The aqueous extract (420 g) was sequentially partitioned into hexane (10 g), EtOAc (115 g), BuOH (101 g), and H_2O (194 g) portions for subsequent bioassay. The organic solvent portions were concentrated to dryness by rotary evaporation (EYELA Autojack NAJ-100 Japan) at 35°C, and the water portion was freeze-dried. The EtOAc portion (20 g) was chromatographed on a silica gel column (Merck 70–230 mash, 600 g, 5.5 i.d.× 68 cm) and successively eluted with a gradient step of EtOAc:MeOH (5:1 to 0:1, v/v) giving the six fractions (E1-E6). This step was repeated six times for the EtOAc portion (115 g). The bioactive E2 fraction (4.6 g) was rechromatographed on a silica gel column and successively eluted with EtOAc:MeOH (7:3, v/v). The column fractions were analyzed by TLC and fractions with similar TLC patterns were pooled. In this step, the six fractions (E21-E26) were obtained and bioassayed at 80 μg/cm^2. The active E25 fraction (512 mg) was purified by Prep. HPLC (Spectra System P2000, Thermo Separation Products) for separation of the biologically active constituent. The column was Asahipak ODP-50 (150 mm i.d.×4.6 mm, Waters), using EtOAc:MeOH (75:25) at a flow rate of 0.5 ml/min and detection at 250 nm. In this step, the five fractions (E251-E255) were obtained and bioassayed at 80 μg/cm^2. The active E253 fraction (347 mg) was rechromatographed under the same condition. Finally, an active compound (264 mg) with a retention time of 32.5 min was isolated. The structure of the active isolate was determined by instrumental analysis. ^{1}H and ^{13}C NMR spectra were recorded in deuteriochloroform with a JNM-LA 400F7 spectrometer, at 400 and 100 MHz (TMS as an internal standard), respectively, and chemical shifts are given in δ (parts per million). UV spectra were obtained in methanol with a Jasco V-550 spectrometer, and EI-MS spectra on a JEOL GSX 400 spectrometer.

Dust Mites and Bioassay

Cultures of *D. farinae* and *D. pteronyssinus* were maintained for 5 years without exposure to any known acaricide. They were reared in plastic containers (15 cm×12 cm×6 cm) containing 30 g of sterilized diet (fry feed No. 1/dried yeast, 1:1 by weight) at 25±1°C and 75% relative humidity in the dark. The fry feed (Miropa) was purchased from Korea Special Feed Meal Co. Ltd., Chonju, South Korea. Mites were observed with a video microscope (pictured by ICS-305B video microscope system (100×) sometch, Seoul, Korea) for the following parameters: walking/active, slow movement, no movement/dead, and discoloration.

An impregnated fabric disc bioassay was used for acaricidal activity of test materials. Amounts (150, 100, 80, 40, 20, 10, 5, 2.0, 1.0, 0.5, and 0.2 μg/cm^3) of each test material dissolved in 100 μl of acetone were applied to discs of black cotton fabric (0.5 g, 5 cm diameter: 700 mesh). Control fabric discs received 20 μl of acetone. After the discs were dried in a fume hood (19°C) for 30 s, each piece was placed in the bottom of a Petri dish (5 cm diameter×1.2 cm). Then, 30 individuals of *D. farinae* (7–10 days old) and *D. pteronyssinus* (7–10 days old) were placed in each Petri dish and covered with a lid. Treated and control mites were held at 25±1°C and 75% relative humidity in the dark. Mortalities were determined 24 h after treatment under a binocular microscope (20×). Mites were considered to be dead if appendages did not move when prodded with a pin. All treatments were replicated three times. LC_{50} values were calculated by probit analysis [20]. The percentage mortality was determined and transformed to arcsine square-root values for analysis of variance (ANOVA). Treatment means were compared and separated by Scheffe's test at P=0.05 (SAS Institute) [20, 21].

When the aqueous extract of *P. ussuriensis* fruits was bioassayed by the impregnated fabric disc method, acaricidal activity was observed against *D. farinae* and *D. pteronyssinus* (Table 1). The dust mite species were equally susceptible, and the extract of *P. ussuriensis* fruits showed a clear dose-response relationship for both species. Concentrations of 80 μg/cm^3 or greater caused complete mortality in both species. The acaricidal activity of the extract was compared with that of benzyl benzoate against *D. farinae* and *D. pteronyssinus* adults (Table 1). The commonly used synthetic acaricide, benzyl benzoate, served as positive control for acaricidal activity. The LD_{50} values of the aqueous extract and the ethyl acetate fraction derived from the aqueous extract of *P. ussuriensis* fruits were 12.36 and 9.51 μg/cm^3

Table 1. Acaricidal activities of quinone, its congeners, and synthetic acaricide against *D. farinae* and *D. pteronyssinus*, using the impregnated fabric disc bioassay method.[a]

Compound	Mite species	LD_{50} mg/cm^3	95% Confidence limit	RT[b]
Aqueous extract	*D. farinae*	12.36	12.01–12.75	0.8
	D. pteronyssinus	11.19	10.89–11.47	0.8
Ethyl acetate fraction	*D. farinae*	9.51	9.43–9.92	1.0
	D. pteronyssinus	8.59	8.13–9.14	1.0
Quinone	*D. farinae*	1.19	0.99–1.24	7.8
	D. pteronyssinus	1.02	0.89–1.10	8.4
Quinine	*D. farinae*	89.16	86.80–90.20	0.1
	D. pteronyssinus	82.33	81.09–83.02	0.1
Quinidine	*D. farinae*	–	–	–
	D. pteronyssinus	–	–	–
Quinaldine	*D. farinae*	1.46	1.03–1.68	6.4
	D. pteronyssinus	1.29	1.12–1.48	6.6
2-Quinolinol	*D. farinae*	91.13	89.75–92.18	0.1
	D. pteronyssinus	86.24	85.89–87.89	0.1
4-Quinolinol	*D. farinae*	86.55	85.61–93.84	0.1
	D. pteronyssinus	78.63	77.75–79.15	0.1
Benzyl benzoate	*D. farinae*	9.32	8.79–9.92	1.0
	D. pteronyssinus	8.54	8.39–8.93	1.0

[a]Exposed for 24 h.
[b]Relative toxicity=LD_{50} value of benzyl benzoate/LD_{50} value of each chemical.

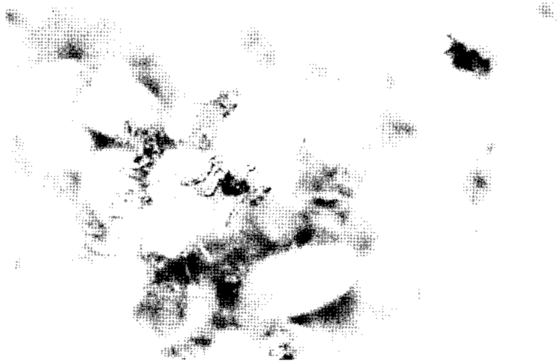
against *D. farinae* adults and 11.19 and 8.59 μg/cm^3 against *D. pteronyssinus* adults, respectively. On the basis of LC_{50} values, the ethyl acetate fraction was comparable to that of benzyl benzoate against *D. farinae* and *D. pteronyssinus* adults. There was no mortality in the untreated controls. This study is the first to report the acaricidal function of *P. ussuriensis* fruit-derived materials against *D. farinae* and *D. pteronyssinus* adults. Very little work has been done with respect to managing arthropod pests including house dust mite.

Owing to the potent activity of the ethyl acetate fraction from the aqueous extract of *P. ussuriensis* fruits, the isolation of the active component was pursued. Bioassay-guided fractionation of the ethyl acetate fraction afforded an active constituent identified by spectroscopic analyses, including EI-MS, and ^{13}C and ^{1}H NMR, by direct comparison with an authentic reference compound. The biologically active constituent was characterized as quinone. This compound was identified on the basis of the following evidence. Quinone as a yellow orange microcrystalline solid ($C_6H_4O_2$, MW: 108.09); EI-MS (70 eV) *m/z* (% relative intensity) M^+ 138 (2), 134 (1), 108 (100, base peak), 95 (2), 82 (40), 80 (28), 54 (90); ^{1}H NMR (CD_3OD, 400 MHz); δ 6.78 (6H, *s*), 6.78 (5H, *s*), 6.78 (3H, *s*), 6.78 (2H, *s*); ^{13}C NMR (CD_3OD, 100 MHz); δ 188.77, 137.67.

Quinone is well known as its metabolite is produced through phenolic metabolism [8, 13, 18, 21, 24]. In addition, quinone possesses the antibacterial activity causing melanin synthesis metabolism if tissues are injured [21]. Furthermore, its congeners (quinoline, quinaldine, quinidine, 4-quinolinol, and 2-quinolinol) also show various biological activities. In this regard, the acaricidal activities of quinone and its congeners were compared with that of benzyl benzoate against *D. farinae* and *D. pteronyssinus* by the impregnated fabric disc bioassay (Table 1). Responses varied according to compound and dose. On the basis of LD_{50} values, the compound most toxic against *D. farinae* was quinone (1.19 μg/cm^2), followed by quinaldine (1.46 μg/cm^3), benzyl benzoate (9.32 μg/cm^3), 4-quinolinol (86.55 μg/cm^3), quinine (89.16 μg/cm^3), and 2-quinolinol (91.13 μg/cm^3). Against *D. pteronyssinus*, these were quinone (1.02 μg/cm^3), followed by quinaldine (1.29 μg/cm^3), benzyl benzoate (8.54 μg/cm^3), 4-quinolinol (78.63 μg/cm^3), quinine (82.33 μg/cm^3), and 2-quinolinol (86.24 μg/cm^3). However, no activity was observed for quinidine at 80 μg/cm^3. These results indicate that the acaricidal activity of the aqueous extract from *P. ussuriensis* fruits can be mostly attributed to quinone. Furthermore, quinone was about 7.8 and 8.4 times more toxic than benzyl benzoate against *D. farinae* and *D. pteronyssinus*, respectively. Quinaldine was about 6.4 and 6.6 times more toxic than benzyl benzoate against *D. farinae* and *D. pteronyssinus*, respectively.

The color alteration of house dust mites between untreated (Fig. 1A) and treated (Fig. 1B) with quinone was studied through a microscope (100×). On the untreated discs the dust mites were colorless and transparent. After treatment with quinone, however, skin discoloration of dust mites occurred, exhibiting a dark brown-black color in the whole body. The color change of dust mites by the treatment of quinone allows to easily distinguish the dust

A. Untreated



B. Treated

Fig. 1. Color change of dust mites by quinone.

mites with the naked eyes. The allergens of house dust mites are caused not only by the dust mite themselves but also by their excrements, involving the mite eggs [3, 8]. Accordingly, the treatments of the common acaricides are often caught up in a vicious cycle, because it is actually impossible to remove allergens in the house. These problems have highlighted the need for the development of new strategies for the control of dust mite allergens. In this regard, our research for the indicator of dust mites is unique, owing to a color alteration of dust mites by quinone. For this reason, we need a new concept for the acaricides involved in both the indicator of dust mites and acaricidal activity. To the best of our knowledge, we could suggest quinone, having the ability to discolor dust mites and excellent acaricidal activity.

This discoloration is likely to be related the phenolic metabolism in plant defense reaction. In phenolic metabolism, polyphenol oxidase catalyzes two basic reactions: hydroxylation to the *o*-position adjacent to an existing hydroxyl group of the phenolic substrate (monophenol oxidase activity), and oxidation of diphenol to quinones (diphenol oxidase activity). Both reactions utilize molecular oxygen as a co-substrate. Diphenol oxidases have received much attention owing to their high catalytic rate and their association with the formation of quinones, which lead to production of the brown or dark pigment melanin [4, 11, 18, 21, 24]. A similar reaction may happen in a mite body with the treatment of quinone. Because polyphenol oxidase exists in both insects and mites as the propolyphenol oxidase form, it is thought to confer disease resistance in insects [4].

In this study, I have found a color change of dust mites and an acaricidal effect by the aqueous extracts from *P. ussuriensis* fruits against *D. farinae* and *D. pteronyssinus*. In particular, quinone had the highest acaricidal activity with a $LD_{50}\ll 1.5\ \mu g/cm^3$. Moreover, similar results have been exhibited with its congener (quinaldine). In contrast, little or no activity was observed for quinine, quinidine, 2-quinolinol, and 4-quinolinol. From this point of view, quinone and quinaldine are the most promising for the possible use against *D. farinae* and *D. pteronyssinus* owing to the low doses required to produce a high mortality in the dust mites. Interestingly, the skin color of dust mites was changed from colorless-transparent to dark brown-black by the treatment of quinone. These results indicate that quinone can be very useful in identifying and removing allergens. Our current data are just a first step in unraveling the complex mechanisms of discoloration of the dust mites by this compound. Further research should be done on the safety issues of this compound for human health, the discoloration mechanisms, and formulations to improving the acaricidal potency and stability.

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