ABSTRACT

The fungicidal activity of herbal-extracted lotions; KU Natural Miticide®, KU herbmalacide®, KU Thong-phan-chang®, KU Ta-Kai-Hom®, KU Ta-Kai-Kaeng®, Betel lotions, and 3% organic acids (acetic, citric, lactic, malic, oxalic, succinic and tartaric acids were in vitro tested against the Malassezia pachydermatitis yeast. Ten tubes of yeast growth on potato dextrose agar (PDA) were soaked with each lotion and shook for 30 seconds, poured the lotion out and left it dry for 90 min. The control was sterile water. The subculture of treated yeast were done twice at after dry and subsequently after incubation at 37°C for 2 weeks. The subculture yeast from the control tubes grew normally in both two subcultures. While the both two subculture from the KU Natural Miticide®, KU herbmalacide®, KU Thong-phan-chang®, KU Ta-Kai-Hom® and KU Ta-Kai-Kaeng® lotion-treated tubes did not grow, except in nine tubes of the first subculture from the KU herbfungusmite® and acetic acid lotion-treated tubes were no yeast growth. These effective lotions were two times diluted by Sabouraud dextrose broth containing yeast (2X10^5 colonies/ml), shook for 5 second, left contact for
5 min and poured on PDA. All of them showed no yeast growth, exception from acetic acid. This indicated that these KU herbal-extracted lotions might be fungicide for *M. pachydermatitis*.

Key words: *In vitro*, *Malassezia pachydermatitis*, Herbal-extracted, organic acids
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INTRODUCTION

The *Malassezia pachydermatitis* is a commensal, lipophilic yeast that is frequently isolated from the external ear canal and from the skin of about 50% healthy dogs. The excessive sebum and moisture skin and immunological factors enhance its multiplication and the dermatitis development. Erythema and pruritus are always present and keratoseborrheic disorders with scaling, crusting, alopecia and greasiness of the hair and skin to lichen-like skin on ear pinnae, lips, muzzle, neck, axillae, ventrum, inguinal area, peri-anal area and forearms, caudal thighs and feet (Reberg and Blakemore, 1999; Muse, 2000). The topical therapy is often used in combination or alternative with a systemic anti-fungal medication. Which the anti-fungal drugs are hepatotoxicity effects for long term treatments (Tilley and Smith, 2000; Plumb, 2002). Therefore, the safety of topical application drugs are high demand, especially the herbal-extracted products. The essential oils from *Cymbopogon citratus* and *C. nardus* exhibited potent antifungal properties on human and animal microflora (Koba, et al., 2003). The 1% concentration alcohol tincture of Betel leaves was effective against dermatophytes, *Candida albicans*, *Microsporum gypseum* and *Trichosporon beigelii* (Rahman et al., 2005). The KU Natural Miticide of our research for sarcoptic mange treatment (Chungsamarnyart, et al., 2003) had been shown the fungicidal activity against the *Microsporum canis*, *M. gypseum*, and *T. mentagrophyte in vitro* (Chungsamarnyart, et al., 2006). However, the fungicidal activity of the medicinal plants lotions and organic acids for *M. pachydermatitis* in dogs and cats has rarely reported.

MATERIALS AND METHODS

The *Malassezia pachydermatitis* was cultured in 150 test tubes containing potato dextrose agar and incubated them at 37°C for 1 weeks. Each ten tubes of *M. pachydermatitis* were soaked by KU Natural Miticide®, KU herbfungusmite®, KU herbmalacide®, KU Thong-phan-chang®, KU Ta-Kai-Hom® (Citronella glass oil), KU Ta-Kai-Kaeng® (Lemon glass oil), Betel leaf-extracted lotions, and 3% organic acids (acetic, citric, lactic, malic, oxalic, succinic and tartaric acids) lotions and gently shook for 30 seconds, poured the lotion out and left them drying at room temperature for 90 min. The control tubes were soaked by sterile distilled water. The subculture of the yeast detaching from agar of each treated tubes and control tubes were first done just after leaving them dry for 90 min. The second subculture were done after incubation at 37°C for 2 weeks. The yeast growth of each subculture
tubes were observed every week. The high effective lotions were diluted by Sabouraud dextrose broth (SDB) containing yeast (2×10^5 colonies/ml) in the same volume (0.2 ml), shook for 5 second and left contact for 5 min. Each tube of them was cultured on slant PDA tube (0.2 ml/tube) and observed the growth of yeast after 7 days.

RESULTS

The agar-detached of the *M. pachydermatitis* of each treated tube was cultured twice at after dry and subsequently after incubation at 37º C for 2 weeks. The both subcultures of yeast from the control tubes were grew normally (Figures 1a and 1b). While the both subcultures from the tubes of the KU Natural Miticide®, KU herbmalacide®, KU Thong-phan-chang®, KU Ta-Kai-Hom® and KU Ta-Kai-Kaeng® lotion-treated were no yeast growth in all of 10 tubes (Figures. 2a, 2b, 4a, 4b, 5a, 5b, 6a, 6b 7a and 7b). That meant 100% fungicidal activity against *M. pachydermatitis* in 90 min.

The first subculture of *M. pachydermatitis* from the KU herbfungusmite®, Betel leaf-extracted lotions, 3% acetic acid, oxalic and tartaric solution-treated tubes could not grow 9/10, 7/10, 9/10, 6/10, and 1/10 tubes as shown in figures 3a, 8a, 9a, 10a, 11a, respectively. That meant 90%, 70%, 90%, 60% and 10% fungicidal activity in 90 min of these lotions, respectively. But the second subculture of these treated tubes showed no growth of *M. pachydermatitis* from all of each 10 tubes (Figures. 3b, 8b, 9b, 10b, and 11b). That meant 100% fungicidal activity against *M. pachydermatitis* if the contact time were longer than 90 min.

The colonies of *M. pachydermatitis* in each tubes detached from an agar and could not re-grow after soaking with all lotions except in the sterile water-treated tubes having some re-growth of *M. pachydermatitis*.

The subculture of the *M. pachydermatitis* from the two times diluted-lotion tubes of the KU Natural Miticide®, KU herbfungusmite®, KU herbmalacide®, KU Thong-phan-chang®, KU Ta-Kai-Hom®, and KU Ta-Kai-Kaeng® showed no growth in all ten tubes. While the yeast from the 3% acetic acid-diluted solution tubes grew normally in all ten tubes.

DISCUSSION

The colonies of *M. pachydermatitis* in each tube was detached from an agar and could not re-grow after soaking with all lotions. It indicated that the *M. pachydermatitis* were non-survivability (fungicidal activity) or the cultured media were not properly for continuing growth (anti-fungal activity).

The *M. pachydermatitis* did not grow in the first subculture from the KU Natural Miticide®, KU herbmalacide®, KU Thong-phan-chang®, KU Ta-Kai-Hom® and KU Ta-Kai-Kaeng® lotion-treated
tubes. It indicated that these lotion-treated yeast were 100% non-survivability after soaking and drying for 90 min. While the first subculture from the KU Herbfungusmite® and 3% acetic lotion-treated tubes

*Figure 1-4.* The first subculture of *M. pachydermatitis* after shaking with lotion and dry (Fig. a) and the second subculture after incubation at 37°C for 2 weeks (Fig. b) from the sterile water-treated tubes (Figs. 1a and 1b), the KU Natural Miticide® lotion-treated tubes (Figs. 2a and 2b) and the KU herbfungusmite® lotion-treated tubes (Figs. 3a and 3b) and the KU herbmalacide® lotion-treated tubes (Figs 4a and 4b) showing normal growth of both subculture of *M. pachydermatitis*.
from the sterile water-treated tubes (Figs. 1a and 1b) and one tube of the first subculture from the KU herbfungusmite® lotion-treated tubes (Fig. 3a, in the first tube).

**Figure 5-8.** The first subculture of *M. pachydermatitis* after shaking with lotion and dry (Fig. a) and the second subculture after incubation at 37° C for 2 weeks (Fig. b) from the KU Thong-phan-chang® lotion-treated tubes (Figs. 5a and 5b), the KU Ta-Kai-Hom® lotion-treated tubes (Figs. 6a and 6b) and the KU Ta-Kai-Kaeng® lotion-treated tubes (Figs. 7a and 7b) and Betel leaf extracted lotion-treated tubes (Figs. 8a and 8b) showing normal growth of *M. pachydermatitis* in
3 tubes of the first subculture from the Betel leaf-extracted lotion-treated tubes (Fig. 8a in the seventh, ninth and tenth tubes).

Figure 9-12. The first subculture of *M. pachydermatitis* after shaking with lotion and dry (Fig. a) and the second subculture after incubation at 37°C for 2 weeks (Fig. b) from the 3% acetic solution-treated tubes (Figs. 9a and 9b), 3% oxalic solution-treated tubes (Figs. 10a and 10b), 3% tartaric acid solution-treated tubes (Figs. 11a and 11b), and 3% malic acid solution-treated tubes (Figs. 12a and 12b), showing the normal growth of *M. pachydermatitis* in 1, 4, 9 and 10 tubes of the first subculture from 3% acetic, oxalic, tartaric and malic acid lotion-treated tubes, respectively. The fungal
contamination in the first tube of the subculture from the oxalic acid solution-treated tube showed after 3 days. The *M. pachydermatitis* in second subculture could not grow exception one tube from the malic acid solution-treated tubes (the eighth tube of fig. 12b.).

Figure 13-14. The first subculture of the *M. pachydermatitis* after shaking with lotion and dry (Fig. a) and the second subculture after incubation at 37°C for 2 weeks (Fig. b) from the 3% citric acid solution-treated tubes (Figs. 13a and 13b) and the 3% succinic acid solution-treated tubes (Figs. 14a and 14b), showing the normal growth of the *M. pachydermatitis* in all ten tubes of the first subculture and one tube of the second subculture from the 3% citric and 3% succinic acid solution-treated tubes (Figs. 13a, 14a, 13b in the third tube and 14b in the fifth tube).

tubes showed no growth of yeast in nine tubes. It meant that the KU Herbfungusmite® and 3% acetic lotion-treated yeast were 90% non-survivability. The second subculture of yeast from all of these lotion-treated tubes after incubation at 37°C for 2 weeks also did not grow. Even in the two times dilution of these lotions by yeast containing broth, the yeast also did not grow exception of 3% acetic acid solution. It indicated that these KU herbal-extracted lotions were 100% fungicide for the *M. pachydermatitis* in vitro. It might be the alternative topical application drug for the Malassezia dermatitis in dogs and cats by wiping the lesion with the lotion-soaked cotton, since a few clinical trials were successful treatment. However, it is being trial in a large number of cases.

The two times dilution of 3% acetic acid (1.5%) showed no fungicidal activity on *M. pachydermatitis*. The acetic acid appears to serve as a mild antibacterial agent in a component of the vaginal lubrication of humans and other primates (Wikipedia, 2007), and a component of ear cleaning lotion (Medleau and Hnilica, 2006). However, this *in vitro* testing exhibited that the 3% acetic acid
lotion-treated *M. pachydermatitis* was 90% non-survivability. It indicated that 3% acetic acid might be the effective fungicidal dose for *M. pachydermatitis*.

The active substances of these herb-extracted lotions are unknown. The objective point of this work is to develop the herbal crude-extracted lotion for alternative veterinary medicine. Some plants had been reported the antifungal substances; the KU Thong-phan-chang® is the *Rhinacanthus nasutus* Kurz extracted lotion which this plant has a new antifungal naphthopyran derivative. (Kodama, et al., 1993). The *Annona squamosa* extraction in KU Natural Miticide® and KU herbfungusmite®, has antifungal substance, Anonaine against *Candida albicans* (Oliver-Bever, 1986).

### LITERATURE CITED


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