

## ***In Vitro* Fungicidal Activity of Herbal Extracted Lotion, KU Natural Miticide® on the Dermatophytes of Dogs and Cats**

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### **ABSTRACT**

*In vitro* study the fungicidal activity of herbal extracted lotion, KU Natural Miticide®, was bioassayed on 3 species of dermatophytes in dogs and cats; *Microsporum canis*, *M. gypseum* and *Trichophyton mentagrophyte*. Each fungus was cultured in 20 potato dextrose agar test tubes for 2 weeks. Ten tubes of each fungus were soaked with herbal lotion for 10 second and pour it out. The control tubes were soaked with sterile distilled water. Two subcultures of each treated fungus were done after leaving it dry for 90 min and after leaving in incubation at 30°C for 6 weeks. The subcultures of each fungi from the control tubes could normally grew. While there were no fungal growth of all lotion treated subcultures. This study indicated that the KU Natural Miticide® might be fungicide for all three main dermatophytes of dogs and cats, *Microsporum canis*, *M. gypseum* and *T. mentagrophyte*.

**Key words:** herbal extract, KU Natural Miticide®, *In vitro*, fungicide, dermatophytes, dogs, cats

### **INTRODUCTION**

The dermatomycoses in dogs mostly are caused by *Microsporum canis* (70%), *Microsporum gypseum* (20%) and *Trichophyton mentagrophyte* (10%) (Fraser, 1986). While in cats are caused by *Microsporum canis* (98%) and about 2% by *Microsporum gypseum* and *Trichophyton mentagrophyte*. (Fraser, 1986). The successful treatment is the combination of systemic and topical treatments. The systemic treatment has used griseofulvin (Siegmund, 1979) or ketoconazole (Plumb, 2002). However, the side-effects of those drugs are risks for long term treatments (Tilley and Smith, 2000; Plumb, 2002). Therefore, the safety topical application drugs are highly demand, especially the herbal extracted

products. There are many traditional uses and research works in the medicinal plants showing the antifungal activity in human and animals; *Rhinacanthus nasutus* Kurz. (Kodama *et al.*, 1993), *Tamarindus indicus* Linn. (Useh *et al.* 2004.), *Allium sativum* (Pai and Platt, 1995), *Boerhavia diffusa* (Agrawal *et al.*, 2004) and *Curcuma Longa* Linn. (Sawada *et al.*, 1971; Banerjee and Nigam, 1978; Venkitraman, 1978; Apisariyakul *et al.* 1995; Packiyasoth and Kyle, 2002; Garg and Jain, 2003; Singh *et al.*, 2003), etc. However, the fungicidal activity for dermatophyte in dogs and cats had rarely reported. The active antifungal principle of *Cassia tora* Linn. was against *T. rubrum*, *T. mentagrophytes*, *M. canis*, *M. gypseum* and *Geotrichum candidum* (Acharya and Chatterjee, 1975). The aqueous extracts of

*Capparis spinosa* and *Juglans regia* completely prevented growth of *M. canis*. (Ali-Shtayeh and Abu Ghdeib, 1999). The chloroform extracts of *Alpinia galanga* and *Boesenbergia pandurata* had antifungal activity against *M. gypseum* (Phongpaichit *et al.*, 2005). Some of above mentioned plants containing in the KU Natural Miticide® of our research for sarcoptic mange treatment (Chungsamarnyart *et al.*, 2003) has been treated as fungicide for *Aspergillus* spp. and *Curvularia* spp. in dogs (not yet publication), but it has not yet study the fungicidal activity on dermatophytes causing the dermatomycoses of dogs and cats. Therefore, this study is the *in vitro* bioassay the efficacy of the KU Natural Miticide® against those three main dermatophytes of dogs and cats.

## MATERIALS AND METHODS

The dermatophytes of dogs and cats, *Microsporum canis*, *M. gypseum* and *T. mentagrophyte* were cultured in test tubes containing potato dextrose agar. Each fungus was cultured in 20 test tubes and incubated them at 30°C for 2 weeks. Ten tubes of each fungus were soaked with KU Natural Miticide® for 10 second, poured the lotion out and left them drying in room temperature for 90 min. The other ten fungal tubes of each fungus were soaked with sterile distilled water as the control groups. The subculture of each fungus from each treated fungal tubes and control tubes were first done just after leaving them dry for 90 min. The second subculture were done after leaving them in incubator at 30°C for 6 weeks. The fungal growth of each subcultured tubes were observed every week. Each fungus were confirmed by lactophenol staining and light microscopic observation.

## RESULTS

The mycelial colony of *Microsporum*

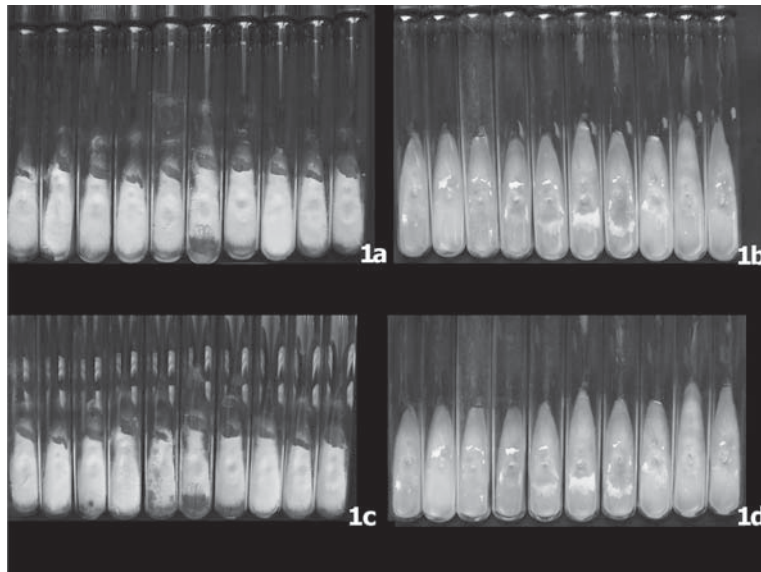
*canis*, *Microsporum gypseum* and *Trichophyton mentagrophyte* in each cultured tubes were collapsed on agar after soaking with sterile water (Figure 1a, 2a and 3a) and KU Natural Miticide® lotion (Figure 1b, 2b and 3b) but the KU Natural Miticide® lotion treated groups showed more collapsed and more dense color of mycelium colony than the sterile water treated groups (comparing between Figure 1a and 1b, 2a and 2b, 3a and 3b). They were no distinctly difference in colony appearance of these fungi after leaving in 30°C incubator for 1 week (Figure 1a, 1b, 2a, 2b, 3a and 3b) and up to 6 weeks (Figure 1c, 1d, 2c, 2d, 3c and 3d), respectively.

The first subculture of each treated fungi after leaving 90 minutes for drying the excess lotion were no growth of all three fungi species (Figure 4b, 5b and 6b) even in incubating for 6 weeks (Figure 4d, 5d and 6d). While the control groups of three fungi, overflowing of sterile water, grew normally within 1 week (Figure 4a, 5a and 6a) and up to 6 weeks (Figure 4c, 5c and 6c).

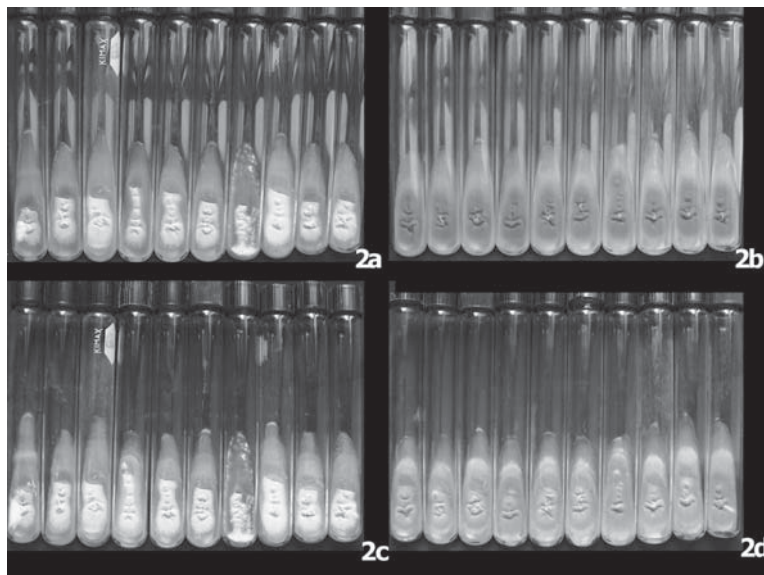
The second subculture of all three fungi from each treated fungi after leaving in 30°C incubator for 6 weeks also could not grew up (Figure 4f, 5f and 6f). While all the second subculture of each fungi from the control groups were normally grew (Figure 4e, 5e and 6e).

## DISCUSSION

The mycelial colony of *Microsporum canis*, *M. gypseum*, and *T. mentagrophyte* in each tubes were collapsed on agar and could not regrew after soaking with KU Natural Miticide® lotion (Figure 1b, 2b, and 3b). These results might be those fungi were not survived (fungicidal activity) or the cultured media were not properly for continuing growth (anti-fungal activity) after overflowing of KU Natural Miticide® lotion. The first subculture just after soaking and drying of all three fungi showed no fungal growth (Figure 4b, 5b and 6b). These exhibited that the fungi were



**Figure 1** The mycelial colony of *Microsporum canis* in ten tubes after soaking with sterile water (Figure 1a) and KU Natural Miticide® lotion (Figure 1b) showing the color change from white to brown exception some dense mycelial area. They were no distinctly difference in colony appearance after leaving in 30°C incubator for 1 week (Figure 1a and 1b) and up to 6 weeks (Figure 1c and 1d).



**Figure 2** The mycelial colony of *Microsporum gypseum* in ten tubes after soaking with sterile water (Figure 2a) and KU Natural Miticide® lotion (Figure 2b) showing the pale brown color colony and dark brown, respectively. They are no distinctly difference after leaving in 30°C incubator for 1 week (Figure 2a and 2b) and up to 6 weeks (Figure 2c and 2d).

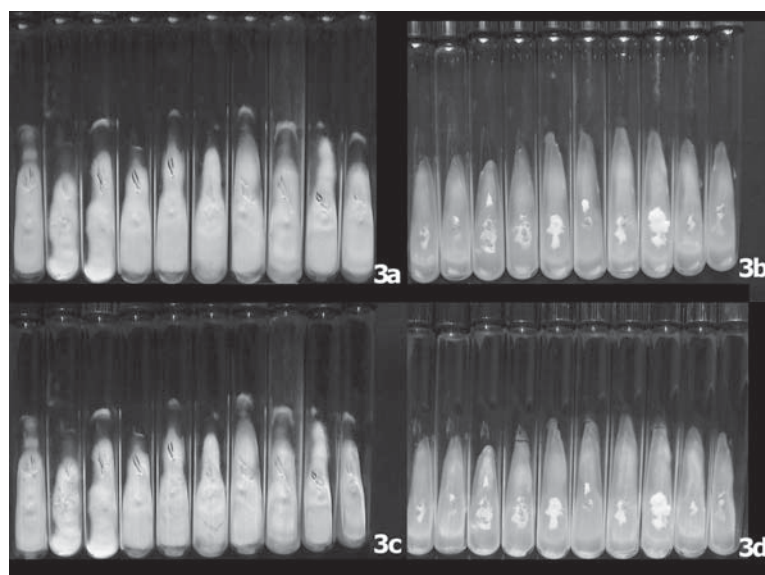
not survived after socking with KU Natural Miticide®. The second subcultures were done after leaving the socked and dried fungi in 30°C incubator for 6 weeks. These second subculture fungi socking with KU Natural Miticide® also could not grew (Figure 4f, 5f and 6f). These confirmed that the treated fungi were not survived after socking with KU Natural Miticide® and they could not recovered after incubating up to 6 weeks. While the first and second subcultures of control groups overflowing of sterile water were normal growth (Figure 4a, 5a, 6a, 4e, 5e and 6e). These *in vitro* results indicated that the KU Natural Miticide® lotion might be the fungicide for the *M. canis*, *M. gypseum*, and *T. mentagrophyte*. It might be the alternative drug for topical application of dermatomycoses in dogs and cats. However, the *in vivo* bioassay and clinical trial will be further studies.

The active substances of this lotion are unknown substances but the point of this work are

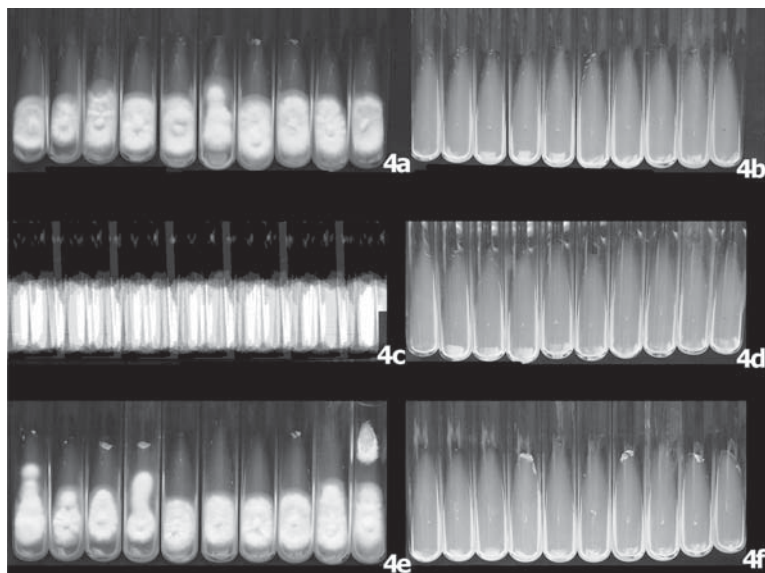
developing the herbal crude-extract lotion for alternative veterinary medicine. However, the fungicidal activity of each plants mixing in this lotion are further study. Some plants had been reported the antifungal activity on human and animals dermatophytes. The *Rhinacanthus nasutus* Kurz had a new antifungal naphthopyran derivative. (Kodama *et al.*, 1993). The partially purified methanolic extracts of stem barks of *Tamarindus indicus* Linn. inhibited the neuraminidase from *Clostridium chauvoei* (Useh *et al.* 2004.).

### ACKNOWLEDGEMENT

The authors wish to thank Miss Wanida Passaduruk, scientist of Department of Veterinary Microbiology and Immunology, Faculty of Veterinary Medicine, Kasetsart University, for her offer the pure cultured fungi.

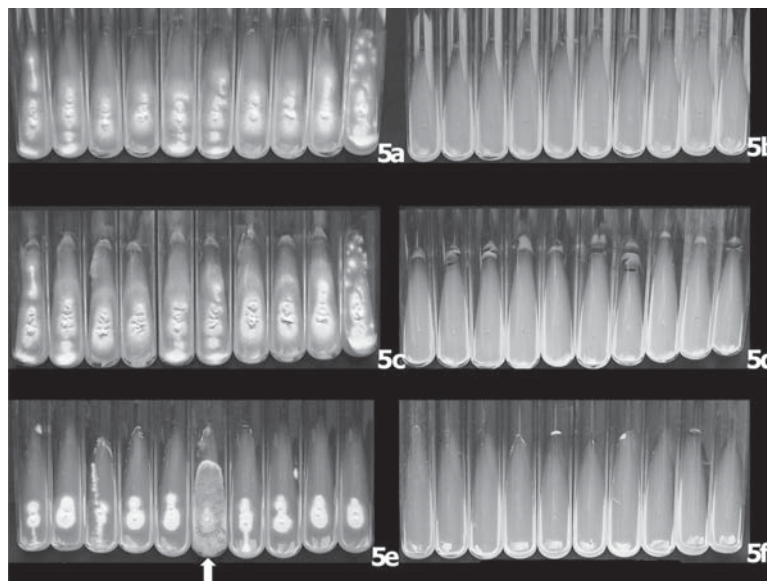


**Figure 3** The mycelial colony of *Trichophyton mentagrophyte* in ten tubes after socking with sterile water (Figure 3a) and KU Natural Miticide® lotion (Figure 3b) showing the white color mycelial colony and brown color colony exception the dense mycelium area surrounding the subculture point, respectively. They are no distinctly difference after leaving in 30°C incubator for 1 week (Figure 3a and 3b) and up to 6 weeks (Figure 3c and 3d).

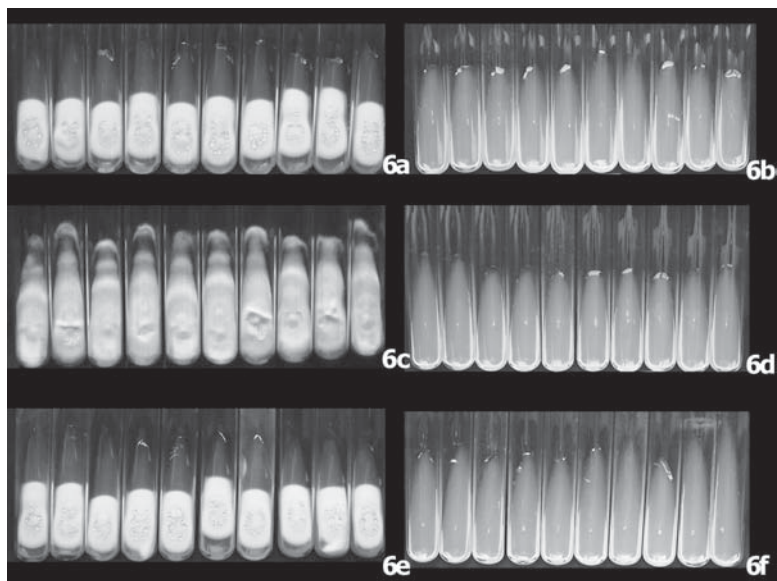


**Figure 4** The mycelial colony of *Microsporium canis* in ten tubes after subculture from each tubes which soaked with sterile water (Figure 4a and 4e) and KU Natural Miticide® lotion (Figure 4b and 4f). Figure 4c and 4d are continuous incubation up to 6 weeks of Figure 4a and 4b, respectively. Each subculture tubes from sterile water soaked tubes exhibit normal growth of *M. canis* including the first subculture just after soaking and drying, and leaving in 30°C incubator for 1 week (Figure 4a) and up to 6 weeks (Figure 4c). The second subculture from soaked, dried and left in incubator for 6 weeks (from each tube of Figure 1c) were also normal fungal growth after incubating 1 week (Figure 4e). While each subculture tubes from KU Natural Miticide® lotion soaked tubes exhibit no growth of *M. canis* in all the same condition as sterile water respectively; the first subculture just after soaking and drying, and incubating for 1 week (Figure 4b), incubating up to 6 weeks (Figure 4d) and the second subculture from each tube of Figure 1d after incubating for 1 week (Figure 4f).





**Figure 5** The mycelial colony of *Microsporium gypseum* in ten tubes after subculture from each tubes which soaked with sterile water (Figure 5a and 5e) and KU Natural Miticide® lotion (Figure 5b and 5f). Figure 5c and 5d are continuous incubation up to 6 weeks of Figure 5a and 5b, respectively. Each subculture tubes from sterile water soaked tubes exhibit normal growth of *M. gypseum* including the first subculture just after soaking and drying, and leaving in 30°C incubator for 1 week (Figure 5a) and up to 6 weeks (Figure 5c). The second subculture from soaked, dried and left in incubator for 6 weeks (from each tube of Figure 2c) were also normal fungal growth after incubating for 1 week (Figure 5e) exception the contamination of the 6<sup>th</sup> tube (arrow). While each subculture tubes from KU Natural Miticide® lotion soaked tubes exhibit no growth of *M. gypseum* in all the same condition as sterile water, respectively; the first subculture just after soaking and drying, and incubating for 1 week (Figure 5b), the first subculture incubating up to 6 weeks (Figure 5d) and the second subculture from each tube of Figure 2d after incubating 1 week (Figure 5f).



**Figure 6** The mycelial colony of *Trichophyton mentagrophyte* in ten tubes after subculture from each tubes which soaked with sterile water (Figure 6a and 6e) and KU Natural Miticide® lotion (Figure 6b and 6f). Figure 6c and 6d are continuous incubation up to 6 weeks of Figure 6a and 6b, respectively. Each subculture tubes from sterile water soaked tubes exhibit normal growth of *T. mentagrophyte* including the first subculture just after soaking and drying, and leaving in 30°C incubator for 1 week (Figure 6a) and up to 6 weeks (Figure 6c). The second subculture from soaked, dried and left in incubator for 6 weeks (from each tube of Figure 3c) were also normal fungal growth after incubating for 1 week (Figure 6e). While each subculture tubes from KU Natural Miticide® lotion soaked tubes exhibit no growth of *T. mentagrophyte* in all the same condition as sterile water, respectively; the first subculture just after soaking and drying, and incubating for 1 week (Figure 6b), the first subculture incubating up to 6 weeks (Figure 6d) and the second subculture from each tube of Figure 3d after incubating 1 week (Figure 6f).

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