



ORIGINAL ARTICLE

Antifungal Effects of Bee Venom Components on *Trichophyton rubrum*: A Novel Approach of Bee Venom Study for Possible Emerging Antifungal Agent

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Background: Bee venom (BV) has been widely investigated for potential medical uses. Recent inadvertent uses of BV based products have shown to mitigate signs of fungal infections. However, the component mediating the antifungal effect has not been identified. **Objective:** This investigation compares bee venom in its whole and partial forms to evaluate the possible component responsible for the antifungal effect. **Methods:** Forty-eight plates inoculated with *Trichophyton rubrum* were allocated into four groups. The groups were treated with raw BV (RBV), melittin, apamin and BV based mist (BBM) respectively and each group was further allocated accordingly to three different concentrations. The areas were measured every other day for 14 days to evaluate the kinetic changes of the colonies. **Results:** The interactions of ratio differences over interval were confirmed in groups treated with RBV and BBM. In RBV, the level of differences were achieved in groups treated with 10 mg/100 μ l ($p=0.026$) and 40 mg/100 μ l ($p=0.000$). The mean difference of ratio in groups treated with RBV was evident in day 3 and day 5. The groups that were treated with melittin or apamin did not show any significant interaction. In BBM groups, the significant levels of ratio differences over time intervals were achieved in groups treated with 200 μ l/100 μ l

($p=0.000$) and 300 μ l/100 μ l ($p=0.030$). **Conclusion:** The the bee venom in its whole form delivered a significant level of inhibition and we concluded that the venom in separated forms are not effective. Moreover, BV based products may exert as potential antifungal therapeutics. (Ann Dermatol 30(2) 202~210, 2018)

-Keywords-Antifungal agents, Apamin, Bee venoms, Melittin, *Trichophyton*

INTRODUCTION

Bee venom (BV) extracted from *Apis mellifera* L. has been utilized for centuries as a suitable pain killer and an anti-inflammatory agent for various chronic diseases¹⁻⁵. A number of recent studies further state the anti-mutagenic, anti-nociceptive, radio-protective, anti-tumorous and antibiotic properties of the BV⁵⁻⁸. Extensive research has been carried out to evaluate the effects of BV and the components of BV in that manner have been elucidated. Bioactive substances including melittin, apamin, mast cell degranulating peptides with histamine, serotonin, dopamine, norepinephrine and a number of enzymes listing phospholipase, hyaluronidase and histidine decarboxylase^{7,9-11}. Various pathways including inhibition of toll like receptors and translocation of nuclear factor kappa B (NF- κ B) and activator protein-1 signaling are suggested mechanism to the noticeable anti-inflammatory effects of BV^{12,13}. Two major components of BV, melittin and phospholipase A₂, are generally thought to play an important role in the induction of irritation and allergic reaction associated with the bee stings⁷. Melittin, a 26 amino acid polypeptide, has

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been known to have antibacterial effects⁷⁻¹². Recently, melittin-loaded perfluorocarbon nanoparticles possessed the ability to safely deliver significant payloads of melittin intravenously and to target and kill tumor cells¹⁴.

In respect, medical and commercial application targeting anti-inflammatory effects of BV has been prevalently manufactured in different fields of Korea. Common products manufactured with BV include anti-acne sprays, anti-blemishes, moisturizers, and nutrient-providing gels. Additionally, antibacterial use of BV based mist (BBM) has been inadvertently used and engendered attention for alleviating signs of fungal infections. Antifungal effects of BV have generally been less underlined compared to other inflammatory oriented diseases. Articles regarding antifungal use of BV include species of Candidal origin and *Trichophyton* species¹⁵. The antifungal activities of BV and sweet BV against 10 clinical isolates of *Candida albicans* that were cultured from blood and the vagina showed antifungal activity determined by using the disk diffusion assay, the broth micro-dilution assay and the killing-curve assay¹⁵. Moreover, antifungal activity of BV against *T. rubrum* and *T. mentagrophytes* showed stronger effect than that of fluconazole¹. However, the underlying mechanism and the principal component from BV that elicits the antifungal effect needs to be determined.

In this investigation, the components of BV including melittin and apamin, previously known to generate the anti-inflammatory effect, along with the BV as in whole raw form and mist based product were separately applied to the colonies of *T. rubrum* to evaluate the possible component responsible for the antifungal effect.

MATERIALS AND METHODS

BV collection and preparation of the components

The Colonies of natural honeybees (*Apis mellifera* L.) were maintained at the National Academy of Agricultural Science, Korea and the BV was collected by the collecting device (Chung Jin Biotech Co., Ltd., Ansan, Korea). An electric current was generated to the hive to cause the bees to sting at the glass plate. The venom which was later dried and was scraped off. The collected venom was diluted in cold sterile water and was centrifuged at 10,000g for 5 minutes at 4°C to eliminate residues from the supernatant. BV was lyophilized by freeze dryer and refrigerated at 4°C for later use. The BV used in this experiment was confirmed with size exclusion gel chromatography (AKTA Explorer; GE Healthcare, Pittsburgh, PA, USA) by dissolving in 0.02 M phosphate buffer with 0.25 M NaCl adjusted to pH 7.2 using a Superdex Peptide column (Amersham Biosciences; GE Healthcare)¹⁴. Other components including melittin

and apamin were manufactured products at Sigma (St. Louis, MO, USA) and BBM (A.C. Care Bee's water essence) of Dongsung Pharmaceuticals (Seoul, Korea).

Preparation of the organism

The medium used to culture the fungus was composed of potato dextrose cornmeal agar (PDACC; Catholic Skin Clinic, Daegu, Korea) with peptone, Tween 80 and antibiotics (chloramphenicol 500 mg L⁻¹ and cycloheximide 500 mg L⁻¹). Standard sized inoculums of *T. rubrum* derived from a spore suspension were applied to PDACC plates. The spore suspension was prepared by applying 5 ml of distilled water (DW) to a 3-week-old *T. rubrum* culture that was later gently withdrawn with a sterile pipette. Each PDACC plate was divided in half by scrapping off the midline in a sterile manner to retain identical conditions for both the experimental and the control groups. Using a sterile spreader, the spore suspension was applied on both sides of the PDACC plate. Forty eight plates were divided into four groups according to the specimen applied which were RBV, melittin, apamin and BBM. Every twelve plates were allocated and were further divided into three groups and labeled accordingly to the differently concentrated components with DW. The components were applied to the margins of the colonies and the amount of each component was variable as the areas of the colonies were different among the groups (Group 1: RBV 0.1 mg/DW 100 μl, Group 2: RBV 10 mg/DW 100 μl, Group 3: RBV 40 mg/DW 100 μl, Group 4: melittin 0.5 mg/100 DW μl, Group 5: melittin 1.0 mg/DW 100 μl, Group 6: melittin 1.5 mg/DW 100 μl, Group 7: apamin 0.5 mg/DW 100 μl, Group 8: apamin 1.0 mg/DW 100 μl, Group 9: apamin 1.5 mg/DW 100 μl, Group 10: BBM 100 μl/DW 100 μl, Group 11: BBM 200 μl/DW 100 μl, Group 12: BBM 300 μl/DW 100 μl).

Evaluation of the antifungal effect

The antifungal activity was measured by evaluating the area changes of each group for 14 days in an interval of two. Digital photography was taken every day using Canon EOS 750D (Canon Inc., Japan) and was followed until the 14th day. The lighting, position, and the background of the shooting were kept consistent throughout the experiment. The area was then converted into numerical values using Image Processing and analysis in Java (Image J version 1.50i; National Institutes of Health, Bethesda, MD, USA) which was then recalculated into ratios in order to calculate the kinetic interval changes of the areas observed in the colonies. The ratio at each interval indicates the area ratio to the previously calculated area in order to evaluate interval changes and the interaction

among the intervals.

Statistical analysis

All data was gathered and transformed in numerical values. Primary efficacy endpoint was the area difference of the colonies from the baseline to day 13. This was calculated with a repetitive measurement linear-effects model, which included the study groups, baseline value, scheduled follow-up intervals, and the interaction of study group with scheduled intervals. The descriptive data are

expressed in mean \pm standard deviation. All analysis was performed using IBM SPSS Statistics ver. 19.0 (IBM Co., Armonk, NY, USA) with p -value of less than 0.05 as statistically significant value. The analysis evaluated the interaction of the experimental group and the control over time intervals.

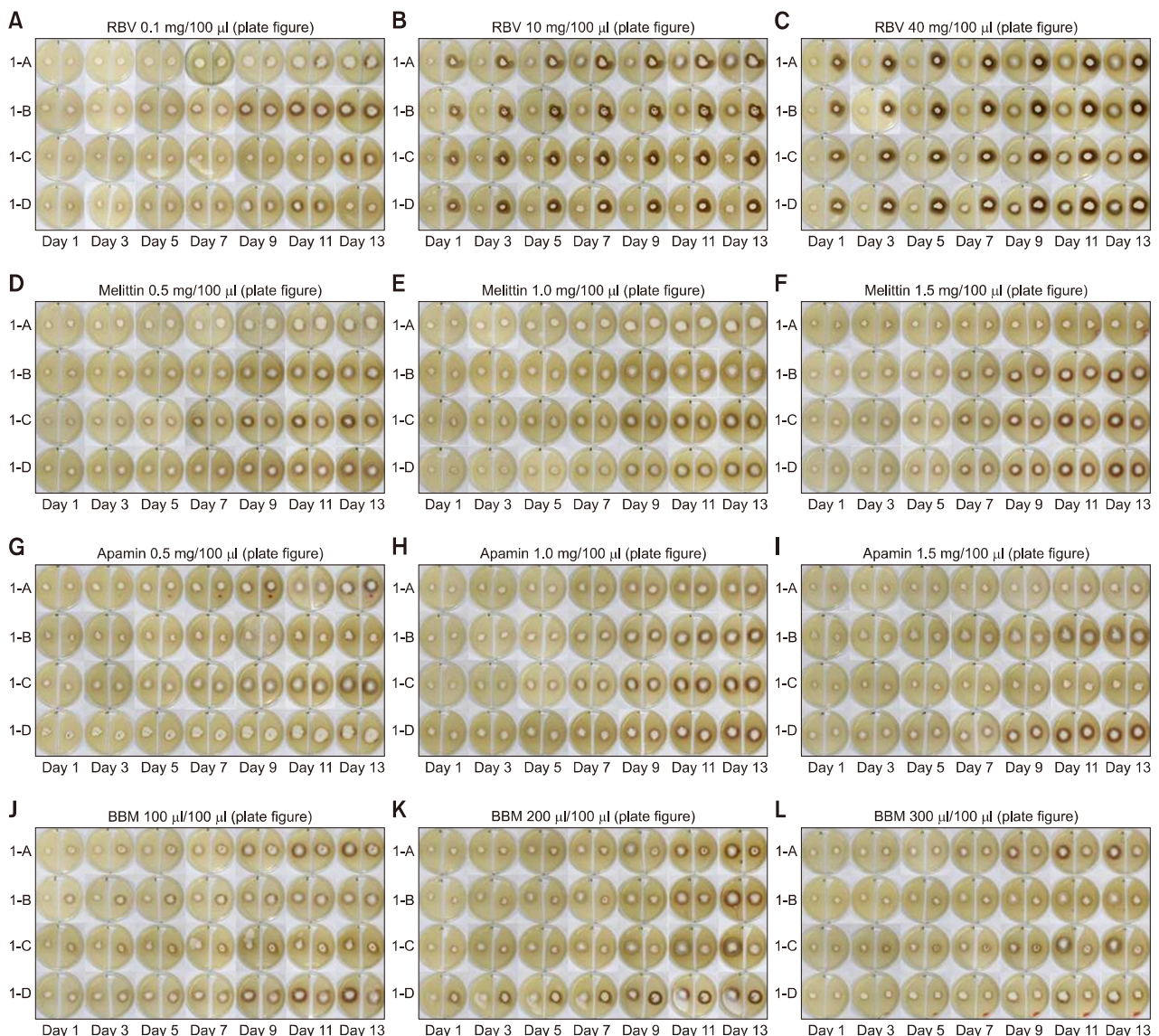


Fig. 1. Serial photographs of *Trichophyton rubrum* colony plates from day 1 to day 13. The colony plates varying each component group. Each plate field is divided in half to inoculate the colonies of experimental (right side of field) and control (left side of field) groups. (A) 0.1 mg/100 μ l of raw bee venom (RBV) treated group, (B) 10 mg/100 μ l of RBV treated group, (C) 40 mg/100 μ l of RBV treated group, (D) 0.5 mg/100 μ l of melittin treated group, (E) 1.0 mg/100 μ l of melittin treated group, (F) 1.5 mg/100 μ l of melittin treated group, (G) 0.5 mg/100 μ l of apamin treated group, (H) 1.0 mg/100 μ l of apamin treated group, (I) 1.5 mg/100 μ l of apamin treated group, (J) 100 μ l/100 μ l of bee venom based mist (BBM) treated group, (K) 200 μ l/100 μ l of BBM treated group, (L) 300 μ l/100 μ l of BBM treated group.

Table 1. Ratio interaction of groups treated with RBV, melittin, apamin, BBM

Component (concentration)	Group	Interval										F (p-value)		
		Day 1	Day 3	Day 5	Day 7	Day 9	Day 11	Day 13	Time	Group	Time × group			
RBV (0.1 mg/ 100 µl)	Ex.	1.000±0.000	1.114±0.097	1.146±0.094	1.156±0.158	1.153±0.089	1.093±0.101	1.031±0.028	6.834 (0.000)*	5.711 (0.054)	2.050 (0.084)			
	Co.	1.000±0.000	1.369±0.269	1.326±0.059	1.241±0.093	1.131±0.050	1.117±0.072	1.045±0.036						
RBV (10 mg/ 100 µl)	Ex.	1.000±0.000	1.152±0.050	1.122±0.046	1.235±0.114	1.149±0.053	1.102±0.070	1.165±0.157	7.906 (0.005)*	3.834 (0.098)	4.849 (0.026)*			
	Co.	1.000±0.000	1.425±0.164	1.403±0.227	1.186±0.090	1.099±0.048	1.071±0.054	1.056±0.055						
RBV (40 mg/ 100 µl)	Ex.	1.000±0.000	1.075±0.035	1.055±0.039	1.090±0.080	1.135±0.088	1.157±0.108	1.221±0.066	8.525 (0.000)*	59.847 (0.000)*	9.386 (0.000)*			
	Co.	1.000±0.000	1.514±0.116	1.322±0.151	1.124±0.113	1.147±0.087	1.104±0.035	1.157±0.065						
Melittin (0.5 mg/ 100 µl)	Ex.	1.000±0.000	1.127±0.120	1.294±0.238	1.182±0.063	1.124±0.085	1.084±0.060	1.279±0.385	2.234 (0.062)	0.298 (0.605)	0.621 (0.712)			
	Co.	1.000±0.000	1.201±0.142	1.184±0.091	1.213±0.132	1.231±0.076	1.150±0.081	1.176±0.042						
Melittin (1.0 mg/ 100 µl)	Ex.	1.000±0.000	1.263±0.077	1.283±0.112	1.118±0.067	1.198±0.059	1.131±0.078	1.110±0.127	5.828 (0.493)	0.106 (0.756)	0.897 (0.130)			
	Co.	1.000±0.000	1.311±0.147	1.140±0.133	1.184±0.038	1.202±0.063	1.162±0.063	1.145±0.204						
Melittin (1.5 mg/ 100 µl)	Ex.	1.000±0.000	1.052±0.043	1.303±0.355	1.158±0.071	1.077±0.049	1.067±0.018	1.057±0.033	3.013 (0.093)	0.018 (0.898)	1.377 (0.290)			
	Co.	1.000±0.000	1.114±0.056	1.089±0.073	1.228±0.202	1.085±0.040	1.109±0.084	1.056±0.027						
Apamin (0.5 mg/ 100 µl)	Ex.	1.000±0.000	1.288±0.159	1.098±0.081	1.108±0.042	1.087±0.022	1.102±0.055	1.101±0.065	5.991 (0.000)*	1.195 (0.316)	2.621 (0.133)			
	Co.	1.000±0.000	1.136±0.094	1.171±0.076	1.234±0.126	1.142±0.032	1.126±0.036	1.108±0.063						
Apamin (1.0 mg/ 100 µl)	Ex.	1.000±0.000	1.447±0.197	1.337±0.259	1.149±0.028	1.121±0.030	1.088±0.048	1.078±0.138	2.649 (0.134)	0.009 (0.929)	2.021 (0.192)			
	Co.	1.000±0.000	1.153±0.074	1.240±0.156	1.130±0.035	1.080±0.056	1.107±0.083	1.466±0.683						
Apamin (1.5 mg/ 100 µl)	Ex.	1.000±0.000	1.175±0.101	1.211±0.073	1.181±0.055	1.133±0.055	1.145±0.062	1.114±0.044	6.941 (0.015)*	0.727 (0.426)	0.187 (0.798)			
	Co.	1.000±0.000	1.180±0.179	1.186±0.091	1.127±0.035	1.089±0.057	1.117±0.080	1.094±0.037						
BBM (100 µl)	Ex.	1.000±0.000	1.079±0.082	1.075±0.021	1.166±0.105	1.229±0.256	1.160±0.155	1.146±0.134	4.531 (0.026)*	12.531 (0.012)*	3.429 (0.052)			
	Co.	1.000±0.000	1.419±0.118	1.137±0.084	1.284±0.088	1.151±0.040	1.126±0.051	1.049±0.038						
BBM (200 µl)	Ex.	1.000±0.000	1.072±0.028	1.225±0.150	1.090±0.055	1.219±0.099	1.297±0.072	1.167±0.061	6.476 (0.000)*	1.311 (0.296)	7.387 (0.000)*			
	Co.	1.000±0.000	1.387±0.103	1.168±0.101	1.218±0.083	1.181±0.067	1.095±0.100	1.160±0.081						
BBM (300 µl)	Ex.	1.000±0.000	1.130±0.098	1.016±0.008	1.109±0.076	1.165±0.098	1.144±0.170	1.163±0.068	3.978 (0.004)*	5.201 (0.063)	2.683 (0.030)*			
	Co.	1.000±0.000	1.284±0.107	1.186±0.094	1.113±0.578	1.090±0.061	1.140±0.077	1.078±0.054						

Values are presented as mean±standard deviation. RBV: raw bee venom, BBM: bee venom based mist, Ex.: experimental group, Co.: control group, Time×group, Time×group: group interaction over time interval. *Statistically significant with $p < 0.05$.

RESULTS

Gross inspection of the colonies

The antifungal efficacies of various components were treated against *T. rubrum* as shown in (Fig. 1). The PDACC plates were divided and spore suspension was applied on both sides of the plates. The left indicates the control group while the right side indicates experimental group. The experimental groups that were treated with raw BV shows a slower growth difference compared to than that of the control group at 10 mg/100 μ l and 40 mg/100 μ l concentrations (Fig. 1B, C). Noticeably growth rate difference in BBM treated groups between the experimental groups at 200 μ l and 300 μ l concentrations were also observed on gross inspection (Fig. 1K, L).

Efficacy of RBV, melittin, apamin, BBM between experimental groups and control groups

For every component, three differently graded concentrations were treated on each colony and the areas were measured every other day for 14 days. The numerical values were then transformed into ratios in order to evaluate the actual level of area difference and interaction of the growth nature throughout the study.

1) Raw bee venom

The main effect between the groups while ignoring the interval variable was only statistically significant in the group with 40 mg/100 μ l concentration ($p=0.000$), which was noticeable compared to no statistically significant values observed in groups with 0.1 mg/100 μ l concentration ($p=0.054$) and 10 mg/100 μ l ($p=0.098$). The main effect among the intervals, while ignoring the group variable showed statistically significant value within all three concentration groups. The interaction effect between the groups over time interval was not statistically significant in the group treated with 0.1 mg/100 μ l concentrated of RBV. However the values were different as the concentrations of RBV were increased to 10 mg/100 μ l and 40 mg/100 μ l with respective p -values of 0.026 and 0.000 (Table 1). The mean difference of ratio in groups treated with RBV of 10 mg/100 μ l was evident in day 3 and day 5 (Fig. 2B). The mean ratio was 1.152 in the experimental group compared to 1.425 in the control group at day 3 and 1.122 in the experimental group compared to 1.403 in the control group at day 5 (Table 1). Similar result was shown in the group treated RBV with 40 mg/100 μ l. The evident difference was seen in day 3 and day 5 (Fig. 2C). At day 3, 1.075 ratio of area increment in the experimental group was observed compared to 1.514 in the control group (Table 1). At day 5, 1.055 ratio of area increment in the

experimental group was observed compared to 1.322 in the control group (Table 1).

2) Melittin & apamin

All of the groups that were treated with melittin or apamin, regardless of concentration gradient, the significant main effect of ratio differences were not observed in any of the measures within the intervals, the groups or within the groups over specific time intervals (Table 1).

3) Bee venom based mist

In BBM, similar pattern to RBV was observed. While, the group treated with BBM of 100 μ l exhibited no significant difference of ratio difference between the groups over time intervals ($p=0.052$), the higher concentration groups showed significant levels of ratio differences within the groups over time intervals ($p=0.000$ in 200 μ l, 0.030 in 300 μ l), (Table 1). The mean difference of ratio in the group treated with 200 μ l of BBM was noticeable at day 3 and at day 7 (Fig. 2K). At day 3, the mean ratio difference was 1.072 in the experimental group and 1.387 in the control group (Table 1). Additional the mean ratio difference was 1.090 in the experimental group and 1.218 in the control group at day 7 (Table 1). Moreover, the mean difference of ratio in the group treated with 300 μ l of BBM was noticeable at day 3 (Fig. 2L). The mean ratio difference was 1.130 in the experimental group and 1.284 in the control group (Table 1).

DISCUSSION

BV or its series of components have shown a broad array of effects from anti-inflammatory agents, anti-nociceptive, antibacterial, to anti-tumorous effects¹⁻⁸. Extensive *in vitro* studies have confirmed the versatile properties that BV retains. In respect to the wide-ranging studies performed with BV, information regarding the antifungal effect of BV is still scarce. However, continual scope of research and aim conducting BV in relation to antifungal properties has stated BV as a possible agent for its medicinal application. Moreover, off-label uses of BBM products have been targeted to control acne eruptions. Based on the reports of robust antibacterial activities of BV against both Gram negative and Gram positive bacteria, in addition to the role of BV in inflamed lesions, a number of BV based acne care products have been manufactured. However these lines of beauty products are inadvertently used and alleviation of lesions with tinea pedis have been suggested by these products.

Therefore, the objective of this study was to identify the possible underlying component in BV that hinders the

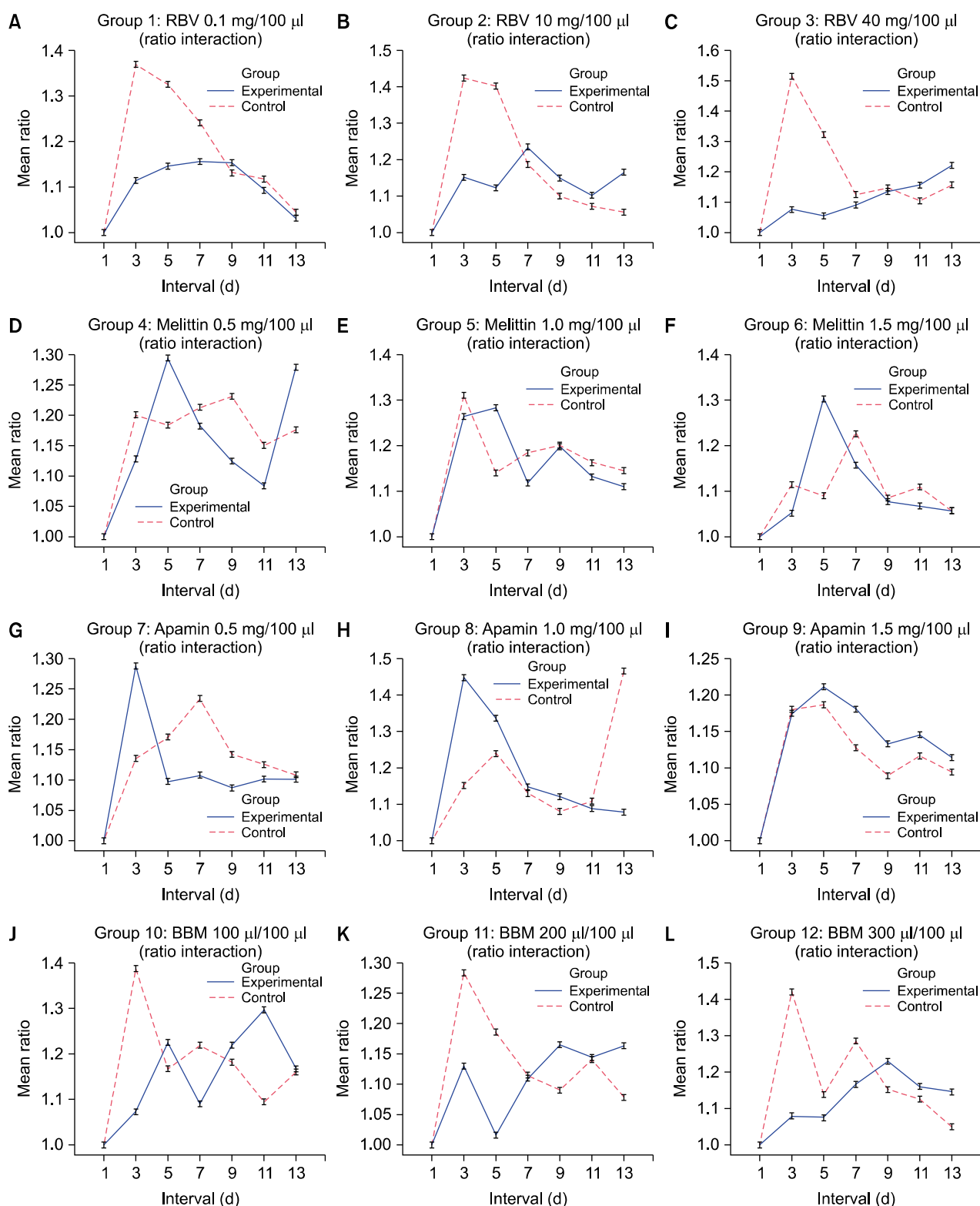


Fig. 2. Graphical interpretation of ratio interaction over time in experimental and control groups. Graphical interpretation of ratio difference interaction over time (interval). (A) 0.1 mg/100 μ l of raw bee venom (RBV) treated group, (B) 10 mg/100 μ l of RBV treated group, (C) 40 mg/100 μ l of RBV treated group, (D) 0.5 mg/100 μ l of melittin treated group, (E) 1.0 mg/100 μ l of melittin treated group, (F) 1.5 mg/100 μ l of melittin treated group, (G) 0.5 mg/100 μ l of apamin treated group, (H) 1.0 mg/100 μ l of apamin treated group, (I) 1.5 mg/100 μ l of apamin treated group, (J) 100 μ l/100 μ l of bee venom based mist (BBM) treated group, (K) 200 μ l/100 μ l of BBM treated group, (L) 300 μ l/100 μ l of BBM treated group.

growth and spreading of the most common fungal pathogen, *T. rubrum*. In the study comparing the antifungal effects between the experimental groups and the control groups at certain time interval, only BV in its whole form regardless of raw nature or in a mist based product showed statistically significant values. In elaboration, the time point regarding the antifungal effect exhibited by RBV was dose dependent as 10 mg/100 μ l ($p=0.026$) and 40 mg/100 μ l ($p=0.000$) concentrated specimen showed statistically significant area difference compared to that of 0.1 mg/100 μ l ($p=0.084$). Based on this finding, some level of fungicidal or fungistatic properties produced by raw bee venom can be inferred. Furthermore, as the antifungal effect of bee venom generally withered after day 5, a time span of 5 days for its effectiveness of RBV was also postulated. Prominent difference according to time interval is exhibited through graphical values (Fig. 2). The antifungal effect was not observed in colonies that were treated either in melittin or apamin. The analysis between the experimental groups and the control groups did not show any statistically significant difference regarding the fungal growth on any concentration level. Similar results were exhibited in the colonies treated with apamin. Melittin is known to inhibit platelet-derived growth factor and vascular smooth muscle cell proliferation by suppressing NF- κ B, Akt activation, and the mitogen-activated protein kinase pathway¹⁴⁻¹⁸. Downward reaction inhibits the transcription of inflammatory cytokine which exerts various protective events induced from melittin. No parameter regarding cell growth or antifungal effect in that manner was observed.

Interestingly, the BBM has shown similar effectiveness to RBV in terms of growth retardation. The concentration levels effective for in hindering the colony growth were prominent in the ones treated with 10 mg/100 μ l ($p=0.000$) and in 40 mg/100 μ l ($p=0.030$). The underlying factor may be due to a variety of component contained in mist. Extracts of bee venom, extracts of royal jelly, propolis, grapefruit, Beta-glucan, phytoncide, and portulaca oleracea extracts were in the material. In addition to bee venom, propolis may have also played a role in the antifungal effect. Farghaly¹⁷ reported the use of propolis on tested microscopic fungi, *Aspergillus fumigatus* (19.2 ± 0.63 mm) showed the most sensitive results to the component^{16,17}. However, the sensitivity decreased in other microscopic fungi including *Geotricum candidum*, *C. albicans* (16.9 ± 0.58 , 16.4 ± 1.2), respectively, which yet remain to be determined¹⁷. However since many of the bee venom based products are already available through highly refined beauty products such as the one used in this study, it may be relevantly useful to directly apply such products to the

affected area upon more safety and clinical evaluation. Numerous studies have been investigated to evaluate the antifungal properties of bee venom. Lee reported that anti-candidal activities of bee venom and sweet bee venom were observed by using the disk diffusion method and the broth microdilution method, confirming that the compounds have a potential for use as anti-candidal agents^{13,15}. Phytochemicals exhibiting antifungal effects against *T. rubrum* and *T. mentagrophytes* were evaluated in different studies. Turmeric oil from *Curcuma longa* had minimum inhibitory concentrations (MICs) in a range of 229.8 ~ 919.2 ppm (parts per million)¹⁸. Four phenolic amides, dihydro-N-caffeoyltyramine, trans-N-feruloyloctopamine, trans N-caffeoyltyramine, and *cis*-N-caffeoyltyramine isolated from *Lycium chinense* were reported to have anti-fungal activity in a range of 5 ~ 10 ppm¹⁹. Other molecules such as 6 α -O-(β -D-xylopyranosyl-(1 \rightarrow 3)- β -D-quinovopyranosyl)-(25,S)-5 α -spirostan-3 β -ol had IC50 values of 25 μ g/ml had also shown antifungal effect against *T. mentagrophytes* and *T. rubrum*²⁰. Limonene was also shown to exert a potent antifungal effect against *T. rubrum* with MIC value of 0.5%²¹.

In respect to these findings, this experiment strived to assess the antifungal effect from each component underlying cause of effect and interesting result is that only with the bee venom in its whole form delivered a significant level of inhibition and we concluded that the venom in separated forms are not effective.

One theory is that while mellitin induces reactive oxygen species to generate the antifungal effect, other components such as phospholipase A2, hyaluronidase which respectively raise the permeation of the cell membrane and the capillary membranes are necessary to give the whole antifungal effect^{1,13,14,22,23}. Additionally, studies state that the lipids and carbohydrates in the venom may act as an opsonin for further inflammatory responses to repeatedly generate the antifungal effect^{22,23}.

Although there are a number of studies evaluating the antifungal effect of bee venom, component-specific evaluation within this mixture has not been evaluated before this study. In addition, the use and effect of bee venom based products was also conducted to evaluate the antifungal effect exerted from these lines of products. The investigation was conclusive with the specified followings. First of all, the antifungal effect was observed in bee venom regardless of raw or manufactured form. The colonies that were treated with only the whole form of bee venom have proven the antifungal effect throughout our study. Separated forms of bee venom components showed no value in hindering the growth of the colonies. Secondly, the effects were dose dependent as statistically significant values

were observed within the differently concentrated groups at different time period. Thirdly, the effect showed a lasting period as the colony sizes were mostly regressed up till the 5th day. It was conclusive that the 5th day was the critical time point as the effect started to wear off after day 5 and further re-application of the material seems necessary in order to generate the continual antifungal effect. Moreover, in terms of bee venom based product, successive research must be considered before recommending its use against various fungal infections. However, the already prevalent use of these manufactured products along with approved safety concerns may ease the commercialization of these manufactured products for targeting fungal infections.

Even though, this study demonstrated the antifungal effects by the components of bee venom along with bee venom based beauty product, further experiments with larger sample sizes, more fungal species and more components of the bee venom should be carried out to evaluate the molecular interaction and possible underlying mechanisms among the components. Further *in vivo* efficacy studies are warranted for clinical application.

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CONFLICTS OF INTEREST

The authors have nothing to disclose.

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