Original Article

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The efficacy of handwashing gel containing banana peel extract in reducing bacterial load on dentists' hands

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Objective: This study aimed to investigate the efficacy of handwashing gel containing banana peel extract in reducing bacterial load on dentists' hands.

Materials and Methods: Twenty-four volunteers participated in this crossover study using 4% chlorhexidine gluconate, gel with banana peel extract, and gel without banana peel extract. At each round of the trial, the volunteers washed their hands with 4% chlorhexidine gluconate, put on gloves, and provided dental treatments, i.e. operative treatment, scaling and root planing, endodontic work, or prosthodontic procedures. After 30 minutes, the volunteers took off gloves and firmly put thumbs for 5 seconds on the first nutrient agar plates (baseline). The volunteers in each group then washed their hands with the assigned handwashing products and firmly put thumbs for 5 seconds on the second agar plates (immediately washing the hands). The volunteers put on gloves and continued to do their work. After one hour, the volunteers took off gloves and firmly put thumbs for 5 seconds on the third agar plates (one hour after washing the hands). After 24-hours incubation at 35°C, the bacterial colonies on agar plates were observed and counted. Bacterial loads on hands were compared using Wilcoxon Signed Ranks test and Mann-Whitney U test.

Results: The results showed that the number of bacterial colonies was significantly decreased from baseline to immediately washing the hands with 4% chlorhexidine gluconate and gel with banana peel extract but not for the gel without banana peel extract. However, the number of bacterial colonies was significantly increased from immediately to one hour washing the hands for all assigned handwashing products.

Conclusion: The data from this study suggests that handwashing gel containing banana peel extract has the promising results in reducing bacterial load on dentist's hands. This finding supports the utilization of banana peels, a vast agricultural residue in Thailand, as anti-bacterial agent.

Keywords: bacterial load, banana peel extract, dentists' hands, handwashing gel

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Introduction

During dental treatments, dentists' hands are the common carriers to provide chances for transmitting microorganisms among individuals. Cross-contamination is preventable, and it needs responsibility from all healthcare workers (HCWs). Hand hygiene is regarded as one of the most important elements of infection control activities. Improper hand hygiene by HCWs is responsible for about 40% of nosocomial infections [1]. Previous study has been reported that bacteria were the most prevalent microorganism on hands (>80% relative abundance), then viruses, and fungi being least prevalent (<5% relative abundance). The hands of HCWs were more likely to harbor pathogenic microorganisms, such as *Staphylococcus aureus (S.aureus*), Enterococcus spp., or *Candida albicans* [2]. Total bacterial colony- forming units (CFUs) on the hands of HCWs have ranged from 3.9×10^4 to 4.6×10^6 CFU/cm^2 while fingertip

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contamination ranged from 0 to 300 CFUs when sampled by agar contact methods [3]. *S.aureus* has been reported as a major type of bacteria on hands and it is one of the most common causes of both community and hospital acquired infections, e.g., impetigo, folliculitis, septic arthritis, osteomyelitis, septicemia, pneumonia, and meningitis [4]. World Health Organization (WHO) issued guidelines regarding specific steps and procedures to be followed during handwashing [5]. Routine handwashing with water and nonantimicrobial soap aims to remove unsanitary and transient microorganisms while handwashing with antimicrobial agents (e.g. chlorhexidine, iodine and iodophors, phenol derivatives) aims to kill or reduce pathogens, both transient and resident flora. The one of antimicrobial agents generally accepted and used nowadays is 4% chlorhexidine gluconate (CHG) [6].

Banana, "Kluai" in Thai word, belongs to the genus "*Musa*". The parents of nowadays banana cultivars are *Musa acuminata* and *Musa balbisiana*. The first one gives bananas sweet taste while the latter gives starch and texture [7]. Kluai Namwa, previously belonging to *Musa sapientum*, is commonly found throughout Thailand. According to the increasing popularity in consuming Kluai Namwa, its peels are vast agricultural residue. Banana peels have been utilized for various industrial applications including bio-fuel production, bio-sorbents, pulp and paper, cosmetics, energy-related activities, organic fertilizer, environmental clean-up and biotechnology-related processes. Banana peels are composed of glycosides, alkaloids, saponins, flavonoid, volatile oil, and tannins which are found in high amount and can inhibit the growth of *S.aureus*. In addition, banana peels contain polysaccharide which has moisturizing effect. It helps moisturize skin and is added to cosmetic products. Extract of banana peels also has the antioxidant activity due to glycosides and monosaccharide components [8-11]. In order to increase the utilization of Kluai Namwa peels, the present study aimed to investigate the efficacy of handwashing gel containing banana peel extract in reducing bacterial load on dentists' hands. According to its antimicrobial activities, stage-2 ripeness of banana peels (green with a trace of yellow) was chosen in this study [12].

Materials and methods

Banana peel extract preparation

"Kluai Namwa" peels used for the investigation were agricultural residue kindly given from the sweet banana crisps store in Chiang Rai. Two hundred grams of banana peels were washed with running tap water, surface sterilized with 70% ethanol, cut into small pieces, rinsed with sterile distilled water, put into 1000 ml of boiling distilled water, then left to macerate at room temperature. After 24 hours, the contents were mixed by the blender and filtered with straining cloth. The filtrate was spun at 7500xg for 15 minutes and the collected supernatant was re-filtered with 0.45 µm paper filter. The obtained product was stored at 4°C until use.

Antibacterial activity assay

The determination of antibacterial activity was done using the agar well diffusion technique. Nutrient agar plates (peptone 0.5 g, meat extract 0.25 g, sodium chloride 0.125 g, agar 0.2 g, and H_2O 50 ml) with the holes volume of 100 µl were prepared and inoculum containing 10^7 CFU/ml of *S.aureus* (No.TISTR746) was spread on the solid agar plates. Banana peel extract was mixed with sterile distilled water (V/V) to get the concentration of 60%, 65%, 70%, 75%, 80% and 85% respectively. 100 µl of each tested solution was placed in the well made in inoculated plates. Control was set up with sterile distilled water. The plates were incubated at 35°C for 24 hours and zones of

inhibition, if any, around the wells were evaluated in millimeters. Mean of the clear zone of each tested solution was calculated from triplicate experiments. The lowest concentration that gave the widest clear zone was selected for further experiments.

Handwashing gel preparation

Three grams of Carbopol ulterz® 940 was suspended in 60 ml of the banana peel extract with constant stirring to get homogeneous mixture, then gently added 3 grams of triethanolamine (TEA) with slow stirring to avoid formation of possible air bubbles in the mixture. After kept aside the prepared mixture for 24 hours, 95% ethanol was added to provide 100 ml mixture. The prepared product was thoroughly mixed until getting the homogeneous gel [13]. Gel base (negative control) was prepared with the same formula using sterile distilled water instead of banana peel extract. The prepared gels were kept in the airtight containers until use.

WHO handwashing protocol

Start from wetting hands with water, apply the handwashing product in a palm to cover all hand surfaces. Then follow these steps (I) rub hands palm to palm (II) right palm over left dorsum with interlaced fingers and vice versa (III) palm to palm with fingers interlaced (IV) backs of fingers to opposing palms with fingers interlocked (V) rotational rubbing of left thumb clasped in right palm and vice versa (VI) rotational rubbing, backwards and forwards with clasped fingers of right hand in left palm and vice versa (VII) rinse hands with water and dry thoroughly with a towel or napkin [5].

Handwashing product testing

Twenty-four dental students at School of Dentistry, Mae Fah Luang University participated in this randomized crossover study. They were randomly distributed into 3 groups (n = 8) which subsequently underwent a rotation of washing hands with 4% CHG solution, gel with banana peel extract, and gel without banana peel extract. Before starting the trial, the volunteers washed their hands following WHO protocol with 4% CHG solution *(primary handwashing),* put on gloves, and provided dental treatments, i.e. operative treatment, scaling and root planing, endodontic work, or prosthodontic procedures. After 30 minutes, the volunteers took off gloves and firmly put thumbs for 5 seconds on the first agar plates *(baseline).* The volunteers in each group then washed their hands with the assigned handwashing product, one pump pressed by the researcher, and firmly put thumbs for 5 seconds on the second agar plates *(immediately after washing the hands).* The volunteers put on new gloves and continued to do their work. After one hour, the volunteers took off gloves and firmly put thumbs for 5 seconds on the third agar plates (one hour after washing the hands). The volunteers rotated to wash their hands with the second and third assigned handwashing products in the next second and third week, respectively. After completing three rotations of the trial, the volunteers were asked to fill out a satisfaction questionnaire regarding the quality of the handwashing gels.

The study protocol was approved by Mae Fah Luang University Research and Ethics Committee (REH-62054).

Bacterial count

All agar plates were incubated at 35°C for 24 hours. The bacterial colony morphology was observed. The number of CFUs was counted twice by a single rater who had been standardized intra-rater reliability.

Statistical analyses

Statistical analyses were performed with the RStudio program version1.2.5033. The difference of the number of bacterial colonies was compared

within group by using Wilcoxon signed-ranks test. Mann-Whitney U test was used to compare the difference of the number of bacterial colonies between groups at each studied time. Statistically significant difference was considered if *p - value* was < 0.05.

Results

Mean of the clear zones from triplicate agar well diffusion experiments for antibacterial activity of banana peel extract showed no difference among the tested concentrations, ranging from 60 - 85%. Subsequently, 60% (V/V) banana peel extract was used to prepare the studied handwashing gel.

The efficacy of handwashing products in reducing bacterial load on twenty-four volunteers' hands was demonstrated by the reduction of the number of CFUs at the studied time points; before washing the hands (baseline), immediately after washing the hands and one hour after washing the hands (Figure 1).

The results demonstrated that when using 4% CHG and gel with banana peel extract, the number of bacterial colonies was significantly decreased from baseline to immediately after washing the hands. There was no significant difference in bacterial reduction in gel without banana peel extract group. After one hour washing the hands, the number of bacterial colonies was significantly increased from immediately after washing the hands for all assigned handwashing products. In addition, the number of bacterial colonies was not significantly increased from baseline to one hour after handwashing in 4% CHG and gel with banana peel extract groups, but significantly increased in gel without banana peel extract group. (Wilcoxon signed-ranks test; *p*-value <0.05; Table 1).

When compared the efficacy among the three handwashing products in reducing bacterial colonies from baseline to immediately after handwashing, the results demonstrated that there were significant difference between 4% CHG and gel without banana peel extract as well as gel with banana peel extract and gel without banana peel extract, but there was no significant difference between 4% CHG and gel with banana peel extract. However, the results showed no significant difference on the number of bacterial colonies when compared immediately after handwashing to one hour after handwashing of all studied handwashing products. (Mann-Whitney U test; *p*-value <0.05; Table 2).

Figure 2 shows the results of results of satisfaction questionnaire regarding the quality of handwashing gels. The volunteers rated their high satisfaction in scent, moisture, and color of banana peel extract gel, respectively. While gel without banana peel extract was rated high satisfaction in color, viscosity and scent, respectively. Four and three volunteers felt slightly itchy after handwashing with banana peel extract gel and gel without banana peel extract, respectively. However, this feeling disappeared within a few seconds and there were no rash or other symptoms. In overall aspects, the volunteers had more satisfaction of gel without banana peel extract than gel with banana peel extract.

Discussion

Dentists may incur the chance of cross-contamination between patients during providing dental treatments. The standard protocol in reducing pathogen transmission is washing hands with effective antimicrobial handwashing products. In this preliminary study, handwashing gel containing banana peel extract demonstrated the efficacy in reducing bacterial load on dentists' hands in a similar pattern as 4% CHG.

Figure 1 The number of colony forming units (CFUs) after using 4% chlorhexidine gluconate, gel with banana peel extract and gel without banana peel extract at the studied time; before washing the hands (baseline), immediately, and one hour after washing the hands.

Table 1 The efficacy of the handwashing products in reducing bacterial load on hands

	4% Chlorhexidine gluconate	Gel with banana peel extract	Gel without banana peel extract	*p*-value[d, e, f] *p*-value[dd, ee, ff]
Before handwashing (Baseline)				
CFUs: range	0-16	0-19	0-11	
CFUs: median (mode)	3.5 (2)	4 (5)	2 (2)	
Immediately after handwashing				
CFUs: range	0-13	0-11	0-5	
CFUs: median (mode)	1 (0)	2 (0)	3 (5)	0.42[d], 0.04[e]*, 0.01[f]*
One hour after handwashing				
CFUs: range	0-15	0-18	1-20	
CFUs: median (mode)	2 (2)	3.5 (0)	4 (4)	0.73[dd], 0.23[ee], 0.38[ff]
p-value[a]	0.00*	0.00*	0.48	
p-value[b]	0.00*	0.02*	0.00*	
p-value[c]	0.47	0.99	0.00*	

* Statistically significant difference at $p < 0.05$

[a] Intragroup comparison of baseline and immediately after handwashing (Wilcoxon signed-rank test)

[b] Intragroup comparison of immediately after handwashing and one hour after handwashing (Wilcoxon signed-rank test)

[c] Intragroup comparison of baseline and one hour after handwashing (Wilcoxon signed-rank test)

[d] Intergroup comparison of 4% chlorhexidine gluconate to gel with banana peel extract between baseline and immediately after handwashing (Mann-Whitney *U*-test)

[e] Intergroup comparison of gel with banana peel extract to gel without banana peel extract between baseline and immediately after handwashing (Mann-Whitney *U*-test)

[f] Intergroup comparison of 4% chlorhexidine gluconate to gel without banana peel extract between baseline and immediately after handwashing (Mann-Whitney *U*-test)

[dd] Intergroup comparison of 4% chlorhexidine gluconate to gel with banana peel extract between immediately after handwashing and one hour after handwashing (Mann-Whitney *U*-test)

[ee] Intergroup comparison of gel with banana peel extract to gel without banana peel extract between immediately after handwashing and one hour after handwashing (Mann-Whitney *U*-test)

[ff] Intergroup comparison of 4% chlorhexidine gluconate to gel without banana peel extract between immediately after handwashing and one hour after handwashing (Mann-Whitney *U*-test)

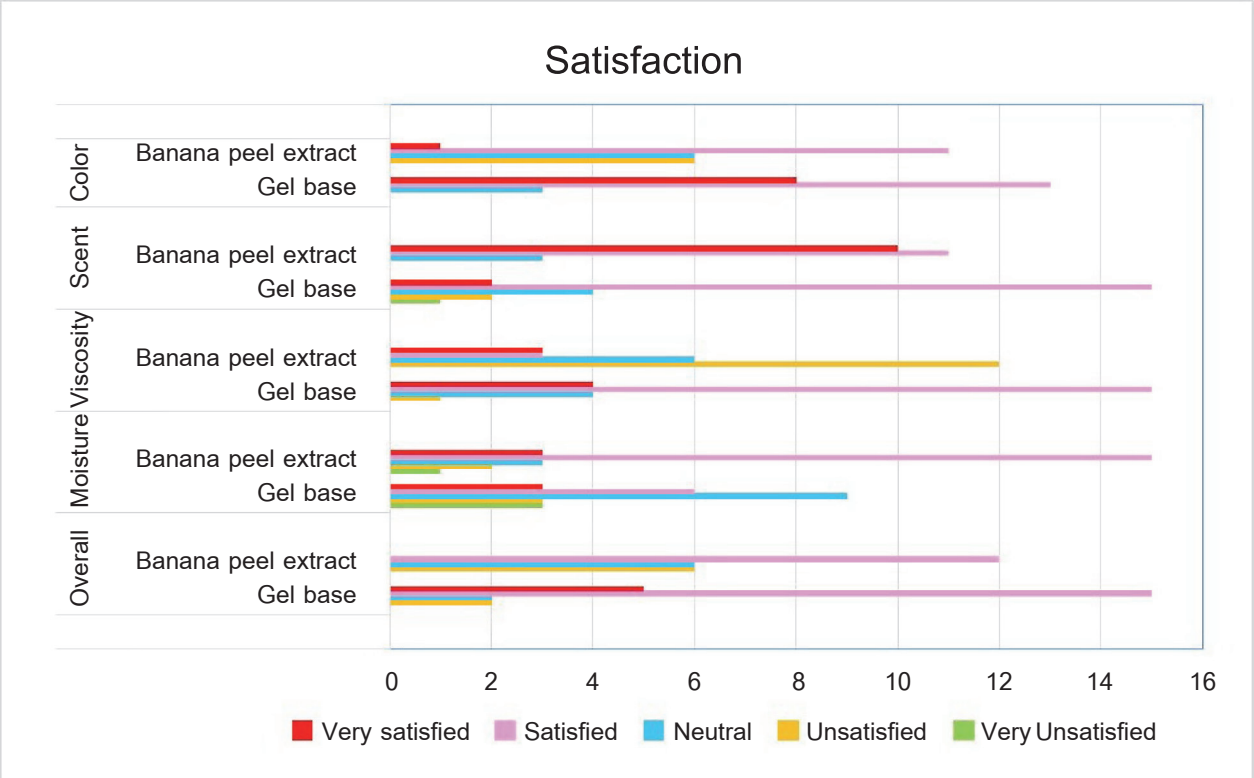

Figure 2 Satisfaction of gel with banana peel extract and gel without banana peel extract (Gel base)

The efficacy of handwashing gel containing banana peel extract in reducing bacterial load was significantly better than that of gel without banana peel extract. This effectiveness may originate from the extracted active ingredients from banana peels which had been reported for antimicrobial properties [8-11]. The result was in agreement with previous studies which reported that tannins, found in high quantities in banana peel, could inhibit the growth of *S.aureus* [10]. Though, in this study, we did not intend to classify bacterial species, we found that almost bacterial colonies on nutrient agar plates (75%) were circular, smooth, fine, small and rounded with entire margin which referred to the characteristics of *S.aureus* [14].

Regardless of dental treatments, the bacterial counts were increased from immediately after handwashing to one hour after hand washing for all studied handwashing products. Dentists' hands can transiently carry potential pathogens coming from the environment, or from patients who possibly carry bacteria, in addition, opportunistic pathogens may multiply rapidly in moist environment underneath gloves and multiplication rate increases along with the duration of glove use [15]. In fact, 4% CHG had an excellent residual effect that could reduce the skin microflora and prevent its colonization, but there was the study reported that bactericidal efficacy of 4% CHG decreased within 3 hours after the application. The wide use of 4% CHG as standard antiseptic among HCWs has made the concern regarding the evolution in the development and selection of resistance genes of nosocomial bacteria such as *S.aureus* [6]. Moreover, repeated washing using 4% CHG can lead to skin irritation, the development of abrasions and further colonization with other organisms [16]. Thus, it seems reasonable to assume that the search for new active ingredients that aim to kill or reduce pathogens on hands, especially natural products, is of interest.

The major determinants for handwashing product selection are antimicrobial profile, user acceptance and cost. In recent years, handwashing products, such as gel, liquid, foam or soap have been developed, produced and widely sold. The previous studies reported that the hand rub formulations, being in gel or liquid form was one factor influencing HCWs compliance to hand hygiene. The use of gel versus liquid formation was compared in terms of skin tolerability and product acceptability [17-18]. There were 4 factors to assess the skin tolerability including appearance, integrity, moisture, and skin sensation. While the parameters to obtain the product acceptability were color, smell, sticky feeling, irritation, skin dryness, ease of use, speed of drying of the skin after application, and pleasant feeling on application. The study concluded that the gel was associated with better skin tolerance and superior acceptance as compared to the liquid formulation. In this study, the satisfaction of handwashing gel containing banana peel extract was its scent and moisture. After washing, the smell of banana stuck on hands for hours as well as the pleasant feeling of skin. However, some volunteers dissatisfied with the stickiness of the studied product. Banana peels contain latex, in which polyterpenes is mainly the cause of stickiness. There were reports of banana latex hypersensitivities, also known as latex-fruit syndrome. The two major allergens involved in latex-fruit syndrome were the 32-33 and 34-37 kD Class I chitinase [19-20]. It is noteworthy to mention that hypersensitive users should avoid using the fruit latex products such as the products from banana, avocado, chestnut, kiwi, peach, tomato, potato and bell pepper.

The previous study has been reported that the thumb showed highest degree of contamination compared to the other fingertips during orthopaedic surgery [16]. It is also noticeable from this study that thirty minutes after routine handwashing with 4% CHG before providing dental treatment (primary handwashing), bacteria still existed at a certain level. To reduce contamination that may occur, it is recommended that dentists should wash hands with effective antimicrobial agents as frequent as possible during dental treatments. Dentists should also be aware and emphasized that wearing gloves does not automatically replace the need for handwashing.

Conclusion

The data from this study suggests that handwashing gel containing banana peel extract has the promising result in reducing bacterial load on dentist's hands. This finding supports the utilization of banana peels, a vast agricultural residue in Thailand, as antibacterial agent.

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References

1. Anargh V, Singh H, Kulkarni A, Kotwal A, Mahen A. Hand hygiene practices among health care workers (HCWs) in a tertiary care facility in Pune. ***Med J armed forces India*** 2013; 69: 54-56.
2. Pittet D, Dharan S, Tauveneau S, Sauvan V, Perneger TV. Bacterial contamination of the hands of hospital staff during routine patient care. ***Arch Intern Med*** 1999; 159: 821-26.

3. Anitha M, Hema priya J, Swathy SR, Pavithra GB. Prevalence of hand hygiene among various categories of healthcare workers in hospital setting. ***Inter J App Res*** 2015; 1: 96-99.
4. Watutantrige RDA, Premalatha P, Lum WS, Evelyn CX. A study on hand contamination and hand washing practices among medical students. ***Inter scholarly Res Net*** 2012; 1-5.
5. World Health Organization. WHO Guidelines on Hand Hygiene in Health Care: a Summary. WHO Press, 20 Avenue Appia, 1211 Geneva 27, Switzerland.
6. Baraldi MM, Rizzo JG, Padoveze MC. Risks and benefits of using chlorhexidine gluconate in handwashing. A systematic literature review ***Am J Infect Control*** 2019; 47: 704-14.
7. Simmonds NW, Shepherd K. Taxonomy and origins of cultivated bananas. ***Bot J Lin Soc*** 2008; 55: 302–12.
8. Aboul-Enein AM, Salama ZA, Gaafar AA, Aly HF, A bou-Elella F, Ahmed HA. Identification of phenolic compounds from banana peel (*Musa paradaisica L.*) as antioxidant and antimicrobial agents. ***J Chem Pharm Res*** 2016; 8: 46-55.
9. Ehiowemwenguan G, Emoghene AO, Inetianbor JE. Antibacterial and phytochemical analysis of banana fruit peel. ***IOSR J Pharm*** 2014; 4: 18-25.
10. Surojanamethaku V. Extraction of tannin from banana peel. ***Bangkok:Kasetsat university*** 2004 [accessed 2020 April 8]. Available from: http://agris.fao.org/agris-search/search.do%3FrecordID%3DTH2001000960
11. Mokbel MS, Hashinaga F. Antibacterial and antioxidant activities of banana (*Musa, AAA cv. Cavendish*) fruits peel. ***Am J Biochem Biotechnol*** 2005; 3: 125-31.
12. Taper AR, Jain RK. Study of advanced maturity stages of banana. ***Int J Adv Eng Res Stu*** 2012; 1: 272-74.
13. Wani NS, Bhalerao AK, Ranaware VP, Zanje R. Formulation and evaluation of herbal sanitizer. ***Int J Pharm Tech Res*** 2013; 5: 40-43.
14. Habib F, Rind R, Durani N, Bhutto AL, Buriro RS, Aijaz N, *et al*. Morphological and cultural characterization of *Staphylococcus aureus* isolated from different animal species ***J App Environ Biol Sci*** 2015; 5: 15-26.
15. Hernandes SE, Mello AC, Sant'Ana JJ, Soares VS, Cassiolato V, Garcia LB, *et al*. The effectiveness of alcohol gel and other hand-cleansing agents against important nosocomial pathogens. ***Braz J Microbiol*** 2004; 35: 33-39.
16. Hajipour L, Longstaff L, Cleeve V, Brewster N, Bint D, Henman P. Hand washing rituals in trauma theatre: clean or dirty? ***Ann R Coll Surg Engl*** 2006; 88: 13–15.
17. Stauffer F, Griess M, Pleininger G, Zhumadilova A, Assadian O. Acceptability and tolerability of liquid versus gel and standard versus virucidal alcohol-based hand rub formulations among dental students. ***Am J Infect cont*** 2013; 41: 1007-11.
18. Traore O, Hugonnet S, Lübbe J, Griffiths W, Pittet D. Liquid versus gel handrub formulation: a prospective intervention. ***Crit care*** 2007; 11: 1-8.
19. Wagner S, Breiteneder H. The latex-fruit syndrome. ***Biochem Soc Trans*** 2002; 30: 935-40.
20. Delbourg MF, Guilloux L, Moneret-Vautrin DA, Ville G. Hypersensitivity to Banana in Latex-Allergic Patients. Identification of Two Major Banana Allergens of 33 and 37 kD. ***Ann allergy asthma immunol*** 1996; 76: 321-26.