



Original Research

Evaluation on the Antibacterial Activity and Biocompatibility Natural Soap Formulated with Edible Bird Nest

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ABSTRACT

Edible bird's nest (EBN) derived from saliva of swiftlet is often consumed for its nutritional benefit. In this study, EBN natural soap was studied based on their morphology, antibacterial activity and cytotoxicity. The surface morphology and the presence of heavy metal in the soaps were investigated through scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDX). The antibacterial activity of EBN natural soap against *Escherichia coli* ATCC 25922 was investigated using disc diffusion and bacterial colony count method while the biocompatibility property was determined with human skin fibroblast cells through MTT assay and morphology observation under an inverted microscope. A consistent surface morphology pattern and no heavy metal were recorded on the soaps. The results of antibacterial activity indicated that the EBN natural soap did not have any antibacterial property but able to retain high cell viability percentage of $91.88 \pm 3.04\%$ with normal cell morphology. Therefore, the EBN natural soap is biocompatible and appropriate to be incorporated in skin products.

INTRODUCTION

Edible bird's nest (EBN) is derived from saliva of certain types of swiftlets. It is consumed for its nutritional and medicinal values (Wong, 2013). Although many claims have been made on the therapeutic and health-promoting effects of EBN, scientific documentations regarding these effects are very limited in published literature. The biological effects of EBN are being investigated and evidence-based studies are being conducted. Several studies have found that EBN may enhance cell proliferation and differentiation where these studies point towards the potential use of EBN in the treatment and prevention of several diseases (Wong, 2013). However, the action mechanism of EBN remains largely unknown and more explorations are needed.

Edible bird's nest contains carbohydrates and glycoproteins as the major components, apart from amino acids, fatty acids and some trace elements such as calcium, sodium, magnesium, zinc, manganese and iron (Marcone, 2005). The composition of EBN makes it esteemed as a nutritional food (Marcone, 2005). Chinese cook this nest material in a double boiler with the addition of sugar to produce gastronomic delicacy known as bird's nest soup (Hobbs, 2004). In this study, the antibacterial and cytotoxicity of EBN natural soap were evaluated. Prior to the evaluations, the EBN soap was viewed under scanning electron microscopy (SEM) equipped with energy dispersive X-ray (EDX). The antibacterial activity of the soap against *E. coli* was investigated using disc diffusion and bacterial colony count method. While the cytotoxicity property was analyzed with the human skin fibroblast cells through MTT assay and the cell morphology was observed under an inverted microscope.

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MATERIALS AND METHOD

Preparation of EBN Soap

An EBN soap was supplied by Pure Natural Resources Sdn. Bhd., Malaysia and prepared using a cold process method by mixing 75% v/v fixed oils, 5% v/v sodium hydroxide (NaOH) and 20% v/v EBN extracts. The fixed oils were used in the preparation of soap composed of 50% extra virgin olive oil, 15% Aloe vera oil, 15% shea butter, 10% unrefined avocado oil, 7% cold press extra virgin coconut oil and 3% neem oil.

Surface Morphology and Surface Elemental Analyses

The surface morphology of the EBN soap was - observed under SEM (Tabletop TM3000, Hitachi, Japan) interfaced with EDX (SwiftED 3000, Oxford Instruments, UK) at an accelerating voltage of 15 kV. The visualization was done at 500× and 1000× magnifications. The analytical analysis element of the soap was then performed using EDX to confirm the presence of heavy metal in the soap.

Antibacterial Activity Analyses

The antibacterial activity of the EBN soap against *Escherichia coli* ATCC 25922 (Gram-negative bacteria) was analyzed through a disc diffusion method. Prior to the disc diffusion method, a disc shape of EBN soaps (10 mm diameter and 5 mm thickness) were produced using a metal puncher. The disc-shaped soaps were sterilized using 70% ethanol solution and immediately dried using an air-gun, prior to the placement of pellets on Luria-Bertani (LB) agar plate that has been smeared with *E. coli*. Previously, the *E. coli* was cultured in 100 mL of LB broth at the bacterial growth log phase of 0.6 optical density (OD), overnight. An amount of 100 μ L was pipetted on each agar plate and the bacteria were homogenously distributed on the agar surfaces for the placement of EBN pellets. The agar plates were then incubated at 37°C for 24 hours and the inhibition zones formed against *E. coli* were observed.

The antibacterial activity of the EBN soap was further assessed through a bacterial colony count test and the percentage of the antibacterial efficacy was determined. The extraction suspension of EBN soap was obtained by dissolving sterilized 0.01 g of EBN natural soap into 5 mL distilled water and then vortexed for 30 seconds. Overnight cultured bacteria with a concentration 1×10^6 cells/mL were added into the EBN natural soap extraction suspension. The mixture suspension was then incubated for 3 hours at 200 rpm and 37°C in a shaking

incubator. Two-fold dilution series of mixture suspension were performed and 10 μ L of each dilution series was dropped on LB agar and incubated at 37°C for 24 hours. The bacterial colonies formed on the agar were counted to obtain the percentage of antibacterial efficacy (Daud et al., 2016). The positive control for the bacterial colony test was set to the addition of 1 mL of 70% ethanol in the extraction suspension without the introduction of soap.

RESULT AND DISCUSSION

SEM-EDX Analyses

The SEM images in Figure 1 (a) and (b) show morphology of EBN natural soap at different magnifications. A consistent surface pattern trend was observed and no visualization of bright metallic particle or compound on the soaps indicating the absence of heavy metal. Further analysis using EDX (Figure 1 (c)) has confirmed the absence of heavy metal where only $82.98 \pm 0.57\%$ C, $12.73 \pm 0.67\%$ O and $4.29 \pm 0.14\%$ Na were contained in the EBN soaps.

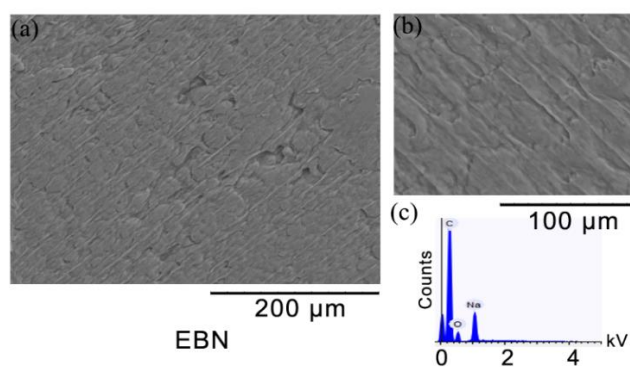


Fig. 1 Morphology of EBN soap at (a) 500× and (b) 1000× magnifications and (c) EDX spectrum of EBN soap.

Antibacterial Activity Analyses

The antibacterial activity of the EBN natural soap against *E. coli* through a disc diffusion method did not show inhibition factor as the diameter of inhibition zones was not clearly seen (Figure 2 (a)). In addition, the dilution of the soap on the agar cause difficulties to determine and measure the diameter of inhibition zone.

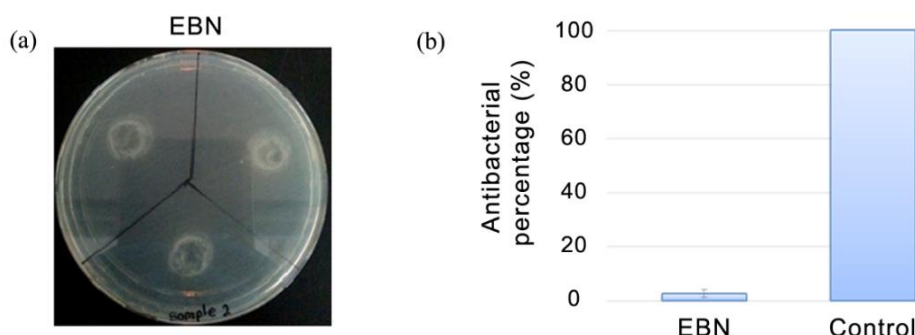


Fig. 2 Antibacterial analysis on EBN soap: (a) Image of inhibition zones and (b) percentages of antibacterial efficacy for EBN soap against *E. coli*.

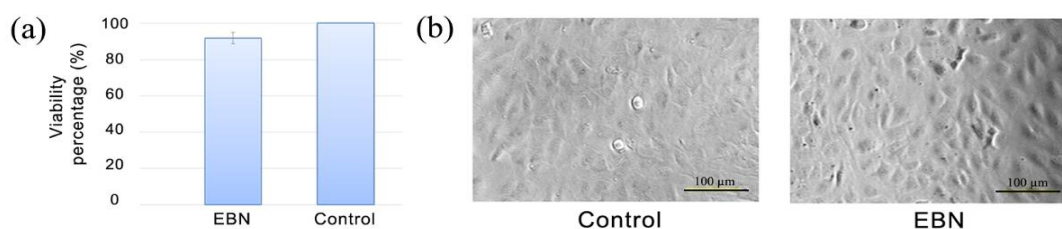


Fig. 3 In-vitro cytotoxicity analyses of (a) cell viability percentages of EBN soap on HSF cells and (b) HSF cells morphology examined under inverted microscope at 20x magnification: Control and EBN natural soap.

The results of inhibition zones were further supported with the bacterial colony count analysis. Figure 2 (b) shows percentage of antibacterial efficacy of EBN natural soap. The EBN soaps were only contributed to $2.67 \pm 1.52\%$ in killing *E. coli* bacteria, indicating very low affinity of antibacterial property. The lack of antibacterial property in the EBN might due to the fact that EBN is a nutritional food material and therefore, might display a similar character towards the tested bacterial strains. However, it may be effective against some other bacteria such as *Pasteurella multocida* (Suriya et al., 2004), and therefore, further studies are needed in this direction (Hun et al., 2007).

In-Vitro Cytotoxicity Analyses

Disclosure of human skin fibroblast cells to the EBN soaps did not lead to cell retardation. High cell viability of $91.88 \pm 3.04\%$ was determined on the EBN soaps as shown in Figure 3 (a). The soaps have produced excellent viability percentage which close to 100%, suggesting its compatibility to be used on the skin. Aswir and Wan Nazaimoon (2011) has also reported that EBN have increased cell proliferation due to the rich content of glyconutrients.

Besides, in this study, the morphology of HSF cell following in contact with the EBN soaps (Figure 3 (b)) showed normal orientation as compared with the control. There was no sign of morphology changes, indicating its compatibility with human skin fibroblast cells.

CONCLUSION

The EBN soap projected no antibacterial activity against *E. coli* bacteria while promoting excellent cell viability close to 100%. Its ability to retain cell morphology, clarifying its potential to be incorporated in soap products for skin nourishment and wound healing.

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