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Nano-Berberine As a Fungicide and Bactericide: An *In Vitro* Evaluation on Vaginal Isolations

Nguyen Nhi Ha[a,b], Ha Thi Thanh Huong[b], Le Quang Huan[c], Pham Thi Van[a], Phan Van Hau[a], Dang Thi Ngan[b], Le Ngoc Thanh[a,b], Vu Thi Thom[b]*

[a]Department of Microbiology, E Hospital, 89 Tran Cung, Cau Giay, Hanoi, Vietnam
[b]VNU-School of Medicine and Pharmacy, Vietnam National University-Hanoi, 144 Xuan Thuy, Cau Giay, Hanoi, Vietnam
[c]Institute of Biotechnology, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam

*For correspondence: thomtbk5@gmail.com

ABSTRACT: Background: Herbal-based products contribute a part in feminine hygiene ones. Several plants extracts have been components of different washing solutions. Berberine nanoparticles had a potential minimum inhibitory concentration against some microbes in comparing to standard antibiotics. Objective: Our studies aimed to evaluate the activities of nano berberine against some microbial strains isolated from vaginal samples. Materials and Methods: The antifungal and antibacterial activity of nano berberine extract 10%, 5% and 2.5% against *Candida albicans*, *Enterococcus faecalis*, *Escherichia coli* and *Klebsiella pneumoniae* was determined by agar dilution agar method. Results: *Candida albicans* and *Enterococcus faecalis* did not yield on nano berberine containing plates while *Escherichia coli* and *Klebsiella pneummoniae* grew well. Conclusion: Nano berberine solution might be used as a potential washing product supporting vulvovaginitis candidiasis control.

Keywords: Antibacterial, antifungal, nano-berberine, vaginitis

INTRODUCTION

Herbal-type preparations are traditional hygiene products which are still in use [1]. The effectiveness of herbal extract on vaginitis treatment and vaginitis symptom control has been studied years ago. Chamomile extract as a douche product may ameliorate vaginitis symptoms with few side-effects [2]. The herbal extract of various plants in Iran had favorable antitrichomonas effects [3]. A combination of *Azadirachta indica*, *Cichorium intybus*, and *Trigonella foenum-graecum* extracts demonstrated *in vitro* synergistic broad-spectrum antimicrobial activities on *Staphylococcus aureus*, *Streptococcus agalactiae* and *Candida albicans* [4]. A commercial intimate care product based on *Arctium majus*, *Chamomilla recutita* and *Aloe barbadensis* used either alone or with antimicrobials

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decreased the frequency and intensity of vulvovaginitis signs and symptoms but remained vaginal pH [5].

In addition to the above extracts, berberine, an herbal-based chemical, should be concerned. Berberine is a quaternary ammonium salt from the protoberberine group of benzylisoquinoline alkaloids. Berberine is usually found in the roots, rhizomes, stems, and bark of some plants as *Berberis*, such as *Berberis vulgaris*, *Berberis aristata*, *Mahonia aquifolium*, *Hydrastis canadensis*, *Xanthorhiza simplicissima*, *Phellodendron amurense*, *Coptis chinensis*, *Tinospora cordifolia*, *Argemone mexicana*, and *Eschscholzia californica* [6].

Besides the beneficial effects of berberine, some limitations including poor aqueous solubility, slight absorption, and low bioavailability have hindered its applications. To overcome these limitations, nanotechnology has been considered as the main strategy and help to the problems of solubility, dissolution rate, and bioavailability of berberine [7].

Berberine nanoparticles demonstrated increasing solubility and dissolution rate and decreasing minimum inhibitory concentration against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, and *Candida glabrata* in comparing to berberine [7].
Our study aimed to evaluate the in vitro antifungal and antibacterial activity of a nano berberine solution against some fungal and bacterial strains isolated from vaginal samples.

MATERIALS AND METHODS

Preparation of nano-berberine: Nano formulation of berberine was provided by Professor Pham Huu Ly from Vietnam Academy of Science and Technology prepared as following protocol: Adding berberine to a mixture of aqueous solutions of 2% Acetic Acid: Formic Acid 2% (v / v = 1/1) and Chitosan. The temperature was maintained around 65-70 ^{o}C, pH ~ 5.6. Mixing time was 16 hours with AD-15 high-speed mechanical agitator yields product A. Neutralizing product A with 0.1% NaOH solution until the pH reached about 7.0. Then, the neutralized product A was spray-dried at 100oC by spray drying machine (BII-S151, Canada) to obtain nano-berberine. The size of nano-berberine was measured by the Fe SEM system as presented in figure 1.

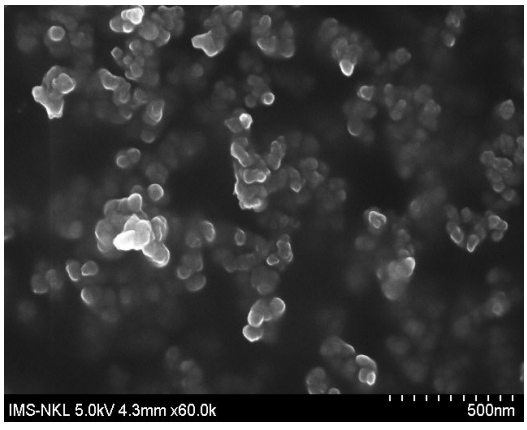

Fig 1. FESEM image of nano-berberine with size from 50-90 nm, scale bar is 500 nm.

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Preparation of fungal and bacterial suspensions: Fungal specimens *Candida albicans* and three bacterial specimens including *Enterococcus faecalis*, *Escherichia coli,* and *Klebsiella pneumoniae* were isolated from clinical vaginal samples in E hospital. They were cultured in blood agar and incubated at 250C (room temperature) in 48 hours with fungi and at 35±20C in 24 hours with bacteria. All specimen suspensions were adjusted to 0.5 McFarland turbidity standards, corresponding to 1.5x108 CFU/ml (Suspension 1). The suspensions 1 then were diluted 100 fold three times serially (Suspension 2, 3, 4).

Preparation of media: The *in vitro* agar dilution assay was used to evaluate antifungal and antibacterial activities of nano-berberine. Four Muller Hilton plate types were diluted with nano berberine at the concentration of 10%, 5%, 2.5%, and 0% (control plate) respectively. Each type of plate was separated into four areas. All plates were incubated at 35±20C in 24 hours before use.

Assay for antifungal activity: Each suspension of *Candida albicans* were respectively inoculated in one area of one plate of each type. All plates were incubated at 250C (room temperature) in 48 hours. The assay was run in triplicates.

Assay for antibacterial activity: Each suspension of each bacterium were respectively inoculated in one area of one plate of each type. All plates were incubated at 35±20C in 24 hours. The assay was run in triplicates.

RESULTS

Antifungal activity of nano-berberine against *Candida albicans*: In our set of experiment, nano-berberine was added in culture media of *Candida albicans* cultured from patient's sample to test whether nano-berberine had anti-fungal activity. The result was shown in figure 2.

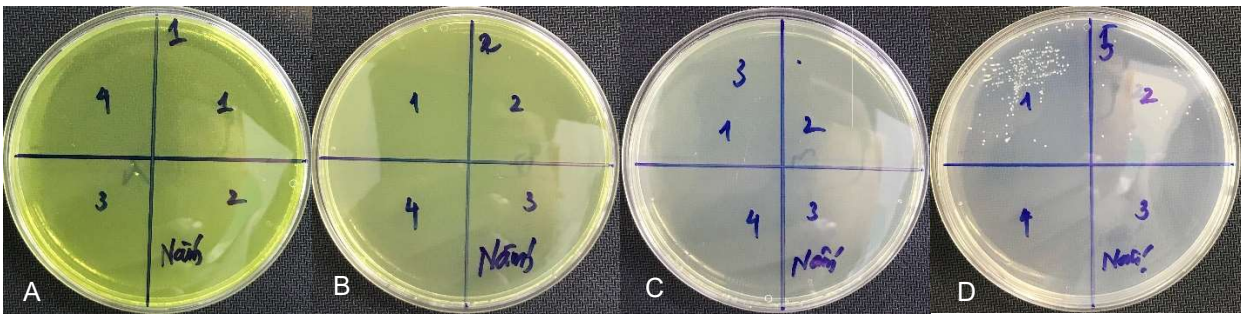

Figure 2: Antifungal activity of nano berberine against C. albicans by using agar dilution assay. (A-C) Nano berberine containing plates with concentration of 10%, 5% and 2.5%, respectively; (D) Control plate. Areas from 1 to 4 were presented for 10^8, 10^6, 10^4, 10^2 CFU per ml concentrations, respectively.

As shown in figure 2D, on the control plate, the number of *C. albicans* colonies yielding in the first and the second areas descended from more than 200 to 18, while *C. albicans* did not yield on the third and fourth areas. In contrast, on 3 nano berberine containing plates (figure 2A-C), *C. albicans* did not grow, even in the first area of the plate with highest concentration of *C. albicans* (10^8 CFU/ml). It meant that all nano-berberine concentrations in this study (from 2.5% to 10%) showed strong inhibition of *C. albicans*. This suggest that low concentration of nano-berberine (2.5%) could be potential used for the vaginal fungi treatment or prevention.

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Antibacterial activity of nano-berberine against *Enterococcus faecalis:* Antibacterial activity was tested on three different bacteria as *E. faecalis, E. coli* and *K. pneumoniae.* Figure 3 expressed the effect of nano-berberine on *E. faecalis*.

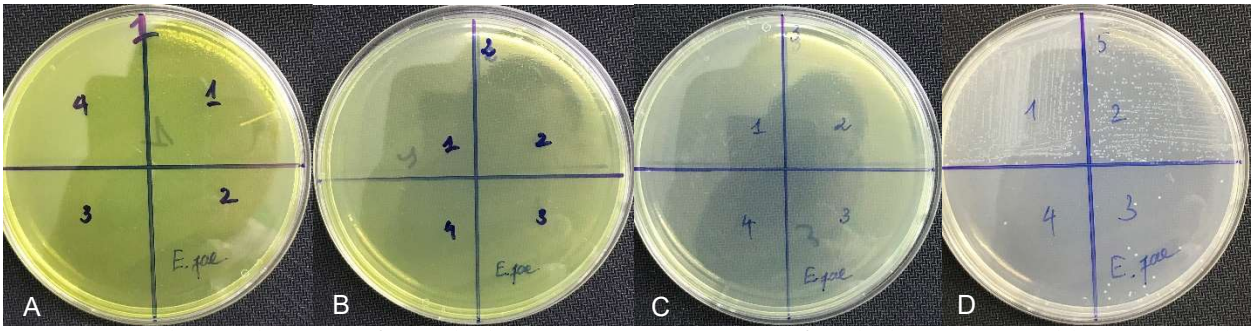

Figure 3: Antibacterial activity of nano-berberine against Enterococcus faecalis by using agar dilution assay. (A-C) Nano-berberine containing plates with 10% in A, 5% in B and 2.5% in C. (D) Control plate without nano-berberine. Number 1 to 4 indicated concentrations of microorganism with 10^8, 10^6, 10^4 and 10^2 CFU per ml.

The numbers of *E. faecalis* colonies yielding in four areas (10^2-10^8 CFU/ml) on control plate D descended according to the concentration of four suspensions with more than 200 colonies in both the first and the second areas (10^8 and 10^6 CFU/ml respectively). In contrast, on 3 nano berberine containing plates (figure 3.A-C), *E. faecalis* did not grow, even the first area with highest concentration of bacteria (10^8 CFU/ml) on the plate containing the lowest concentration of nano-berberine with 2.5%. This indicated that *E. faecalis* isolated from vaginal samples was sensitive to nano-berberine.

Antibacterial activity of nano-berberine against *Escherichia coli* and *Klebsiella pneumoniae:* Two other isolated bacteria tested were *E. coli* and *K. pneumoniae*. The results are shown in table 1 and table 2. In table 1, *E. coli* yielded more than 200 colonies in both the 10^8 and the 10^6 CFU/ml areas, although they did not yield on the 10^2 CFU/ml area. That means choosing appropriate concentration of bacteria was an important point to perform the assay. The numbers of *E. coli* colonies in the 10^4 CFU/ml area of 4 plate types were countable, however, there was no insignificantly difference between nano-berberine groups ($p > 0.05$).

Table 1: Antibacterial activity of nano-berberine against Escherichia coli by using agar dilution assay.

Nano berberine extract concentration (%)	Number of colonies			
	10^8 CFU/ml	10^6 CFU/ml	10^4 CFU/ml	10^2 CFU/ml
10%	UC	UC	3.3 ± 3.21	0
5%	UC	UC	4.7 ± 2.51	0
2.5%	UC	UC	4.7 ± 3.51	0
Control	UC	UC	2.0 ± 1.00	0

UC: Uncountable, number of colonies > 200

Similar result was observed with *K. pneumoniae*. 10^2 CFU/ml concentration of *K. pneumoniae* was too less bacteria to grow in our experiment, however, 10^6 and 10^8 CFU/ml was too high concentrations that let the bacteria over-grown and uncountable in our experiment. 10^4 CFU/ml concentration of *K. pneumoniae* was used to distinguish the effect of different concentrations of nano-

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berberine on bacteria colony growth. Unfortunately, there was no significant different between 2.5%, 5% and 10% of nano-berberine groups in comparison with control group.

Table 2: Antibacterial activity of nano berberine against Klebsiella pneumoniae by using agar dilution assay.

Nano berberine extract concentration (%)	Number of colonies			
	10^8 CFU/ml	10^6 CFU/ml	10^4 CFU/ml	10^2 CFU/ml
10%	UC	UC	4.0 ± 2.64	0
5%	UC	UC	3.6 ± 3.78	0
2.5%	UC	UC	4.0 ± 3.61	0
Control	UC	UC	2.3 ± 2.52	0

UC: Uncountable, number of colonies > 200

From those data, in current study, nano-berberine did not affect on *E. coli* and *K. pneumoniae* isolated from vaginal samples.

DISCUSSION

Vulvovaginitis candidiasis affected about 75% of women at least once during their reproductive life, with 40 – 50% having two or more episodes [8]. *Candida albicans* was the most common pathogenic *Candida*, occupied 58.6% of general isolates [9] and 59% of biofilm-forming multidrug-resistant strains [10]. Berberine demonstrated not only a stable antifungal activity in planktonic conditions but also concentration-dependent inhibitory effects against *Candida* biofilms [11]. Our study showed that *Candida albicans* did not grow on nano berberine-containing plates, suggesting the effective antifungal activity of nano berberine.

As for antibacterial activity, nano berberine inhibited completely *Enterococcus faecalis* but insignificantly *Escherichia coli* and *Klebsiella pneumoniae.* During bacterial vaginosis treatment, *Enterococcus* contributed a part in the 5-component panel for bacterial vaginosis recurrence prediction, it could restrain other bacteria, but its negative interaction was much less than of *Lactobacillus* [12]. Whether the *in vitro* inhibitory of nano berberine against *Enterococcus faecalis* could affect the outcome of bacterial vaginosis treatment is still a question demanding further studies. Muhammad et al (2018) proved that berberine nanoparticles expressed multiple *in vitro* antimicrobial activities against *S. aureus, B. subtilis, E. coli*, *P. aeruginosa*, *C. albicans,* and *C. globate* [7]. Berberine itself was used to treat successfully methicillin-resistant *S. aureus* [13]. Moreover, berberine coated with nanogel even showed higher potential anti-bacterial activity in many studies, especially for anti-*E. coli* activity which was not seen in our study [7, 14]. From these current data, some missing information is really needed to be elucidated in further experiments, especially with *E. coli* and *K. pneumoniae*.

CONCLUSION

Nano berberine had a noticeable antifungal and antibacterial activity against clinical vaginal strains of *Candida albicans* and *Enterococcus faecalis*. Nano-berberine solution might be used as a potential washing product supporting vulvovaginitis candidiasis control.

DECLARATION OF CONFLICT OF INTEREST

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No conflict of interest associated with this work.

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