



Main ingredients in herbal formulation may act as preservative agent

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ARTICLE INFO

Type: Original Research

Topic: Medicinal Plants

Received July 24th 2016

Accepted December 16th 2016

Key words:

- ✓ Barij Baby Cream
- ✓ Antimicrobial effectiveness test
- ✓ Natural preservative

ABSTRACT

Background & Aim: Nowadays, the use of chemical preservatives has been limited due to the controversial adverse effects. In this study, we evaluated the preservative potency of product with active herbal ingredients (Barij Baby cream) in contrast with the same products with two different chemical preservatives including methylparaben plus propylparaben and potassium sorbate plus sodium benzoate.

Experimental: The efficacy of herbal formulation against pathogens (*E. coli*, *P. aeruginosa*, *C. albicans*, *A. niger*, *S. aureus*) was evaluated by antimicrobial effectiveness test during 28 days.

Results: 2 log reductions in bacterial counts at 14 days from the initial count, and no growth in bacterial and fungal (molds and yeasts) counts at 28 days from the 14 days is essential for accepting the antimicrobial effectiveness of category 2 products in United States Pharmacopoeia (USP). Our results of antimicrobial effectiveness test showed that the active herbal ingredients in Barij Baby cream passed the USP criteria as chemical preservatives in the same product.

Recommended applications/industries: Therefore, in designing new herbal products, it is essential to evaluate the antimicrobial effectiveness of products before supplemented the product with chemical preservatives.

1. Introduction

Parabens or alkyl esters of p-hydroxybenzoic acid are permitted to use as preservatives in many thousands of cosmetics, foods and drugs. Therefore, by using these products the humans are exposed to daily intake of parabenes. Parabenes are quickly absorbed from gastrointestinal tract, blood and skin (Darbre et al., 2004; Soni et al., 2001). The oestrogenic effects of parabens have been confirmed in 1998 (Routledge et al., 1998). Also topical daily administration of parabens decreased the keratinocytes proliferation and

cell morphology (Ishiwatari et al., 2007). On the basis of research, the oestrogenic stimulation is associated with female breast cancer incidence and development of malignant melanoma in male reproductive system (Darbre & Harvey, 2008; Tavares et al., 2009). Furthermore, the use of parabens in baby and child care products and demonstrating their estrogenic effects in children's blood samples (Purdell et al., 2015) have concerned the health affairs.

In attention to these concerns, replacing the parabens with the suitable ones preferably from natural sources in baby and child care products is one of the most important issues in current years. Essential oils,

and plant extracts are the good sources of natural preservatives (Mahboubi *et al.*, 2014), but confirming their antimicrobial effectiveness along with their efficacy in final products is the subject of this investigation.

For supporting the natural issue in designing of herbal products, in designing of Barij baby cream, we evaluated the antimicrobial effectiveness of this natural Cream with main active ingredients of *Calendula officinalis* extract, *Matricaria chamomilla* essential oil, *Lavandula angustifolia* essential oil and *Aloe vera* gel against the same two products with two different chemical preservatives by antimicrobial effectiveness test. We supposed that the active herbal ingredients in product can act as natural preservative such as chemical ones.

2. Materials and Methods

2.1. Barij Baby cream and its active herbal ingredient

Three kinds of Barij Baby Creams were formulated by Formulation department, Medicinal Center of Barij, Kashan Iran. The differences of three formulas were in their preservative components.

The specifications of creams include:

- 1- Baby Cream with *Calendula officinalis* extract, *Matricaria chamomilla* essential oil, *Lavandula angustifolia* essential oil and *Aloe vera* gel (Barij Baby Cream)
- 2- Barij Baby Cream with potassium sorbate (0.1% w/w) and sodium benzoate (0.1% w/w) (Barij Baby Cream PS)
- 3- Barij Baby Cream with methylparaben (0.18% w/w) and propylparaben (0.02% w/w) (Barij Baby Cream MP)

2.2. Microbial strains

Escherichia coli ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027, *Staphylococcus aureus* ATCC 6538, *Candida albicans* ATCC 10231, *Aspergillus niger* ATCC 16404 were used for antimicrobial evaluations. Bacteria and fungi were cultured on Soybean Casein Digest Agar and Sabouraud dextrose agar. The cultures were incubated at 32.5 ± 2.5 °C and 22.5 ± 2.5 °C for 3-5 and 6-10 days, respectively. One

colony of each microorganism was suspended in normal saline and their turbidities were adjusted to 0.5 McFarland. The CFU/ml of bacteria and fungi were 1×10^8 and 1×10^6 , respectively.

2.3. Antimicrobial Effectiveness Test

For conducting the test, 20 g of each formulation was transferred in five different capped bacteriological containers, aseptically. Each prepared inoculum (0.5-1% of the volume of product) was added to each container. The final concentrations of inoculums were 10^6 and 10^4 CFU/ml for bacteria and fungi, respectively. The containers were incubated at 22 °C and the number of CFU present in each test was determined by plate counts in time intervals 0, 7, 14, 21 and 28 days. The log₁₀ was calculated for each microorganism after determining the CFU/ml. The change in concentration of microorganism in terms of log reduction was defined as the difference between the log₁₀ unit value of each time interval with the others (USP, 2015).

3. Results and discussion

Our results showed that the reduction in log *E. coli* counts in Barij Baby Cream PS was from 7.4 to 3.7 after 28 days, while this decrease was estimated from 7 to 1 for Barij Baby cream PM and from 7.6 to 4.27 for Barij Baby Cream (Figure 1-A).

P. aeruginosa counts reductions were 6.3, 5.7 and 6.7 for Barij Cream Baby PS, Barij Baby cream PM and Barij Baby Cream after 28 days, respectively (Figure 1-B). Our results showed that *P. aeruginosa* was more sensitive than *E. coli* in three formulations.

S. aureus counts reduced from 7.9 to 2.9 in Barij Cream Baby PS and from 6.7 to 1 in Barij Baby cream PM and from 7.6 to 3.6 in Barij Baby cream (Fig. 1-C).

According to the results, Barij Baby cream exhibited the higher antibacterial activity against *P. aeruginosa*, followed by *S. aureus* and then *E. coli*.

Log reduction from the initial counts of bacteria at 14 days were 2.2, 2 and 2.5 for Barij Baby cream PM, Barij Baby Cream PS and Barij Baby Cream, respectively and also a reduction in log CFU of bacterial counts were observed in all groups from the

14 days to 28 days (Fig. 1-D). The antimicrobial effectiveness of three formulations in bacterial count reductions at day 28 were 2.87, 3.32 and 2.2 for Barij Cream Baby PS, Barij Baby cream PM and Barij Baby Cream from 14 days, respectively (Fig. 2).

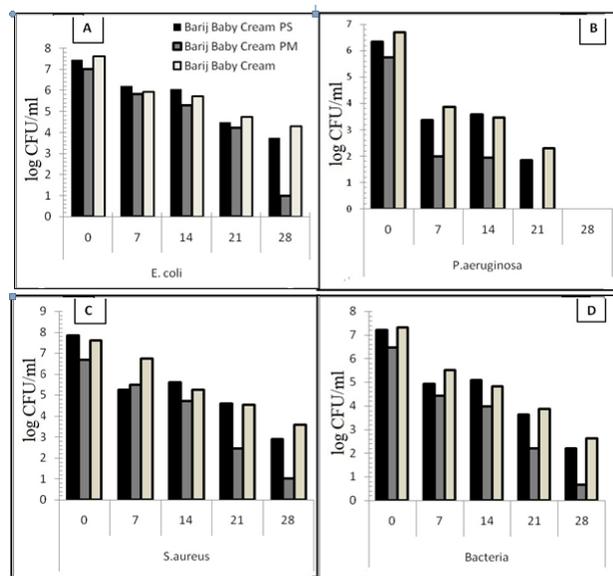


Fig. 1. The Antimicrobial Effectiveness testing of different formulations of baby creams A) *E. coli*, B) *P. aeruginosa*, C) *S. aureus*, D) Bacteria

According to USP criteria, an antimicrobial agent should have the efficacy in controlling of fungi growth. Fungal counts reductions were 1, 1 and 2.6 for Barij Cream Baby PS, Barij Baby cream PM and Barij Baby Cream, respectively. Therefore, fungal count's reduction (yeast and molds) were determined at 28 days from the 14 days (Fig. 2).

Nowadays, the use of parabens and chemical preservatives has been limited in pharmaceutical and healthcare products.

Many scientists have applied many essential oils as preservatives (Kordsardouei et al., 2013; Kumar Tyagi et al., 2014; Mahboubi et al., 2014) but they have not used the results of researches in market. In this study, we prepared a formulation of Barij Baby Creams as baby and child care product with the main active herbal ingredients including *Calendula officinalis* extract, *Matricaria chamomilla* essential oil, *Lavandula*

angustifolia essential oil and *Aloe vera* gel and the antimicrobial effectiveness property of this formula was compared with two other formulations with the same main active ingredients plus chemical antimicrobial agents.

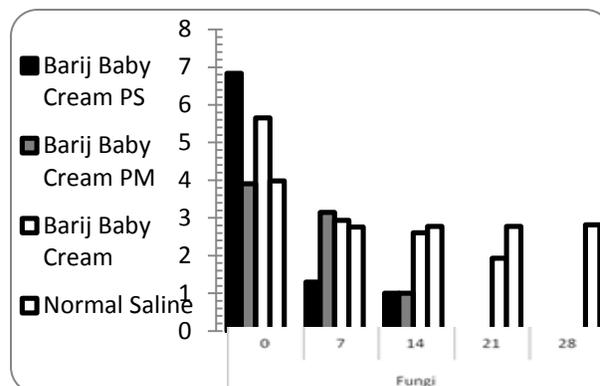


Fig. 2. The antimicrobial effectiveness test of Barij Baby Creams against fungi

According to compendial product categories in USP, Barij Baby cream is belonged to category 2 (topically used products made with aqueous bases or vehicles). On this categorization, the accepted criteria for antimicrobial effectiveness of these products is 2 log reduction in bacterial counts at 14 days from the initial count, and no increase in bacterial and fungal counts at 28 days from the 14 days counts (USP, 2015).

Although, the Barij Baby cream with preservatives (parabens and potassium sorbate plus sodium benzoate) passed the USP criteria for antimicrobial effectiveness test, but the Barij Baby cream with natural active ingredients passed the USP criteria for category 2 products.

The results of our study confirmed the antimicrobial effectiveness of natural products in Barij Baby creams. Our results confirmed the preservative potency of main effective ingredients such *Calendula officinalis* extract, *Matricaria chamomilla* essential oil, *Lavandula angustifolia* essential oil and *Aloe vera* gel.

Investigations on other researches by other scientists exhibit that all main ingredients of Barij Baby cream have antimicrobial activities. The efficacy of *Calendula officinalis* extract (2.5%) in comparison with methyl paraben (0.4%) in cosmetic emulsion was

evaluated against *P. aeruginosa*, *E. coli*, *S. aureus*, *C. albicans* (Herman *et al.*, 2013). The antimicrobial activity of *C. officinalis* extract was 0.8-1.7 times stronger than methylparaben (Herman *et al.*, 2013). The antimicrobial activity of *L. angustifolia* essential oil and its synergistic effects with *M. chamomilla* essential oil were the subject of investigation (de Rapper *et al.*, 2013). Also, the antimicrobial effects of *Aloe vera* gel were confirmed (Goudarzi *et al.*, 2015; Lawrence *et al.*, 2009). Regardless of the antimicrobial properties of these ingredients, plant extracts or essential oils have multifunctional effects, while the chemical preservatives had only antimicrobial efficacy with many known and unknown adverse effects.

M. chamomilla essential oil has been prescribed as inflammatory and analgesic agents for long times and these effects are attributed to inhibition of Prostaglandin E2 and attenuation of COX-2 (Srivastava *et al.*, 2009). Wound healing effect of topical application of *M. chamomilla* was shown on patients under dermabrasion of tattoos (Srivastava *et al.*, 2009). The healing properties, anti-inflammatory action, moisturizing and anti-aging effect of *Aloe vera* gel have been explored (Hashemi *et al.*, 2015; Surjushe *et al.*, 2008). The safe application and therapeutic efficacy of *Calendula officinalis* ointment and *A. vera* cream were shown in treatment of diaper dermatitis in infants (Panahi *et al.*, 2012).

Therefore, the active herbal ingredients in natural herbal products have multifunctional effects and in addition to desired therapeutic efficacy have other biological activity such as preservative potency.

In conclusion, the multifunctional effects of plant extracts and essential oils can donate the other biological activity to products, therefore in designing of herbal products, it is important to evaluate the antimicrobial effectiveness of herbal products before inserting the chemical preservatives in order to remove the chemical ones from pharmaceutical and healthcare products.

4. Acknowledgements

This study is supported by Medicinal Plants, Research center of Barij, Kashan, Iran.

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