Preparation of Antibacterial Lotion of *Carex meyeriana* Kunth

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Abstract: This paper is to prepare lotion of *Carex meyeriana* Kunth (CMK) with antibacterial effect. The lotion is mainly composed of the following materials: CMK, *Leonurus japonicas*, *Sophora flavescens*, *Dictamnus dasycarpus* and polyhexamethylene biguanide. Suspension quantitative germicidal test and toxicity test were used to evaluate the antibacterial effect and safety of prepared lotion. Results showed that the lotion exerts profound inhibitory effects on *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*, all the inhibitory rates exceeded 99.9% after treatment for 20 min, 20 min and 30 min, respectively. After being stored in a 40℃ incubator under strong light for 6 months, the antibacterial effects of prepared lotion were not decreased significantly. Moreover, the lotion has no skin irritation and adverse reactions. Thus the lotion reported in present investigation possesses better applicable potentials due to the excellent antibacterial effects as well as better stability and safety properties.

1 INTRODUCTION

Lotion mainly includes plant medicine and synthetic medicine. Traditional botanical drugs potentiate the onset and rapid efficacy of synthetic drugs. However, synthetic drugs are prone to generate resistance and relapse. Therefore, antibacterial products with Chinese herbal medicine as the main component combined with synthetic drugs come into being and have been widely used. As one of the Northeast Three Treasures in China, *Carex meyeriana* Kunth has a good bacteriostatic effect (Cheng 2020), but its uses are currently limited to the preparation ofmattresses and insoles. High value-added development of *Carex meyeriana* Kunth is urgent to be conducted. *Leonurus japonicas*

Houtt (LjH) is used in the treatment of gynecologic disorders such as menorrhagia, menostasia, and other irregular menstruation disorders (Hyun 2010). *Sophora flavescens* (SF) is used for external treatment of diseases such as red and leucorrhea, Yin swelling and Yin itching, eczema, wet sores, skin itching and trichomonas vaginitis (Sato 2007). *Dictamnus dasycarpus* Turcz (DdT) can dispel wind and relieve itching totreat wind heating and dampness caused by rubella andeczema (Zou 2013). From the theory of compatibility of traditional Chinese medicine, the combination of radix DdT and radix SF can greatly increase the functions of detoxification, heat clearing and dehumidification (Zhang 2015). In this study, extracts of CMK, LjH, SF and DdT were synergized with polyhexamethylene biguanide (PHMB) toprepare the lotion with antibacterial potential. Then, the stability and safety properties were further evaluated.

2 MATERIALS AND METHODS

2.1 Materials

CMK were collected in October 2020 in Longtan District, Jilin City, Jilin Province. LjH, SF and DdT were purchased from Longtan pharmacy (Jilin, China). PHMB was purchased from Shanghai Gaoju Biotechnology Co, LTD. (Shanghai, China), lactic acid was purchased from Tianjin Damao Chemical Reagent Factory (Tianjin, China). All chemical reagents are of 95% analytical pure. All the strains used in the experiment were provided by China

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2.2 Methods

2.2.1 Formula Screening with Bacteriostatic Test

Extracts of LjH, SF and DdT were prepared by hot-water and ethanol extractions, and then the MIC of the extracts against *Escherichia coli* (EC), *Staphylococcus aureus* (SA) and *Candida albicans* (CA) were determined by the doubled and half dilution method.

CMK extract: CMK was cut into 2 - 3 cm and refluxed with distilled water at a solid-liquid ratio of 1: 40 at 90℃ for 3h.

LjH and DdT extracts: LjH and DdT were powdered and refluxed with distilled waterat a solid-liquid ratio of 1: 12 at 90℃ for 1.5 h.

SF extract: SF was crushed and refluxed with 80% ethanol as the solvent by a solid-liquid ratio of 1:20 at 70℃ for 3h.

The lotion was prepared by 1: 1 of MIC of each extract after the extracts were filtered, separately.

2.2.2 Product Appearance and Determination of pH Value

Visual inspection of the product appearance wasperformed under the conditions of ambient temperature of 19℃ and relative humidity of 85%, according to sanitary products standard GB15979-2002.

The pH range of aqueous solution samples was determined by pH test paper, and the pH meter was corrected with the corresponding pH correction liquid, and then the pH value of the sample was determined. The pH value of organic samples was measured by pH test paper.

2.2.3 Determination of PHMB Content

According to GB26367-2010, the absorbance was measured at 545 nm, water was served as solvent.
The calculation formula of PHMB content is as follows:

$$C = \frac{m}{V \times 1000} \quad (1)$$

C—PHMB content in sample solution (g/mL).

m—PHMB mass of the sample solution from the standard curve (g).

V—Volume of sample solution (mL).

2.2.4 Stability Test

The accelerated test method was adopted, and the samples were placed in a 40℃ constant temperature box with strong light for 6 months. The inhibitory effects of the samples on SA, EC and CA were measured before and after storage to judge their stabilities (Aslani 2016).

2.2.5 Toxicity Test

According to the disinfection technical specification 2002 edition, rabbits were selected for vaginal mucosa irritation test. Rabbits were divided into the infected and control groups (n = 3). The average score of the vaginal mucosal stimulation of the experimental group was calculated by the following equation: add up all scores of three animals in the experimental group, and divide by the total numbers of observed animals. (number of animals × 3). The scoring method for control group was the same as above. The average score of the experimental group was subtracted from the average score of the control group to obtain the stimulation index. According to the disinfection technical Specification 2002 edition, guinea pigs were selected for skin allergy test. There were 16 guinea pigs in the control group and the experimental group, respectively. Take 0.5 mL of sample and apply it to the animal hair removal area, then negative control groupwas given tight subjects stimulate processing, and the skin reaction was observed and scored 24 h and 48 h after the treatment. The animals with skin reaction (score≥1) were divided by the number of experimental animals in this group to obtain the sensitization rate (%).

2.2.6 Preparation of Bacterial Suspension

The bacterial stock solution used in the experiment was configured with the corresponding inclined surface culture medium, and the original bacteria was dipped in the sterile operating table with the inoculation stick, and the original bacteria was evenly smeared on the surface of the inclined surface culture medium by rotating the inoculation stick, and then wrapped up and cultured for 24 h. Take out the inclined culture medium, andann wash the inclined plane with 10 mL of sterile water, then make the bacterial suspension and pour it into the sterile conical bottle (Zhang 2011).

2.2.7 Identification Test of Neutralizer

According to Disinfection Technical Specification 2002 edition of ministry of health, the neutralizer

identification test of antibacterial lotion was carried out with TSB of 1% soap base, 6.5% tween 80 and 4.5% lecithin as neutralizer.

2.2.8 Microbial Index Detection

According to the hygienic standard for disposable sanitary products GB-15979-2002, the ambient temperature for inspection was fixed at 19℃ - 21℃. Accurately weigh 10±1 g of the sample, add it into 200 mL of sterilized physiological saline, and mix well to obtain a physiological saline sample solution. Then, take the supernatant and count the colonies, inoculate a total of 5 plates, add 1 mL of washing liquid sample to each plate, and then pour 15 - 20 mL of melted nutrient agar medium cooled to about 45℃, and mixed well in each plate. After the agar was solidified, turned the plate over and incubated at 35±2℃ for 48 h, and then counted the number of colonies on the plate.

The formula calculating the total number of bacterial colonies:

$$X_1 = A \times \frac{K}{5} \quad (2)$$

X_1 — Total number of bacterial colonies.

A — Total number of bacterial colonies on 5 nutrient agar plates.

K — Dilution degrees.

2.2.9 Bacteriostatic Test

According to Disinfection Technical Specification 2002 edition of ministry of health, quantitative bacteriostatic test of bacteria and fungi for lotion was carried out.

The formula killing log value:

$$K_L = No - Nx \quad (3)$$

K_L — Killing log value.

No — Log value of average living bacteria concentration in control group.

Nx — Log value of live bacteria concentration in experimental group.

3 RESULTS AND DISCUSSION

3.1 Screened Formula

The MIC of different plant extracts against different strains were shown in Table 1.

Table 1: MIC of different plant extracts against different strains.

Stran	LJH (mg/mL)	SF (mg/mL)	DDT (mg/mL)	Dandelion (mg/mL)	Mentha (mg/mL)
SA	24	5	5	50	/
EC	/	8	6	/	32
CA	/	5	10	/	/

As shown in Table. 1, MIC of single dandelion and peppermint was high, so LjH, SF and DdT were choose to make antibacterial lotion.

3.2 Preparation of Antibacterial Lotion

Extracts of CMK, LjH and DdT, SF were mixed in the ratio of 1: 1: 1, 95% PHMB was added to the solution at a concentration of 0.8%, mixed well and then 5%lactic acid solution was added, mixed well and left for 48 h, the supernatant was taken, filtered and centrifuged to obtain the antibacterial lotion.

3.3 Appearance and pH Value of Lotion

The lotion was brown liquid, neat in appearance, consistent with the inherent shape of the sanitary product, without abnormal odor and foreign matter. The pH value of the antibacterial lotion was 3.7 after repeated determination for 3 times.

3.4 PHMB Content and Lotion Stability

The content of PHMB was 0.2 ± 0.02%, which meet the hygiene standards of guanidine disinfectants. The bactericidal rate reached 99.9%, showing good stability.

3.5 Vaginal Mucosa Irritation and Skin Allergy

As shown in Table 2, the index of vaginal mucosa stimulation to rabbits was 0.33, and the results intensity test of vaginal mucosa stimulation was no vaginal mucosa irritation. As shown in Table 3, the sensitization rate of the sample was 0%, suggesting none of skin allergy.

Table 2: Vaginal mucosa irritation rating table.

Group	Number	Stimulus response score				
		Epithelial tissue	*Leukocyte infiltration*	*Small vessels*	*Edema*	*Total*
Infected	1	0	0	1	0	1
	2	0	0	1	0	1
	3	0	0	1	0	1
	average score		— —			0.33
contrast	4	0	0	0	0	0
	5	0	0	0	0	0
	6	0	0	0	0	0
	average score		— —			0
Stimulus index			0.33			

Table 3: Skin allergy in guinea pigs.

Group	Quantity	Induction concentration	Stimulate concentration	Time (h)	Erythema response intensity				
					0	1	2	3	4
Test group	16	Sample apply	Sample apply	24 h	16/16	0/16	0/16	0/16	0/16
				48 h	16/16	0/16	0/16	0/16	0/16
Negative control	16	—	Sample apply	24 h	16/16	0/16	0/16	0/16	0/16
				48 h	16/16	0/16	0/16	0/16	0/16
Positive control	16	0.60%	0.30%	24 h	0/16	15/16	1/16	0/16	0/16
				48 h	0/16	12/16	1/16	0/16	0/16

continued table

Edema response intensity				Sensitized animals	Sensitization rate (%)
0	1	2	3		
16/16	0/16	0/16	0/16	0	0
16/16	0/16	0/16	0/16	0	0
16/16	0/16	0/16	0/16	0	0
16/16	0/16	0/16	0/16	0	0
12/16	4/16	0/16	0/16	16	100
8/16	7/16	1/16	0/16	13	81.25

3.6 Neutralizer Identification Test

Table 4 showed the experimental results of neutralizer identification.

Table 4: Results of neutralizer identification test.

Concentration	group	Number of growing colonies(cfu/mL)			Average (cfu/mL)
		1	2	3	
concentrate	1	0	0	0	0
	2	3.06×10^4	4.02×10^4	3.80×10^4	3.87×10^4
	3	2.89×10^7	3.05×10^7	2.95×10^7	2.96×10^7
	4	2.61×10^7	2.95×10^7	2.86×10^7	2.81×10^7
	5	3.02×10^7	3.20×10^7	2.63×1^7	2.95×10^7
Annotation: All the negative controls were sterile					

The Table 4 showed that TSB neutralizer with 1% saponin, 6.5% Tween 80 and 4.5% lecithin could neutralize the bactericidal components in antibacterial lotion, and was suitable for the killing test of fungi and bacteria to be carried out.

3.7 Microbial Index Test

The results of microbial indicators of the lotion were in line with the requirements of the hygienic standard for disposable sanitary products GB15979-2002.

3.8 Bacteriostatic Test

The results in Table 5 showed that the killing logarithms of the lotion against SA and EC for 20 min were greater than 5.00, and the killing logarithm of CA treated for 10 min was greater than 4.00, indicating that the lotion has killing effects on EC, SA and CA.

Table 5: Killing effect of antibacterial lotion on EC, SA and CA.

Test strains	Concentration	Average colony number logarithm and range	Average killing logarithm and range at different time (min)	
			0	*1*
EC	stoste	7.07 (7.03-7.11)	>5.00 (>5.00)	>5.00 (>5.00)
SA	stoste	7.06 (7.02-7.11)	>5.00 (>5.00)	>5.00 (>5.00)
CA	stoste	6.26 (6.20-6.31)	2.47 (>4.00)	>4.00 (>4.00)

PHMB is one of the commonly used antibacterial agent, which exhibits low minimal inhibitory concentration and rapid inactivating efficacy against common microorganisms (Gong 2021). As an antiseptic in the medical field for many years(Zheng 2012), CMK can inhibit EC, SA and CA (Cheng 2020). DdT can inhibit the growth of many pathogenic bacteria *in vitro* (Zhao 1998). The aqueous extract of SF has antimicrobial and anti-inflammatory effects and has been widely used for the treatment of skin problems (Kim 2013). Due to the synergy of the above-mentioned extracts, the experimental results showed that the combinatory use of plant extracts and PHMB can inhibitEC, SA and CA, significantly. The lotion prepared using these components has good compatibility with skin, no irritation and allergic reaction, long-action time and low-recurrence rate, and improves the shortcomings of the previous lotion.

4 CONCLUSIONS

This study innovatively used plant extract of CMK for preparation of antibacterial lotion, expanded the scope of its application, compound CMK lotion was made from natural herbs CMK, LjH, SF, DdT extract as the functional component. The lotion is a brown liquid with no abnormal odour and a pH of 3.7. The lotion possesses good stability, and water was used as solvent and PHMB with content of 0.2% and 0.5% lactic acid were added. The *in vitro* antibacterial test showed that the lotion has antibacterial activity against EC, SA and CA, and the antibacterial effects can last for a long time, and there were no irritation and adverse reactions to vaginal mucosa. The results showed that the lotion has promising market application potential and development value.

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