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The preservative efficacy of Licorice and Rosemary combination in cream formula

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ABSTRACT

Based on our previous results, which proved that each of G. glabra and R. officinalis extracts potentiate the antibacterial effect of the other aganist MRSA when combined together at their sub-MIC. We thought to investigate the capacity of Glycyrrhiza glabra(Licorice) and Rosmarinus officinalis (Rosemary) extracts to act as preservatives for topical formulations, which in our knowledge is the first to be used together. Two Oil/Water(O/W)cream were formulated : using methyl paraben, a common used preservative (MP) and the combination between Licorice and Rosemary (LR) as preservatives, were tested for their Primary skin irritation on Laboratory experimental animals which proved that they were devoid of any primary skin irritation, erythema, or edema even after 48 h of application, and by challenging them with microbial indicators: Bacterial; (Escherichia Coli 25922, Pseudomonas aeruginosa 27853 and Staphylococcus aureus 29737), Yeast; (Candida albicans10231), and fungi; (Aspergillus niger 1015), revealed that the concentration of each test microorganisms decreased during the test period.

Key words: Licorice, Rosemary, Preservatives, Topical formulations.

INTRODUCTION

Natural remedies from medicinal plants are found to be safe and effective. Many plants species have been used in folkloric medicine to treat various ailments. Even today compounds from plants continue to play a major role in primary health care as therapeutic remedies in many developing countries [1].

Licorice, the roots of *Glycyrrhiza glabra(G. glabra)*, it is also known as "sweet root"[2],has been used as a medicinal herb for over 4000 years. The active components of this plant have extensive therapeutic usage throughout the world and are subjected to enormous works in recent years. Rosemary extracts have bioactive properties, but their antimicrobial activities have not been deeply characterized. Antimicrobial activities of plant essential oils have been known for centuries, but their strong flavor limited their use in food [3].

Muhammed Majeed and Prakash [4] recorded that no single natural extract has been found to be comparable in efficacy to conventionally used preservatives such as parabens, so combinations of naturals with synergistic activity have been identified. Such extracts could offer protection against invasive growth of bacteria and fungi. Parabens for example, are poorly water soluble and their action is strongest against gram positive bacteria and fungi and weakest against gram negative bacteria. It is here that combinations of natural extracts may be useful. The aim of the present work was to evaluate the preservative efficacy of Licorice and Rosemary combined extracts.

MATERIALS AND METHODS

Plant Material

Licorice (*Glycyrrhiza glabra* Family :Fabaceae) Dried roots, Rosemary (*Rosmarinus officinalis* Family: Lamiaceae) were collected from local market.

Preparation of extracts

The grounded plant parts were subjected to extraction process by maceration. Dried powder of plant parts (10g) was mixed with 100 ml diethyl ether (10%w/v) at room temperature for 48 hours with occasional stirring [5]. Thereafter, it was filtered through filter paper Wattman filter No. 1.The supernatant was collected and diethyl ether was evaporated in vacuum evaporator to make the final volume about 5ml, transfer to test tube, dissolve in Dimethyl sulfoxid (DMSO) and Stored in dark bottles at 4ºC for further studies.

Test microorganisms

Microbial indicators: Bacterial; (*Escherichia Coli* 25922, *Pseudomonas aeruginosa* 27853 and *Staphylococcus aureus* 29737), Yeast; (*Candida albicans*10231), and fungi; (*Aspergillus niger* 1015). All reference strains were kindly provided from the stock culture of Microbiology Department, National Organization for Drug Control and Research (NODCAR), Cairo, Egypt.

Preparation of O/W cream

The O/W cream based preparations containing aqueous phase and oil phase were kindly prepared in the half productivity unit at Applied Research Centre for Medicinal Plants (ARCMP).Ingredients of oil phase (A) mixed together by melting in a china dish with constant stirring. Components of aqueous phase (B) mixed together and warmed to about the same temperature of oil phase. Aqueous phase was added to oil phase drop by drop with constant stirring. The preservative methyl paraben was added after cooling to 40ºC and in another preparation the plant extracts were added [6].
Ingredients Quantity in gm:

Phase A	
Stearic acid	15.00
Potasssium hydroxide	0.50
Sodium hydroxide	0.18
Propylene glycol	3.00
Phase B	
Glycerin	5.00
Methyl paraben	0.1(or plant extracts)
Purified water	complete to 100.00gm

Organoleptic characters

The effect of the extracts on the organoleptic characters (appearance, colour, texture and odor) of the cream was expressed in the form of scores from 0 to 5, such that (0) means no effect, and as the score increase there is a higher effect.

Evaluation of o/w cream

The o/w cream was evaluated for pH, viscosity, spread ability and primary skin irritation test on experimental animals.

Determination of pH

Weigh accurately 5± 0.01gm of the cream in 100ml beaker. Add 45 ml of water and disperse the cream in it. Determine the pH of suspension at 27ºC using the pH meter [7].

Viscosity

The viscosity of formulated o/w cream was measured by Brookfield Viscometer (DV-III programmable Rheometer) using spindle CP-40 at varying speed and shear rates. The measurements were made over the range of speed setting from 0.10, 0.20, 0.30, 0.40 and 0.50 rpm with 60sec between two successive speeds as equilibration with shear rate ranging from 0.20 sec^{-1} to 1.0 sec^{-1}. Viscosity determinations were performed at room temperature. The viscosity data was plotted for Rheogram [8].

Spread ability

Spread ability is a term expressed to denote the extent of area to which the topical application spreads on application to skin on the affected parts. For the determination of spread ability, excess of sample (3gm) was applied in between two glass slides and compressed to uniform thickness by placing 1000 gm weight for 5 minute. Thereafter weight (50gm) was added to the pan and the top plate was subjected to pull with the help of string attached to the hook. The time in which the upper glass slide moves the lower plate to cover a distance of 10cm is noted. A shorter interval indicates better spread ability. The spread ability (S) can be calculated using the formula[6]:

$$\text{Spread ability} = \frac{\text{Weight tied to upper glass slide (gm)} \chi \text{ Length moved on glass (cm) slide}}{\text{Time (sec)}}$$

Primary skin irritation test on Laboratory experimental animals

The animal selected was rabbits; they were kindly supplied from the animal house of Applied Research Centre for Medicinal Plants. These animals were kept in different cages and supplied with fresh food and water during the test period, 24 h prior to test; hair from the back was shaved to expose sufficient large test area. The test site was cleaned with surgical spirit then o/w cream was applied to test area. The test site was observed for erythema and edema for 24 h and 48 h; after application. This test was conducted to evaluate the irritancy of the prepared cream on the intact skin of animals [9].

Antimicrobial Effectiveness test of plant extracts in cream formulations

Referenced in the current USP <51>[10]: demonstrates the effectiveness of the preservative system in a product, which inoculated with a controlled quantity of microbial indicators, Bacteria; (*E. Coli*, *P. aeruginosa* and *S. aureus)*, Yeast; (*C. albicans)*, and fungi; (*A. niger)*. Microorganism's inoculation was done between 4 x 10^6 to 1.5 x 10^8 colony forming units (CFU) per ml of product. The test then compares the level of microorganisms found on a control sample versus the test sample over a period of 28 days. Indicator microorganisms must be harvested under current USP guidelines to assure viability. At various intervals, the products were tested to determine their ability to control reproduction or destroy the microorganisms.

RESULTS AND DISCUSSION

Organoleptic characters

In this study the effect of the tested extracts on the organoleptic characters of creams during test period was monitored on color, odor and texture and there was no change in their appearances such as colour, nor was there cracking or separation of phases of the creams.

Evaluation of O/W cream

pH of MP and LR formulations

Our data record that the pH of the formulation was shown nearly neutral pH range as shown in Table 1.The pH of human skin typically ranges from 4.5 to 6.0 [11] and 5.5 is considered to be average pH of the skin. Therefore, the formulations intended for application to skin should have pH close to this range.

Table 1: pH of MP&LR creams

pH	MP cream	LR cream
	6.5	6.65

Viscosity

The viscosities of the formulations were measured at varying speed and shear rates. Apparent viscosity and rheological behavior of the formulation lead to consistency. The data of cream formulations has shown shear thinning/pseudo plastic behavior figure1a,1b, at ambient temperature where there is decrease in viscosity by increasing shear rate (When the viscosity decreases with increasing shear rate, we call the fluid shear-thinning. In the opposite case where the viscosity increases as the fluid is subjected to a higher shear rate, the fluid is called shear-thickening. Shear-thinning behavior is more common than shear-thickening. Shear-thinning fluids also are called pseudoplastic fluids) this shear thinning behavior is a desirable property for topical preparations, as they should be thin during application and thick otherwise. This shear thinning behavior is desirable property for topical preparations, as they should be thin during application and thick otherwise [6].

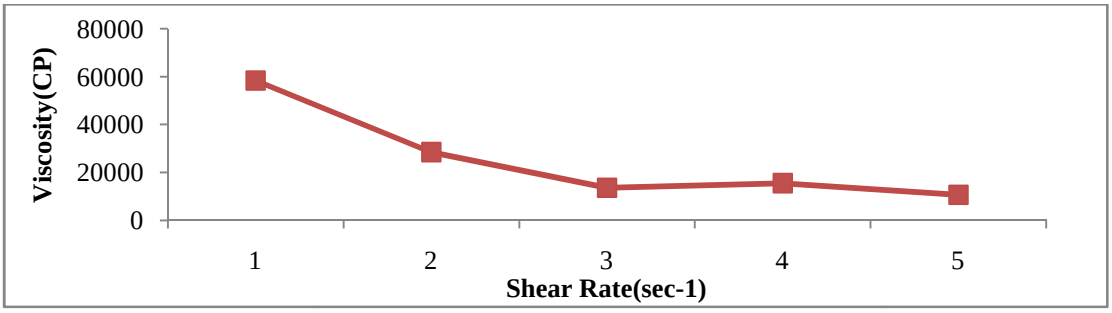

Fig.1a: Viscosity/Shear rate of LR cream

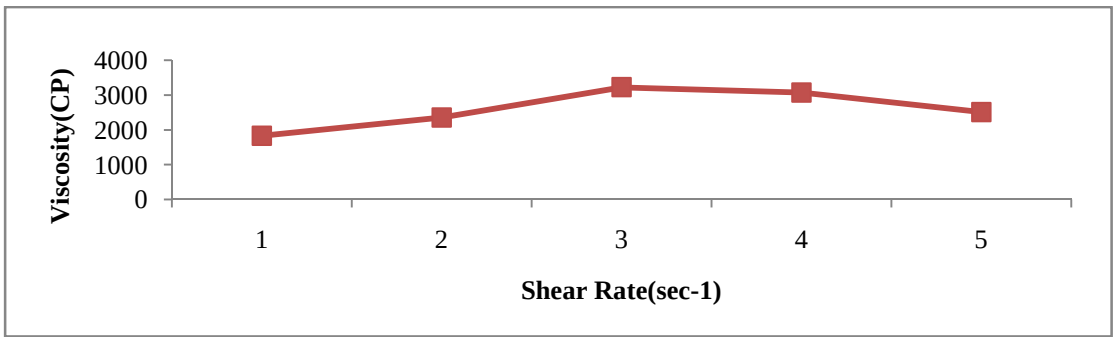

Fig.1b: Viscosity/Shear rate of MP cream

Spread ability

LR cream takes shorter interval than MP cream as shown in table(2).The therapeutic efficiency of the formulations depends upon their spreading value. Hence, determination of spread ability is very important in evaluating topical application characteristics. A shorter interval indicates better spread ability [12].

Table 2: Spread ability of MP and LR creams

Cream	Spread ability
MP	100 gm.cm/sec
LR	250 gm.cm/sec

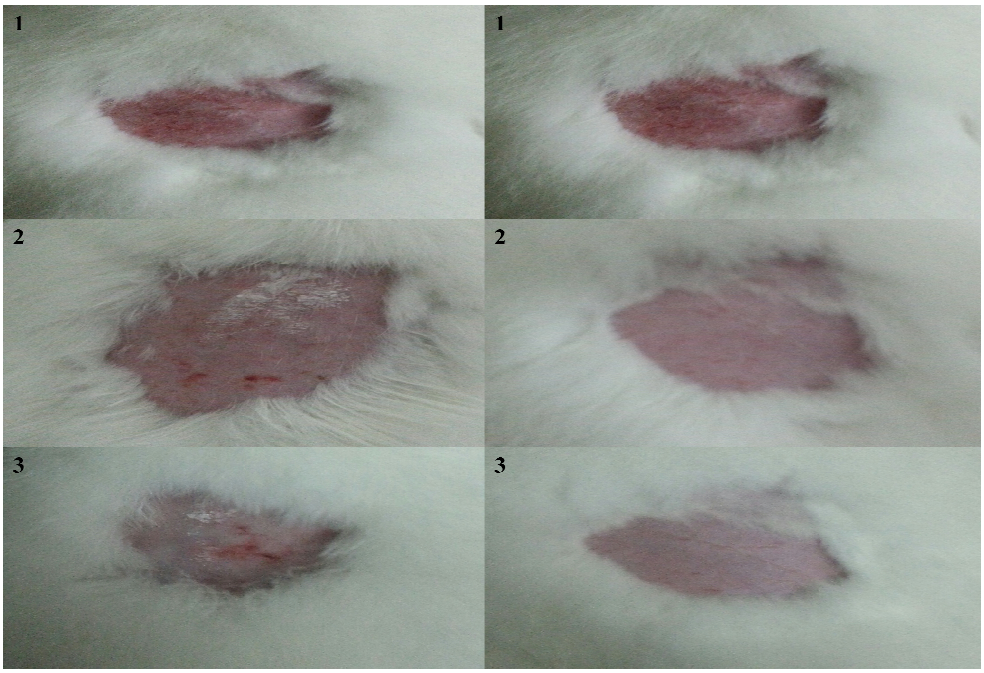

Fig.2:Photograph of Primary skin irritation test :column A, represent MP cream effect on rabbit skin;1) Before treatment,2)After 24h and 3)After 48h, while pictures on column B, represent LR cream effect on rabbit skin;1) Before treatment,2)After 24h and 3)After 48h

Primary skin irritation test on Laboratory experimental animals

In our study, no erythema and edema were observed on the skin of the rabbits even after 48 h of application, as the results in figure(2) that lines with previous findings [6].

Challenge Test: according to USP<51>

The effect of *G. glabra* and *R. officinalis* extracts combination, on total microbial plate count of each corresponding microbial indicator in the creams is shown in Table 3. We can deduce that:

- The concentration of viable bacteria is reduced to not more than 0.1% of the initial concentration by the 14th day;
- The concentration of viable yeasts and molds remains at or below the initial concentration during the first 14 days, and;
- The concentration of each test microorganisms reduced to 100% of its initial concentration during the remainder of 28 days.

Our results comply with previous findings [13] who stated that a cream with good preservative capacity is one that is capable of inhibiting immediate post-production contaminants as well as subsequent low inoculum of in-use contaminants, and thereby maintains acceptable low levels of microorganisms in the preparation.

Table 3: Reduction in total microbial plate count throughout 28 days

Microbial Indicator	Days	Mean Viable count			Number of reduced log cycles		Reduction percent	
		Control	Test MP cream	Test LR cream	Test MP cream	Test LR cream	Test MP cream	Test LR cream
S.aureus	7	$1.5x10^8$	$1x10^4$	$1x10^4$	5	5	99.99	99.99
E.coli		$1.5x10^8$	$1x10^5$	$1.6x10^3$	>3	>2	99.93	99.89
P. aeruginosa		$1.5x10^8$	$1x10^5$	$1x10^5$	>3	>3	99.33	99.33
C.albicans		$9.6x10^6$	$3x10^4$	0	>2	5	99.69	100
A.niger		$4x10^6$	$2x10^4$	0	>2	6	99.5	100
S.aureus	14	$1.5x10^8$	$1x10^5$	$1x10^2$	>3	>6	99.93	99.99
E.coli		$1.5x10^8$	0	0	8	8	100	100
P. aeruginosa		$1.5x10^8$	$1x10^5$	$1x10^5$	>3	>3	99.93	99.93
C.albicans		$9.6x10^6$	$2x10^5$	0	>1	5	97.92	100
A.niger		$4x10^6$	0	0	0	0	100	100
S.aureus	21	$1.5x10^8$	0	0	7	7	100	100
E.coli		$1.5x10^8$	0	0	7	7	100	100
P. aeruginosa		$1.5x10^8$	0	0	7	7	100	100
C.albicans		$9.6x10^6$	0	0	5	5	100	100
A.niger		$4x10^6$	0	0	6	6	100	100
S.aureus	28	$1.5x10^8$	0	0	7	7	100	100
E.coli		$1.5x10^8$	0	0	7	7	100	100
P. aeruginosa		$1.5x10^8$	0	0	7	7	100	100
C.albicans		$9.6x10^6$	0	0	5	5	100	100
A.niger		$4x10^6$	0	0	6	6	100	100

CONCLUSION

Antimicrobial activities of *G. glabra* and *R. officinalis* extracts were investigated against five microbial indicators (*E. coli, S.aureus, P .aeruginosa, A.niger and C. albicans*). The natural extracts showed an evident antimicrobial effect against yeast and bacteria. From the results presented above, we can assume that the natural extracts *G. glabra* and *R. officinalis* are promising natural preservatives, with application in cosmetic industry.

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