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Research Article

FORMULATION AND EVALUATION OF HERBAL CREAM FROM *ZIZIPHUS SPINA* LEAVES EXTRACT

Ali Heyam Saad[1]*, Shehab Naglaa Ahmed[2] and El-ahaj Babiker Mohamed[3]
[1]Department of Pharmaceutics, Dubai Pharmacy College, Dubai, UAE
[2]Department of Pharmacognosy and Phytochemistry, Dubai Pharmacy College, Dubai, UAE
[3]Department of Pharmaceutical Chemistry, Ajman University of Science and Technology, Fujairah, UAE
*Corresponding Author Email: heyam57@hotmail.com

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ABSTRACT
Ziziphus spina Crist leaves extract is well known by its antimicrobial activity and its uses in skin diseases. The aim of this study was to evaluate the antibacterial activity of aqueous extract of *the plant* leaves against *Staphylococcus aureus* and *Escherichia coli* species. Also, to formulate effective, stable herbal antibacterial cream and evaluate its physical & antibacterial properties. Disk diffusion method was used to assess antibacterial activity of aqueous extract using reference disks of antibiotics. The antibacterial cream was prepared by incorporating different amount of ingredients together, and a certain amount of the herbal extract. The prepared cream was evaluated for their Physical, rheological and antibacterial properties. Finally, the efficacy of the herbal antibacterial cream formulation was compared to two commercial products. The antibacterial activity of the aqueous extract *was* found to be effective against both *S. aureus* and *E. coli* especially when the extract concentration increased. All physical and rheological properties of all the preparation were nearly the same as commercial products. Stability studies showed a stable, homogenous appearance and effective during three months storage period at room temperature. It can be concluded that the aqueous extract of *Ziziphus S.Crist leaves* exhibited strong antibacterial activity especially with increase of the extract concentration. The prepared cream was found to be natural, stable and safe. *Ziziphus S. Crist cream* could be used topically in order to treat skin infection. The Present investigation suggests that this cream is a suitable candidate for further clinical trials.
Keywords: *Ziziphus s.Crist* ; aqueous extract; Antimicrobial cream; Physical, rheological evaluation; *S. aureus; E.Coli*.

INTRODUCTION

Therapy with herbal drugs is an old tradition al medicine. Plants have been used over the years for the treatment of numerous health problems including infectious and non-infectious skin disorders.

The antimicrobial effects of some plants were attributed to the number of phytochemical constituents like flavonoids, triterpenes and tannins[1]. The use of conventional medications is often unsatisfactory for many patients with chronic skin disorders because of adverse effects and loss of effectiveness on long term uses[2]. Moreover, the development of drug resistance in human pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substances from other sources. Therefore, it has become necessary to search for an alternative safe, effective medicinal with little side effects.

Ziziphus spina Crist leaves (ZSCL) family Rhamnaceae) is a well-known medicinal plant (Sidr), with leaves, roots, and bark being exploited in traditional medicine in Africa a, India and UAE [3]. The leaves of ZSC are used in traditional medicine treating chest and stomach problems, hair and skin care[4, 5]. Moreover, The Quran mentioned the tree twice (LIII: 13–18; LVI: 28–32); In addition to Sunnah and is highly respected by the Muslims through the Middle East[6]. The lote-tree is commonly identified as *Z. spina-christi*[6]. The antimicrobial properties of ZSCL have been previously investigated against *E.Coli* and *P.aeroginosa*[7]. The plant is known to be active against wide spectrum of bacteria due to presence of flavonoids, saponins and phenolic compounds[8]. Several reports are available on the antimicrobial activity of plant extracts on human pathogenic bacteria[9].

Staphylococcus aureus and *Escherichia coli* are the main pathogens that cause skin infections[9]. Development of microbial resistance to antibacterial is a global concern. However, human and animal studies supported the toxic effect of synthetic chemicals[10]. Therefore, restriction on their use are being imposed[11] and the search for natural antimicrobial as safe alternatives is becoming critical. Skin problems are common among children[12] and the development of new natural & safe therapeutic antimicrobial topical preparation is the plan of our study. The aim of the present study was to investigate antibacterial activity of aqueous extract of ZSCL, to formulate a natural, safe antibacterial cream and to evaluate its physicochemical properties and stability.

MATERIALS AND METHODS

Chemicals and Solvents

Glycerin and Benzyl alcohol were purchased from Avondale Laboratories Ltd., England, Glyceryl monostearate, Cetyl alcohol, from BDH Chemicals Ltd., England, Triethanolamine (AVONCHEM Ltd., UK), Spermaceti (Sigma-Aldrich Chemie GMbH, Germany). All reference disks containing different concentrations of antibiotic: Doxycycline, streptomycin, chloramphenicol, carbeincillin, nalidixic acid, penicillin, novobiocin, methicillin, gentamycin, vancomycin, oxacillin, tetracycline, trimethoprim- sulphamethoxazole were obtained from BDH Chemicals Ltd., England, and gifted from Dubai Research Center. All chemicals were of analytical grade.

Medias

Nutrient agar was purchased from DEFCO Labodratories, USA, Peptone bacteriological from BDH Chemicals Ltd., England, Sabauraud dextrose agar and sheep red blood from OXOID Ltd., England,

Yeast Extract from UNIPATH Ltd., England and Sodium Chloride from East Anglia Chemicals, United Kingdom.

Test Strains and Animals

Staphylococcus aureus (ATCC 6538), *Escherichia coli* (ATCC 25922), Mueller Hinton broth (Merck, Germany),

Mueller Hinton agar (Merck, Germany), all were American Type Culture Collections.

Animals

Healthy Wistar albino rats weighing between 150 -180 g were used. The experiments were carried out per the guidelines of Research and Ethical Committee in Dubai Pharmacy College. The dorsum of each rat was shaved prior the experiments.

Plant collection

Leaves from *Ziziphus spina Crist plant* were collected from Dubai pharmacy College campus, Dubai, UAE in October 2012. Authentication of the plant was done by Taxonomy Department, Dubai Pharmacy College. Voucher specimens were deposited at the Pharmacognosy and Phytochemistry Department, Dubai Pharmacy College, Dubai UAE.

Preparation of plant aqueous extract

The collected leaves were thoroughly washed and dried in shadow and then grounded to powder. 10 g of the powder were macerated in 200 ml of boiling distilled water for 20mins then filtered. The filtrate was then lyophilized and kept for biological study[13].

Formulation and physical properties evaluation

Formulation of the Cream

Base cream containing water and oil phases was prepared. The compositions and the amounts of the formulation ingredients are shown in Table 1. In order to prepare the cream, different amount of ingredients were incorporated together, and then the required amount of the herbal extract was added[14].

Evaluation of physical properties of ZSCL formulations

Physical characteristics

The prepared formula containing ZSCL extract and the commercial preparations were examined for their physical (pH, color, consistency and homogeneity) as well as rheological properties.

Determination of the pH

pH of the prepared formula was measured using digital pH meter.

Homogeneity[15]

Homogeneity of various formulations was tested by visual observation and was ranked as follows:

+++ = Excellent, ++ = Very Good, + = Good, - = Poor.

Consistency

The cone attached to holding rod was dropped from the fix distance of 10 cm such that it should be fall on the centre of measuring cylinder filled with ZSCL cream. The distance travelled by cone was noted down after 10 sec.

Rheological Properties

The prepared formula was evaluated for the following rheological characteristics:

Viscosity measurements

A Brookfield synchroelectric viscometer, Brookfield, MA) was used to measure the viscosity (in cps) of ZSCL creams. The spindle was rotated at 2.5 rpm. Samples of the creams were allowed to settle over 30 min at the temperature of test (25±1 °C) before the measurements were taken.

Extrudability[15]

Extrudability was determined, using an extrudability apparatus. A closed collapsible tube containing formulation was pressed firmly at the crimped end. When the cap was removed, formulation extruded until the pressure dissipated. Weight in grams required to extrude a 0.5-cm ribbon of the formulation in 10 seconds was determined. The average extrusion pressure in grams was reported.

Spreadability[15]

The Spreadability determination: excess of sample was applied in between two glass slides and was compressed to uniform thickness by placing 100gm weight for 5minutes. Weight was added to the pan. The time required to separate the two slides, i.e. the time in which the upper glass slide moved over the lower plate was taken as measure of spreadability.

$S = m * l / t$

Where,

m = weight tide to upper slide

l = length moved on the glass slide

t = time taken.

Spreadability test also was performed by Applying the ointment on the skin and noticing whether spreading was good or not.

Antibacterial activity[16]

Staphylococcus aureus (ATCC No. 29737) and *E. coli* (ATCC No. 8739) were grown in Mueller Hinton broth (Merck, Germany) at 37°C for 24h. Final cell concentrations were 108cfu/ml according to the McFarland turbidometry. 100µl of the inoculum were added to each plate containing Mueller Hinton agar (Merck, Germany). Four different concentrations of the ZSCL extract (5, 10, 15 and 20% equal to 0.05, 0.1, 0.15 and 0.2mg/ml, respectively) were prepared. The sterile filter paper disks (6mm in diameter) were saturated with 50µl of each concentration of the extract. The plates were incubated at 37°C for 24h and the diameters of inhibitory zones were measured. The assay was carried out three times for each concentration. Disks containing different concentrations of antibiotics were used as references to compare the sensitivity of each tested bacterial species[9].

Irritation test

ZSCL cream was applied on normal and non-broken rat skin. The test cream and cotton swab covering it were secured firmly on the applied surface with the help of adhesive tapes. Then observations were made for any sign of erythema and ranked as follows : +++ = Severe erythema, ++ = Moderate erythema, + = Slight erythema, - = No irritation.

Physical stability test

Three sets of 20 g samples of the formulation and the two commercial products, Gentamycin 1% W/W and Chloramphenicol 2% W/W respectively were stored at room temperature 37°C for 3 months. Then, after three months, their stability was checked regarding antibacterial activity and appearance.[10]

Table 1: The Composition and The Amounts Of The Ingredients Used To Make 10 g of ZSCL Antibacterial Cream

	Ingredients	Amount (g)
Oil Phase	Stearic acid	1
	Spermaceti	0.5
	Cetyl alcohol	0.5
Water Phase	Glycerin	0.5
	Triethanolamine	0.2
	Benzyl alcohol	0.2
	Water	7
	Z. spina Crist	0.1

Table 2: Physical Properties and Skin Irritation of *ZSCL* Cream and the Commercial Products

Parameter	*ZSCL Cream*	Comm. Product-1	Comm. Product-2
Colour	Light Green	White	White
pH	5.7±0.57	5.8±0.57	6.3±0.57
Homogeneity	+++	+++	+++
Consistency (60 sec)	5 mm	5 mm	5 mm
Skin Irritation	NIL	NIL	NIL

Comm. Product-1: Gentamycin cream (1% w/v), Comm. Product-2: Chloramphenicol cream (2% w/v)

Key: **Homogeneity** +++ Excellent, ++ Very good, + Good, - Un-satisfactory

Skin Irritation: ++ + Severe erythema, ++ Moderate erythema, + Slight erythema, - No irritation

Table 3: Rheological Properties

Parameter	*ZSCL* Cream	Comm. Product-1	Comm. Product-2
Viscosity (cps)	10×10^6	25×10^6	15×10^6
Spreadability (g.cm/sec)	37	38	35
Extrudability (gm)	520	520	510

Comm. Product-1: Gentamycin cream (1% w/v), Comm. Product-2: Chloramphenicol cream (2% w/v)

Table 4: Inhibition Zone (mm) *ZSCL* Aqueous Extract at Different Concentration (Mean + SD)

Bacterial species	Concentration of extract			
	5%	10%	15%	20%
S. aureus	11.44 + 0.68	13. 55 + 2.40	7.00 + 15	22.14 + 10
E.Coli	14.33 + 2.66	15.68 + 1.16	17 + 1.64	19. 44 + 1.54

Table 5: Inhibition Zone (mm) of Antibiotic Disks (mean + SD)

Antibiotic disks	Inhibition diameter (mm)	
	S.aureus	E.Coli
Doxycyclin 30 µg	10.05 + 0.0	14.5 + 0.07
Stretomycin 10 µg	20 + 0.00	25 + 0.00
Chloramphenicol 30 µg	20.1 + 0.13	24.5 + 0.7
Carbenicillin 100 µg	10 + 0.00	Resistant
Nalidixic acid 30 µg	30.5 + 0.1	30 + 0.00
Penicillin 30 µg	Resistant	Resistant
Novobiocin 30 µg	Resistant	10 + 0.00
Methicillin 5 µg	Resistant	Resistant
Gentamycin 10 µg	19.0 + 0.7	20 + 0.00
Vancomycin 30 µg	Resistant	Resistant
Oxacillin 1 µg	Resistant	Resistant
Tetracycline 30 µg	Resistant	Resistant
Trimethoprim- Sulphamethoxazole 25 µg	20 + 0.00	34.5 + 0.7

Table 6: The Effect of Storage Time on the Physical Properties and Antimicrobial Activity of the *ZSCL* Cream.

Microbial Organisms	Zone of inhibition (mm)			
	Comm. Product-1	Comm. Product-2	Freshly prepared *ZSCL* Cream	3-month prepared *ZSCL* Cream
E.coli	14.25 ± 0.96	13+0.9	13± 0.3	12. 25 ± 0.50
S. aureus	23.05± 0.50	22+0.3	23± 0.3	22.5 + 2.38
pH	5.3±0.57	5.3±0.57		5.3±0.57
Colour	Light Green	White	White	White
Homogeniety	+++	+++	+++	+++
Consistency (60 sec)	5 mm	5 mm	5 mm	5 mm

3-month prepared *ZSCL Cream:* after 3 months at room temp., Comm. Product-1: Gentamycin cream (1% w/v), Comm. Product-2: Chloramphenicol cream (2% w/v)

Figure 1: Rabbit showing application sites

RESULTS AND DISCUSSION

The aqueous extract of *ZSCL* plant was screened for biological activity against different strains of bacteria. The plant leaves were selected on the basis of data obtained from the literatures, reports of their local traditional uses, Quran and Sunnah, for the treatment of various skin disorders. The antibacterial activity-screening was carried out on organisms that are known to be among the most common causative agents of both primary and secondary infectious skin disorders.

The yield of the aqueous extract of *ZSCL* was 3% w/w. The results of the initial antibacterial screening assay of the crude aqueous extract of *ZSCL* plant on the selected microbial strains are shown in Table 4. The results showed that aqueous extract of *ZSCL* effectively inhibited the growth of *E. coli* and *S. aureus*. The antibacterial activity was enhanced with increase of the extract concentration. It was found that the maximum antibacterial activity in 20% concentration of the plant was 22mm and 19.44 mm for *S. aureus* and *E. coli* respectively. The inhibition zones of the antibiotic disks are presented in Table 5, showing that *S. aureus* and *E. coli* were resistant to penicillin, methicillin, vancomycin , oxacillin and tetracycline. On the other hand, *E. coli* was resistant to Carbenicillin. *S. aureus* was resistant to novobiocin while the latter inhibited the growth of *E. coli*. The findings of pH study was found to be between 5.7, 5.8 and 6.2, for *ZSCL* cream and commercial products respectively, even after three months. As shown in Table 2, Physical properties were found to be equivalent to the commercial ones. Homogeneity test showed no turbidity and instability. Consistency showed no sticking and adhesion to skin. Rheological properties (spreadability and extrudability) of *ZSCL* cream were within limits

(Table 3). That indicated suitability of the creams, for application on the skin.

Antibacterial activity of the plant was considerable in comparison with the other reports. Another study had shown that inhibition zone of methanolic extract of *ZSCL* was 26.2 mm for *S. aureus* and 20.3 mm for *E. coli*[8]. In another report, antibacterial activity of chloroform extract of ZSCL against both bacteria was 23.1mm[9]. Our results indicated that the diameters of inhibition zone of the active extract were comparable with the standard antibiotic used as a positive control (Table 5). Moghimipour et.al., 2009, reported that *Escherichia coli* was resistant to carbenicillin, penicillin, methicillin, Vancomycin, oxacillin and tetracycline, while *S. aureus* was resistant to penicillin novobiocin, methicillin, vancomycin, oxacillin and tetracycline[19]. A phytochemical analysis revealed that the active principle responsible for the antibacterial activity was a phenolic compounds[8].

Creams are semisolid dosage forms intended mainly for external use and commonly consist of two immiscible phases, an oily internal phase and an aqueous external phase. Due to emulsified nature of skin surface, drugs formulated as cream more effectively interact with skin and more readily penetrate through biological membranes. Some of plant extracts with antifungal activity have been previously formulated as topical creams.

It has been previously reported by the author that formulation of *ZSCL* extract as topical preparations such as cream or shampoo may lead to enhancement of stability and acceptability of the active ingredient, because it contains self surfactants while the antifungal activity remains considerable[17]. In another report, methanolic extract of Eucalyptus camadulensis has been formulated as an anti-dermatophytic cream preparation[18,19]. Regarding the selection of base formula, it was done after performing several trials. The first contained excess fat which produced a greasy sense on usage, turbidity and its low consistency. Therefore, the formula was modified to overcome the problems. Then, the proportions of the oily phase components were changed and three formulations were made. Finally the best formulation was chosen according to the results of different chemical and physical tests. Control experiments and stability determination showed a stable homogenous appearance during three months storage period and no separation phase occurred.

CONCLUSION

To our knowledge, this is the first study dealing with ZSCL to be formulated as an antibacterial topical formulation. Over all, *ZSCL* showed high antibacterial capability in the tested extract and the cream preparations, due to its higher phenolic contents. This study proved the ability of *ZSCL* antibacterial cream with potential application to reduce skin infection with consequent health benefits. Further clinical studies are needed to validate the therapeutic potential of this herbal antibacterial cream against all skin disorder.

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