Original article

Formulation and evaluation of an antibacterial cream from *Oxalis corniculata* aqueous extract

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Abstract

Introduction and objective: *Oxalis corniculata* is well known because of its anti-dysentery and antidiarrheal effects and also its application in skin diseases. The aim of this study was to evaluate the antibacterial activity of aqueous extract of *O. corniculata* on *Staphylococcus aureus* and *Escherichia coli* species and to formulate a herbal antibacterial cream.

Materials and methods: Disk diffusion method was used to assess antibacterial activity of *O. corniculata* extract. Antibacterial activity of this plant was compared with doxycycline, streptomycin, chloramphenicol, carbeincillin and nalidixic acid. In order to prepare cream, different amount of ingredients were incorporated together, then the required amount of the herbal extract was added. The cream formulations were compared according to their physical and chemical stability. Finally, the best formulation was selected by altering the type and quantity of formulation factors.

Results: Results showed that aqueous extract of *O. corniculata* was effective on *S. aureus* and *E. coli* and the antibacteria were more effective when the extract concentration increased. Control experiments and stability determination showed a stable homogenous appearance during three months storage period and no phase of separation was occurred.

Conclusion: The aqueous extract of *O. corniculata* exhibited strong antibacterial activity especially with increase of the extract concentration. The results of different chemical and physical tests of cream showed that the formation could be used topically in order to protect skin against damage caused by this *S. aureus* and *E. coli*.

Significance and impact of the study: The results of the present investigation suggest that this plant is a suitable candidate for further pharmacological evaluation.

Keywords: *Oxalis corniculata*; Aqueous extract; Antibacterial cream; *Staphylococcus aureus*; *Escherichia coli*

Introduction

Staphylococcus aureus and *Escherichia coli* are the main pathogens that cause skin infections. Development of microbial resistance to antibacterial is a global concern. Plants are important sources of potentially useful constituents for the development of new therapeutic agents because most of them are safe with little side effects. *Oxalis corniculata* (*Oxalidaceae*) is a small shrub [1] that grows in Iran [2]. The leaves contain fat, water, niacin, vitamin C, protein, beta-carotene, calcium, carbohydrate [3], phytosterols, flavonoids, mucilage and phenolic compounds [1]. It is used in traditional medicine treating fever, burns, diarrhea, gastrointestinal disorders and skin diseases and has been used as an anti-inflammatory agent [1].

The antimicrobial properties of this plant have been previously investigated on a plant pathogen *Xanthomonas compestris* [1,4] and some human pathogens [1,5,6]. The development of drug resistance in human pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substances from other sources. Several reports are available on the antimicrobial activity of plant extracts on human pathogenic bacteria [1,4,7]. The aim of the present study was to investigate antibacterial activity of aqueous extract of *O. corniculata* and formulate an antibacterial preparation and evaluate its physicochemical properties and stability.

Materials and methods

Preparation of plant extract

The leaves of plant used in this study were collected from Khuzestan province, Iran. Voucher specimens were deposited at the Botany Department, Faculty of Science, Shahid Chamran University, Ahvaz. Leaf samples were thoroughly washed and dried in shadow and ground to powder. 10g of the powder was macerated in 200ml boiling distilled water for 20mins [8]. The macerate was first filtered through muslin cloth and centrifuged at 3500g for 15mins. The supernatant was removed by evaporation [8].

Antibacterial activity

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 10^8cfu/ml according to the McFarland turbidometry. 100µl of the inoculum was added to each plate containing Mueller Hinton agar (Merck, Germany). Four different concentrations of the *O. corniculata* 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 extract. Disks containing different concentrations of antibiotics were used as reference to compare the sensitivity of each tested bacterial species [9]. Antibiotics disks contain doxycyclin, streptomycin, chloramphenicol, carbencillin, oxacillin, nalidixic acid, penicillin, novobiocin, methicillin, gentamicin, vancomycin, trimethoprim-sulfamethoxazole and tetracycline (Bact Difco, USA).

Formulation and physico-chemical evaluation

Base cream contains water and oil phases. The compositions and amounts of the formulation ingredients are shown in table 1. In order to prepare the cream, different amount of ingredients were incorporated together, then the required amount of the

herbal extract was added. 10g sample of the formulation was placed in a centrifuge tube (1cm diameter) and centrifuged at 2000rpm for 5, 15, 30 and 60mins. Then the phase separation and solid sedimentation of the samples were inspected [10].

Table 1: The composition and amounts of the ingredients used to make 10g *O. corniculata* antibacterial cream

Compound		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
	O. corniculata extract	0.1

Test samples were stored at 5°C for 48h and then at 25°C for 48h. The procedure was repeated three times and then their stability and appearance were inspected [10]. Three set of 20g samples of formulation were stored at 4-6°C, 25°C and 45-50°C. After 24h, one and three months, their stability and appearance were checked [10]. 20g of each formulation was stored periodically at 45-50°C and 4°C for 48h. The procedure was repeated six times and then the samples were checked regarding their appearance and stability [10]. 500mg of the sample was spread on a clean slide and observed using an optical microscope (×10 and ×40) [11]. The pH of formulations was determined at 48h. The extent of volatile and non-volatile composition was determined [9] and also water content of the formulation was measured using Dean-stark method [12].

Results

The yield of the aqueous extract of *O. corniculata* was 3% w/w. Antibacterial activity of aqueous extract of *O. corniculata* is presented in table 2. The maximum antibacterial activity in 20% concentration of the plant was 21mm for *S. aureus* and 19.33mm for *E. coli*. Inhibition zone of antibiotic disks are presented in table 3. *S. aureus* and *E. coli* were resistant to penicillin, methicillin, vancomycin and tetracycline. The results of pH study were 5.3±0.57, 5.3±0.57, 6±0.0, 5.3±0.57, after 48h, one week, one month, and three months, respectively. As indicated in this table, there was no significant change in their pH during storage (p>0.01).

Table 2: Inhibition zone (mm) *O. corniculata* aqueous extract at different concentration (Mean ±SD)

Bacterial species	Concentration of extract			
	5%	10%	15%	20%
S. aureus	10.33± 0.57	13.33 ±2.30	6±1	21±1
E. coli	13.33± 2.88	15.66± 1.15	18± 1.73	19.33± 1.52

Table 3: Inhibition zone (mm) of antibiotic disks (mean ± SD)

Antibiotic disks	Inhibition diameter (mm)	
	S. aureus	*E. coli*
Doxycyclin 30µg	10.05±0.07	14.5±0.70
Streptomycin 10µg	20±0.00	25±0.00
Chloramphenicol 30µg	20.1±0.14	24.5±0.70
Carbenicillin 100µg	10±0.00	Resistant
Nalidixic acid 30µg	30.05±0.07	30±0.00
Penicillin 10µg	Resistant	Resistant
Novobiocin 30µg	Resistant	10±0.00
Methicillin 5µg	Resistant	Resistant
Gentamicin 10µg	19.5±0.70	20±0.00
Vancomycin 30µg	Resistant	Resistant
Oxacillin 1µg	Resistant	Resistant
Tetracycline 30µg	Resistant	Resistant
Trimethoprim-sulfamethoxazole 25µg	20±0.00	34.5±0.70

The samples were stable during and after centrifugation, creaming test, thermal change test, freezing and thawing test and thermal cycle. Homogeneity test showed no turbidity and instability. The extent of volatile and non-volatile composition was 1.23% and 98.7%, respectively. According to Dean-stark experiment, water content of 10g of the formulation was 6ml.

Discussion

Field existences of antibiotic resistant pathogenic bacteria are increasing in recent years. Pharmaceutical companies are now looking for alternatives. Plants have been a rich source of medicines because it is believed that plant based drugs cause less or no side effect and affect a wide range of antibiotic resistant microorganisms. The results of this study (Table 2) showed that aqueous extract of *O. corniculata* effectively inhibited the growth of *E. coli* and *S. aureus*. The antibacterial activity was enhanced with increase of the extract concentration. Antibacterial activity of the plant was considerable in comparison with the other reports.

The results of a study has shown that the zone of inhibition of the methanolic and ethanolic extract of *O. corniculata* were 16.87mm and 13.39mm for *S. aureus* and 1.00mm and 8.10mm for *E. coli* [1]. Another study has shown that antibacterial activities of alcoholic extract of 0.3 and 0.4g/ml concentrations of *Torilis leptophylla* against *E. coli* were 11mm and 12mm, although there was no significant inhibitory effect against *S. aureus* in concentrations less then 0.4g/ml [13]. In another report, antibacterial activity of chloroform extract of some economically important seaweeds against *S. aureus* were 2.1mm for *Ulva lactuca*, 6.56mm for *Padina gymnospora* and 8.8mm for *Gracilaria edulis* [14].

A research has shown that alcoholic and aqueous extract of corn silk had good antibacterial activity in 30, 60 and 80% concentrations [15], while the extract of *O. corniculata* at 5% concentration showed antibacterial activity. Another study has shown that inhibition zone of methanolic extract of stems and leaves of *Tribulus terrestris* was 28.2mm for *S. aureus* and 22.3mm for *E. coli* [16]. Our results indicated that the diameters of inhibition zone of the active extracts were comparable with the standard antibiotic used as a positive control (Table 3).

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. The plant extract showed a broad spectrum of activity at 20% concentration, with the zone of inhibition of 21mm against *S. aureus* and 19.33mm against *E. coli*. A phytochemical analysis revealed that the active principle responsible for the antibacterial activity was a phenolic compound [1].

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 that formulation of *Zataria multiflora* extract as topical cream may lead to enhancement of stability and acceptability of the active ingredient, 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]. Base formula contained excess fat which produced a greasy sense on usage, turbidity and its low consistency. Therefore, the formula was modified to overcome the problems. At first, 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. Also, there was no significant change in the appearance of the samples and the base during centrifugation, thermal cycle and freezing and thawing experiments.

Conclusion

The aqueous extract of *O. corniculata* exhibited strong antibacterial activity and antibacterial activity was enhanced with the increase of the extract concentration. The results of different chemical and physical tests of cream showed that it could be used topically in order to protect skin against damage caused by these pathogens.

Conflict of interest statement: All authors declare that they have no conflict of interest.

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