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Original Article

FORMULATION OF AN ANTI-BACTERIAL CREAM FROM PLANTOXALIS CORNICULATA ANDITS EVALUATION

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ABSTRACT

Objective: Even in areas where modern medicine is available, the interest on herbal medicines and their utilization have been increasing rapidly in recent years. Plant-derived substances and herbal medicines have recently attracted the great interest towards their versatile application as medical plants are the rich source of bioactive compounds used in traditional and modern medicine. The present work is to formulate and evaluate the antibacterial cream of oxalis corniculata extract.

Methods: The ethanolic extracts were prepared by using the maceration method.

Results: The agrochemical potential of methanolic extract, n-hexane, chloroform, ethyl acetate,and n-butanol soluble sfractions showed excellent activites against Escherichia coli, Shigella dysenteriae, Salmonella typhi, and Bacillus subtilis. Similarly the crude n-hexane and chloroform fractions were also found to have significant activity against fungal strains including Fusarium solani, Aspergillus flexneri, and Aspergillus flavus.

Conclusion: Oxalis corniculata is a common medicinal plant widely used against numerous infectious diseases. The two isolated compounds 5-hydroxy-6,7,8,4'-tetra methoxy flavone and 5,7,4'-trihydroxy-6,8-dimethoxyflavone were evaluated for antibacterial and antifungal activities. The results showed that latter compound was more active than that of the former.

Keywords: Oxalis corniculata extract, Antibacterial cream, Anti-fungal

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INTRODUCTION

Oxalis corniculata is an endangered and medicinally important plant indigenous to tropical and sub-tropical regions of the world,its medicinal usage is reported in Indian pharmaceutical codex, the Chinese, British and the American pharmacopoeias and in different traditional systems of medicines such as Ayurveda, Unani and Siddha. Wide ranges of phytochemical constituents have been isolated from the plant like flavanoids, tannins, phytosterols phenol, glycosides, fatty acids galactoglycerolipid and volatile oil. it is rich source of essential fatty acids like palmitic acid,oleic acid, linoleic, linolenic and stearic acids and it possess anti-bacterial, anti-inflammatory anti-oxidant properties [1]. Anti-bacterial cream is a medicated cream which is used to treat certain skin infections caused by bacteria, the topical cream can be used to treat certain skin infections and to prevent infections in burns, skin grafts, minor cuts, wounds [2]. Due to jumbled use of existing anti-microbial drugs pathogenic bacteria have developed resistance against wide range of antibiotics. Microbiologists from all over the world are in search to formulate new anti-microbial drugs and evaluate efficiency of plant products to replace chemical anti-microbial agents [3]. Medicinal plant extracts have shown to serve as a cheap source of anti-microbial agents against pathogenic microbes [4]. Literature review suggests that Handali *et al.* formulated an anti-bacterial cream form Oxalis cornichulata aqueous extract and found that it was effective against S. aureus and E. coli [5]. Raghavendra MP *et al.* formulated an antibacterial cream from Oxalis corniculata leaves and effect was compared with the marketed product [6].

The aim of the present study is to evaluate antibacterial and anti-fungal activities of different fractions of oxalis corniculata including methanol, n-hexane, chloroform, ethyl acetate and n-butanolsoluble fractions.

MATERIALS AND METHODS

Extraction of oxalis corniculata

The fresh leaves of plant oxalis corniculata were collected, washed thoroughly, dried in shadow and ground to powder. Take 10g of the powder was macerated in 200 ml boiling distilled water for 20 min. The macerate was first filtered through muslin cloth and centrifuged at 3500 g for 15 min. the supernant was removed by evaporation and stored in a suitable container. The extracted oxalis corniculata is stored in a suitable container and is added to the further cream base.

Preparation of antibacterial cream

Oil in water (o/w) emulsion-based cream was formulated. The oxalis extract other oil soluble components were dissolved in oil phase and heated to 75 °C. The water-soluble components were dissolves in water and heated to 75 °C. After heating, water phase was added slowly to oil phase with continuous stirring until cooling of emulsion took place. The oil and water phases and their quantities are listed below

Table 1: List of ingredients for formulation

Ingredients	Quantity
stearic acid	1.0g
spermaceti/olive oil	0.5g
Cetyl alcohol	0.5g
Glycerine	0.5 ml
tri ethanolamine	0.2 ml
Benzyl alcohol	0.2 ml
Water	7 ml

Evaluation of creams

Physical properties

Determination of organoleptic properties

The Cream was observed for color, odour and appearance [7].

pH of the cream

The pH of various formulations was determined by using digital pH meter. About 1 g of the cream was weighed and dissolved in 100 ml of distilled water and stored for two hours. The measurement of pH of each formulation was done in triplicate and average values were calculated [7].

Test for thermal stability

The formulated cream was inserting into glass bottle with the help of spatula, and taped to settle to the bottom. Filled up to two-third capacity of bottle and insert plug and tighten the cap. Filled bottle was kept erect inside the incubator at 4 ˚±1 ° for 48 h. The sample passed the test, if on removal from the incubator shows no oil separation or any other phase separation [8, 9]

Irritancy

Test Mark an area (1sq. cm) on the left-hand dorsal surface. The cream was applied to the specified area and time was noted. Irritancy was checked up to 30 min and reported [8].

Viscosity

Viscosity of formulated cream was determined by book field viscometer at 50 rpm [8]

Spreadability

The Spreadability was expressed in terms of time in seconds taken by two slides to slip off from the cream, placed in between the slides, under certain load. Lesser the time taken for separation of the two slides, better the Spreadability. Two sets of glass slides of standard dimensions were taken. The herbal cream formulation was placed over one of the slides. The other slide was placed on the top of the formulation, such that the cream was sandwiched between the two slides weight was placed upon the upper slides so that the cream between the two slides was pressed uniformly to form a thin layer. The weight was removed and the excess of formulation adhering to the slides was scrapped off. The upper slide allowed slipping off freely by the force of weight tied to it. The time taken for the upper slide was noted [10].

$$\text{Spreadability} = m \times l/t$$

m = weight tied to the upper slide (30g) l =length of glass slide (5 cm) t =time taken in seconds.

Phase separation

The formulated cream was kept intact in a closed container at 25–300 °C not exposed tolight. Phase separation was observed carefully every 24 h for 30 d. Any change in phaseseparation was checked [8].

RESULTS AND DISCUSSION

In our work we prepared nine (CC1-CC9) different cream formulations. Among these all formulations are tested for further final selection purpose.

Physical properties

The physical properties and all formulated cream were judged by its Color, Odour and texture.

The results are tabulated below.

pH of the antibacterial cream

The result of pH of prepared creams (CC1–CC9) was found to be around 6 which are suitablefor topical application. Because skin pH in between 4.5-6.

Test for thermal stability

Thermal stability of the formulation was determined by the humidity chamber controlled at 60-70% RH and 37 °C. Finally all the formulations stable and no oil separation was observed.

Irritability

A small amount of gel was applied externally on the skin surface for few minutes and checked for reactions on the skin. It was found to be non-irritant.

Viscosity

Viscosity of formulated antibacterial cream was determined by brook field viscometer at 50 rpm. The viscosity of anti bacterial cream was found in range of 1000 to 3000 cp which indicates that cream was easily spreadable by small amount of shear.

Table 2: Physical properties of cream

Formulation code	Colour	Odour	Texture	Consistency
CC1	Cream white	Characteristic	Smooth	Semi solid
CC2	Cream white	Characteristic	Smooth	Semi solid
CC3	Cream white	Characteristic	Smooth	Semi solid
CC4	Cream white	Characteristic	Smooth	Semi solid
CC5	Cream white	Characteristic	Smooth	Semi solid
CC6	Cream white	Characteristic	Smooth	Semi solid
CC7	Cream white	Characteristic	Smooth	Semi solid
CC8	Cream white	Characteristic	Smooth	Semi solid
CC9	Cream white	Characteristic	Smooth	Semi solid

Table 3: pH of antibacterial cream

Formulation code	Ph
CC1	7.21
CC2	8.65
CC3	7.72
CC4	7.13
CC5	6.65
CC6	5.82
CC7	5.14
CC8	4.89
CC9	5.55

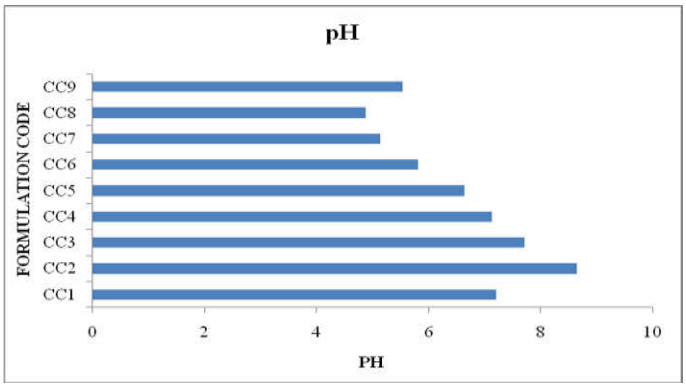

Fig. 1: pH of the antibacterial cream formulations

Table 4: Test for thermal stability of cream

Formulation code	Thermal stability
CC1	Stable
CC2	Stable
CC3	Stable
CC4	Stable
CC5	Stable
CC6	Stable
CC7	Stable
CC8	Stable
CC9	Stable

Table 5: Test for irritability of cream

Formulation code	Irritation
CC1	No
CC2	No
CC3	No
CC4	No
CC5	No
CC6	No
CC7	No
CC8	No
CC9	No

Fig. 2: Irritability test

Table 6: Viscosity of the cream

Formulation code	Viscosity (cp)
CC1	1020
CC2	1050
CC3	1078
CC4	1065
CC5	1155
CC6	1260
CC7	1570
CC8	1885
CC9	1895

cp: centipose

Table 7: Spread ability of antibacterial cream

Formulation code	Spread ability (cm)
CC1	5.6
CC2	5.8
CC3	6.1
CC4	6.5
CC5	6.8
CC6	7.5
CC7	10.6
CC8	12.5
CC9	14.3

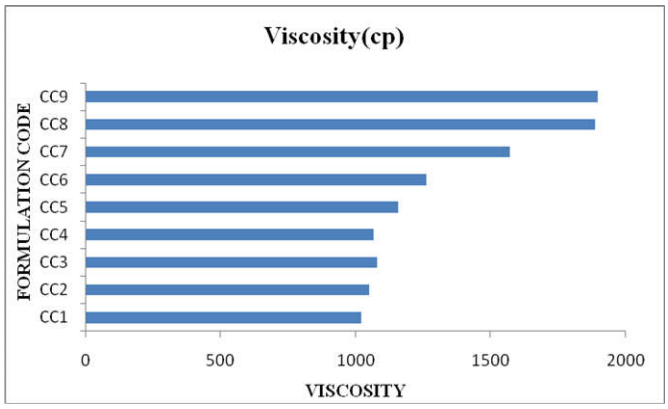

Fig. 3: Viscosity of antibacterial cream

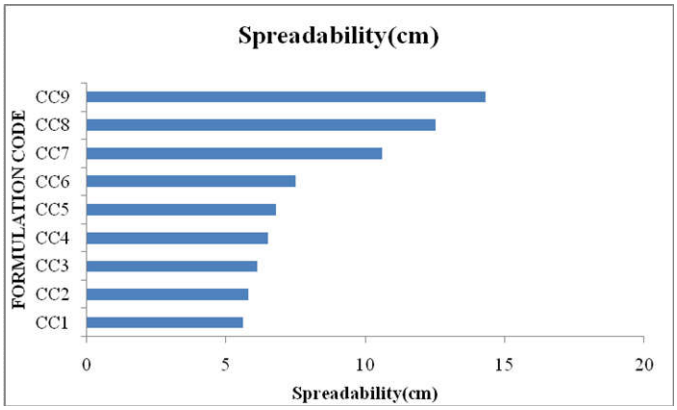

Fig. 4: Spreadability of antibacterial cream

Table 8: Phase separation of antibacterial cream

Formulation code	Phase separation
CC1	No
CC2	No
CC3	No
CC4	No
CC5	No
CC6	No
CC7	Yes
CC8	Yes
CC9	Yes

From the above results, CC6 Formulation was considered as optimized formulation. Which contains stearic acid 1.0 g, cetyl alcohol 0.5, tri ethanol amine 0.2 ml, benzyl alcohol 0.2 ml Olive oil 4 ml.

Spreadability

Spreadability of cream formulations, that is, the ability of a formulated cream to evenly spread on the skin plays an important role. Formulations CC6, CC5 were good spreadability properties. CC7-CC9 Formulations have leaking and overspreading occurred.

Phase separation

In these antibacterial cream formulations (CC1-CC5) no phase separation was observed.

Antibacterial assay

This test is carried out by preparing bacterial inoculums

The bacterial strains were subcultured to get fresh cultures of bacteria. for this purpose, a single colony from bacterial strain was inoculated on nutrient broth. The broth was incubated for 24 h at 37 °C. 14 gm of nutrient agar media was dissolved in 1 L of distilled water at PH 7 and autoclaved for 20 min at 121 °C. The media were allowed to cool down to 45 °C and poured to petri plates for preparing 75 ml of solid media. using sterile cork borer 7 wells per plate were made in the solidified media. Agar diffusion method was used for antibacterial activity. Bacterial culture was inoculated on the surface of solid media. The crude extract of oxalis corniculata and fractions were dissolved in dimethylsulfoxide (DMSO) at the same concentration of 2 mg/ml to prepare stock solutions. from the stock solution, 1000 ul was poured into respective wells [11]. Cefixime was used as a positive control and DMSO was used as a negative control. The zone of inhibition of crude extract and fractions were measured in mm after 24 h of incubation at 37 °C and compared with the zone of inhibition of standard drug cefixime. The maximum antibacterial activity in 20%concentration of the plant was 19.33 mm diameter for E. coli [12].

CONCLUSION

The present study involves formulation and evaluation of antibacterial cream using leaves of Oxaliscorniculata and the study suggests that oxalis corniculata has good antibacterial, antifungal properties and can be used to treat various skin infections. The aqueous extract of oxalis corniculata exhibited strong antibacterial activity, especially with an increase of extract concentration. The results of different physical and chemical tests of cream showed that the formulation could be used topically in order to protect skin against damage caused by S. aureus, and E. coli. Various evaluation parameters have been studied providing satisfactory results and this study revealed that the developed herbal antibacterial cream formulation of Code CC6 i. e the 20% concentration of extract was comparatively better than other formulations. So it is considered as optimized formulation.

DISCUSSION

Our study aimed at formulating an antimicrobial cream for leaves of Oxalis corniculata and evaluating its efficacy. The results showcase antibacterial and antifungal properties of the extract which is similar to the other studies conducted on the same herb. It was found to be useful in skin infections caused by mainly S. aureus and E. coli and fungi as well. This paves way for future studies to be conducted on the same.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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