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Formulation and evaluation of herbal cream using ethanolic extract of *Embllica officinalis* for antibacterial activity

Jitendra Kumar Rai ^{1,*}, Krishanu Samanta ², Shweta Mishra ¹ and Jitendra Jena ²

¹ Department of Pharmaceutics, Pharmacy College Azamgarh, Itaura, Chandrshwar, Azamgarh, U.P. India, 276128

² Department of Pharmaceutical Chemistry, Pharmacy College Azamgarh, Itaura, Chandrshwar, Azamgarh, U.P. India, 276128

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Abstract

Skin infections occur commonly and after present therapeutic challenges to practitioners due to the growing concerns regarding multidrug resistant bacterial, viral and fungal strain. The aim of present study was to formulate topical cream using Ethanolic leaves extract *Embllica officinalis* and evaluate In- vitro study the antibacterial activity of the ethanolic extracts of dried leaves of *Embllica officinalis* was determined by using the Agar cup plate method versus different bacteria such as *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* etc. By blending the ethanolic extract of *Embllica officinalis* (10 % w/w) into aqueous cream we formulated herbal cream. Formulation of herbal cream evaluation their physio-chemical properties, in vitro drug release and in vitro Antibacterial Activity against *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*. The herbal cream formulation A5 show better release and effective against bacterial strain

Keywords: Herbal cream; *In-vitro* release; Antibacterial activity; Irritancy; Spreadability; Diffusion

1. Introduction

The conveying of drugs through the skin has long been an encouraging concept because of the following reasons: ease of access, large surface area, vast exposure to the circulatory and lymphatic networks and protective nature of the treatment [1]. Instead of, the alternative formulation like herbal medicine may also be prepared in the form of ointment. These ointments mention a viscous semisolid preparation applied externally on body surfaces area such as the skin, mucus membranes of the eye, vagina, anus, and nose etc. These ointments have medical properties. The medicated ointments contain a medicinal ingredient mixed, suspended or emulsified in the ointment base. Thus, the ointment has number of aim when applied externally such as antipruritic, keratolytics, protectants, antiseptics, emollients and astringents. Ointment bases are mainly free from water and generally contain one or more chemical in suspension or solution or dispersion form. Hence Ointment bases may be various types like absorption bases, dehydrating hydrocarbon oleaginous and water-soluble type [2].

Amla belongs to the family Euphorbiaceae and possesses antiviral, antibacterial, anticancer properties [3]. *Embllica officinalis* enjoys a sacred position in Ayurveda, an indigenous system of medicine in India. The major principle in *Embllica officinalis* active against microbes includes flavonoids, ascorbic acid, Gallic acid, alkaloids and hydrolysable tannins [4]. In classical Indian medicines, all parts of plant including the fruits, seed, leaves, roots, bark and flowers are used in various formulations. Amla is used in following problems: cold, anemia, dysentery, fever, gravel, sores. Since traditional times, leaves have been used for anti-inflammatory and antipyretic treatments. Today in the modern era, the pathogenic bacteria have developed resistance against existing antibiotics because of the extensive use of antimicrobial drugs against the infectious diseases. So, some of the active compounds prohibit the growth of the disease causing

* Corresponding author: Jitendra Kumar Rai

microbes either singly or in combinations [5]. For a long period of time plants have been a precious source of natural products which are used formalizing the human health, especially in last decades with more extensive studies for natural treatments. There is a continuous and immediate need to invent the new antimicrobials compounds with the varied chemical structure and innovative mechanisms of action for new and re -appearing infectious diseases. So, scientists are increasingly turning their attention to community medicines, looking for new leads to develop better drugs against microbial infections. Considering that extracts of amla (*Emblica officinalis*) show broad spectrum antimicrobial activity. The aim of the study was to show that *Emblica officinalis* has antibacterial activity and also has high potential as antibacterial agent when formulated as cream for topical use ethanolic extract of *Emblica officinalis*.

2. Material and methods

2.1. Identification and authentication

The leaves of *Emblica officinalis* were collected from the Pharmacy College Azamgarh in December, 2022. The plant sample of *Emblica officinalis* (Voucher specimen no. Euphorbia. 2022/3) Euphorbiaceae was diagnosed and authenticated at the Department of Botany by Professor Nawal Kishore Dubey at Banaras Hindu University, (Uttar Pradesh) India. The collected leaves were made lacking of unwanted foreign materials, sun-dried for a week.

2.2. Preparation of methanolic extract of *Emblica officinalis* leaves

The sun-dried leaves of *Emblica officinalis* was powdered using a laboratory mill. 140g of milled leaves of *Emblica officinalis* was extracted with ethanol by maceration for 48 hr. The extract was filtered and concentrated using evaporator at 35°C to obtain semisolid extract. The extract was stored in a refrigerator. A stock concentration of 400mg/ml was prepared from which working concentrations of 500mg/ml, 250mg/ml, 100mg/ml and 50mg/ml were prepared.

2.3. Test microorganisms

The microorganisms used for the study were following: *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*. In this study, multi - drug resistant wound separates bacteria from pathology, Civil Line, Azamgarh were used. The bacterial strains were raised and managed on Mueller Hinton agar at 37 °C.

2.4. Microbiological media

Chemicals and standard drugs Mueller Hinton Agar and Nutrient broth was obtained from the Chemical store of the Pharmacy College, Azamgarh.

2.5. Evaluation of antibacterial activity of leaves Extract *Emblica officinalis*.

The antibacterial activity of the ethanolic extract of the leaves of *Emblica officinalis* at concentrations of 50mg/ml, 100mg/ml, 150mg/ml and 200mg/ml were determined using the cup plate method. A molten Mueller Hinton agar stabilized at 45 °C was seeded with 0.1 ml of a 24 h broth culture of the test organism (*Bacillus cereus*, *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*) containing approximately 10⁸cfu/ ml in a sterile petri dish and allowed to set. Wells of 6mm diameter were created with a sterile corn borer and filled to about three-quarters full with solutions of the methanolic extract of the leaves of *Emblica officinalis*. The plates were pre-incubated for 1 h at room temperature to allow for diffusion of the solution and then incubated for 24 h. The zones of inhibition were measured (mean, n=2). Streptomycin and Gentamycin were used as positive and negative controls respectively. The in vitro bacterial response to the extract was evaluated using the diameter of the zones of inhibition as follows; resistant: 10 mm and below, intermediate: 11-15 mm and susceptible: 16mm and above [7].

2.6. Preparation of Cream

The herbal cream prepared by fusion method. In this method the constituents of the base were placed together in a melting pan and allowed to melt together at 70°C. After melting, the ingredients were stirred gently maintaining temperature of 70 °C for about 5 minutes, incorporating different ethanolic extract of *Emblica officinalis* into the various bases by triturating in a ceramic mortar and then cooled with continuous stirring. [8]. The prepared herbal cream was put in cream jars, labeled and were stored at room temperature.

2.7. Evaluation of herbal Cream

The evaluations were carried out on the herbal cream by using the following parameters:

2.7.1. Colour and odour

Colour and odour of prepared ointment was examined by visual examination.

2.7.2. Loss on drying

1 g of cream was placed in the petri-dish and heated in the water bath at 105°C every 30 min until it get constant weight.

2.7.3. pH

The pH of cream was determined by digital pH meter. 1 g of cream was dissolved in 50 ml of distilled water and the pH was measured.

2.7.4. Diffusion study

In vitro drug release studies of samples were carried out by using Modified Franz diffusion cell. Dialysis membrane previously soaked in pH 7.4 phosphate buffer was taken and placed in between donor and receptor compartments. In the donor compartment 10mg of formulation was added. Volume of the diffusion medium was maintained 25 ml in receptor compartment and temperature maintained at $34 \pm 0.5^\circ\text{C}$, and rpm was maintained at 25 by using hot plate magnetic stirrer. Aliquots were withdrawn at intervals of 15min, 30min, 45min, 1hr up to 6hours and replaced by equal volumes of diffusion medium. Aliquots were suitably diluted with pH 7.4 and analyzed by UV Spectrophotometer at 220 nm. F4 shows 98% of drug release within 6 hours [9].

2.7.5. Spreadability

The spreadability was determined by placing excess of sample in between two slides which was compressed to uniform thickness by placing a definite weight for definite time. The time required to separate the two slides was measured as spreadability. Lesser the time taken for separation of two slides results better spreadability [10]. Spreadability was calculated by following formula:

$$S=M \times L / T$$

Where as

S= Spreadability

M= Weight tide to the upper slide

L= Length of glass slide

T= Time taken to separate the slides It was found to be 5 seconds.

2.7.6. Extrudability

The formulation was filled in collapsible tube container. The extrudability was determined in terms of weight of ointment required to extrude 0.5cm of ribbon of ointment in 10 seconds [11].

2.7.7. Loss of Drying (LOD)

LOD was determined by placing the formulation in Petri dish on water bath and dried for the temperature 105°C. It was found to be 20%.

2.7.8. Solubility

Solubility of the sample was tested against various solvents.

2.7.9. Washability

Formulation was applied on the skin and then ease extend of washing with water was checked.

2.7.10. Non irritancy

Test Prepared herbal ointment was applied to the skin of human being and observed for the effect.

2.7.11. Stability study

The stability study was carried out for the prepared ointment at temperature of 37°C for 2 months.

3. Results and discussion

3.1. Antimicrobial study

Table 1 Antimicrobial activity of the ethanolic extract on different bacteria

S. no	Concentration(mg/ml) extract of <i>Emblica officinalis</i>	Staphylococcus aureus	Bacillus cereus	Escherichia coli	Pseudomonas aeruginosa
1.	50	10.67 ± 3.05	12.00 ± 3.00	8.00 ± 2.00	13.33 ± 3.22
2.	100	8.67 ± 1.53	13.33 ± 1.53	11.00 ± 2.00	16.33 ± 4.16
3.	150	13.00 ± 2.00	18.67 ± 2.09	10.33 ± 3.06	17.67 ± 5.03
4.	200	30.33 ± 3.06	28.67 ± 3.22	25.33 ± 4.06	30.33 ± 5.03

3.2. Formulation development of herbal cream

Table 2 Formulation development of herbal cream using ethanolic extract of *Emblica officinalis*

S. no	Formulation code	Using methanolic extract of <i>Emblica officinalis</i> (gm)	Wool Fat (gm)	Cetostearyl alcohol (gm)	Hard paraffin (gm)	White soft paraffin (gm)
1.	A1	0.5	0.5	0.5	0.5	8.5
2.	A2	1.0	0.5	0.5	0.5	8.5
3.	A3	1.5	0.5	0.5	0.5	8.5
4.	A4	2.0	0.5	0.5	0.5	8.5
5.	A5	2.5	0.5	0.5	0.5	8.5

3.3. Evaluation of herbal cream

Table 3 Evaluation of herbal cream

S. no	Evaluation parameter	Observation
1.	Colour	Pale white
2.	Odour	Characteristic
3.	Consistency	Smooth
4.	pH	7.2
5.	Spreadability (seconds)	5 seconds
6.	Extrudability	0.5 g
7.	Diffusion study (after 6 hours)	98%
8.	Loss on drying	20%
9.	Solubility	Soluble in water, alcohol and chloroform
10.	Washability	Good
11.	Non irritancy	Non irritant
12.	Stability study	Stable at 20°C, 25°C and 35°C

3.4. Drug release study

These results were stated that most of the infections are caused by the gram-positive bacteria such as *Staphylococcus aureus* and *Streptococcus pyogenes*. Less common cause by the gram-negative bacteria such as *Escherichia coli* and *Pseudomonas* species. The ethanolic extract of *Embllica officinalis* showed significant antibacterial activity against all the tested microorganisms. This observation indicates that the activity due to the presence of large varieties of phytoconstituents present in the extract. This results also well correlates with the earlier report. Hence, the observed antibacterial activity of the cream was due to the presence of active constituents of the extract and the activity also well maintained when it was converted to ointment. This was good sign to do further studies on that to make it as one of the commercial herbal creams for the treatment of bacterial infections.

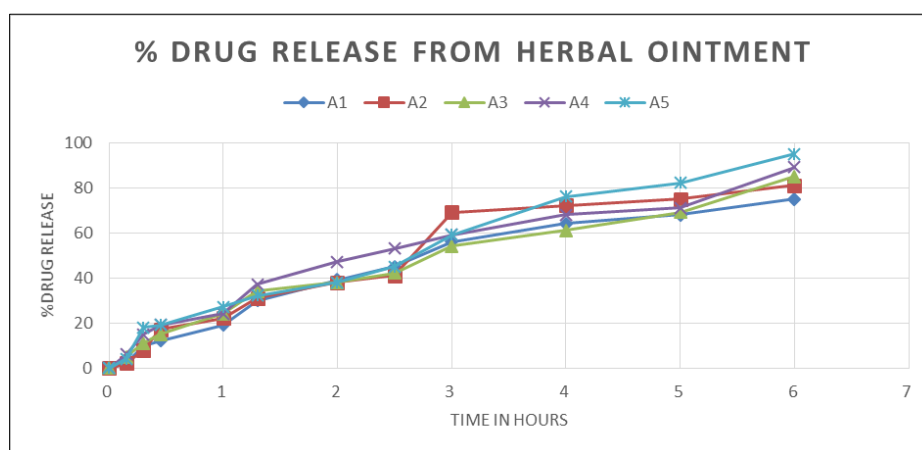


Figure 1 % drug release from herbal cream at 6 hrs.

4. Conclusion

This study shows that *Embllica officinalis* has antibacterial activity and has high potential as antibacterial agent when formulated as cream for topical use. On the basis of antibacterial efficacy Ethanolic extract of *Embllica officinalis* were incorporated in different ratio into appropriate base the final herbal cream readily spread on skin surface. It shows no irritancy, easy washable, good extradubility, better release profile and was stable at different temperature.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest.

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