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Research Article

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Formulation and Characterization of an Antibacterial Cream from *Lantana camara* Leaf Extract

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Abstract Purpose: The aim of the study was to evaluate the antibacterial activity of the ethanolic extract of the plant *Lantana camara* leaves against *Staphylococcus aureus* and *E. coli* species. Also to formulate effective and stable herbal antibacterial cream evaluates its physical and antibacterial properties.

Methods: Disk diffusion method was used to assess antibacterial activity of ethanolic extract using reference disk of antibiotics. The antibacterial cream was prepared by incorporating different amount of ingredients together and a certain amount of the herbal extract.

Results: The prepared cream was evaluated for their physical, rheological and antibacterial properties. Finally the efficacy of the herbal cream formulation was compared to two commercial products. The antimicrobial activity of the ethanolic extract was found to be effective against both *Staphylococcus aureus* and *E. coli.* All the 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.

Conclusion: The prepared cream was found to be natural, stable and safe. *Lantana camara* cream could be used topically in order to treat skin infections.

Keywords *Lantana camara*, ethanolic extract, antimicrobial cream; physical, rheological evaluation, *Staphylococcus aureus*, *E. coli*

Introduction

The rate of skin infections due to bacterial and fungal organisms is on the increase. This has become a significant health problem in many underdeveloped and developing countries and is particularly predominant in overpopulated areas with high humidity and poor hygienic conditions [1]. *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. The issue of resistance of dermatological infections to some medicaments available in the market has sparked up interest in the research of the antimicrobial properties of drugs from natural sources which are active against the major causative organisms of skin infections [2, 3]. Drug resistant strains are causing severe problems in many infections including skin infections such as carbuncles, folliculitis, impetigo, and burn wound sepsis. Antimicrobial resistance prolongs the duration of hospitalization, thereby increasing the cost of patient care [4]. One of the ways to prevent antibiotic resistance of pathogenic species is by using new compounds that are not based on existing synthetic antimicrobial agents. Apart from the problem of resistance, environmental degradation, cost and pollution associated with irrational use of orthodox medicines have necessitated renewed interest in nature as a source of effective and safer alternatives in the management of human infections [5]. Plants

possess a wide range of bioactive principles, which make them potential sources of antimicrobial agents and different types of medicines.

Lantana camara Linn. Family Verbenaceae commonly known as wild sage, is a flowering shrub native of tropical America and is cultivated throughout the world as an ornamental [6]. Different parts of the plant are used in folklore remedies and traditional systems of medicine for the treatment of various human ailments. Over the last twenty-five years a large number of plant species have been evaluated for their antibacterial activity. One of the plants known for having many medicinal uses in traditional system of medicine is *Lantana camara* [7].

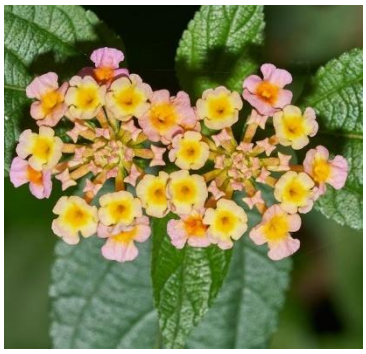

Figure 1: Lantana camara leaves and flowers

The leaves are used in the treatment of itches, cuts, ulcers, swellings, bilious fever, eczema and rheumatism. It has received attention due to its role in economy and ecology. It is serious weed in several countries that causes toxicity in grazing animals and is rapidly disturbing the ecological balance due to its luxuriant growth [8]. Many pharmacological investigations indicated that extracts of the leaves of exhibit antibacterial properties.

Materials and Methods

Plant Material

The specimen of the plant was collected from medicinal garden of Ujjain Institute of Pharmaceutical Sciences, Ujjain (M.P.) India.

Preparation of Extracts

The leaves of the plants were air dried at room temperature before grinding them to powdered form with the help of mechanical grinder. Several solvents of different polarity i.e. benzene, hexane, petroleum ether (40-60 ºC), chloroform, ethanol and ethyl acetate were used respectively to get extracts of the previously dried and powered leaves. The powdered leaves were extracted by Soxhlet apparatus [9]. Each extract was first filtered through Whatman Filter Paper No. 1 to clarify and then pass through a 0.45µm membrane filter. The filtrate was evaporated under reduced pressure in vacuum evaporator. The dried crude extracts were sterilized overnight by UV radiation and then stored at room temperature in amber color glass vials until used for antibacterial testing.

Preparation of Concentrations

In the study of the antibacterial activity, all the extracts were diluted in dimethylsulfoxide (DMSO). The concentrations corresponding to the extracts given in Table-1 are expressed in terms of mg/ml.

Test Bacterial Strains

Staphylococcus aureus and *Escherichia coli.* Both the bacterial strains were obtained from Department of Microbiology, R.D. Gardi Medical College, Ujjain, M.P., India.

Screening of Extracts for Antibacterial Activity

The antibacterial effects were tested by the disc diffusion method [10-13]. Firstly the bacteria to be tested were inoculated into Mueller Hinton broth (HiMedia®) and incubated for 3-6 h at 35 ºC. Petri dishes containing Mueller

Hinton Agar (HiMedia®) were impregnated with these bacterial suspensions. Discs of 6mm diameter (Sterile blank, HiMedia®) were impregnated with different concentration of each extracts. Blank disc impregnated with DMSO was used as negative control and disc of chloramphenicol (10 µg/disc, HiMedia®) as positive control. All test plates were incubated at 37ºC for 24h and the diameter of zones of inhibition 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 [10]. Antibiotics disks contain streptomycin, penicillin.

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 60min. Then the phase separation and solid sedimentation of the samples were inspected [11].

Table 1: Composition of making 10 gm antibacterial cream

Ingredients	Phase	Amount (for 10gm)
Stearic acid	Oil phase	1.0gm
Spermaceti		0.5gm
Cetyl alcohol		0.5gm
Glycerine	Water phase	0.5gm
Triethanolamine		0.2gm
Benzyl alcohol		0.2gm
Water		7.0gm
Extract		0.1gm

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. 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. 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. 500mg of the sample was spread on a clean slide and observed using an optical microscope (×10 and ×40) [12]. The pH of formulations was determined at 48h. The extent of volatile and non-volatile composition was determined.

Results

Antibacterial activity of extracts is presented in Table-2. The maximum antibacterial activity in 20% concentration of the ethanolic plant extract was 17mm for *S. aureus* and 14 mm for *E. coli*. Inhibition zone of antibiotic disks are presented in Table-3. *S. aureus* and *E. coli* were resistant to penicillin. The results of pH study were 5.5±0.57, 5.34±0.57, 5.9±0.0, 5.6±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 ($p>0.01$). Formulations containing the extracts had slightly darker colour. This was carried out so as to evaluate the compatibility of formulated cream with the skin in terms of pH. A high pH value indicating alkalinity could affect the pH balance of the skin, thereby causing negative skin reactions such as rashes while a pH value lower than that of the skin would be termed too acidic for the skin. This can also lead to sensitivity problems and hyper reaction. A pH value of 5.5 is the ideal pH for pharmaceutical products for skin application [14-16]. The formulation possessed good spreadability. The formulated cream readily spread when applied on the skin topically and rubbed gently. In creams applied topically for therapeutic effect, it is desirable that the active substance should be released at the skin surface and should penetrate at a suitable rate in sufficient amounts to maintain an effective concentration at the site of action [16].

Table 2: Antibacterial activity of various extracts of *Lantana camara* leaves

Extract	Conc. *mg/ml*	Diameter of zone of inhibition (mm)	
		S. aureus	***E. coli***
Ethanolic extract	20	17	14
	15	15	12
	10	12	11
	05	10	08
Benzene extract	20	8	5
	15	6	5
	10	5	4
	05	3	2
Chloroform extract	20	15	12
	15	13	10
	10	9	6
	05	8	5
Petroleum ether extract	20	13	11
	15	10	9
	10	8	7
	05	6	4

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

Antibiotic disks	*Zone of inhibition mm*	
	S. aureus	***E. coli***
Streptomycin 10µg	20±0.00	25±0.00
Penicillin 10µg	Resistant	Resistant

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 antibacterial activity of the *Lantana camara* extracts varied with the solvents used for the extraction. It is suggested that the crude preparations of the leaves of the plant containing both the active and non-active components to have higher efficacy than semi-crude or pure plant substances [14]. The results of this study (Table-2) showed that ethanolic extract of *Lantana camara* effectively inhibited the growth of *E. coli* and *S. aureus*. The antibacterial activity was increased with increase of the extract concentration. Antibacterial activity of the plant was considerable in comparison with the other reports. 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* and *S. aureus* was resistant to penicillin while with streptomycin it shows activity. The plant extract showed a broad spectrum of activity at 20% concentration, with the zone of inhibition of 17mm against *S. aureus* and 14 mm against *E. coli*. A phytochemical analysis revealed that the active principle responsible for the antibacterial activity was a phenolic compound. 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 [15]. In another report, methanolic extract of *Eucalyptus camadulensis* has been formulated as an anti-dermatophytic cream preparation [16]. 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 ethanolic extract of *Lantana camara* 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.

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Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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