

***In vitro* and *in vivo* Efficacy of a Herbal Formulation against Skin Infections**

Padma Deshmukh*, Pooja Gupta and Ravi Shankar

Department of Microbiology, Smt. C.H.M. College, Ulhasnagar - 3, Thane, India.

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Skin infections and resistance development are major problems in India. An herbal gel consisting of six medicinal plants viz: *Curcuma longa*, *Berberis aristata*, *Citrus reticulata*, *Ficus reliogosa*, *Mangifera indica* and *Mesua ferrea* was screened for its antibacterial activity against resistant strains of *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Corynebacterium* spp., the most implicated organisms in case of skin infections. Skin toxicity tests performed revealed that the herbal gel did not possess any toxic effects. Bactericidal efficacy of the herbal gel was found to be 100% within 2 hours of contact as compared to 3 hours in case of Benzoyl peroxide. Complete clearance of infection with no scaly or dry skin remnants was observed after 20 days in case of animal simulation models.

Key words: Skin infections, Herbal gel, Skin toxicity, Benzoyl peroxide.

In India, bacterial infections of skin constitute a large proportion of skin diseases. Regarding the quantum of dermatological problems in the community, a reliable estimate is that one in twenty people has a skin disease in India ¹.

These skin infections can result into severe topical and systemic complications. Hence, it is essential to treat these infections. The most commonly used topical chemotherapy includes Benzoyl peroxide, isotretinoin etc, while the systemic drugs commonly prescribed are erythromycin, clindamycin etc. Though effective, these drugs have their own side effects. Another problem being faced in treatment of these diseases is development of antibiotic resistance by the implicated organisms. Antibiotic resistance is a global public health problem. Overuse of antibiotics and consequent antibiotic selective pressure is thought to be the most important factor contributing to the appearance of different kinds of resistant bacteria. The more often a drug is used, the more likely bacteria are to develop resistance to it ². In the wake of these facts the mainstream medicine is becoming increasingly receptive to the use of antimicrobial and other drugs derived from plants.

* To whom all correspondence should be addressed.

Early humans recognized their dependence on nature in both health and illness. Led by instinct, taste, and experience, primitive men and women treated illness by using plants, animal parts, and minerals that were not part of their usual diet. Medicinal plants, since time immemorial, have been used in virtually all cultures as a source of medicine. The widespread use of herbal remedies and healthcare preparations, as those described in ancient texts such as the Vedas and the Bible, and obtained from commonly used traditional herbs and medicinal plants, have been traced to the occurrence of natural products with medicinal properties. Herbs also play an important role in Ayurvedic medicines. The principal Ayurvedic book on internal medicine, the Charaka Samhita, describes 582 herbs ³.

The present study was undertaken with a view to determine the efficacy of a herbal formulation prepared using the combination of the six medicinal plants (*Berberis aristata*, *Citrus reticulata*, *Curcuma longa*, *Ficus religiosa*, *Mangifera indica* and *Mesua ferrea*) which have shown to exhibit antibacterial activity against organisms causing skin ailments ⁴.

MATERIAL AND METHODS

Microorganisms

The samples, for the present study were collected from patients suffering from different types of skin infections. Various hospitals and clinics were approached for the same. Human volunteers were also the source for the samples.

The identification of the isolates was done on the basis of their Gram nature, cultural characteristics observed on the selective media, pigment production, haemolytic activity and their biochemical properties. The reference used for the identification was Bergey's Manual of Determinative Bacteriology, 8th Edition.

Antibiotic sensitivity test ⁵:

The antibiotic sensitivity of the organism was tested using the "Agar disc diffusion" method. *Herbal gel preparation* ⁶:

Creams/ Ointments/ Gels are semisolid preparation, usually containing medicament, used for application to the skin. An herbal gel was prepared using water based gel (*Aloe vera*-Unprocessed) of medicinal grade as the base, by

the following procedure:

The required amount (2 gms) of the herbal formulation was weighed and mixed with a weighed quantity (100 gms) of sterile base to give a homogenous product.

Testing the herbal gel

1. In vitro studies:
 - Antibacterial activity
 - Bactericidal efficacy
2. In vivo studies:
 - Skin toxicity
 - Healing power

1. In-vitro studies: -

The in-vitro testing of the herbal gel was performed in two steps:

1. Antibacterial activity of the herbal gel.
2. Determination of the bactericidal efficacy of the herbal gel.

Antibacterial activity of the herbal gel ⁷⁻¹⁰

The basic principle underlying the test is to determine the antibacterial activity of the herbal gel against the isolates by measuring the zone size of inhibition. The method used for this test is agar well diffusion method in which the activity of the test compound in the test is measured by correlating with the inhibition zone around it.

Determination of the bactericidal efficacy of the herbal gel ¹¹

The bactericidal efficacy time of the herbal gel was determined by carrying out the following test.

In-vivo studies

The information provided by the in-vitro studies are not sufficient to compare the practical usefulness of a compound as a potential antimicrobial and healing agent, especially, when comparison is to be made for evaluating a new topical agent.

The microbial flora found in various types of skin ailments in-vivo; respond altogether differently to the antimicrobial agents when they are tested in-vitro. Efficiency of any topical agent, herbal or chemotherapeutic, must therefore be necessarily studied in-vivo using proper animal models. In-vivo tests, thus become imperative for evaluating the antimicrobial and healing qualities of the topical agent.

Skin toxicity test

Though intact skin is relatively an effective barrier to the penetration of many substances, studies reveal that the skin is permeable in some degree to a variety of compounds. The skin toxicity test is based on this concept¹². The tests carried out are based on primary irritation, cumulative irritation and sensitization reactions. Primary irritation of the skin can be defined as a local inflammatory reaction which does not produce tissue destruction or irreversible change at the site of contact; the macroscopic manifestations of irritation are edema and erythema.

Healing power

Cutaneous healing may be defined broadly as the interaction of a complex series of phenomena that eventuates in the resurfacing, reconstitution and proportionate restoration of tensile strength of wounded skin¹³.

RESULTS AND DISCUSSION

23 samples were collected and each case yielded identifiable bacteria, out of which 10 were found to be dominated by single organisms. The causative agents of the remaining 12 were found to be associated along with *Micrococcus*. This high incidence of *Micrococcus* can be explained by the fact that *Micrococcus* forms a part of the normal flora of the skin. Pie diagram (Fig-1a) represents the type of infections encountered and Fig-1b illustrates the percentage distribution of the causative agents.

The organism mainly associated with skin infections like impetigo, carbuncle, furuncle etc is *Staphylococcus aureus*^{14,15}. *Pseudomonas* is found to be the main causative agent of acute cellulitis, Hot tub folliculitis etc^{16,17}. The present study confirms the presence of these respective organisms in different types of skin ailments.

Table 1.1. Antibacterial activity

S. No.	Organisms	Gel Base	Standard ointment	Herbal Cream
1.	<i>Staphylococcus aureus</i> (S1)	Not inhibited	14 mm	24 mm
2.	<i>Corynebacterium sp.</i> (C1)	Not inhibited	12 mm	14 mm
3.	<i>Pseudomonas aeruginosa</i> (P1)	Not inhibited	14 mm	24 mm

Table 1.2. Bactericidal efficacy

	<i>Corynebacterium</i> sp.(C1)			<i>Staphylococcus Aureus</i> (S1)			<i>Pseudomonas Aeruginosa</i> (P1)		
No. oF Ours	Gel	BP	Herbal gel	Gel	BP	Herbal gel	Gel	BP	Herbal gel
0	+++	++	++	+++	+	+	+++	++	++
1	+++	+	++	+++	+	+	+++	++	+
2	+++	+	++	+++	+	-	+++	+	-
3	+++	-	++	+++	-	-	+++	-	-
4	+++	-	+	+++	-	-	+++	-	-
5	+++	-	-	+++	-	-	+++	-	-
6	++	-	-	++	-	-	++	-	-
24	+	-	-	+	-	-	+	-	-
Bp	→ Benzoyl peroxide gel,				+++	→Heavy growth			
++	→ Moderate growth,				+	→Very slight growth			
-	→No growth								

Antibiotic sensitivity profile

The antibiotic sensitivity of the organisms was determined using the Kirby and Bauer chart. The most resistant isolates (*Staphylococcus aureus* –S1, *Pseudomonas aeruginosa* –P1 and *Corynebacterium* spp. –C1) were used for further study.

Antibacterial activity

The antibacterial activity of the Herbal gel, gel (Base) and a standard ointment (Benzoyl peroxide) was studied using the Agar cup method. The results were inferred on the basis of the size of the zone of inhibition. As shown in Table no.1.1, the Herbal gel was found to be more effective as compared to the standard ointment for all the three selected isolates.

Bactericidal efficacy

The bactericidal efficacy of the Herbal gel along with the base and a commonly available chemotherapeutic agent was performed. The results are presented in Table no.1.2.

The gel (base) was found to be non inhibitory as growth of all the three organisms was observed even after 24 hours.

The Herbal gel was found to be very effective against *Staphylococcus aureus* &

Pseudomonas aeruginosa. A decrease in the load of organisms was observed after 1 hour of contact while complete inhibition was observed after 2 hours. However, *Corynebacterium* sp. was found to resist the action of the Herbal gel even after 4 hours. Its complete inhibition was observed after 5 hours.

Benzoyl peroxide was found to be less effective against *Staphylococcus* & *Pseudomonas* compared to the herbal preparation. Its bactericidal efficacy was observed only after 3 hours of contact. In case of *Corynebacterium* spp., Benzoyl peroxide was observed to be more effective as compared to the Herbal gel.

Skin toxicity test

Guinea pigs were selected for the in vivo studies. Six guinea pigs were selected and divided into three groups viz Control, Test and Standard. The Herbal gel was tested for its toxic effects on the experimental animals using the skin toxicity test. Since the idea was to use the Herbal gel for topical application, its ill effects, if any, were studied. Results presented in Table 2.1 show that the Herbal gel had no toxic effects like necrosis, erythema or edema on the skin even after 72 hours of application of 100 mg. No cumulative irritation

Table 2.1. Skin Toxicity

Test Compound	Dose	Erythema			Edema			Necrosis		
		24 Hrs	48 Hrs	72 Hrs	24 Hrs	48 Hrs	72 Hrs	24 Hrs	48 Hrs	72 Hrs
Extract	100 Mg	-	-	-	-	-	-	-	-	-
Gel	100 Mg	-	-	-	-	-	-	-	-	-
Herbal Gel	100 Mg	-	-	-	-	-	-	-	-	-
Face Pack	100 Mg	-	-	-	-	-	-	-	-	-

+ → Positive Reaction, - → Negative Reaction

Table 2.2. Skin Toxicity

Test Compound	Daily Dose	Erythema			Edema			Necrosis		
		7 Days	14 Days	21 Days	7 Days	14 Days	21 Days	7 Days	14 Days	21 Days
Extract	100 Mg	-	-	-	-	-	-	-	-	-
Gel	100 Mg	-	-	-	-	-	-	-	-	-
Herbal Gel	100 Mg	-	-	-	-	-	-	-	-	-

+ →Positive Reaction, - → Negative Reaction

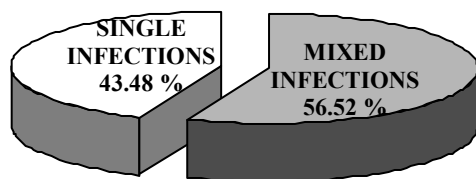


Fig. 1a. Percentage representation of the type of skin infections encountered

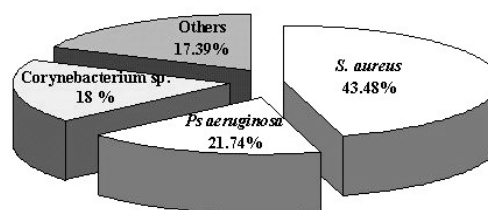


Fig. 1b. Percentage distribution of the etiological agents in various skin ailments

was observed for the Herbal gel even after 21 days (Table 2.2).

Animal tests

The In vitro tests and the toxicity tests performed using the herbal formulation reported to give very promising and encouraging results. Hence, a step was taken forward to test the efficacy of the herbal gel in healing infection. The experimental animals (Guinea pig) were depilated and lacerated. The site was inoculated with the pathogenic isolates. A complete infection was established in 5 days. The control animals were left untreated. The other two groups of animals were treated with the herbal gel and standard ointment respectively. The experimental animals were examined every five days for the clearance of infection. Complete clearance of the site of infection treated with the herbal gel was observed after twenty days. Though effective, the standard ointment left the skin dry and scaly.

CONCLUSION

The following conclusions can thus be drawn from the present study:

The plants under study have got the potential to treat the resistant micro organisms. The herbal preparation can be screened for antifungal activity against dermatophytes. These plants being effective in their crude form can further be explored to study the efficacy of their phytochemical constituents.

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