Contents lists available at UGC-CARE

International Journal of Pharmaceutical Sciences and Drug Research

[ISSN: 0975-248X; CODEN (USA): IJPSPP]

Available online at www.ijpsdronline.com

Research Article

Anti-microbial Activity of a Polyherbal Formulation Containing *Ocimum Sanctum*, *Rubia Cordifolia* and *Glycirrhiza Glabra* Extracts against Different Microbial Species

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ARTICLE INFO

Article history:

Received: 18 October, 2020

Revised: 17 December, 2020

Accepted: 30 December, 2020

Published: 30 January, 2021

Keywords:

Anti-microbial,
Glycyrrhiza glabra,
Polyherbal cream,
Ocimum sanctum,
Rubia cordifolia,
Topical cream.

DOI:

10.25004/IJPSDR.2021.130106

ABSTRACT

In the present investigation, anti-microbial evaluation of individual creams of *Ocimum sanctum, Rubia cordifolia, Glycyrrhiza glabra* and their optimized polyherbal cream was carried out using commonly used gram-positive bacteria (*Staphylococcus aureus and Bacillus subtilis*) gram negative bacteria *(Escherichia coli and Pseudomonas aeruginosa and fungi (Candida albicans* and *Aspergillus niger)*. The study reported that, both individual extract creams and polyherbal creams have shown anti-microbial activity. The *O. sanctum* extract-based cream was more effective against gram-positive bacteria having more zone of inhibition (20 ± 0.57, 15 ± 0.05) than the rest 04 species, while *Glycyrrhiza glabra* extract-based cream was more effective against gram negative bacteria having more zone of inhibition (20 ± 1.0, 11 ± 0.46) and fungi (10 ± 0.46, 13 ± 0.15). The *Rubia cordifolia* extract-based cream reported less strong action against all species than above mentioned both the creams. However, all 3 creams were less potent than the reference standard cream. The optimized polyherbal formulation showed enhanced zone of inhibition significantly compared to individual extract-based creams. The effect was even more than the reference standard cream against gram positive (22 ± 0.28, 18 ± 0.20) and gram negative (19 ± 0.15, 22 ± 0.35) bacteria, suggesting synergistic combination of ingredients,

Thus, study validated the usefulness of polyherbal cream consisting of *O. sanctum*, *Glycyrrhiza glabra* and *R. cordifolia* against most common pathogens in dermatology, suggesting possible role to treat multidrug-resistant mixed infections. This action may be attributed to the synergistic actions of various phytoconstituents involved.

Introduction

In general, skin infection represents a group of infections often occurs due to pathogenic invasions of the layers of the skin and underlying soft tissues. Skin infections are becoming a major health concern due to the continuous increase in its number in both settings, i.e., OPD and IPD.[1] It is also reported that, at any given point of time, the number of skin infection patients across the globe is between 20-30% of total patients. Almost 14% of these patients need hospitalization as emergency patients, which accounts for a huge financial burden on patients or the government as the case may be.[2]

Many synthetic anti-microbial agents are available for topical and systemic use; however, the rapid development of resistance and associated duration dependant side effects restrict their utilization. These restrictions keep on demanding new drugs periodically. Unfortunately, the rate of infection rates and the rate of mutations observed in pathogens is more than the pace of drug development.[3] This in turn, makes the research and development efforts to find ideal and cost-effective therapy a matter of priority.[4]

These concerns suggest the need to develop potent, economical, patient-friendly, and time tested treatment with

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Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

a reduced rate of development of resistance.[3] On the other hand, World Health Organization reported that about 80% of the world's population relies chiefly on traditional medicine for primary health care needs, including dermatological treatment may be in its crude form.[5] It is also well known that most natural remedies are inherently safer compared to synthetic therapies irrespective of its mode of administration and duration of use.[6] These medicines have widespread effects especially useful in the management of disease with pathogenesis attributed to multi-component composition. The synergistic action produced by various phyto-constituents present in a particular plant or its part is an important advantage to address complex conditions commonly seen in dermatology.[7] The literature survey revealed that several plants or their specific parts are mentioned or used in the Indian traditional system of medicine for the treatment of various skin complaints. Many of these have also been scientifically validated using established preclinical and clinical methods of modern medicine.[5] These reports are always useful to plan related study. The extensive literature survey revealed that the plants, namely *O. sanctum* (Leaves), *R. cordifolia* (Roots), *Glycirrhiza glabra* (Roots) have been scientifically reported for their effectiveness in numerous skin infections and other conditions which can aggravate existing skin complaints.[8-10]

The delivery of drugs through the skin has long been a promising concept because of the ease of access, large surface area, vast exposure to the circulatory and lymphatic networks, and non-invasive nature of the treatment.[1] Along with other topical dosage forms, herbal drugs are also being formulated in the form of creams. Creams being homogeneous, semi-solid or viscous preparations with a relatively fluid consistency and are widely intended for topical application for better results.[11]

Literature survey also revealed another important outcome, i.e., weak action of plant product when consisting of single active ingredient while on the contrary, it is reported that polyherbal preparations show enhanced effectiveness by many folds. This demands for suitable modification.[7] Moreover, the high degree of complex nature of most skin complaints demands preparation of preparation with multiple components that can act in various ways. This is also a common practice in allopathy.[12]

Considering all the aforementioned aspects the present investigation entitled "Anti-microbial activity of a polyherbal formulation containing *O. sanctum*, *R. cordifolia* and *G. glabra* extracts against different microbial species" was conducted.

Materials and Methods

Materials

Drugs and Chemicals

All the drugs and chemicals of AR grade were procured from local vendor.

Microorganisms

The microorganisms *S. aureus*, *E. coli, B. subtilis*, *Pseudomonas aeruginosa, C. albicans* and *A. niger* were obtained from Dr. D. Y. Patil Institute of Pharmaceutical Sciences & Research, Pune, India.

Collection and Identification of Medicinal Plants

Fresh leaves of *O. sanctum and* roots of *R. cordifolia and Glycyrrhiza glabra* were procured and subjected to the process of extraction as described below.

Methods

Preparation of Extracts

Air-dried coarse powders of *O. sanctum* leaves, *R. cordifolia* roots, *Glycyrrhiza glabra* roots, were extracted separately by the soxhlet extraction process and petroleum ether. These extracts were further successively extracted with hydroalcoholic (60:40) solution to obtain their respective hydroalcoholic extracts. The extracts were then concentrated to dryness under reduced pressure and controlled temperature, respectively, and they were preserved in a refrigerator for further study.[8-10]

Formulation of Topical Creams of Individual Extracts[13]

Individual creams containing extracts *O. sanctum* leaves, *R. cordifolia* roots and *Glycyrrhiza glabra* roots were prepared separately using the optimized formula mentioned in Table 1. To formulate these creams, the first two phases, namely aqueous and oil were prepared separately. The aqueous phase was prepared by incorporating that is dispersing 1gm of methylcellulose polymer in hot water (at around 75°C) and then cooled down to around 5°C under continuous stirring in 100 mL of water, with continuous stirring, and then 1ml of glycerin and 1 ml of propylene glycol was added with constant stirring. Then this prepared aqueous phase was then added to the three individual extracts separately. The second oil phase was prepared, by melting the 0.9 g of beeswax at 70°C, with intermittent stirring and 1 ml of almond oil mixed. After

Table 1: Optimized composition of topical creams *O. sanctum* leaves extract

Ingredients (% w/w)	*Cream of OS leaves extract*	*Cream of RC roots extract*	*Cream of GG root extract*
Extract	2	2	2
Glycerin	1	2	1.5
Propylene glycol	1	1	1
Zinc oxide	0.8	0.8	0.8
Methylcellulose	2	2	1
Beeswax	0.9	0.9	0.9
Almond oil	1	1	1
Sodium benzoate	0.1	0.1	0.1
Purified water	q.s.	q.s.	q.s.

preparation of both the above phases, these were mixed together to get a mixture. Preparing the above mixture, the mixture of 0.1 g of sodium benzoate as a preservative and 0.8 g of zinc oxide as a skin whitener was added, with continuous stirring.

Formulation of Topical Creams of Individual Extracts

The polyherbal cream using all three extracts was formulated in the similar way as that of the individual creams using the optimized formula mentioned in Table 2.

Anti-microbial activity of Extracts Individual Creams and polyherbal Cream

Test Microorganisms

A panel of common pathogenic microorganisms was used in the study, which includes two gram-positive bacteria (*S. aureus* and *B. subtilis*); two gram-negative bacteria (*E. coli* and *Pseudomonas aeruginosa*), and two fungi (*C. albicans* and *A. niger*).[14]

Preparation of Nutrient agar Plates for Cup Plate Method

Nutrient Agar medium was prepared according to the manufacturer's instructions and autoclaved for 20 minutes at 20 psi pressure. After autoclaving, the agar medium was cooled to 40-45°C. The respective bacterial suspension's turbidity was adjusted to a 0.5 McFarland standard (10^8cfu/ml), which is further used as inoculum. Around 20 mL of the cooled agar medium was added with 50 µL of respective inoculum and then poured onto the sterile Petri plate and plates were allowed to solidify for 5 minutes. On each petri dish, five perforations/wells were made with a metal tube/borer with a 4 mm diameter to receive individual plant extracts, topical creams of individual extracts, polyherbal cream and Soframycin cream as a reference standard. The extracts, creams, and reference standard Soframycin cream were added immediately into the wells and kept for incubation at 37°C for 24 hours to allow the microorganism to grow and anti-microbial agents to diffuse through the culture medium. At the end of the incubation, the zone diameter measured with the help of a zone reader. All the experiments repeated 3 times and mean results were noted down.[15]

Table 2: Optimized composition of polyherbal topical creams using the combination of extracts

Ingredients	*Quantities (% w/w)*
O. sanctum extract	1
R. cordifolia extract	1
Glycyrrhiza glabra extract	1
Glycerin	1.5
Propylene glycol	1
Zinc oxide	0.8
Methylcellulose	1.5
Beeswax	0.9
Sesame oil	1
Sodium benzoate	0.1
Purified water	Qs

Results

Anti-microbial activity

Anti-microbial Activity of Individual Extracts

This anti-microbial activity was performed that showed significant activity. The details of this are as tabulated in Table 3. The results of the study revealed that *O. sanctum* showed very strong anti-microbial activity against *S. aureus* and *B. subtilis*, strong inhibitory effects against *P. aeruginosa*, *E. coli* and *A. niger* and moderate inhibitory effects against *C. albicans*. Similarly, *R. cordifolia* showed very strong anti-microbial activity against *S. aureus*, strong inhibitory effect against *P. aeruginosa* and *E. coli* but the moderate effect on *B. subtilis* and *A. niger*. However, *Glycyrrhiza glabra* extract showed very strong anti-microbial activity against *E. coli* and strong inhibitory effect against remaining all microbes. Reference standard showed potential activity against all selected microorganisms. Thus all three extracts were capable of inhibiting the microbial growth of all the microbes showing varied potency in a zone of inhibition (Fig. 1).

Anti-microbial Activity of Optimized Topical Cream of Individual Extracts and Polyherbal Topical Cream

O. sanctum leaf extract based topical cream showed very strong inhibitory effect against *B. subtilis* and *P. aeruginosa*, strong inhibitory effect against *S. aureus, E. coli* and *A. niger* (Table 4). *R. cordifolia* root extract-based topical cream showed very strong inhibitory effect against *P. aeruginosa*

Table 3: Anti-microbial activity of individual extracts

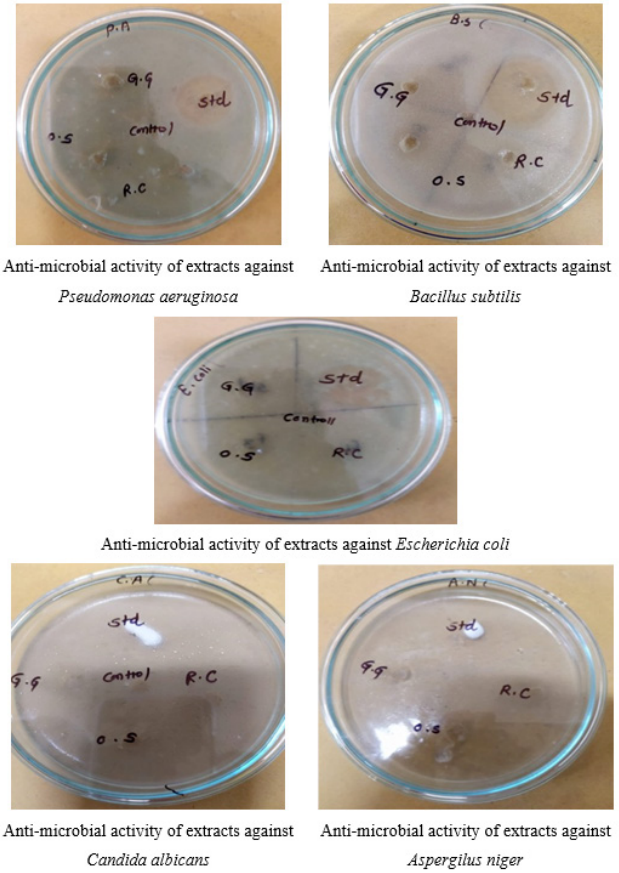
	*Zone of Inhibition (mm)**					
Name of Extract	*S. aureus*	*B. subtilis*	*E. coli*	*P. aeruginosa*	*C. albicans*	*A. Niger*
O. sanctum	20 ± 0.57	15 ± 0.05	12 ± 0.55	10 ± 0.57	09 ± 0.55	10 ± 0.57
R. cordifolia	16 ± 0.57	09 ± 0.57	10 ± 0.11	10 ± 0.05	09 ± 0.25	09 ± 0.1
Glycyrrhiza glabra	13.5 ± 0.11	14 ± 1.15	20 ± 1.0	11 ± 0.46	10 ± 0.46	13 ± 0.15
Ref. Standard (Soframycin cream)	25 ± 0.05	29 ± 0.64	30 ± 0.11	23 ± 0.25	25 ± 0.25	26 ± 0.15

*Mean value of the diameter of zone of inhibition with a standard deviation of three replicates
Degree of inhibition: 1. Moderate inhibition zone (9 mm); 2. Strong inhibition zone (10-14mm); 3. Very strong inhibition zone (15-18mm)

Table 4: Anti-microbial activity of optimized topical cream of individual extracts and polyherbal topical cream

Sr. No	Name of Formulation	Zone of Inhibition (mm)*					
		S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*	*C. albicans*	*A. niger*
1	*O. sanctum* leaf extract based topical cream	14 ± 0.40	15 ± 0.20	14 ± 0.15	24 ± 0.28	10 ± 0.43	11 ± 0.20
2	*R. cordifolia* root extract based topical cream	10 ± 0.17	08 ± 0.55	13 ± 0.20	17 ± 0.15	11 ± 0.20	21 ± 0.05
3	*Glycyrrhiza glabra* root extract based topical cream	14 ± 0.11	14 ± 0.40	15 ± 0.1	11 ± 0.20	10 ± 0.58	21 ± 0.15
4	Polyherbal topical cream	22 ± 0.28	18 ± 0.20	19 ± 0.15	22 ± 0.35	13 ± 0.20	19 ± 0.17
5	Reference Standard (Soframycin cream)	25 ± 0.05	15 ± 0.20	15 ± 0.15	21 ± 0.20	15 ± 0.26	15 ± 0.47

*Mean value of the diameter of zone of inhibition with a standard deviation of three replicates
Degree of inhibition: 1. Moderate inhibition zone (9 mm); 2. Strong inhibition zone (10-14mm); 3. Very strong inhibition zone (15-18mm)



Anti-microbial activity of extracts against *Pseudomonas aeruginosa*

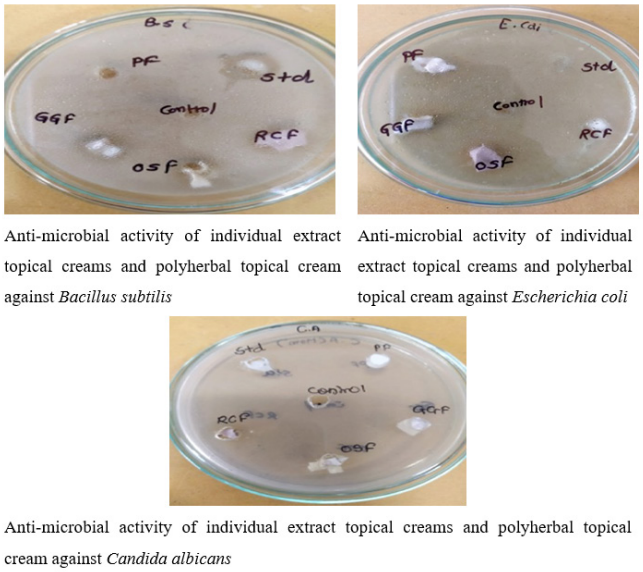
Anti-microbial activity of extracts against *Bacillus subtilis*

Anti-microbial activity of extracts against *Escherichia coli*

Anti-microbial activity of extracts against *Candida albicans*

Anti-microbial activity of extracts against *Aspergilus niger*

Fig. 1: Anti-microbial activity of extracts against different microbial species
Note: GG: *Glycyrrhiza glabra*; OS: *O. sanctum*; RC: *R. cordifolia*



Anti-microbial activity of individual extract topical creams and polyherbal topical cream against *Bacillus subtilis*

Anti-microbial activity of individual extract topical creams and polyherbal topical cream against *Escherichia coli*

Anti-microbial activity of individual extract topical creams and polyherbal topical cream against *Candida albicans*

Fig. 2: Anti-microbial activity of creams of individual extracts and polyherbal topical cream against various microbial species
***Note:** GGF:*Glycyrrhiza glabra* root extract based topical cream; OSF: *O. sanctum* leaf extract based topical cream; RCF: *R. cordifolia* root extract based topical cream

and *A. niger,* strong inhibitory effect against *S. aureus, E. coli* and *C. albicans.* While, *Glycyrrhiza glabra* root extract-based topical cream showed very strong inhibitory effect against *E. coli* and *A. niger,* strong inhibitory effect against *S. aureus, B. subtilis* and *P. aeruginosa*. *O. sanctum* and *Glycyrrhiza glabra* extracts were found to be ineffective against *C. Albicans,* while *R. cordifolia* extract was unable to produce inhibitory effect against *B. subtilis*. Polyherbal topical cream and reference standard showed potential activity against all selected microorganisms. These results showed that polyherbal formulation was more potent and efficacious in inhibiting the growth of all the microbes compared with the individual extracts (Fig. 2).

Discussion

Skin infections are the 4th leading cause of nonfatal disease burden and present a foremost health concern especially due to its recurrence and need for costly long-term treatment. It has been reported that these skin infections with complex pathology adversely affect the quality of personal and professional life. [16] Several skin diseases are associated with long-term disfigurement, disability, and stigma. Various bacteria can cause skin infections, viruses, fungus, or parasites accounting for primary or secondary infections, leading to wide variation from patient to patient.[17]

S. aureus, *E. coli*, *Pseudomonas aeroginosa*, *C. albicans*, *A. niger* and *Bacilus subtilis* are emerging as the main pathogens that cause skin infections in larger populations. [14] Though many anti-microbial agents for skin infections have been produced, the rapid development of microbial resistance towards these agents by microorganisms has been a major concern.[3] Simultaneously appearance of newer infections, recurrence of some infections with varied presentations and related consequences are the major limiting factors. [16] Apart from this the conventional anti-microbial agents are also associated with several untoward effects, which further may result in the cessation of therapy.[3] The patients' ultimate concern is to provide them with efficient anti-microbial therapy with minimal side effects and microbial resistance pace.[18]

Plants have been documented to possess a wide variety of natural bioactive compounds with their usefulness in maintaining human health and well being.[19] They have also been proved for their efficacy in several ailments by virtue of their variety of pharmacological actions, including anti-microbial effects.[6] The utilization of plants and plant products for anti-microbial efficacy is of great significance in concern with therapeutic outcomes.[20] Hence the research interest regarding the anti-microbial efficacy of natural products against skin infections induced by major causative microbial species has been continuously increasing among scientists.[21]

In light of this, we have prepared polyherbal cream using *O. sanctum* leaves extract, *R. cordifolia,* and *Glycyrrhiza glabra* root extracts. The reduction in untoward effects without any alteration in untoward effects without any alteration in pharmacological activity is the formulation's basic aim.[6] Accordingly, we decided to go for topical preparation and cream being the most compliant is selected so that one can bypass the untoward effects caused due to systemic administration.[22] As per the principle of general pharmacology, oral administration of drugs usually elicits more side effects; hence the preparation of cream itself is the first positive outcome of the study.[18] It has been well documented that polyherbal formulation usually offers a better effect than its individual component; hence same approach has been implemented here. Further, looking at the involvement of various pathogenic strains either individually or together, it is quite desirous to have a remedy that can combat a variety of mixed infections.[7] In accordance with it, four common species have been used for anti-microbial screening. In order to examine the pharmacological efficacy, the polyherbal cream prepared with *O. sanctum* leaves extract, *R. cordifolia* and *Glycyrrhiza glabra* root extracts was compared against the creams of the individual extracts. The results showed significant improvement with the polyherbal formulation as compared to creams of individual extracts.

The polyherbal formulation deals with the phytochemical interactions, which may result in either a synergistic effect or a strong untoward effect. The former makes it worth being useful with possible added advantage of patient-friendly nature while later one leads to rejection of formulation. In some cases, neutral effect may also be observed.[7] Collectively speaking, polyherbal formulation is useful only if it is more potent and offers lesser side effects than the formulation of individual ingredients.[23]

According to this objective, the optimization followed by in vitro evaluation clearly showed that the polyherbal cream is synergistic. Thus polyherbal cream with synergistic therapeutic action as per the prescribed standards is a noteworthy outcome of this study, which perhaps is an essential requirement for any formulation to be an ideal one.[24]

The most important and desired expectation from any polyherbal formulation preparation is its widespread action.[23] The allopathic medication has a high degree of potency but is usually limited to a particular indication, which may not be fruitful in complex pathogenesis. In such cases, the addition of other drugs to make it multidrug formulation is an option but has many limitations while preparing such fixed-dose combinations.[25] On the other hand, one plant material being natural has multiple components that are usually compatible with each other and with the biological system.[23] This multi-phytocomponent approach, when used in combinations, can enhance its spectrum of activity by multiple folds, which is priority need in certain conditions.[26] The skin infection is one such condition, where mixed infection is commonly noted either in the beginning or after due course of time.[17] The current synergistic anti-microbial results of polyherbal cream having ingredients which are widely used as edible stuff over individual creams not only second the formulation optimization but also can emerge as a revolutionary remedy, especially for multidrug-resistant mixed infections in dermatology.[7]

The other documented pharmacological uses of these plant materials can be of great help in specific coexisting conditions that are rarely possible with modern medicine.

The study confirmed the broad-spectrum anti-bacterial and antifungal activity of polyherbal cream consisting of *O. sanctum* leaves extract, *R. cordifolia* and *Glycyrrhiza glabra* root extracts. The findings suggest possible use of this polyherbal cream to combat co-existing conditions and reduce the need for additional drugs. The significantly improved action of polyherbal formulation compared to creams of individual extracts is attributed to the synergistic action of various phytochemicals.

Acknowledgement

Authors are thankful to Dr. N. B Ghiware Sir, Head, Center for Research in Pharmaceutical Sciences, Nanded Pharmacy College Nanded, for Providing research facilities.

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HOW TO CITE THIS ARTICLE: Shafi S, Shendarkar GR. Anti-microbial activity of a polyherbal formulation containing *Ocimum Sanctum*, *Rubia Cordifolia* and *Glycirrhiza Glabra* extracts against different microbial species. Int. J. Pharm. Sci. Drug Res. 2021;13(1):36-41. **DOI:** 10.25004/IJPSDR.2021.130106