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In *Vitro* Antimicrobial Activity of A Ghamra Based Polyherbal Cream

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ABSTRACT

In this study, in vitro antimicrobial activity of Polyherbal cream comprising of various plant herb extracts of leaves viz. *Aloe barbadensis, Calendula officinalis, Lawsonia inermis, Carica Papaya and Azadirachta indica* along with *tridax procumbens* as a key Plant was evaluated. Polyherbal formulations (F1 & F2) were designed and developed as ointment based semisolid cream. Disc diffusion method and agar well diffusion method were used for *in vitro* antibacterial and antifungal screening for both the formulations. Zone of inhibition were observed as a measure of investigation. The Formulation-1 (F1) showed efficient zone of inhibition at concentration of 4% than the formulation-2 (F2) at lower concentration 2% of *tridax procumbens.* In antifungal screening, the compound showed significant zones of inhibition against tested fungi.

Keywords: Antimicrobial activity, Antifungal activity, Polyherbal cream.

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INTRODUCTION

Herbal medicines are popular as remedies for diseases by vast majority of world's population. WHO estimates about three quarters of world population currently use herbs and other forms of traditional medicines to treat their diseases. Herbal formulation is popular among rural and urban community in India[1]. Many plants have been studied for their medicinal and antimicrobial properties[2-5] and some of them resulted in development of drugs[6]. Most of the skin infections are caused by fungi and bacteria[7]. Herbal remedies for skin care with antibacterial and antifungal activities are prepared from a variety of plant parts such as leaves, stem, root, bark and fruits. These may be applied in the form of cream, lotion, soap, sap, solvent extract and ointment to establish antimicrobial properties[8]. 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[9]. Numerous studies have been conducted with the extracts of various plants, screening antimicrobial activity as well as for the discovery of new, antimicrobial compounds[10-11]. *Tridax procumbens* (family-*Asteraceae*) is a perennial plant. They are available in all seasons and is found to be effective as an antimicrobial agent[12]. *Carica Papaya* leaves may hinder the growth of wound infection causing pathogens in invitro conditions. All the extracts prepared from leaf (acetone, water, and methanol) exhibited highest antibacterial activity against gram negative organism[13]. Aqueous extracts of *Lawsonia inermis* inhibits the skin pathogens *S. aureus*, *S. mutans* and *P.aeruginosa* potentially at 100% concentration. These extracts control the bacterial and fungal pathogens which cause skin disease[14]. A processed *Aloe vera* gel preparation inhibited the growth of fungus *Candida albicans*, acts as bactericidal against *Pseudomonas aeruginosa*, antiseptic, anti-inflammatory and skin moisturizing agent[15]. *Azadirachta indica* leaves have antibacterial properties and could be used for controlling airborne bacterial contamination in the residential premise[16-17]. *Calendula* has antibacterial and antifungal activity[18,19].

MATERIALS AND METHOD

Plant materials

Leaves from various plants as *Tridax procumbens* (Ghamra), *Lawsonia inermis* (Henna), *Aloe barbedensis* (Aloe Vera), *Carica papaya* (Papaya), *Calendula officinalis* (Marigold), and *Azadirachta indica (Neem)* were selected on the basis of their frequent traditional usage and antimicrobial potency. All the plants for present study were collected from campus of AKS University, Satna (MP).

Preparation of Plant Extract

Each powdered plant part was subjected to Soxhlation process using the various solvents. 30 gm of powdered samples and 300 ml of solvents were used for each extracts. The extracts were collected and concentrated using Rotary Vacuum Evaporator. The crude semi-solid extracts were collected and stored in small vial. The extracts were stored at 4°C until further use for preparation of polyherbal cream.

Formulation of Polyherbal Cream:

The formula was based on the concentration of *Tridax Procumbens* as it was 4% in F1, 2% in F2 and F3 (Cream base only) was considered as positive control. The cream base constituted of white bees wax, white petroleum, PEG 300, and cetosteryl alcohol. The semi dried extracts in the percentage specified (Table-1) were used for the preparation of formulation 1 & 2 using the ointment base. Standard trituration method was used where required quantity of the ointment base was melted at a temperature of about 70°C using hot plate. Further, the extracts were respectively added to the melted base at 40°C and mixed. The preparation stirred gently and continuously until a homogeneous dispersion was obtained.

Table: 1 Formula for formulation

S.No.	Name of Extract	Formulation 1 (F-1) Quantity (%)	Formulation 2 (F-2) Quantity (%)	Formulation 3 (F-3) Quantity (%)
1.	Ghamra (*Tridax Procumbens*)	4%	2%	--------
2.	Papaya	1%	1%	--------
3.	Heena	1.5%	1%	--------
4.	Neem	1%	1%	--------
5.	Marigold	1%	1%	--------
6.	Aloe vera	1%	0.5%	--------
7.	Ointment base	93%	91.5%	100%

Test Organisms

Bacteria: *Staphylococcus aureus* (Gram +ve), *Pseudomonas aeroginosa* and *E. Coli* (Gram -ve).

Fungus: *Candida albicans* (Aerobic, Incubation period- 48hours, Temperature 25^0C)

All the microbial strains were procured from Sapience Bio-analytical Research Lab., Bhopal (M.P)

Standards Used

Antibacterial: Cefotaxime (For gram +ve)

Gentamicin (For gram -ve)

Antifungal: Miconazole

Culture media

For Bacteria, the medium used for the activation of the microorganisms was nutrient broth. The nutrient agar media was used for the antimicrobial test. All the culture media were prepared and treated according to the manufacturer guidelines (HiMedia Laboratories Ltd., Mumbai, India). The media for Fungus was prepared using Yeast extracts, Peptone, Dextrose, Agar in a quantity of 3 gm, 10 gm, 20 gm and 15 gm respectively in an amount of 1liter.

Preparation of disc

The Discs were prepared using the disc of Whatmann filter paper no.1 of same diameter (0.5mm) with the help of punching machine. The discs were first autoclaved in a Petri dish for sterilization. The preparation of each concentration of discs was done by dipping the disc in different concentration. They were dried and preserved in the petri dishes at 2-8°C.

Antibacterial Activity

Microorganisms were inoculated in nutrient broth by streaking with loop and incubated at 35 ºC for 12 hour. Nutrient agar media was prepared and poured in Petri plates and kept for drying. Replaced the plate top and allow 3 to 5 minutes, but no longer than 15 minutes, for any excess surface moisture to be absorbed before applying the test samples disks. Disks were dipped in concentration of samples (10mg/ml) and air dried in laminar air flow to remove the solvent before this step. Placed the appropriate discs evenly on the surface of the agar plate by using a sterile forceps. Inverted the plate and placed them in an incubator at 35°C within 15 minutes after disks were applied. After 12 hours of incubation, the diameter of the disk was examined along with the zone of inhibition[20].

Antifungal Activity

The different samples were screened for antifungal activity by agar well diffusion method. The cultures of 48 hours old grown on potato dextrose agar (PDA) were used for inoculation of fungal strain on PDA plates. An aliquot (0.02ml) of inoculum was introduced to molten PDA and poured in to a petridish by pour plate technique. After solidification, the appropriate wells were made on agar plate by using cork borer. In agar well diffusion method 0.05ml of nine different compounds were introduced serially after successful completion of one compound analysis. Incubation period of 24-48 hours at 28^0C was maintained for observation of antifungal activity of compounds. The antifungal activity was evaluated by measuring zones of inhibition of fungal growth. The complete antifungal analysis was carried out under strict aseptic conditions. The zones of inhibition were measured with antibiotic zone scale in mm.

RESULTS AND DISCUSSION

The results of the antifungal and antibacterial assays of the formulated creams are shown in Table 2,3,4 which showed excellent growth inhibition against Gram positive S. aureus, Gram negative *E. coli*, *P. aeruginosa* and a fungal species *C. albicans* respectively. The highest activity (zone of inhibition 23.66mm) was demonstrated by the sample 1 against P. aeruginosa while the lowest activity (zone of inhibition 0mm) was demonstrated by the sample 3 against all four organisms. The results of present investigation clearly indicated that the antibacterial activity vary with sample used.

Table 2: Antibacterial activity of test samples against gram positive bacteria.

S. No.	Name of microorganisms	zone of inhibition (mm) Mean±SD			
		Cefotaxime	F-1	F-2	F-3
1.	*S. aureus*	26.0±2.0	21.0±1.0	20.0±1.5	0

Table 3: Antibacterial activity of test samples against gram negative bacteria.

S. No.	Name of microorganisms	Zone of inhibition (mm)			
		Gentamicin	F-1	F-2	F-3
1.	*E. coli*	20.33±1.5	17.33±1.5	14.66±2.0	0
2.	*P. aeruginosa*	27.0±1.0	23.66±2.5	17.0±1.0	0

Table 4: Antifungal activity of test samples against *candida albicans*.

S. No.	Name of microorganisms	Zone of inhibition (mm)			
		Miconazole	Sample-1	Sample-2	Sample-3
1.	*C. albicans*	16.66±1.5	14.33±1.0	13.33±1.5	0

The polyherbal cream was prepared with an objective to possess enhanced antimicrobial activity as close to the frequently used antibiotics. This formulation, especially, *tridax procumbens* based also includes the extracts of various herbs with natural antimicrobial activity.

The cream was tested against pathogenically important microbes for its antibacterial and antifungal activity. The antibiotics used in this study include cefotaxime as standard against gram positive bacteria and gentamicin against gram negative bacteria.

Whereas miconazole is used as standard against *candida albicans*. The activity observed was concentration dependent as revealed by the zone of inhibition. Formulation-1 showed highest zone of inhibition indicating enhanced anti-microbial activity which may be due to more concentration of the *tridax procumbens* extract. The F-3, which is cream base, did not show any activity against selected microbes.

CONCLUSION

The cream was formulated by using herbal extracts of selected plants available locally which possess comparatively significant antibacterial and antifungal activity against *S. aureus, E. coli, P. aeruginosa* and *C. albicans* which causes skin infections. On the basis of prior research studies, these plants have potentials for antimicrobial activity and hence all these were formulated in the form of cream for topical use, which could be used for herbal management of various skin ailments improved therapeutic activity.

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