International Journal of Advanced Research in Engineering Technology & Science ISSN: 2349-2819
Email: editor@ijarets.org Volume-10 Issue-8 August-2023 www.ijarets.org

Design and Assessment of Polyherbal Gel in contradiction of Staphylococcus Aureus Bacteria CausingSkin Disease

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Abstract

Over past few years, herbal drugs have been gradually shown a revival of interest for the use of medicinal plants to be used against various disorders. Not limited to developing countries but developed areas are also inclined towards the usebecause herbal medicines as they have been reported to be safe with least adverse effects especially in comparison to the synthetic drugs. Aim of this research is to formulate and evaluate polyherbal gel against bacteria. In the present study we have prepared a topical gel formulation which is comprised of combined drug aqueous extract of Murraya Koeniggi, Eucalyptus globulus, Dodonaea viscosa and Mentha spicata in a concentration of 19.2% respectively in a base. The base was prepared by using carbapol 940, propylene glycol-400, glycerine, honey, propylparaben, EDTA, triethanolamine and required amount of water in a quantity sufficient to prepare 100g. The prepared formulation wereevaluated for its antimicrobial activity by agar well diffusion technique against S. aureus, Gram positive bacteria by using agarwell diffusion method. Further, the formulation was also undergone physical evaluation for appearance and homogeneity, pH,viscosity and rheological studies, spreadability, skin irritation test (Patch test) and wash ability.The results of the study revealed that the formulated gel has shown better zone of inhibition against staphylococcus aureus bacteria causing skin disease. Based on our research, it could be concluded that this formulations possess a positive antimicrobial activity and can be used safely on human skin.

Keywords: *Murraya Koeniggi, Eucalyptus globusus, Dodonaea viscose and Mentha spicata, Antibacterial Activity, Polyherbal gel, Staphylococcus aureus,*

1. Introduction:

The purpose of topical distribution is to localise the pharmacological or other effects of the medicine to the skin's surface or deeper layers in order to treat cutaneous disorders like psoriasis or the cutaneous

symptoms of more systemic illnesses like acne. Although foams, sprays, medicated powders, solutions, and even medicated adhesive systems are employed, the method for topical distribution is dominated by semi-solid formulations in all of their varieties. [1]

Topical delivery encompasses two fundamental product categories:

- External topical that are applied to the cutaneous tissues by spreading, spraying, or another method to cover the afflicted area.
- Internal topical used for localised activity that are given to the mucous membrane orally, vaginally, or on anorectal tissues.[2]

Benefits of topical drug delivery

- Preventing first-pass metabolism.
- Convenient and simple to use
- The ability to quickly terminate taking the drugs if necessary.
- The ability to more precisely target a specific spot for medicine delivery.
- Providing short biological half-life drug usage.
- Patient compliance will be improved.[3]
- Drug discovery process is in need because of prevalence of many diseases for which right medicines are notavailable. Role of natural products have always been in trend form ancient times throughout the ages and 80

% humans still rely on the natural products for primary health care and relief from various diseases.[4].

Staphylococcus (S.) aureus silently stays as our natural flora, and yet sometimes threatens our life as atenacious pathogen. In addition to its ability to disturbed our immune system, its multi-drug resistant gene makes it one of the most intractable pathogenic bacteria in the history of antibiotic chemotherapy. Itconquered practically all the antibiotics that have been developed since 1940s. In 1961, the first MRSA wasfound among S. aureus clinical isolates. Then MRSA prevailed throughout the world as a multi-resistant hospital pathogen. In 1997, MRSA strain susceptible to vancomycin was isolated. Vancomycin-intermediate

S. aureus (VISA), so named according to the CLSI criteria, was the product of adaptive mutation of S. aureusagainst vancomycin that had long been the last resort to MRSA infection. Here, we describe the genetic basis for the remarkable ability of S. aureus to acquire multi-antibiotic resistance, and propose a novel paradigm for future chemotherapy againstthe multi-resistantpathogens [5].

2. MATERIALS AND METHOD

2.1 Collection & Authentication of plants: The leaves of Murraya koenigii, Eucalyptus globulus, Dodonaea viscosa & Mentha spicata were collected from district Deharadun Uttrakhand in the month of September 2020. All plants leaf washed thoroughly with distilled water. The cleaned plant parts are then allowed forthe complete shade drying and then made to fine powder with a mechanical grinder and stored in an airtightcontainer.

2.2 Chemicals: Carbopol 974 P(Merck Ltd.), PEG 400,Methanol, Ethanol ,Glycerine, Honey, PPS, EDTA, DMWater, Triethanolamine

2.3 Preparation of extract:

Using a soxhlet device, 20 gm of four plants powder was extracted with high polarity solvents such as water,methanol, and ethanol. The water, methanol, and ethanol extracts were dark in colour, yielding 2.21, 3.61, and

2.98 percent, respectively. The extraction temperature of 40 ^{o} C being determined to be the most effective.[7,8]

2.4 Development of polyherbal gel: As an anti-microbial agent, use the optimal quantity of poly herbal extract as various formulation were prepared. Among all the formulations. The best formulation was selected forfurther studies.[9,10]

Formulation of Gel

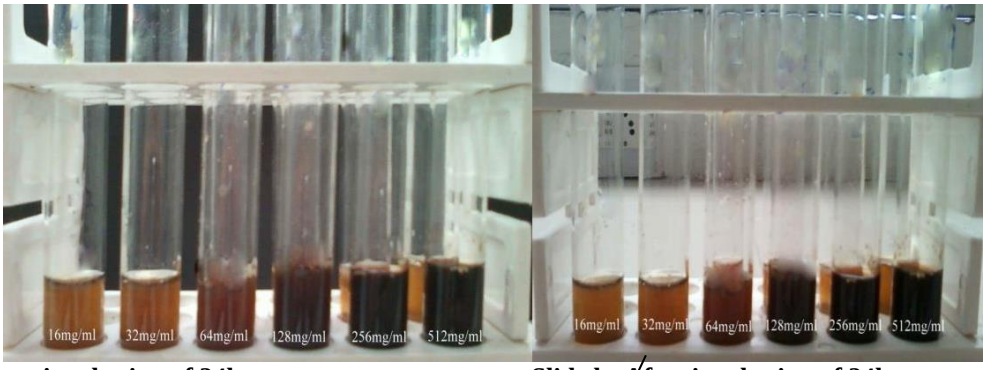
S.No	Content	Concentration (%)
1.	Drug Extract	19.2
2.	Carbopol 940	0.8
3.	Triethanolamine	0.5
4.	Glycerine	1
5.	Honey	2 ml
6.	Propyl Paraben Sodium	0.02
7.	EDTA	0.03
8.	DM Water	Q.S

Table no.1: Formulation of Gel

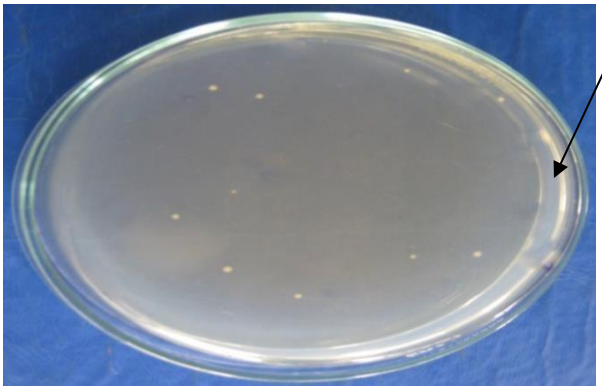
The exact amount of polymer Carbopol 934 (5gm) was weighedand dispersed in 50 ml of distilled water and mixed by continuous stirring in a magnetic stirrer at 800rpm for 1hr. Triethanolamine 0.5% a gelling agent, Glycerine (1%) as a moistening agent and honey (2ml) was added to the mixture with continuous stirring until a transparent gel was formed and set aside for 24 hours. Further, EDTA and PPS were added into the formulation as a preservative to keep the preparation for longer period of time. The next day plant extract incorporated into the gel base and mixed continuously for uniformity. Three gel formulations were prepared by given concentrationsof plant extract.

Determination of minimum inhibitory concentration (MIC) [11,12]:

MIC is defined as the lowest concentration of extracts that completely inhibits the growth of the microorganism in 24 hrs (Thongson C, 2004). MIC of four medicinal plants of aqueous extract were determined. Murraya koenigii(32mg/ml),Eucalyptus globulus(32mg/ml), Dodonaea viscose (64mg/ml) andMentha spicata. (64mg/ml)as shown below.



Slide a. Before incubation of 24hrs **Slide b: After incubation of 24hrs**



Slide c : MIC of aqueous extract of Murraya koenigii(32 mg/ml)

2.6 Polyherbal formulation[13-16]

Aqueous extract of Murraya koenigii(32mg/ml),Eucalyptus globulus(32mg/ml), Dodonaea viscose (64mg/ml) andMentha spicata. (64mg/ml) is highly effective against recovered isolates. So polyherbal formulation is formulated by using aqueous extract of Murraya koenigii(32mg/ml), Eucalyptus globulus(32mg/ml), Dodonaea viscose (64mg/ml) andMentha spicata. (64mg/ml) against multidrug resistant strain (MRSA).

MIC of four medicinal plants		
Plants	**Extract**	**MIC**
Murraya koenigii	Aqueous	32 mg/ml
Eucalyptus globulus	Aqueous	32 mg/ml
Dodonaea viscosa	Aqueous	64 mg/ml

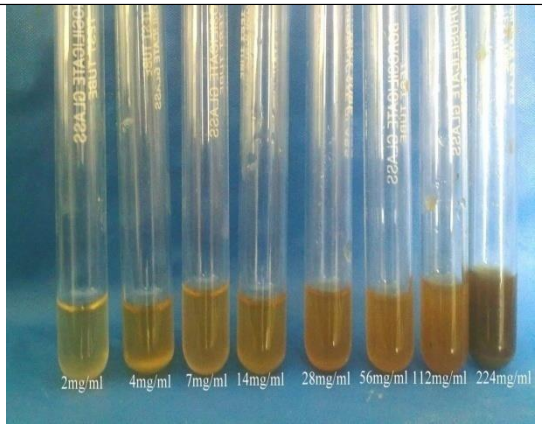
Mentha spicata.	Aqueous	64 mg/ml

Table 2: MIC of four medicinal plants

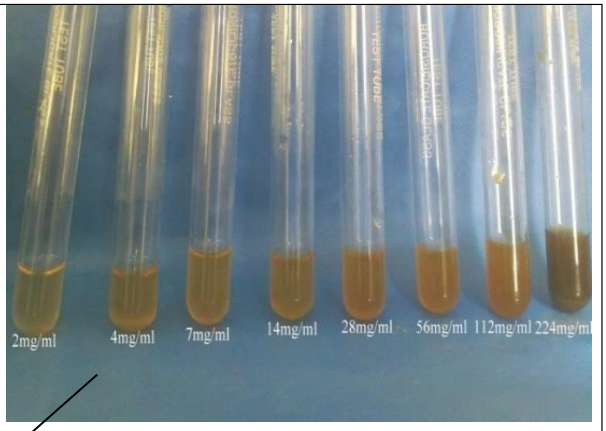
32 mg + 32 mg + 64 mg + 64 mg = 192mg

By combining the above MIC of four medicinal plants we formulated the drug against MRSA.

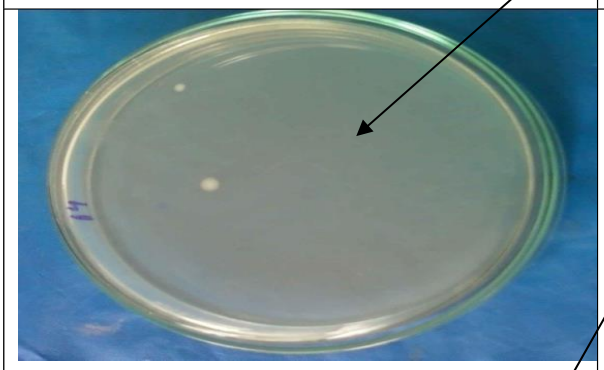
Table 3: By combining the MIC of four medicinal plants



Slide d : Before 24hrs of incubation

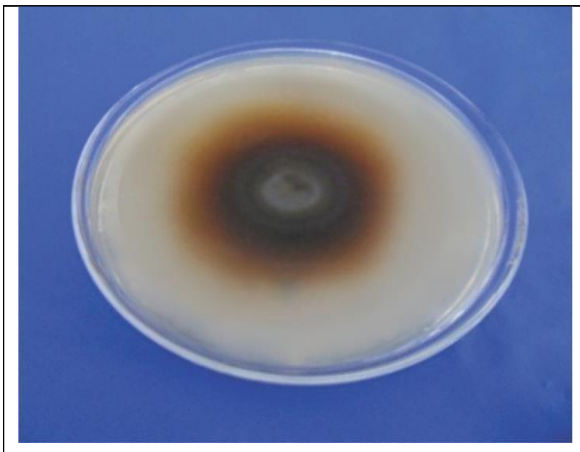


Slide e: After 24hrs of incubation



Slide f: MIC (14mg/ml)

Slide g: 14mg/ml



Slide h: Zone of inhibition of 14mg of herbal extracts (20 mm)

2.5 Evaluation Parameters of Poly Herbal Gel

Evaluation of herbal gels the formulated polyherbal gel was evaluated of itsall-standard parameters

Type of Formulation	Parameters						
	Clarity and appearance	Color homogeneity	presence of fiber and particles	pH	Viscosity(cps)	Spreadability((g. cm/sec))	Hardness (kg/cm^2)
Gel	Good	Uniform	Clear	6.94±0.2	6505 ± 110	7.1±0.1	-------

Table no.4: Evaluation of Polyherbal Gel

The organoleptic properties, including physical appearance, color, texture, phase separation, homogeneity, and immediate skin feel of the ointment, gel and creams formulations, Results showed that all formulations have a good appealing appearance and smooth texture, and they were all homogenous with no signs of phase separation. All formulations were white in color and aromatic odor, pH of all dosage form formulations was found from 6.94±0.2 and 7.0±0.4 that is within the range and compatible with skin pH. The pH of all formulations lies in thenormal pH range of the skin, Hardness test is indicative of strength of ointment formulations and the results are found 121±2.35 kg/cm^2. Which indicating that ointment has adequate strength of hardness which is compatible with skin surface and produce the best result. Rheological property of the semisolid formulations gel can be assessed by spreadability. Spreadability test is a qualitative tool to evaluate physical state as well as the bioavailability of the formulation. The spreadability value was found in the range from6.99±0.12 to7.3±0.14 (gm. cm/sec)

which indicates the better spreadability of the formulations.Viscosity is another most important aspect of any semisolid dosage form which indicate the maximum retention time of formulation over the skin surface. In all these formulations the viscosity was found between 6505 ± 110 to 6862± 98, which almost same and indicatingthe good retention time over the skin surface. so all these formulations has shown the good flowability as per given data and official standards.

In vitro release studies: Comparative In vitro release profile of formulation
Release of drug from dosage form is very important aspects of any dosage form in order to induce its pharmacological and therapeutic effect. The result of release of drug from different dosage form are given in TableNo.

S.No.	Time (in min)	% Drug release in gels
0	0	0
1.	5	15.437±0.945
2.	10	22.553±3.085
3.	15	30.967±0.447
4.	20	42.333± 1.480
5.	30	52.780±0.435
6.	45	64.220±1.446
7.	60	72.173±1.615
8.	90	79.533±0.996
9.	120	85.627±1.873
10.	180	91.333±1.564
11.	240	94.667±1.987
12.	300	98.257±0.765

Table No 5:- cumulative % release of drug from various dosage form

Result and discussion

In vitro studies of polyherbal formulations against MDR Staphylococcus aureus: -

Poly herbal formulation was formulated by using different concentration of medicinal plants as per zone of inhibition observed against MDR Staphylococcus aureus. Antibacterial activity of polyherbal formulation and MIC will be determined against MDR bacterial by using Agar well diffusion and broth dilution method.

Development and Evaluation of Gel formulations: Gel was prepared by using the optimum concentration of selected plant extract for polyherbal formulation.

In vitro antibacterial activity of Gel: - Antibacterial activity of formulation was determined against MDR Staphylococcus.

Evaluation of all different formulation

Physicochemical parameters like color homogencity, presence of fiber and particles, washability, pH and viscosity are evaluated. The visual inspection of the prepared formulation indicated no lumps and to have uniformcolor dispersion, free from any fiber and particle, easy washable, pH was found to be 6.94, it is near to the skin pH which indicates that the prepared formulation can be compatible with skin and viscosity

In-vitro drug release: Release of drug from the dosage form was found to be released to 98 % within 300minutes

Conclusion :

Based on our research, it could be concluded that gel formulation possess a positive antimicrobial activity and can be used safely on human skin. Hence, drugs from natural origin are more acceptable in the belief that they are safer with fewer side effects than the synthetic ones. According to latest trend observed is that herbal formulations are in demand in the worldwide. Herbal gel was formulated using varying concentration of all fourherbal

extracts. It was further evaluated for physicochemical parameters and antimicrobial activity and the polyherbal gel was found to be efficacious . From the above study we found that the combination of all four herbs in the form of gel had a synergistic affect and shown the maximum zone of inhibition against staphylococcus aureus.

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