Formulation and evaluation of herbal antimicrobial Gel of different plant extract

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***Abstract*- The increasing prevalence of microbial infections, especially those associated with impaired wound healing and biomedical implant failure has spurred the development of new materials having antimicrobial activity. Hydrogels are a class of highly hydrated material finding use in diverse medical applications such as drug delivery, tissue engineering, as wound fillers and as implant coatings, to name a few. The biocompatible nature of many gels makes them a convenient starting platform to develop selectively active antimicrobial materials. Hydrogels with antimicrobial properties can be obtained through the encapsulation or covalent immobilization of known antimicrobial agents, or the material itself can be designed to possess inherent antimicrobial activity. Antimicrobial hydro gels are extremely attractive materials for use as wound dressings and fillers. Due to their high water content, gels provide a moist, heavily hydrated environment to the wound area, facilitating cellular immunological activity essential to the wound healing process. However, this same hydrated environment can also facilitate microbial infection. Thus, gels capable of imparting antimicrobial action in addition to serving their primary functional role (e.g. wound healing, drug, delivery, etc…) are desirable.**

***Keywords*- Hydrogels, encapsulation, immunological, immobilization, gelling agent.**

1. Introduction:

Antibacterial activity is the ability of a substance to inhibit or kill bacterial cells. Different types of antibiotics and chemotherapeutic agents are being used in the treatment of one form of disease or the other. Most of these antibiotics were originally derived from micro-organisms while the chemotherapeutic agents are from plants. However, nowadays these antibiotics and chemotherapeutic agents are obtained by various synthetic processes. Nepal is richly blessed with forests containing arrays of different herbs, shrubs and trees. The leaves, stems, bark and roots of these plants are being used by the local populace and people with thin income for incurring different types of ailments because of the inadequate medical facilities across the country.

Herbal medicine, also called botanical medicine or phytomedicine, refers to the use of any plant's seeds, berries, roots, leaves, bark, or flowers for medicinal purposes. Long practiced outside of conventional medicine, herbalism is becoming more mainstream as up-to-date analysis and research show their value in the treatment and prevention of disease. Plants had been used for medicinal purposes long before recorded history. Traditional medicine is an important source of potentially useful new compounds for the development of chemotherapeutic agents. The first step towards this goal is the screening of plants used in popular medicine. Thus antimicrobial research is geared towards the discovery and development of novel antibacterial and antifungal agents. Plant drugs are frequently considered to be less toxic and freer from side effects than the synthetic ones. In an earlier study, medicinal plants have been reported to be very beneficial in wound care, promoting the rate of wound healing with minimal pain, discomfort, and scarring to the patient.

1.2 Topical Drug Delivery System: The goal of any drug delivery system is to provide a therapeutic amount of drug to the proper site in the body to promptly achieve and then maintain the desired drug concentrations. The route of administration has a significant impact on the therapeutic outcome of a drug. Skin is one of the most readily accessible organs on human body for topical administration and is main route of topical drug delivery system. Topical delivery can be defined as the application of a drug containing formulation to the skin to directly treat cutaneous disorders (e.g. acne) or the cutaneous manifestations of a general disease (e.g. psoriasis) with the intent of containing the pharmacological or other effect of the drug to the surface of the skin or within the skin. Semi-solid formulation in all their diversity dominate the system for topical delivery, but foams, spray, medicated powders, solutions, as well as medicated adhesive systems are also in use.

- External topical that is spread, sprayed, or otherwise dispersed on to cutaneous tissues to cover the affected area.
- Internal topical that are applied to the mucous membrane orally, vaginally or on anorectal tissues for local activity.

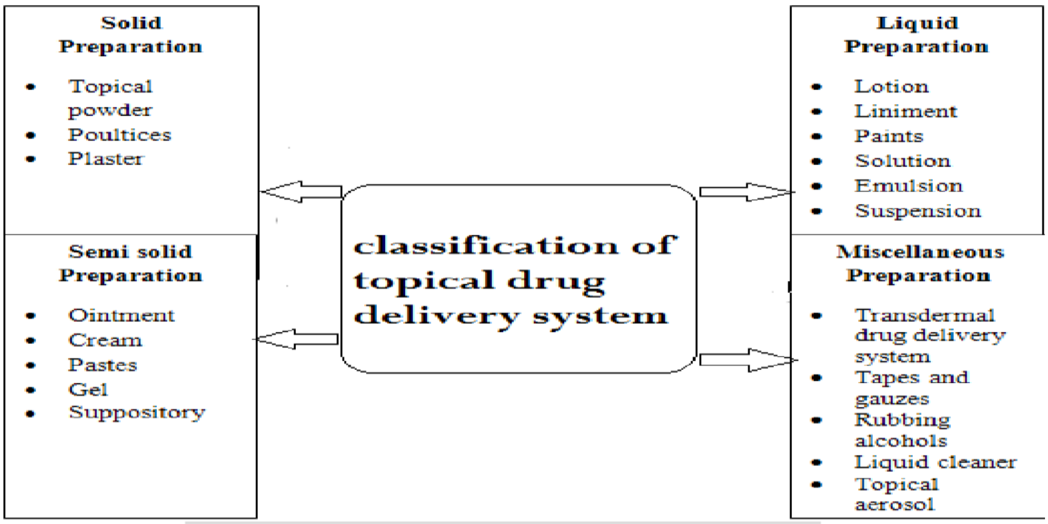
1.3 Advantages of Topical Drug Delivery System :

- ❖ Avoidance of first pass metabolism.
- ❖ Convenient and easy to apply.
- ❖ Avoidance of the risks and inconveniences of intravenous therapy and of the varied conditions of absorption, like pH changes, presence of enzymes,
- ❖ Achievement of efficacy with lower total daily dosage of drug by continuous drug input. •Avoids fluctuation in drug levels, inter- and intrapatient variations.
- ❖ Ability to easily terminate the medications, when needed.
- ❖ A relatively large area of application in comparison with buccal or nasal cavity
- ❖ Ability to deliver drug more selectively to a specific site.
- ❖ Providing utilization of drugs with short biological half-life
- ❖ Improving physiological and pharmacological response.
- ❖ Improve patient compliance.
- ❖ Provide suitability for self-medication.

1.4. Disadvantages of Topical Drug Delivery System:

- ❖ Skin irritation of contact dermatitis may occur due to the drug and/or excipients.
- ❖ Poor permeability of some drugs through the skin.
- ❖ Possibility of allergenic reactions.
- ❖ Can be used only for drugs which require very small plasma concentration for action Enzyme in epidermis may denature the drugs
- ❖ Drugs of larger particle size not easy to absorb through the skin

Classification of Topical Drug Delivery System



1.5 Gel: A gel is a solid or semisolid system of at least two constituents, consisting of a condensed mass enclosing and interpenetrated by a liquid. Gels and jellies are composed of small number of solids dispersed in relatively large amount of liquid, yet they possess more solid-like than liquid-like character. The characteristic of gel and jelly is the presence of some form of cutaneous structure, which provides solid-like properties**.**

1.6 Human skin

The skin is the outer covering of the body. It is the largest organ of the integumentary system. The skin has multiple layers of ectodermal tissue and guards the underlying muscles, bones, ligaments and internal organs. Human skin is similar to that of most other mammals, except that it is not protected by a pelt. Though nearly all human skin is covered with hair follicles, it appears hairless. There are two general types of skin, hairy and globous skin.

Fig no.1 structure of human skin

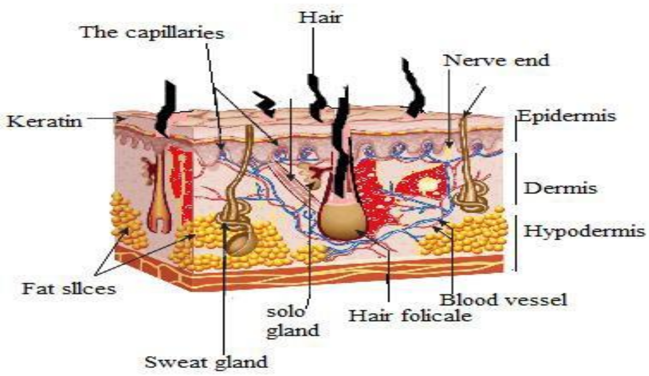


Fig no.1 structure of human skin

The adjective cutaneous literally means "of the skin". Skin plays a key role in protecting (the body) against pathogens and excessive water loss. Its other functions are insulation, temperature regulation, sensation, synthesis of vitamin D, and the protection of vitamin B folates. Severely damaged skin will try to heal by forming scar tissue.

1.6.2The layers are:
a) Stratum Corneum (Horny layer)
b) Stratum Lucidum (Clear layer)
c) Stratum Granulosum (Granula layer)
d) Stratum Spinosum)
e) Stratum Basal (Basal layer)

1.6.3: Dermis: - Dermis (vascular) is a thick layer consists chiefly of connective tissues. It is involved in the regulation of body temperature and provides mechanical resistance. Its rich vascular network, dermis supplies nutrients to the epidermis layer. This layer also consists of senseorgans for pain, pressure, touch, sebaceous and sweat glands, temperature, nerve fibers, blood vessels, and hair follicles.

1.6.4 Hypodermis: - Subcutaneous tissue below the dermis, subcutaneous layer acts as a protective cushion and insulates the body. It consists of adipose and loose connective tissues as well as blood vessels and nerves.

The skin has mainly three functions namely protection, regulation and sensation

PROTECTION: The skin acts as a barrier and provides protection against mechanical shocks, pressure, variations in temperature, micro-organisms attack, radiation and chemicals exposure. The protection from environmental hazard is the primary function of the skin.

REGULATION: The skin plays important roles in regulates several aspects of physiology including maintaining of body temperature via sweat and hair. The synthesis of Vitamin D takes place in skin. It is also acts as a reservoir for the synthesis of Vitamin D.

SENSATION: The skin contains extensive framework of nerve cells that detect and relay changes in the environmental factors. There is different separate receptors for heats, cold, touch etc.

1.7 Pathways of drug penetration:

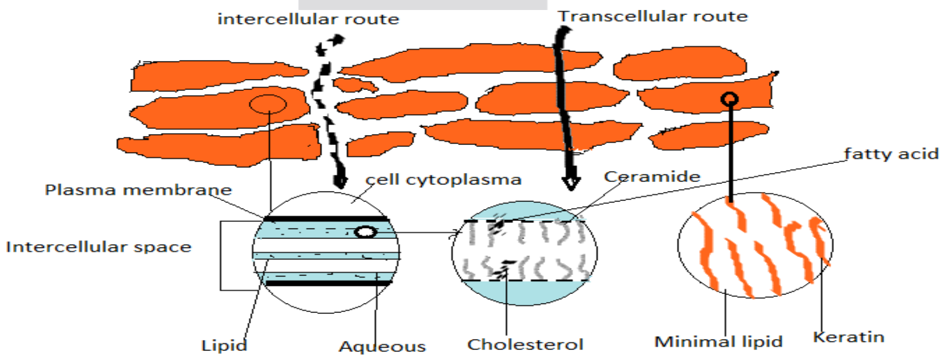


Fig no: 2 mechanism of drug penetration pathway

2. PLANT PROFILE:

S. no	Biological name	Chemical constituent	Parts of use	Pharmacological use

1	Piper nigrum (black pepper)	Piperine, beta-carotene, lauric-acid, palmitic acid, and pepper phellandrene.	seeds, leaves	analgesic, antipyretic antioxidant,antibacterial, antifungal
2	*Aegle marmelos*	Marmesin, psoralin umbelliferone, marmeline and dictamine.	leaves, seed, bark	Diarrhoea, dysentery, diabetes, antibacterial, antiprotozoal and antifungal properties

3.1 Material and method:

S. No	Material
1.	Black pepper extract
2.	Bel leaves extract
3.	Carbopol 940
4.	Propylene glycol
5.	Methyl paraben
6.	Propyl Paraben
7.	Triethanolamine
8.	Water

3.2 Formulation of Gel base: Gelling agent was dispersed in sufficient quantity of water. Propylene glycol400 which is used as humectants or plasticizer was added to the dispersion. Other excipients such as methylparaben and propyl paraben were added with continuous stirring. In Carbopol gels, pH of the vehicle was brought to neutral by using TEA (Triet hanolamine).The final weight of the gel was adjusted to 50 gm with distilled water. Then the mixture was stirred by using propeller for 2 hours at 500 rpm. After stirring, this homogenous gel appeared to be free of bubbles. It was kept at room temperature for 24 hours to check the consistency and stability of gel.

Formulation of Herbal gel containing black pepper and Bael leaves: Herbal gel was prepared by incorporating ethanolic extract of black pepper and Bael leaves extract liquid in to optimized Carbopol gel. Bel leaves extract liquid is included along with water required for dispersing Carbopol. On the other hand, accurate amount of ethanolic extracts of black pepper (1%, 1.5% and 2%), propylene glycol400, methylparaben and propylparaben were added to the Carbopol dispersion. Triethanolamine was added drop wise to the formulation for adjustment of required skin pH (6.8-7) and to obtain the gel at required consistency. It was then stirred by using propeller for 2 hours at 500rpm. After stirring, the prepared gel appeared to be homogenous and devoid of air bubbles. The prepared gel was kept at room temperature for 24 hours.

4. **EVALUATION OF GEL:**

Physical properties: In this parameter evaluate to morphology of gel such as colour, odour, taste and physical appearance through direct visualisation.
Rheology test: In this parameter determine the rheology properties of gel such as viscosity and consistency of gel. **Viscosity** determinations of the prepared *in situ* gels as well as sols were carried out on a cone and plate geometry viscometer (Brookfield, Massachusetts, USA), using spindle No 40. Viscosity of *in situ* gelling solutions was measured at different angular velocities at a temperature of 37°. A typical run comprised changing of the angular velocity from 0.0 to 100 rpm. The averages of two readings were used to calculate the viscosity.
Determination of gel strength: The experiment was done by placing the gels in standard beaker below the probe. In this an analytical probe is then immersed into the sample. The Texture Analyzer was set to the 'gelling strength test' mode or compression mode with a test-speed of 1.0 mm/s. An acquisition rate of 50 points per seconds and a trigger force of 5 g were selected. An aluminium probe of 7.6 cm diameter was used for all the samples. The study was carried out at room temperature. The force required to penetrate the gel was measured as gel strength in terms of g.
Determination of pH: pH values of 1% aqueous solutions of the prepared gels were measured by a pH meter
Spreadability Test: For the determination of spreadability, excess of sample was applied in between two glass slides and was compressed to uniform thickness by placing 1kg weight for 5 min. weight (50 g) was added to the pan. The time in which the upper glass slide moves over to the lower plate was taken as measure of spread ability.

S = M.L / T
M- Weight tied to the upper slide
L - Length moved on the glass.
T - Time Taken

Compatibility test and Content uniformity: Drug content of gel was determined by dissolving accurately weighed 1gm of gels in Methanol. After suitable dilution absorbance was recorded by using UV- visible spectrophotometer at 222 nm. Drug content was determined using slope of standard curve.
Skin Irritation test: skin irritation test can be determined by ether small portion of gel applied direct to skin or invitro test on mice model method.
Test for stability: The stability study for the topical herbal gel formulation was done as per ICH guidelines in a stability chamber for a period of 6 months. The selected topical herbal gel formulation was loaded in a humidity chamber (Floor standing model, 3 units in one with individual humidity and temperature controller at 25°C ± 2°C/60% RH ± 5% RH, 32°C ± 2°C/60% RH ± 5% RH and 40°C ± 2°C/75% RH ± 5% RH. Samples were withdrawn at an initial, first, second, third and sixth months and evaluated for change in color, odor, homogeneity, pH, viscosity.
Antibacterial activity test: variety of laboratory methods can be used to evaluate or screen the *in vitro* antimicrobial activity of an extract or a pure compound. The most known and basic methods are the disk-diffusion and broth or agar dilution methods.

a) **Agar diffusion well-variant**
b) **Agar diffusion disc-variant.**
c) **Bioautographic method direct-variant (chromatogram layer).**
d) **Bioautographic method indirect-variant (agar diffusion Minimum inhibitory**
e) **Concentration (MIC) determination**. **Statistical evaluation**:

5. RESULT AND DISCUSSION:

5.1 Preformulation study:

Organoleptic Characterization for black pepper seed and bell leaves powder:

Table No.5.1: Organoleptic characteristics of extract

Leaves powder	Characteristics	seed powder	Characteristics
Colour	Light green	Color	Brownish
Odour	Carminative	Odor	Pungent
Taste	Slightly pungent	Taste	Slightly bitter to pungent

5.2 table: Extraction of Beal leaf and pepper seed:

Sample	Extraction method	Solvent use
Beal leaves	Solvent extraction	Ethanol
Black pepper	Solvent extraction	Ethanol

5.3 Phytochemical investigation of the extract

Phytochemical investigation of extract of *black pepper and Beal leaves* revealed the presence of flavonoids, phenolics and other constituents. Many of these compounds have been shown to produce potent antimicrobial, antidiabetic and antihyperlipidemic activities. The results were mentioned in Table no: 5.3

Constituents	**Test**	**End point**	**Results**
Flavonoids	Ferric chloride	Green color	++
	Lead acetate	Yellow precipitate	++
Protein	Xanthoprotein	Yellow precipitate	++
	Ninhydrin	Blue color	++
Amino acids	Ninhydrin	Purple or bluish color	++
	Tyrosine	Dark red color	++
Phenol	Ferric chloride	Blue or red color	++

Organic acid	Phosphoric acid	Light yellow precipitate	++

EVALUATION OF POLYHERBAL GEL

5.4.1 Physical appearance

The formulated gel was checked visually for color, appearance and homogeneity and the results were listed.

Table No. 5.4.1: Physical appearance of formulated gel.

S.no	F1 [1% ethanolic extract of *blackpepper*]	F2 [1.5% ethanolic extract of *blackpepper*]	F3 [2% ethanolic extract of *black pepper*]
Physical appearance	Transparent yellowgel	Transparent yellowgel	Transparent yellowgel
Color	Slightly black	Slightly black	Slightly black
Homogeneity	Absence of aggregates	Absence of Aggregates	Slight aggregates

5.4.2 Measurement of pH

Table no: 5.4.2

Formulation Code	**pH**
F1	5.9
F2	5.7
F3	5.8

5.4.3 Determination of Viscosity

Table No. 5.4.3: Measurement of viscosity

Formulation Code	**Viscosity [cps]**
F1	1428 ± 0.1
F2	1425 ± 0.75
F3	1358 ± 0.25

5.5 Spreadability

Table No. 5.5: Measurement of spreadability

Formulation Code	**Spreadibility** (gm.cm/sec)
F1	19.37
F2	21.35
F3	22.13

5.6 Gel strength

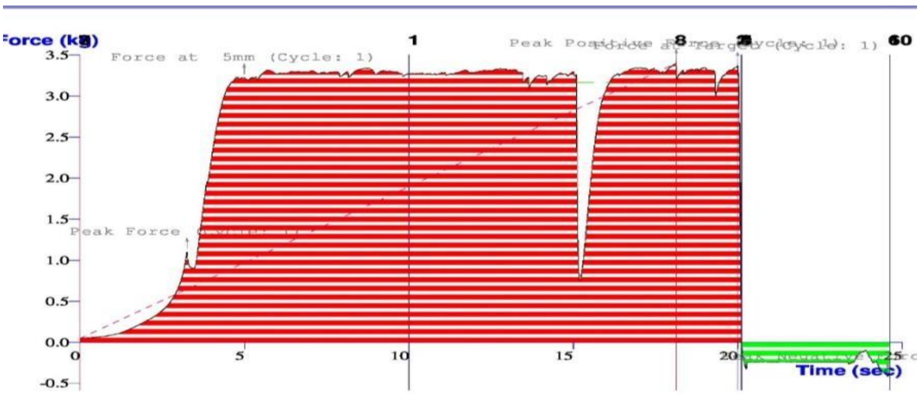
Table No. 5.6: Test data of gel strength

Gel strength / omh value (g)Force 1	Force target(cycle:1) (kg)	Gradient to positive peak (cycle:1) kg/sec
0.693	0.016	0.004

5.7 Extrudability

Table no: 5.7

Firmness (g)	Force at target (cycle :1) (kg)	Force at 5mm (cycle :1) (kg)
3168.854	3.369	3.228



5.8Antimicrobial activity testing by cup plate method.

The antimicrobial activity testing was performed by measuring and comparing the diameter of zones of inhibition (in mm). The zone of inhibition can be defined as the clear region around the well that contains an antimicrobial agent. It is known that the larger the zone of inhibition, the more potent the antimicrobial agent. The formulated polyherbal gel (F2) was observed for its antimicrobial property towards the organism such as Staphylococcus aureus (ATCC-6538P), Escherichia coli (ATCC-8739), it was also compared with standard such as Gentamicin(10-mcg) and Fluconazole(25-mcg). From the result it was observed that it showed good zone of inhibition but lesser when compared to standard. Zone of inhibition of the formulated gel were mentioned in Table No.5.8 and Figure.

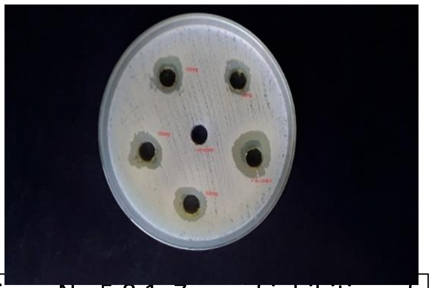
Table No. 5.8: Zone of inhibition of the formulated Polyherbal gel

	Organism						
	Staphylococcus aureu **(ATTC-6538P)**			**Mean (in mm)**	***Escherchia coli*** **(ATCC-8739)**		
	1	**2**	**3**		**1**	**2**	**3**
Polyherbal gel (F2)	**12.2**	**12.4**	**12.2**	**12.3 ± 0.1**	**13.2**	**13.3**	**13.2**
Gentamicin (10-mcg)	**21.1**	**23.2**	**21.1**	**22.2 ± 0.06**	**22.2**	**22.5**	**22.3**
Fluconazole (25-mcg)	-	-			-	-	

6. CONCLUSION

All these investigations have brought out ultimate factors which lead to the following conclusions:

- This study targets the chronic skin condition acne with the aim of formulating an effective and safe Polyherbal gel by using black pepper and Beal leave.
- The ethanolic extract of black pepper and collected Beal leaf extract gel were incorporated in to optimized Carbopol gel base.
- The combination of these two herbal constituents may produce an effect to minimise the bacterial infection problem.
- Antimicrobial study shows that there was no microbial contamination observed and it showed good zone of inhibition and *in vivo* skin irritation study results showed that there was no skin lesions like defeating of skin, adverse skin reactions, and local systemic change.
- Overall, this study reports concluded that the formulation of polyherbal gel may offer an effective and safe dosage form which leads to patient adherence and compliance to the therapy.



FigureNo.5.8.1: Zone of inhibition of *black pepper and Beal leaves* extract towards *Staphylococcus aureus*

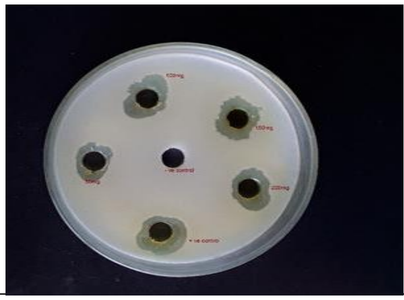


Figure No. 5.8.2: Zone of inhibitionof *black pepper and Beal leaves* extract towards *Escherichia coli*

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