FORMULATION AND EVALUATION OF HERBAL ANTI-MICROBIAL TOPICAL PREPARATION

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Abstract

Background: Herbal medicine is the sign of modern medicine and drug development. In this study we use two herbal drug as an active pharmaceutical ingredient first is an Azardichata indica second one is Occimum sanctum i.e. neem and tulsi respectively. Using this two active ingredient, we formulated anti-microbial herbal topical preparation. **Materials and Methods:** In this formulation, neem oil and tulsi soft extract used as anti-microbial agent and honey, camphor, lemon juice, bees wax used as pharmaceutical excipient which act as anti-oxidant, penetrating agent, pH modifier, ointment base respectively. Topical preparation was formulated using hot diffusion method. The anti-microbial topical preparation was evaluated for various evaluation parameters which include colour, odor, pH, viscosity, spredablility, homogeneity, texture, after feel, removal study, type of smear, stability studies and in-vitro anti-microbial stability. **Results:** Significant antimicrobial effect was observed with

formulated topical preparation. **Conclusion:** Formulated herbal anti-microbial topical formulation was associated with significant reduction in microbial growth, which could account for the efficacy of the folk use of the plant in the treatment of microbial infection.

Key Word: *Anti-microbial*, Tulsi, *Neem oil, Herbal Preparation.*

Introduction

Herbal medicine formulated from plants and their extract refers to a dosage form consisting to provide definite nutritional cosmetics and other health care needs. Herbal medicine is sign of modern medicine and drug development. In this modern era why we study the phytochemical properties of the plant again, why many research scholar give first preference to the herbal medicine because herbal drug may enable targeted delivery of high concentration of antimicrobial infection.[1] According to the World Health Organization, 80% of the population in developed countries relies on plant-based traditional medicines to maintain their primary health care needs. High treatment cost and side effects along with drug resistance are major problems associated with synthetic drugs.[2] Herbal drug has less potential for systemic side effects & toxicity.[3]

T – Bact 2% ointment, soframycin 1% cream, fucidin cream, Neosporin Skin ointment, Nadoxin Cream, Candid Lotion etc. many topical allopathic cream are in market but they show some side effect like itching, swelling, redness, to avoid such side effect we can employ herbal drug in novel approach to treat the microbial infection.[4]

Raw materials used in formulation of herbal medicine are easily available in huge quantity. In India, great biodiversity is available so we have number of herbal plants which act as medicine.[5]

In this study, we used two herbal drug as an active pharmaceutical ingredient first is an Azardichata indica & second one is Occimum sanctum i.e. neem and tulsi respectively. In Indian mythology both plant have greatest weightage. The Vedas called neem "SARVA ROGA NIVARINI" which means one that cures all ailments and ills. According to Indian mythology, Amrita (ambrosia or the elixir of immortality) was being carried to heaven and a few drops of it fell on the neem, such believes we hear from ancient Indians and think that is it true…?, if yes then why its happen, what the mechanism behind that this several questions was arising in my mind from that movement I was started study on this two plants to solve a

questions whatever in mind. After viewing many literatures, we feel that it's true so I was done the phytochemical study of the neem and tulsi. This both plants are generally planted in front of every house and they have many more medicinal use like anti-inflammatory, anti-fungal, anti-bacterial, anti-septic, anti-depressant, anti-pyretic, analgesics and anti-oxidant.[6-9] But if we use leaves, root, flowers, steam of the plant as it is for medicinal purpose without processing then there is no proper dosage regimen, dosage uniformity is not occur so we should formulate proper dosage form of that herbal drug is essential which give the uniformity in dosage form, and which shows the safety, efficacy, and potency of the drug.

The objective of the study was to formulate and evaluate the antimicrobial herbal ointment from the local medicinal plants.

Materials and Methods:

Collection of plant material:

Tulsi Soft Extract was obtained from local market. Neem oil was obtained from Himalaya herbals Pvt Ltd., Honey was obtained from Dabur India limited, Lemmon juice obtained from fresh fruits of citrus limon. Camphor and Bees Wax were obtained from SD Fine Chem Pvt. Ltd.

Test microorganisms:

The microorganisms used for the study were *Staphylococcus aureus, Streptococcus Spp and Pseudomonas Aeruginosa*. The bacterial strains were grown and maintained on agar at 37 °C.

Methodology

The herbal anti-microbial ointment preparation was prepared using hot diffusion method using the formula on Table 1.

Table No. 1: Formula for Herbal Anti-microbial Ointment Preparation

Sr. No	Name of Ingredients	Quantity	Role
1	Tulsi Soft Extract	3 gm	Anti-microbial Agent
2	Neem oil	2.8 ml	Anti-microbial Agent
3	Camphor	0.5 gm	Penetrating Agent

4	Bees wax	1.5 gm	Ointment base
5	Honey	1.5 ml	Antioxident
6	Lemon Juice	0.75 ml	pH modifier

These herbal materials were selected because the individual plant materials have shown antibacterial effect against the bacteria which were able to cause wound infection.[10]

Procedure:

- ✓ Herbal antimicrobial ointment was prepared using white bees wax as ointment base.
- ✓ The pH of Neem Oil, Tulsi Soft Extract, Honey and Comphor was adjusted to weakly acidic by using lemon juice.
- ✓ Take the given quantity of bees wax on hot water bath and also warm the mixture of Neem oil, Tulsi soft Extract, Honey and Powdered Comphor.
- ✓ Add the mixture of Neem oil, Tulsi soft Extract, Honey and Powdered Comphor in molten bees wax at the temperature rang 70-72^{0}C.
- ✓ Mix the all ingredient uniformly in uniform direction.
- ✓ Dispense the formulation in wide mouth container with proper label
- ✓ Formulation should be stored in cool and dry place.

Physicochemical evaluation

The physicochemical parameters such as colour, odor, pH, viscosity, spredablility, homogeneity, texture, after feel, removal study, type of smear and stability studies were pre-meditated to ensure satisfactory results for the formulated herbal cream (Table 1). The physicochemical properties were evaluated using existing techniques as previously described [11-14]. The stability of formulated formulation was assessed at different temperature conditions of 25°C and 40°C within 3 months.

Color: Color variation is one of the organoleptic characteristics of actives medication which can indicate the presence of contaminations, impurities or degradation products

Odor: There were in six main odor qualities fruty, flowery, resinous, spicy, characteristic, and burned.

pH: The pH of preparation was determined by using Digital pH meter. 0.5g of the weighed formulation was dispersed in 50 ml of distilled water and its pH was measured.

Viscosity: Viscosity of formulation was measured by using Brookfield Viscometer Model DV2T with spindle #64 at 50 RPM for 120 Sec.

Homogeneity: The formulations were tested for the homogeneity by touch and visual appearance.

Spreadability: Spreadability was calculated using the spreadability apparatus made of wooden board with scale and two glass slides having two pans on both sides mounted on a pulley. Excess sample (2 g) was placed between the two glass slides and 100 g weight was placed on the glass slide for 5 min to compress the sample to a uniform thickness. Weight (250 g) was added to the pan. The time in seconds required to separate the two slides was taken as a measure of spreadability.[15]

Spreadability was measured manually by using following formula.

$$S = M*L/T$$

(S: Spreadability, M: Weight in the pan tied to the upper slide, L: Length moved by the glass slide, T: Time taken to separate upper slide from the ground slide.)

After feel: Emolliency, slipperiness and amount of residue left after the application of fixed amount of preparation was checked.

Type of smear: After application of preparation, the type of film or smear formed on the skin were checked.

Removal: The ease of removal of the preparation applied was examined by washing the applied part with tap water.

Stability study:

The stability study was performed as per ICH guidelines the formulated preparation was filled in open mouth container and stored at different temperatures and humidity conditions, i.e. 25 ± 2^0C/60±5% RH, 40 ± 2^0C/75±5% RH for a period of three months and studied for appearance, pH and spreadability.

In-Vitro Antimicrobial Activity Study:

In 1940, the Agar disk-diffusion testing method was developed to assess routine antimicrobial activity of various clinical microbiology laboratories[16]. (N.G. Heatley) Nowadays, many accepted and approved standards are published by the Clinical and Laboratory Standards Institute (CLSI) for bacteria and yeasts testing.[17,18] Although not all fastidious bacteria can be tested accurately by this method, the standardization has been made to test certain fastidious bacterial pathogens like streptococci, Haemophilus influenzae, Haemophilus parainfluenzae, Neisseria gonorrhoeae and Neisseria meningitidis, using specific culture media, various incubation conditions and interpretive criteria for inhibition zones.[17]

In this well-known procedure, agar plates are inoculated with a standardized inoculum of the test microorganism. Then, filter paper discs (about 6 mm in diameter), containing the test compound at a desired concentration, are placed on the agar surface. The plate was left at +4 C for 2 h to facilitate the diffusion of the extracts in the agar, and then incubated at 37 C for 24 h for bacterial strains and at 30 C for 4–7 h for fungal strains. The Petri dishes are incubated under suitable conditions. Generally, antimicrobial agent diffuses into the agar and inhibits germination and growth of the test microorganism. Antimicrobial activity was determined by measuring the diameter of inhibition zone around the well.[19,20]

In-Vitro Antimicrobial activity of tested by using Disc Diffusion Assay (Quantity per disc–10µl).

1. Inoculum used: 1×106 CFU/ml.
2. Incubation Temperature: 37^0C
3. Growth Media: Sterile Nutrient Agar at pH 6.7
4. Test bacterial culture: *Staphylococcus aureus, Streptococcus spp, Pseudomonas Aeruginosa*.
5. Incubation Time: 24 hrs.

Results and Discussion:

In this study, the physicochemical property of the herbal ointment was evaluated (Table 2). The prepared formulations show a smooth and homogeneous appearance.

Table No. 2: Physicochemical assessment of the herbal ointment preparation

Assessed Parameter	Recorded Physical Observation
Color	Brown
Odor	Characteristic
pH	5.25
Spreadability	Good
Homogeneity	Homogenous
Texture	Smooth
After Feel	Emollient
Removal	Easy
Type of Smear	Non greasy
Stability at 25^{o}C and 40°C	Stable

Viscosity:

The viscosity of herbal ointment preparation shows good spreadable property which indicates that the preparation is easily spreadable by small amounts of shear. The results are given in Table no 3.

Table No. 3: Viscosity of the herbal ointment preparation at different RPM

RPM	Viscosity (cps)
50	22910
100	15260

Spreadability:

Spreadability denotes the extent of area to which the formulation readily spreads on application to skin or hair. The bioavailability efficiency of a formulation also depends on its spreading value.

Table No. 4: Spreadability of the herbal ointment preparation

Weight tied to the upper slide (M)	Length of glass slide moved (L)	Time taken (T)	Spreadability M x L/T
250 gm	0.36	10 Sec	9.00
250 gm	0.37	10 Sec	9.25
250 gm	0.34	10 Sec	8.50

Stability Studies:

There was no change in organoleptic properties such as color, odor and texture when the samples were re-assessed after 3 months of storage condition. Also, there was no change in the pH and spreadability of the preparation and this showed that the stability of the formulated preparation was maintained.

Table No. 5: Stability studies of the herbal ointment preparation

Storage Condition	Appearance	pH	Spreadability
After 3 months at 25±2℃ /60±5%RH	NCC	5.46	8.57
After 3 months at 40±2℃ /75±5%RH	NCC	5.59	8.81

NCC: Not change in colour

Anti-microbial Activity Testing:

The antimicrobial activity was determined by measuring the diameter of zone of inhibition recorded. The results obtained in the evaluation of the antimicrobial activity of the formulated herbal ointment against selected pathogens are shown in Table No. 6 and Figures 1;

Table No. 6: Zones of inhibition of the formulated ointment on test organisms.

Sample ID	Inhibition Zone Diameter (mm) against pathogen		
	Streptococcus Aureus	Streptococcus Spp	Pseudomonas Aeruginosa
Formulation	14	9	8
Control	7.5	7	7.5

Petri-Plate showing zone of inhibition

Staphylococcus Aureus *Streptococcus Spp* *Pseudomonas Aeruginosa*

Figure no. 1: Anti-microbial activity of herbal ointment preparation

Discussion:

Many synthetic anti-microbial medicinal formulations are in market but many microbial agents become resistance to anti-microbial drug, therefore invention of new formulation to give anti-microbial activity are requires. Drug resistance is the reduction in effectiveness of a drug in curing a disease. When the drug is not intended to kill or inhibit a pathogen, then the term is equivalent to dosage failure or drug tolerance. Antibiotic resistance is the ability of bacteria to resist the effect of an antibiotic, i.e. the bacteria is not killed or their growth is not stopped. Resistance bacteria survive exposure to the antibiotics and continue to multiply in the body, potentially causing more harm and spreading to other animals or people.

Development of resistance to newly introduced antimicrobials Agent Year of FDA approval First reported resistance Penicillin 1943 1940 Streptomycin 1947 1947 Tetracycline 1952 1956 Methicillin 1960 1961 Nalidixic acid 1964 1966 Gentamycin 1967 1969 Vancomycin 1972 1987 Cefotaxime 1981 1981(AmpC) 1983(ESBL) Linezolid 2000 1999.

Microbes that carry resistance genes survive to replicate themselves. The progeny of these resistant microbes will eventually become the dominant type. Mutation: when microbes replicate themselves, genetic mutations can occur. Sometimes, these mutations can lead to the creation of a microbe with genes that aid it in surviving exposure to antimicrobial agents. Gene transfer: microbes can also acquire genes from other microbes. Genes that have drug-resistant qualities can be transferred between microbes easily.

Therefore we again return to wards the herbal medicine which give in new combination i.e. azardichata indica oil, tulsi soft extract, honey and Comphor to achieve anti-microbial activity. Phytoconstituents present in azardichata indica oil are azardichtin, nimbin, and nimbidine which give antimicrobial activity. Tulsi soft extract contains eugenol, rosmarinic acid aspigenin, myretenal, lueteolin, β-sitosterol and carnosic acid which gives antimicrobial activity. Comphor enhance the penetrating ability of formulation which further helps to increase bioavailability of drug. In this formulation we use undiluted honey which act as anti-oxidizing agent as well it act as antimicrobial agent. Antimicrobial activity of honey are due to the two mechanisms are as follow, because the enzymatic production of hydrogen peroxide. However, another kind of honey, called non-peroxide honey (*viz.*, manuka honey), displays significant antibacterial effects even when the hydrogen peroxide activity is blocked. Its mechanism may be related to the low pH level of honey and its high sugar content (high osmolarity) that is enough to hinder the growth of microbes. Bees wax is use as ointment base. Evaluation of prepared formulation also done.

Conclusion:

Based on the results of this study, it can be concluded that the formulated herbal anti-microbial topical formulation was associated with significant reduction in microbial growth, which could account for the efficacy of the folk use of the plant in the treatment of microbial infection. According to this study, formulated herbal topical preparation may be employed as anti-microbial agents for a variety of infectious diseases for many infectious disease caused by the tested microorganism. The final preparation was stable at various temperatures and easily spread on skin.

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Conflict of interests:

The authors have not declared any conflict of interests.

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