

FORMULATION AND EVALUATION OF ANTIMICROBIAL HERBAL OINTMENT

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ABSTRACT

Most of the antibiotics were originally derived from micro-organisms while the chemotherapeutic agents are from plants. Herbal medicine refers to the use of any plant's seeds, berries, roots, leaves, bark, or flowers for medicinal purposes. Along with other dosage forms, herbal drugs are also formulated in the form of ointment. An ointment is a viscous semisolid preparation used topically on a variety of body surfaces. The objective of the study was to formulate and evaluate the antimicrobial herbal ointment from the local medicinal plants. The ethanolic extracts of the selected plants were taken in different ratio randomly and the antimicrobial tests of the combinations were carried out. The most effective combination was then determined by comparing the results of the zone of inhibition given by the 10 different extract ratios on *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella species*. Then the minimum inhibitory concentration of the effective combination was found out. The ointment base was prepared and formulation of ointment was done by incorporating the active ingredients in most effective ratio in the base by trituration. After the completion of the formulation, quality of the ointment was assessed in terms of irritancy, spreadability, diffusion and stability.

Key words: Herbal ointment, minimum inhibitory concentration, irritancy, spreadability, diffusion, stability

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 (Reiner, 1984). 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 (Sule and Agbabiaka, 2008).

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. Indigenous cultures (African and Native American) used herbs in their healing rituals, while others developed traditional medical systems (Ayurveda and Traditional Chinese Medicine) in which herbal therapies were used systematically. Scientists found that people in different parts of the globe intended to use the same or similar plants for the same purposes. Recently, the World Health Organization estimated that 80% of people worldwide rely on herbal medicines for some aspect of their primary healthcare. For most herbs, the specific ingredient that causes a therapeutic effect is not known. Whole herbs contain many ingredients, and it is likely that they work together to produce the desired medicinal effect. Herbalists prefer using whole plants rather than extracting single components from them. Whole plant extracts have many components. These components work together to produce therapeutic effects and also to lessen the chances of side effects from any one component. Several herbs are often used together to enhance effectiveness and synergistic actions and to reduce toxicity (Hawkins and Ehrlich, 2007).

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 (Momin, 1987).

Along with other dosage forms, herbal drugs are also formulated in the form of ointment. An ointment is a viscous semisolid preparation used topically on a variety of body surfaces. These include the skin and the mucus membranes of the eye, vagina, anus, and nose. An ointment may or may not be medicated. Medicated ointments contain a medicament dissolved, suspended or emulsified in the base. Ointments are used topically for several purposes, e.g. as protectants, antiseptics, emollients, antipruritic, keratolytics and astringents. Ointment bases are almost always anhydrous and generally contain one or more medicaments in suspension or solution or dispersion. Ointment bases may be hydrocarbon (oligeanous), absorption, water removable and water soluble type. On the basis of their level of action, they are classified as: epidermatic, endodermatic and diadermatic (Carter, 1987). An antiseptic ointment is aimed to destroy or inhibit the growth of bacteria.

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 (Odimegwu *et al.*, 2008). The objective of the study was to

formulate and evaluate the antimicrobial herbal ointment from the local medicinal plants.

MATERIALS AND METHODS

1. The ethanolic extracts of following plants were taken in different ratio randomly and the antimicrobial tests were carried out for all the combination of extracts. The most effective combination was then determined by comparing the results of the Zone of inhibition. The different combinations tried were:

Table 1. Combination of plant extract in different ratio.

Extracts Combination no.	1	2	3	4	5	6	7	8	9	10
Extracts of Plants										
<i>Azadiracta indica</i> (Neem)	1	1	3	2	1	1	1	2	2	2
<i>Elsholtzia fruticosa</i> (Chhiuki)	1	1	1	3	2	2	1	1	1	2
<i>Eucalyptus globulus</i> (Essential oil) (Masala)	1	2	1	1	3	1	2	1	0	0
<i>Ocimum Santrum</i> (Tulsi)	1	3	2	1	1	1	3	2	2	1
<i>Rhododendron setosum</i> (Sunpati)	1	1	1	1	1	3	2	3	1	3

These plants were selected because the individual plant extracts have shown antibacterial effect against the bacteria which were able to cause wound infection.

2. Zone of inhibition measured in 10 different extract ratio on *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella species* were noted. These tests were carried out by cup plate method. These bacteria were selected for their potential to cause skin and wound infections.
3. Cup plate method was employed to evaluate the antibacterial efficacy of the extract combination. The diameter of the borer used was 6 millimeter. The combination having the biggest zone of inhibition was taken to incorporate in the ointment base.
4. Then the minimum inhibitory concentration (MIC) of the effective combination was found out. To find MIC, double strength broth, 50% extract solution, sterilized distilled water were taken in different ratio to obtain the final concentration 0%, 5%, 10%, 15%, 20% and 25%. The effective concentration was checked by observing the solution when it showed turbidity. In case of colored test solutions visual observation is not possible. So, the mixture solution was swabbed in suitable media and incubated at 37°C for 48 hours. MIC was indicated by the concentration at which there was no growth.
5. The ointment base was prepared by fusion method. In this method the constituents of the base were placed together in the basin and allowed to melt together at 70°C. After

melting, the ingredients were stirred gently maintaining temperature of 70°C for certain periods and then cooled with continuous stirring.

6. Formulation of ointment was done by incorporating the active ingredients in the base by trituration using mortar and pestle. The prepared ointment was filled in tubes and was stored at room temperature.
7. The formulated ointment was evaluated by using the parameters like: spreadability, irritant effect, diffusion, and physical stability. Spreadability test was performed by applying the ointment on the skin and noticing whether spreading was good or not. For the irritancy test, the ointment was applied to the normal and broken skin of human beings. Diffusion test was carried out by the cup plate method and the final product was compared with branded marketed products. Finally, the physical stability test of the final product was carried out at various temperatures i.e. at 2°C, 25°C and 37°C for four weeks.

RESULTS AND DISCUSSION

Zone of inhibition given by the 10 different combination ratio (Table 1) were noted and the result obtained is as follows:

Table 2: Zone of inhibition of the combined extract of plants

Extract combination no.	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Klebsiella species</i>
1	0 cm	2.1 cm	0 cm
2	3.6 cm	1.9 cm	4.7 cm
3	3.6 cm	2.4 cm	3.1 cm
4	3.6 cm	2.2 cm	2.6 cm
5	4.4 cm	2.1 cm	5.2 cm
6	3.2 cm	1.8 cm	2.4 cm
7	3.4 cm	2.0 cm	3.6 cm
8	2.1 cm	2.0 cm	2.5 cm
9	1.6 cm	2.1 cm	2.2 cm
10	2.2 cm	2.0cm	2.1 cm

Of the 10 different combinations tried, the combination number 5 was selected as the most effective combination based on the antimicrobial efficacy [Table 2]. The MIC of the most effective combination was found to be at 10% extract concentration.

Different formulations of ointment bases were considered for optimum desirable characteristics like: compatibility with extracts, penetration, spreadability, and irritant effects. The following two ointment bases [Formulation 1 and 2] were selected as the final base for the preparation of ointment because these bases were found to be compatible with the extracts at 10% concentration as well as possessing other optimum characteristics such as rate of release of medicament.

Formulation 1

Stearic acid	15 g
White wax	2 g
Yellow Vaseline	8 g
Triethanolamine	1g
Propylene glycol	8 g
Purified Water quantity sufficient to	100 g

Formulation 2

Polyethylene glycol 200	32g
Polyethylene glycol 4000	32g
Propylene glycol	7.5 g
Purified water quantity sufficient to	100 g

Based on these studies the composition of the final formulation of the herbal ointment was like this:

Euclyptus oil	3 g
Elsholtzia extract	2 g
Ocimum extract	1 g
Azadiracta extract	1 g
Rhododendron extract	1 g
Methyl paraben	0.2 g
Propyl paraben	0.1 g
Base (formulation 1 or 2) quantity sufficient to	100 g

In the formulation, eucalyptus oil also acts as flavoring agent. After the completion of the formulation, quality of the ointment was checked in terms of irritancy, spreadability, diffusion and stability.

When the ointment was applied to the normal and broken skin, it showed no irritant effect but a slight burning sensation was caused in open wounds. The burning sensation was due to the eucalyptus oil used in the formulation. The ointment readily spread when applied on the skin topically and rubbed gently. The product well diffused in the media around the cup of agar media and the diffusion of the product was comparable to similar marketed ointment of reputed brands. Similarly the ointment was found to be physically stable at different temperatures i.e. 2^oC, 25^oC and 37^oC within a test period of four weeks. There were no changes in the spreadability, diffusion and irritant effect even after the exposure to different temperatures.

CONCLUSIONS

The purpose of the study was to prepare antimicrobial herbal ointment using locally available plants. On the basis of antimicrobial efficacy, five different local plants were taken and their ethanolic extracts were incorporated in the most effective ratio in appropriate base. The final product readily spread on skin surface, showed no irritant effect, diffused well and was stable at different temperatures.

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