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FORMULATION AND EVALUATION OF HERBAL BASED ANTI MICROBIAL CREAM USING COW GHEE AS BASE

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ABSTRACT

Essential oils like eucalyptus and neem are well known for its anti microbial activity. The aim of this study is to evaluate the anti-bacterial activity of two essential oils (eucalyptus oil,neem oil)by incorporating them in cream containing cow ghee as base and evaluate its physical and anti microbial properties.cow ghee here not only acts as base but also as penetration enhancer.creams are prepared by fusion method.Agar diffusion method” was used to see anti bacterial activity of cream using reference disk of antibiotics. Anti bacterial cream was prepared by incorporating different amount of ingredients together and certain amount of oils in different concentrations and anti microbial activity was carried out on Escherichia coli,Staphylococcus aureus,Pseudomonas aeruginosa,Bacillus subtilis.zone of inhibition was measured. Finally efficiency was compared with standard product. Anti bacterial activity was found to be in limits. The prepared cream was evaluated for their physical and rheological studies.Stability studies showed stable, homogenous apperance over period of 3 months at room temperature. The prepared formulations showed anti bacterial activity as concentration of essential oils were increased. Formulation which has 2.5% essential oils has shown zone of inhibition almost equal to standard. Further invivo studies are to be performed.

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INTRODUCTION

India has an ancient heritage of traditional medicinal system. Herbal medicines have been used since the beginning of civilization to maintain health and treat disease¹. Various literatures provides lots of information on the folklore practices in different parts of country and traditional aspect of therapeutically important natural products. Nowadays people increasingly prefer alternative to conventional medicine. The reasons are it is safe and it works.

Anti microvial activity is the ability of substance to inhibit or kill bacterial cells. 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.

Semisolid dosage forms are dermatological products of semisolid consistency applied to skin for therapeutic or protective or cosmetic action. Topical semi-solid dosage forms are normally presented in the form of creams, gels, ointments, or pastes. They contain one or more active ingredients dissolved or uniformly dispersed in a suitable base and any suitable excipient such as emulsifiers, viscosity-increasing agents, antimicrobial agents, antioxidants, or stabilizing agents.

In present work different formulations of creams were prepared by using different concentrations of oils and anti microbial activity was seen. cow ghee was used in this work as base as well as penetration enhancer. The main aim of this work is to formulate and evaluate herbal base anti microbial cream.

OBJECTIVE:

1. To evaluate anti-microbial activity of some essential oils.
2. To evaluate the capacity of essential oils to form cream
3. To evaluate physical and rheological properties of formulated cream
4. To evaluate anti microbial activity of formulated cream.

EXPERIMENTAL PROCEDURE

MATERIALS

Essential oils (eucalyptus oil, neem oil) and cow ghee was brought from ayurveda store (Hyderabad, India). All of the chemicals was procured from vijaya college of pharmacy.

METHODS

Preparation of cream²:

Cream was prepared using fusion method. Different concentrations of essential oils and cow ghee for F1 to F4 were used. In the preparation, wool fat, bees wax, cetosteryl alcohol were melted in china dish and cow ghee was added. later oils were added when creams are in warm condition. Four formulations were prepared by taking different concentrations of oils as shown in table 1. Weight was 20g for all formulations. Preparation of cream was shown in figure no:1



Figure no:1: formulation of cream.

EVALUATION OF PHYSICAL PROPERTIES OF HERBAL FORMULATION

PHYSICAL CHARACTERISTICS

The prepared herbal formulations were examined for their physical (pH, color, consistency and homogeneity) as well as rheological properties.

DETERMINATION OF PH³

pH of the prepared formula was measured using digital pH meter.

HOMOGENEITY⁴

Homogeneity of various formulations was tested by visual observation and was ranked as follows: +++=Excellent, ++=Very Good, +=Good, -=Poor.

CONSISTENCY⁵

The cone attached to holding rod was dropped from the fix distance of 10cm such that it should be fall on the centre of measuring cylinder filled with herbal cream. The distance travelled by cone was noted down after 10 sec.

RHEOLOGICAL PROPERTIES⁶

The prepared formula was evaluated for the rheoloical characteristics:

VISCOSITY⁷

A Brookfield synchroelectric viscometer, Brookfield, MA was used to measure the viscosity (in cps) of herbal creams. The spindle was rotated at 2.5 rpm. Samples of the creams were allowed to settle over 30 min at the temperature of test (25±1 °C) before the measurements were taken.

EXTRUDABILITY⁸

Extrudability was determined, using an extrudability apparatus. A collapsible tube containing formulation was pressed firmly at the crimped end. When the cap was removed, formulation extruded until the pressure dissipated. Weight in grams required to extrude a 0.5 cm ribbon of the formulation in 10 seconds was determined. The average extrusion pressure in grams was reported.

SPREADABILITY⁸

The Spreadability determination: excess of sample was applied in between two glass slides and was compressed to uniform thickness by placing 100gm weight for 5minutes. Weight was added to the pan. The time required to separate the two slides, i.e. the time in which the upper glass slide moved over the lower plate was taken as measure of spreadability.

$$S = m \times l/t$$

where, m = weight tied to upper slide

l = length moved on the glass slide

t = time taken.

Spreadability test also was performed by applying the cream on the skin and noticing whether spreading was good or not.

IRRITATION TEST

Herbal cream was applied on normal and non-broken rat skin. The test cream and cotton swab covering it were secured firmly on the applied surface with help of adhesive tapes. Then observations were made for any sign of erythema and ranked as follows: +++ = Severe erythema, ++ = Moderate erythema, + = Slight erythema, - = No irritation

PHYSICAL STABILITY

Four sets of 20 g samples of the formulation and the one commercial products, soframycin 1% W/W were stored at room temperature 37°C for 3 months. Then, after three months, their stability was checked regarding antibacterial activity and appearance.

ANTIMICROBIAL TEST

Agar well-diffusion method was followed to determine the antimicrobial activity. Nutrient agar (NA) plates were swabbed (sterile cotton swabs) with 8 hour old - broth culture of respective bacteria. Wells (10mm diameter and about 2 cm a part) were made in each of these plates using sterile cork borer. Formulations prepared (1%, 1.5%, 2% and 2.5%) were added using sterile syringe into the wells and allowed to diffuse at room temperature for 2hrs. Control experiments comprising inoculums without formulation were set up. The plates were incubated at 37°C for 18-24 h for bacterial pathogens. The diameter of the inhibition zone (mm) was measured and compared with standard. Triplicates were maintained and the experiment was repeated thrice, for each replicates the readings were taken in three different fixed directions and the average values were recorded.

RESULTS AND DISCUSSION**FORMULATION OF CREAMS:**

Different formulation trails were performed using different concentrations of neem and eucalyptus oil and shown in formulation table 1.

Table 1: The composition and the amount of the ingredients used to make anti bacterial cream.

INGREDIENTS	F1	F2	F3	F4
Bees Wax	1.06g	1.06g	1.06g	1.06g
Wool Fat	1.06g	1.06g	1.06g	1.06g
Cetostearyl Alcohol	1.06g	1.06g	1.06g	1.06g
Cow Ghee	18.13g	18.00g	17.9g	17.8g
Oils(Eucalyptus+Neem)	1%	1.5%	2%	2.5%

PHYSICAL PARAMETERS:

All physical parameters like colour pH, homogeneity, consistency, skin irritancy test were performed. All formulations are light yellow color. All creams showed good homogeneity. pH and consistency of all formulations are in limits. Results are shown in table 2.

Table 2: Physical Properties.

PARAMETER	F1	F2	F3	F4
Colour	Light Yellow	Light Yellow	Light Yellow	Light Yellow
pH	5.8±0.52	5.9±0.59	6.2	6.0
Homogeneity	+++	+++	+++	+++
Consistency	5mm	4.5mm	5mm	5mm
Skin Irritancy	nil	nil	nil	nil

KEY:-Homogeneity +++ Excellent, ++ Very good + Good, - unsatisfactory.

RHEOLOGICAL PROPERTIES:

All formulations were tested for viscosity, spreadability, extrudability and they were in limits. Results are shown in table 3.

Table 3: Rheological Properties of formulations.

PARAMETER	F1	F2	F3	F4
Viscosity	120	90	100	110
Spreadability(cm)	39	38	39	40
Extrudability(g)	615	610	610	605

ANTI MICROBIAL ACTIVITY:

All herbal formulations were tested for anti microbial activity against selected strains and compared with standard. F4 formulation shown best result which is almost equal to standard.

Results are shown in table:4 and in fig:2.

Table 4: Inhibition zone of herbal formulation at different concentrations.

Microorganism	Standard	F1	F2	F3	F4
Escherichia.Coli	20mm±0.01	10mm±.20	14mm±1.5mm	16±2.0mm	22mm±1.5mm
Staphylococcus Aureus	22mm±0.1	9mm±1.21mm	14mm±1.52	19±1.5mm	21mm±1.4mm
Pseudomonas Aeruginosa	24±0.13mm	6mm±1.21mm	14mm±1.42mm	22±1.4mm	25mm±1.1mm
Bacillus Subtilis	28±0.1mm	18mm±0.2mm	21mm±0.3mm	25mm±0.4mm	29mm±0.2mm

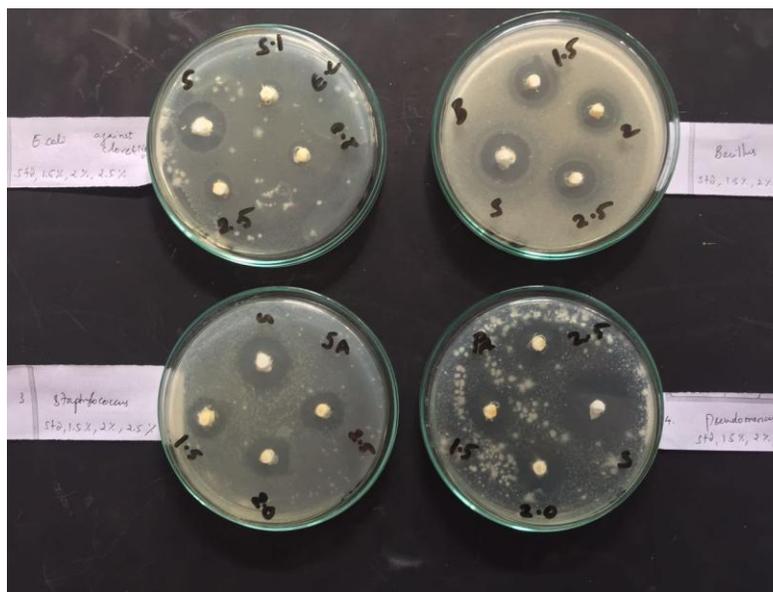


Figure2: antimicrobial activity of cream:

PHYSICAL STABILITY:

Four sets of 20 g samples of the formulation and the one commercial products, soframycin 1% W/W were stored at room temperature 37°C for 3 months. there was no change in appearance, consistency and anti microbial activity.

CONCLUSION

To our knowledge, this is the first study using combination of 2 essential oils and cow ghee as base to be formulated as anti microbial topical formulation over all cream with increasing concentration of essential oils showed increase anti bacterial capability. This study proved anti bacterial cream with potential application to reduce skin infection with consequent health benefits.

Further clinical research is recommended in future to validate the therapeutic potential of this anti bacterial cream against all skin disorder.

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CONFLICT OF INTEREST

All authors declare that they have no conflict of interest

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