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FORMULATION AND EVALUATION ANTISEPTIC ACTIVITY OF POLYHERBAL CREAM

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ABSTRACT

Herbal formulations have gained significant importance due to their safety, cost-effectiveness, and minimal side effects when compared to synthetic products. The present study was aimed at the formulation and evaluation of a polyherbal cream with antiseptic activity using natural plant extracts of *Azadirachta indica* (Neem), *Plectranthus amboinicus* (Mexican mint), and *Aloe barbadensis* (Aloe vera). These plants are well known for their antimicrobial, anti-inflammatory, and wound-healing properties. The polyherbal cream was prepared using suitable excipients by incorporating hydroalcoholic extracts of the selected plants. The formulated cream was evaluated for physicochemical parameters such as appearance, pH, viscosity, spread-ability, homogeneity, wash-ability, and stability. The antiseptic activity of the formulation was assessed using the agar well diffusion method against selected pathogenic microorganisms, including Escherichia coli and Staphylococcus aureus, by measuring the zone of inhibition. The results demonstrated that the polyherbal cream showed satisfactory physicochemical properties and good stability. The formulation exhibited significant antimicrobial activity, indicating a synergistic effect of the combined herbal extracts. The study concludes that the developed polyherbal cream possesses effective antiseptic activity and can serve as a safe and natural alternative to conventional antiseptic creams.

KEYWORDS: *Azadirachta indica, Plectaranthus amboinicus, Aloe barbadensis.*

1. INDRODUCTION

Cream is defined as semisolid emulsions which are oil in water (o/w) or water in oil (w/o) type and these semisolid emulsions are intended for external application. Cream is classified as oil in water and water in oil emulsion.[11] It is applied on outer part or superficial part of the skin and its main ability is to remain for a longer period of time at the site of application. The function of a skin cream is to protect the skin against different environmental condition, weather and gives soothing effect to the skin. There are different types of creams like cleansing, cold, foundation, vanishing, night, massage, hand and body creams.[7]

The skin is the largest organ of the human body and serves as the primary protective barrier against physical injury, chemical exposure, and microbial invasion. Breaches in skin integrity due to cuts, wounds, burns, or infections create favourable conditions for the growth of pathogenic microorganisms, leading to delayed healing and secondary infections. Antiseptic preparations are therefore essential in preventing microbial contamination and promoting wound healing. Conventional synthetic antiseptics, although effective, are often associated with drawbacks such as skin irritation, hypersensitivity reactions, toxicity on prolonged use, and the development of microbial resistance. These limitations have increased global interest in herbal and polyherbal formulations as safer, cost-effective, and eco-friendly alternatives.[17]

Azadirachta indica (Neem) is a well-known medicinal plant extensively used for its potent antimicrobial, antifungal, anti-inflammatory, and immunomodulatory activities.[18] Neem leaves contain active phytoconstituents such as nimbin, nimbidin, azadirachtin, and flavonoids, which are effective against a wide range of Gram-positive and Gram-negative bacteria. Due to its strong antiseptic and wound-healing properties, neem has been traditionally used in the management of skin infections, ulcers, and burns.[20]

Plectranthus amboinicus (Mexican mint or Indian borage) is an aromatic medicinal plant known for its antimicrobial, antioxidant, and anti-inflammatory activities. The leaves are rich in essential oils, thymol, carvacrol, and phenolic compounds that exhibit significant antibacterial activity against pathogenic microorganisms. Its soothing and anti-irritant properties make it suitable for topical formulations aimed at skin protection and infection control.[6]

Aloe barbadensis (Aloe vera) is widely recognized for its excellent wound-healing, moisturizing, soothing, and antimicrobial properties.[19] Aloe vera gel contains bioactive compounds such as aloin, aloe-emodin, polysaccharides, vitamins, enzymes, and amino acids, which promote tissue regeneration, reduce inflammation, and inhibit microbial growth. Additionally, aloe vera improves skin hydration and enhances the penetration of active ingredients, making it an ideal base component for topical creams.[4]

Incorporating these three medicinal plants into a polyherbal cream offers a rational approach to developing an effective antiseptic formulation with enhanced therapeutic benefits. The formulation of a polyherbal antiseptic cream aims to provide antimicrobial protection while maintaining skin compatibility, stability, and patient acceptability. Evaluation of the formulated cream through physicochemical parameters and antimicrobial activity studies is essential to ensure its quality, safety, and efficacy.

Thus, the present study focuses on the formulation and evaluation of the antiseptic activity of a polyherbal cream containing *Azadirachta indica*, *Plectranthus amboinicus,* and *Aloe barbadensis,* with the objective of developing a natural, safe, and effective topical antiseptic preparation suitable for the prevention and management of skin infections.

Antiseptics

Antiseptics are agent which applied to living tissue/skin to reduce the possibility of infection, sepsis, or putrefaction. Antiseptics are antimicrobial substance that are generally distinguished from antibiotics by the latter's ability to be transported through the lymphatic system to destroy bacteria within the body, and from disinfectants, which destroy microorganisms found on non -living objects. Antibacterial are antiseptics that have the proven ability to act against bacteria. Microbicides which destroy virus particles are called vermicides or antiviral. Antisepsis was recommended by Hungarian physician Ignaz Semmelweis in 1847 but, tragically, he was ignored (and worse). The wide spread introduction of antiseptic surgical methods followed the publishing of the paper Antiseptic principle of the practice of surgery in 1867 by Joseph Lister, inspired by Louis Pasteur's germ theory of putrefaction In this paper, Lister advocated the use of carbolic acid (phenol) as a method of ensuring that any germs present were killed.[17]

PLANT COLLECTION AND AUTHENTIFICATION

Fresh and healthy leaves of *Azadirachta indica* and *Plectaranthus amboinicus* were collected from the local areas of Thirumittacode, Palakkad and is identified at botany department, NSS Arts and Science College, Parakkulam.

DRYING OF LEAVES

The leaves were washed with water to clean the adhering dust particles. The leaves were dried in shade at room temperature for 31 days and made in coarse powder by using grinder, stored in air tight container.

Extraction of Neem leaves

Neem leaves were collected and washed with distilled water and dried in hot air oven. After proper drying, leaves were powdered. Then 5g Neem leaves powder, 80 to 100 degree Celsius. Dimethyl sulfoxide was taken in a volumetric flask and shaken for 3 d on REMI RSB-12 mechanical shaker. Then the solution was heated on a water bath at 80-100 ℃ and concentrated up to 20 ml and then filtered using muslin cloth to remove impurities. Then the filtrate or filter product obtained, which is a clear solution or clear extract of Neem leaves, was used in the preparation.

Extraction of Mexican mint leaves

Mexican mint leaves were collected and washed with distilled water and dried in shade. Then after proper drying, the leaves were powdered. Then 1g Mexican mint leaf powder+10 ml dimethyl sulfoxide was taken in a volumetric flask and then shaken for 3 d on REMI RSB-12 mechanical shaker. Then the solution was heated on water bath at 80 to 100 degree Celsius. for few minutes and then concentrated up to 5 ml and filtered using a muslin cloth to remove impurities. Then the filtrate or the filter product in which a clear solution or clear extract of Mexican mint leaves was used in the preparation.

Extraction of Aloe Vera gel

Mature, healthy and fresh aloe Vera leaves were collected and washed with distilled water. Then after proper drying of leaves in hot air oven, the outer part of the leaf was dissected longitudinally using a sterile knife. Then the aloe Vera gel that is the colourless parenchymatous tissue was removed using the sterile knife. Then it is filtered using muslin cloth to remove the fibres and impurities. Then the filtrate or the filter product which is a clear aloe Vera gel was used in the preparation.

PHYTOCHEMICALS	PRESENT (+) OR ABSENT (-) (NEEM)	PRESENT (+) OR ABSENT (-) (MEXICAN MINT)
Carbohydrates	-	-
Alkaloids	+	+
Glycosides	+	+
Protein and Amino acids	+	+
Flavanoids	+	+
Saponins	+	+
Phytosterols and Triterpenoids	+	+
Phenolic compounds and Tannins	+	+
Volatile oil	-	+

Formulation of cream

- **Preparation of oil phase**

➢ Heat liquid paraffin and beeswax in a borosilicate glass beaker at 75 ℃ and maintain that heating temperature. (Oil phase).

- **Preparation of aqueous phase**

➢ In another beaker, dissolve borax, methylparaben in distilled water and heat this beaker to 75 ℃ to dissolve borax and methylparaben and to get a clear solution. (Aqueous phase).

- **Addition of aqueous phase to oil phase:**

➢ Then slowly add this aqueous phase to heated oily phase.

➢ Then add a measured amount of aloe Vera gel, Neem extract, and Mexican mint extract and stir vigorously until it forms a smooth cream.

➢ Then add few drops of rose oil as a fragrance.

➢ Put this cream on the slab and add few drops of distilled water if necessary and mix the cream in a geometric manner on the slab to give a smooth texture to the cream and to mix all the ingredients properly.

➢ This method is called as slab technique or extemporaneous method of preparation of cream.

Sl.No.	Ingredients	Formulation F1H	Formulation F2H	Formulation F3H
1	Aloe vera gel	3ml	4.5ml	3ml
2	Neem extract	3ml	4.5ml	3ml
3	Mexican mint extract	0.6ml	1.5ml	1.2ml
4	Beeswax	10.5g	9g	9.6g
5	Liquid paraffin	45ml	30ml	36ml
6	Borax	1.2g	0.6g	0.9g
7	Methyl paraben	0.12g	0.06g	0.09g
8	Distilled water	Q. S	Q. S	Q. S
9	Rose oil	Q. S	Q. S	Q. S

Physical evaluation

In this test, the cream was observed for colour, odour, texture, state.

Irritancy

Mark the area (1 cm^2) on the left-hand dorsal surface. Then the cream was applied to that area and the time was noted. Then it is checked for irritancy, erythema, and oedema if any for an interval up to 24 h and reported.

Wash ability

A small amount of cream was applied on the hand and it is then washed with tap water.

pH

0.5 g cream was taken and dispersed in 50 ml distilled water and then p^H was measured by using digital p^H meter.

Viscosity

Viscosity of cream was done by using Brooke field viscometer at a temperature of 25 ℃ using spindle No. 63 at 2.5 RPM.

Phase separation

Prepared cream was kept in a closed container at a temperature of 25-100 ℃ away from light. Then phase separation was checked for 24 h for 30 d. Any change in the phase separation was observed/checked.

Spread ability

The spread ability was expressed in terms of time in seconds taken by two slides to slip off from the cream, placed in between the slides, under certain load. Lesser the time taken for separation of the two slides better the spread ability. Two sets of glass slides of standard dimension were taken. Then one slide of suitable dimension was taken and the cream formulation was placed on that slide. Then other slide was placed on the top of the formulation. Then a weight or certain load was placed on the upper slide so that the cream between the two slides was pressed uniformly to form a thin layer. Then the weight was removed and excess of formulation adhering to the slides was scrapped off. The upper slide was allowed to slip off freely by the force of weight tied to it. The time taken by the upper slide to slip off was noted.

$$\text{Spread ability} = m \times l/t$$

Where, m= Standard weight which is tied to or placed over the upper slide (30g)

l= length of a glass slide (5 cm)

t= time taken in seconds.

Greasiness

Here the cream was applied on the skin surface in the form of smear and checked if the smear was oily or grease-like.

5.13.1 Microbial evaluation

Preparation of agar well diffusion method

Agar well diffusion method performed to determine the zone of inhibition. Nutrient agar is used for determining antibacterial activity and potato dextrose agar for antifungal activity. In this to above inoculated agar plates well of 5 mm diameter were prepared by using a sterile cork borer. The definite quantity of herbal cream and control was placed into each well. The plates were then incubated for 72 hours at 37^0C and observed for zone of inhibition. All the experiments were repeated 3 times to get accurate results.

RESULT AND DISCUSSION

Physical evaluation

SL. NO	PARAMETER	F1H	F2H	F3H
1	Appearance	Smooth, uniform and consistent cream	Smooth, uniform and consistent cream	Smooth, Uniform and consistent cream
2	Colour	Faint green	Faint green	Faint green
3	Odour	Pleasant	Pleasant	Pleasant
4	Texture	Semi-solid	Semi-solid	Semi-solid

Irritancy

SL.NO	Formulation	Irritant effect	Erythema	Oedema
1	F1H	Nil	Nil	Nil
2	F2H	Nil	Nil	Nil
3	F3H	Nil	Nil	Nil

Wash-ability study observation

SL.NO	FORMULATION	WASHABILITY
1	F1H	Easily Washable
2	F2H	Easily Washable
3	F3H	Easily Washable

pH observation table

SL.NO	FORMULATION	pH
1	F1H	6.3
2	F2H	6.2
3	F3H	6.4

Viscosity

Herbal Antiseptic Cream	F1H	F2H	F3H
Viscosity (cPs)	2160	2003	2168

Phase separation

SL.NO	FORMULATION	PHASE SEPARATION
1	F1H	No phase separation
2	F2H	No phase separation
3	F3H	No phase separation

Spread-ability

SL.NO	FORMULATION	SPREAD-ABILITY (gm.cm/sec)
1	F1H	24.5
2	F2H	30.8
3	F3H	18.9

Greasiness

SL.NO	FORMULATION	GREASINESS
1	F1H	Non-greasy
2	F2H	Non-greasy
3	F3H	Non-greasy

Result of antimicrobial study

ANTIBACTERIAL STUDY BY AGAR WELL DIFFUSION METHOD

SL.NO	SAMPLE	ZONE OF INHIBITION (mm)
		Staphylococcus aureus
1	F1H	17
2	F2H	19
3	F3H	14
4	Gentamicin	23

ANTIFUNGAL STUDY BY POTATO DEXTROSE AGAR PLATE METHOD

SL.NO	SAMPLE	ZONE OF INHIBITION (mm)
		Candida albicans
1	F1H	18
2	F2H	22
3	F3H	16
4	Fluconazole	26

SUMMARY AND CONCLUSION

The result of the study confirm that a polyherbal antiseptic cream formulated with *Azadirachta indica*, *Plectaranthus amboinicus* and *Aloe barbadensis* exibits promising antiseptic activity. Making it an effective alternative to synthetic antiseptic creams. The optimized formulation (F2H) showed better spread-ability, stability, and overall efficiency in combating skin infection causing microorganisms.

The study support the potential use of herbal ingredients in pharmaceutical and cosmetic formulations, promoting safer and more sustainable skin care solutions. Further clinical evaluations and long term stability studies are recommended to enhance the product's commercial viability and ensure broader consumer safety.

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