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FORMULATION AND *IN VITRO* EVALUATION OF POLYHERBAL ANTI-ACNE CREAM

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ABSTRACT

Acne vulgaris is a common pilosebaceous skin condition that primarily affects areas of the face, trunk, and back that have large oil glands. Lesions, come dones, and inflammatory seborrhea are frequently formed as a result of acne. Pus in acne is caused by Propionibacterium acnes and Staphylococcus epidermidis, which in turn causes inflammation. The antibacterial properties of a polyherbal anti-acne cream that contains plant extracts of clove oil, Ocimum sanctum (leaves), and Azardiachata indica (leaves) were extracted through a maceration process using ethanol solvent. The invitro method was utilized to assess the antibacterial potential of the ethanol extract of tulsi and neem leaves, which were used to make an anti-acne cream (like agar cup method)

When compared to a single plant extract, the polyherbal cream's antibacterial action produced. a larger zone of inhibition (ZI). Consequently, it should be noted that the polyherbal anti-acne cream, which was created with a focus on Propionibacterium, the common bacteria that causes acne, has the potential to have an antibacterial effect in any acne treatment. Many people now prefer to use herbal products over synthetic ones because of the increased side effects of allopathy cream. It is commonly used to treat acne and has additional properties such as anti-aging, anti- inflammatory, and antibacterial properties. Additionally, the prepared polyherbal anti-acne cream met every requirement of the pharmaceutical evaluation. To determine whether a cream is suitable for human skin, certain evaluation tests are carried out. This tests Physical assessment, Spreadability, pH, Viscosity, Homogeneity and Washability test determine whether the cream causes irritation after being applied to the skin or not.

Keywords: Acne Vulgaris, Propionibacterium acne, Azardiachata indica, Ocimum sanctum, polyherbal cream.

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INTRODUCTION

Neem and Tulsi, generally referred to as holy plants and herbs, have been traditionally used for many kinds of skin problems since ancient times. In this study, tulsi (Ocimum sanctum) and neem (Azadirachta indica) leaves were mixed with various ingredients to formulate a polyherbal cream ⁽¹⁾

Neem is a species of tree in the Meliaceae family, Azadirachta indica. Medicine is made from the seeds, bark, and leaves. Tulsi (Ocimum sanctum Linn) is the most important herb in Ayurveda, and scientific studies are now verifying its health benefits. There is growing evidence that tulsi's special combination of pharmacological actions can address stress related to the body, mind, metabolism, and chemicals. Several medicinal plants have been used as skincare since ancient times, and they demonstrate promising results on many kinds of skin types ^(2,3)

Since the beginning of time, many medicinal plants have been used as cosmetics. These plants have shown promise in treating a variety of skin conditions, including wrinkles, acne, blackheads, age spots, rashes, and allergies. Some common plants include Phyllanthus emblica, Psidium gujava, Curcuma longa, Mangifera indica, Annona squamosa, Aterocarpus heterophyllus, Carica papaya, Aloe vera, Azadirachta indica, Annona squamosa, Ocimum sanctum, Terminalia arjuna, Terminalia chebula, and Vitis vinifera, among others ^(4,5)





Figure No.1: Neem

Figure No.2: Tulsi

HOWACNE IS COMMON:

The most prevalent kind of skin condition is acne. Teens, young adults, and older children are the age groups most affected $^{(6)}$

About 80% of people between the ages of 11 and 30 have acne. For girls, the most common age range for acne cases is 14 to 17, while for boys, the most common age range is 16 to 19. Most people with acne will go through years of recurrent bouts, or flare-ups, before noticing that as they age, their symptoms progressively get better.

Acne symptomstypically go away by the time a person reaches their twenties.Yet, 1% of men and 5% of women over 25 still have acne symptoms, suggesting that acne can sometimes persist into adulthood. ^(7,8) Causes of Acne:

Hyperkeratosis (accelerated keratinization) at the hair infundibulum.

Hyperactive sebaceous glands (overactive lipid secretion).

Propionibacterium acnes activity that promotes comedogenesis. Women's cyclic hormone levels.⁽⁹⁾

Occupational risks include prolonged exposure to chemicals and air pollutants, as well as elevated humidity levels. Seasonal effects, excessive sexual activity, emotional or psychological stress, mechanical skin surface manipulation, and some medications, suchas corticosteroids, are additional triggers and events linked to acne.⁽¹⁰⁾



Figure No.3: Types of Acne

Treatment of acne:

Mild acne: Benzoyl peroxide

Moderate acne: Topical retinoids/ antibiotics

Severe acne: Hormonal therapy

To overcome this problem there are several medications prepared by the Pharmaceutical Industry like, antiacne pills, lotion, moisturizer, creams etc. Cream is the One form of semisolidemulsion that is intended for external application is cream, that's can be either water in oil (w/o)or oil in water (o/w).^(11,12)

Cream is categorized as an emulsion of water and oil. It is applied to the outermost or most superficial layer of the skin, and its main benefit is that it lasts longer at the application site. The cream's role is to soothe the skin, heal infections, remove tans and acne, and protect the skin from various environmental conditions⁻ The cream is applied on the skin which have topical drug delivery system. ^(13,14)

MATERIALS AND METHODS:

Collection and Authentication of Plant Material:

Collect fresh Neem and Tulsi leaves or other plant parts.

The leaves of Neem and Tulsi was collected in the month of February 2024 from Sangola Solapur district Maharashtra. The plant was authenticated by Dr. Tembharne R. R. Dept. of Botany from Sangola college, Sangola.

Wash the plant material thoroughly with water to remove dirt and impurities.

Allow the plant material to air dry or use a drying oven at a low temperature (e.g., 40-45°C) to remove excess moisture.

Extraction Process:

Grind the dried plant material into a coarse powder using a grinder or mortar and pestle.

Weigh a specific amount of the powdered plant material (e.g., 100 grams) accurately.

Extraction Procedure:

Neem and Tulsi leaves were both dried in sunlight for a duration of four days, and after being crushed into a coarse powder with a mixer grinder, they were sieved through number $22^{(15)}$. For upcoming studies, the coarse powdered form was kept in storage. then extracted in a soxhlet device using ethanol. Just three hours were spent on the extraction process using 100 grammes of plant material and 500 milliliters of ethanol. The extract changed into an ethanolicextract by evaporated it in a rotating evaporator. ^(15,16)



Figure No.4: Extraction of Neem and Tulsi

Filtration and Concentration

After the extraction period, filter the extract using a filter paper or cloth to remove solidparticles. Concentrate the filtered extract using a rotary evaporator or other suitable method toremove the solvent and obtain a concentrated extract.

FORMULATION TABLE:

Part A (oil phase)Part B (Aqueous phase)			
Ingredients	Quantity	Ingredients	Quantity
Stearic acid(Emulsifier)	2w/v	Polyethylene glycol(Base)	4w/v
Lecithin (Surfactants)	0.5w/v	Sodium laurelsulphate (Surfactant)	2w/v
Almond oil	3v/v	Triethanolamine	Q.S.
Clove oil	1v/v	Propyl paraben(Preservative)	2w/v
Neem leaves ethanolic extract	4w/v	Mentha oil (Perfume)	2v/v
Tulasi leaves ethanolic extract	4w/v	Water upto 100%	Q. S

	Table	No.1:	Formula	for cream
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Preparation and formulation of poly-herbal cream: -

The poly-herbal cream was developed with extracts of ethanol from the leafy parts of Azadirachta indica (Neem leaf extract; NLE) and Ocimum sanctum (Tulsi leaf extract; TLE). The phase of water (Part B) and the oily component (Part A) were used in the formulation of the oil in water O/W cream. A cream with an oil-in-water (O/W) emulsion base (semisolid formulation) was created. The emulsifier (stearic acid) was dissolved in the oil phase (Part A) and heated to 75° C along with different oil soluble ingredients (lecithin, almond oil, clove oil, and fraction of ethanolic extract of Azadirachta indica leaf and Ocimum sanctum leaf ethanol extract).^(17,18)The aqueous phase was utilised to dissolve the preservatives and other water solubleingredients (PLG, SLS, triethanolamine, propyl parabene, Mentha oil, and water (Part B) and heated to 75°C. next to heating, the phase of water was slowly added to the oil phase while stirring continuously until the emulsifier cooled.⁽¹⁹⁾

PREPARATION OF NUTRIENT MEDIA:

Sr. No	Ingredient	Quantity
1	Beef extract	0.3gm
2	Peptone	0.5gm
3	Sodium chloride	0.05gm
4	Distilled water	100ml

Table No.2: Nutrient media for microorganisms (Bacteria)

First, autoclave all glassware at 121°C for 30 minutes to sterilize it.

Fill 100ml of distilled water with 0.3g of beef extract, 0.5g of peptone, and 0.05g of sodium chloride. Boil for thirty minutes, then let the mixture cool.

Transfer the bacteria to culture media, making sure that the process is limited to aseptic areas to avoid microbiological contamination.

Incubate the culture media at 37°C for 48 hours^{. (20,21)}



Figure No.5: Prepared culture media

Antibacterial activity testing (Agar cup plate method):

Once melted, the agar is cooled and then added to a petri dish. Spread the 0.2 ml inoculum onto the petri dish that has solidified. A sterile borer is used to drill four cups. Agar plate cups were filled with three different concentrations of test extract (0.5, 1 and 2 milliliters) and then the remaining stirred solution was added. For 24 hours, the petri dish is incubated at 37°C. Zone of inhibition antibacterial activity is seen. ^(22,23)



Figure No.6: Agar media

EVALUTION TEST:

Physical assessment: The colour, texture, and condition of the cream were visually assessed during this assessment test. ⁽²⁴⁾

Spreadability: The spreadability of the cream was decided using the parallel plate method. In this study, about one grammes of the cream manufacturing was placed on a 20x20 cm glass a slide. ⁽²⁵⁾ Another slide of the same size was placed on top of the cream, and a 125-grams weight was applied to the upper slide to evenly press and spread the cream into a thin layer. After the weight removed, the spread cream was measured to find out itsspreadability. ⁽²⁶⁾

pH: The pH paper was used to measure the cream's pH.⁽²⁷⁾

Viscosity: The cream's viscosity was assessed at regular intervals. The viscometer was used to measure changes in viscosity. Viscosity is measured at room temperature as well as at 45 °C. ^(28,29)

Homogeneity: After the cream was set in a container, homogeneity was assessed by visual inspection of the cream formulation. They were examined for appearance and the existence of any aggregates in this homogeneity test. ^(30,31)

Washability test: The hand is treated with a small quantity of cream and then rinsed with tap water.⁽³²⁾

RESULT:

Formulation was assessed in terms of certain physical parameters. The created cream had a pH range of 6.7 to 6.9 was transparent, and was homogenous. Additionally, this formulation displayed appropriate spreadability qualities along with acceptable rheological behavior.

Sr.no	Parameters	Formulation (F1)	Formulation (F2)	Formulation (F3)
1	Colour	Dark greenish	Dark greenish	Dark greenish
2	Texture	Smooth	Smooth	Smooth
3	Spreadability	5.46±0.01	5.55±0.05	6.40±0.02
4	pH	6.7	6.9	6.8
5	Homogeneity	Good	Good	Good
6	Washability	Easily washable	Easily washable	Easily washable

 Table No.3: Results of Evaluation Test

In vitro study (Antibacterial Activity):

Sr. no.	Concentration of gel	Dimeter of Zone of inhibition
1	2ml	5mm
2	4ml	7mm
3	6ml	9mm

Table No.4: Antibacterial Activity



Figure No.7: Antibacterial activity (zone of Inhibition)

DISCUSSION:

The selection of Neem and Tulsi is based on their well-documented therapeutic properties. Neem and Tulsi are renowned for their potent antibacterial activity, essential for combating acne-causing bacteria such as Propionibacterium acnes and Staphylococcus aureus. Neem and Tulsi contribute significantly to reducing inflammation and preventing the development of new acne lesions. Neem offers soothing and healing properties, aiding in skin repair and reducing irritation. Liquorice adds an anti-inflammatory and skin-brightening effect, which helps in reducing post-acne hyperpigmentation. The formulation of the polyherbal anti-acne cream involves creating a stable emulsion that incorporates the active herbal extracts effectively.

The use of stearic acid, cetyl alcohol, and glycerin ensures the cream base has the desired consistency and moisturizing properties. The emulsifying agents stabilize the formulation, preventing phase separation and ensuring a uniform distribution of the active ingredients. The choice of preservatives like methylparaben is crucial to ensure the cream remains free from microbial contamination over its shelf life. Following these the formulation was prepared successfully which passes over all expectations in evaluation parameter.

CONCLUSION:

A polyherbal anti-acne cream formulated with a synergistic combination of herbal extracts WI enhanced efficacy in inhibiting acne-causing bacteria, reducing inflammation, and promoting skinhealing compared to single-herb or conventional formulations. In vitro evaluation will reveal the cream's potential through assessments of antimicrobial activity, anti-inflammatory effects, and wound healing properties.

Incorporating clove oil with cream formulations and analyzing the anti-acne cream formulations is the present work's scientific review of the widely used anti-acne agent. The outcomes revealed how well the clove oil-containing Antiacne cream works to fight the bacteria that lead to acne. Since clove oil functions as an effective anti-acne agent, using itto anti-acne preparations may be effective. A variety of essential oils and their various parts have been the focus of thorough studies in recent years investigating their antimicrobial properties against specific bacteria and fungi.

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