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HERBAL ANTIACNE GEL FORMULATION AND EVALUATION USING ALOEVERA, NUTMEG, AND VIGNA RADIATA

Sampada G.Shrawankar*, Dr. Manjeet Singh, Dr. Rajesh Z. Mujariya, Lokesh I. Patle.

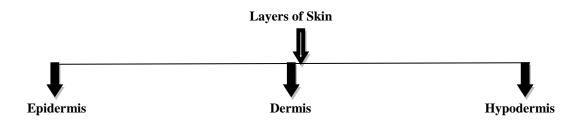
Student, Executive Director, Principal, HOD Pharmaceutics. Department of Pharmaceutics, Sardar Patel University, Balaghat

Abstract: Skin is the most common mode of transmission of pathogens to patient proper hygiene can prevent to health care associated infections are the spread of antimicrobial resistances. The aim of the study is to prepare and evaluate Antiacne gel. The objective of the study was to prepare and herbal Anti-acne Gel prepare from ethanol extract Nutmeg & Vigna radiata seeds and collect gel from Aloebarbadensis leaves. Formulate polyherbal gel containing Vigna radiata Nutmeg extract and Aloebarbadensis gel & perform physical characterization, stability study, and antimicrobial activities against various bacterial strains such as Staphylococcus aureus, Escherichia coli. To evaluate the safety of prepared polyherbal gel by skin irritation study. The main goal of polyherbal gel was for reduce the oil secretion & cleaning the skin from acne. It is a vital principle in the prevention, Control and required infections. Polyherbal Antiacne gel was avoiding adverse effects like itching, irritation, dermatitis etc. So maintaining skin clean as the prime criteria of same polyherbal formulations an attempt has made to formulate as formulation.

Key Words: AloeBarbedeions, Myristica fragrans, Vigna Radiata MIC: Minimum Inhibitory Concentration, Staphylococcus aureus, Escherichia coli.

I. INTRODUCTION:

Skin - Skin is the biggest organ of the body, accounting for about 15% of the total adult body weight. It performs many vital functions, including protection against external physical chemical, as well as prevention of excess water loss from the body and a role in thermoregulations. Humans shed around 500 million skin cells each day. In fact, the outermost parts of the epidermis consist of 20–30Trusted Source layers of dead cells. The epidermis constantly makes new cells in its lower layers. Over the course of around four weeks, these cells make their way to the surface, become hard, and replace the shedding, dead cells. Keratinocytes are the most common type of cells within the epidermis. Their job is to act as a barrier against bacteria, parasites, fungi, viruses, heat, ultraviolet (UV) rays, and water loss



Epidermis

Outer layer that is barrier to infection "superficial" Made up of Stratified sqaumous epithelial cells. A vascular

Epidermis divided into different regionsStratum basale

- Stratum spinosum
- Stratum granulosum
- Stratum lucidium
- Stratum corneum

Dermis

It lies between the epidermis and subcutaneous layer and contains many type of sensory receptor for touch, pressure, pain vibration, temperature etc.

The types of cells located in the dermis are

- Fibroblasts
- Mast cells
- Histocytes
- Lymphatic vessels
- Hair follicles
- Sweat Glands

Hypodermis

It is very deep to skin and also known as subcutaneous layers It consists of loose connective tissue.

Types of cells in Hypodermis are

- Fibroblast
- Adipose tissue
- Macrophages

Skin Relating Problems

- Dry Skin
- Pigmentation
- Prickly Heat
- Atopic dermatitis
- Melanoma
- Psoriasis
- Scabies
- Acne

ACNE

As oil and dead skin cells clog pores, sebum can accumulate inside the pores and cause acne, an inflammatory skin condition. It is a chronic skin condition that develops when dead skin cells clog hair sacs. Acne vulgarism is the common term for the condition. The age range of the patients who are afflicted by this is between 16 and 25. A mild form of acne is common during adolescence, but a severe case can leave scarring long after therapy and can give an unpleasant look. Practically speaking, acne symptoms can be divided into three categories: mild, moderate, and severe.

- Acne is affected by two major factors:
- Heredity
- Hormones

Types of acne

Spots or pimples appear when the skin generates excessive amounts of oil, which encourages the growth of germs that clog the skin's pores and cause swelling and redness.

In no way are pimples infectious. Whiteheads: These little bumps that remain under the skin's surface.

Although having a strikingly black appearance and rising to the skin's surface, blackheads are not caused by dirt. Black skulls don't have a black hue due of dirt; they are just black. The keratin protein is often oxidized by air.

Papules: These little, pink pimples on the skin are visible and painful to the touch.

Pustules: (pimples or zits) can be seen on the surface of the skin. They are red at the lowest level and contain pus at their top.

Nodules: Prominent growths on the skin's surface. These are painful, huge, solid pimples that are visible on the skin's surface as well as deep into the skin.

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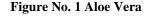
Cysts: Clearly discernible growths on the skin's surface. They are firmly embedded, painful, packed with pus, and extremely susceptible to scarring.

Hormone Adjustments

Environmental influences, genetic vulnerability, and hormones hormone adjustments Acne may be brought on by hormones, environmental factors, genetic predisposition, and other causes. Acne develops when sebaceous glands create sebum, a sticky material, and hair follicles become blocked with dead skin cells. Skin cells inside the follicles become clumped together due to the excess sebum, obstructing the flow. Bacteria that have settled inside a plugged pore or comedown emit substances that trigger inflammation. Comedowns develop into pimples and pustules. Some acne lesions swell up to the point of rupture, when nodules are created. Nodules develop cysts as a result of the convergence of the afflicted glands, which may lead to the production of scars after healing.

PLANT PROFILE OF ALOEVERA:-

A species of succulent plant in the genus Aloe is called Aloevera. Aloe, which has 500 species, is wide spread and is regarded as an invasive plant in many parts of the world. It is an evergreen perennial that is native to the Arabian Peninsula but thrives untamed in dry, subtropical, and tropical environments all over the world. It is grown for commercial purposes, mostly as a centuries-old topical remedy. The species works well inside as a potted plant and is interesting for ornamental purposes. It is utilized in several consumer goods, including as drinks, lotion for the skin, cosmetics, ointments, and gel for sunburns and small burns. Clinical proof of Aloevera extract's efficacy or safety as a topical medication or cosmetic is scant. The word Aloevera is Latin term which means "aloe" and "Vera" ("true"). Aloevera is a plant with no stems or very short stems that can reach heights of 60 to 100 cm (24 to 39 inches) and spreads through offsets. The thick, meaty leaves range in color from green to grey-green, with white specks visible on certain types' top and lower stem surfaces.





The leaf's edge is serrated and features tiny white teeth. Each bloom is pendulous and has a yellow tubular corolla that is 2-3 cm (34-1 14 in) long. The flowers are produced in the summer on a spike that can grow up to 90 cm (35 in) tall.

Aloe Vera produces arbuscular mycorrhiza, a symbiosis that gives the plant better access to mineral nutrients in soil, just as other Aloe species. Aloevera leaves include phytochemicals such acetylated mannans, polymannans, anthrones, and other anthraquinones like emodin and different lectins that are being investigated for their potential bioactivity.

Classification scientific

Kingdom: Planate Clade: Tracheophytes Clade: Angiosperms Clade: Monocots Order: Asparagales Family: Asphodelaceae Subfamily: Asphodeloideae Genus: Aloe Species: A. Vera

Pharmacological Properties of Aloevera gel

- Wound Healing properties
- Effects on skin exposure to UV and gamma radiation
- Antibacterial properties
- Antiseptic effect
- Anti-acne effect
- Moisturizing and anti-aging effect

PROFILE OF NUTMEG SEED:-

Two separate spices, nutmeg and mace, which include the seed kernel within, are mostly derived from the nutmeg. Nutmeg, which comes from the fruit and mace, which comes from the dried meat around the seed, both come from the same plant. The bark, leaves, and flower are also used to make mace oil. The output of nutmeg oil ranges from 500 to 1200 kg per hectare, and it is distinguished by its distinct, warm flavor and strong aroma. Despite being the greatest producer, India's output is insufficient to meet local demand. The majority of the world's nutmeg production throughly 50%—comes from Indonesia. Due to the varied pharmacological actions, nutmeg extracts and essential oil (EO) are employed in the creation of novel drugs in India, China, and other tropical nations. In traditional medicine, nutmeg has been used as an antibiotic, antioxidant, psycho stimulant, and antithrombotic among other things. The seeds have been utilized in Ayurveda to treat urinary in continence, sleeplessness, and poor digestion. Moreover, nutmeg has been shown to have antibacterial, insecticidal, and fungicidal properties.

Figure No.2 Nutmeg



It is also known to reduce spasms anti-inflammatory effect, it has been used externally. The seeds are applied topically to cure tooth aches and rheumatic pain, While the seeds have been eaten orally to treat diarrhoea Dysentery, vomiting and stomach distension. Escherichia coli, Salmonella Choleraesuis, and Staphylococcus Aureus are just a few of the Gram positive and Gram negative.Microorganisms that nutmeg has demonstrated antibacterial efficacy against. At least 85% of people have acne a common skin condition Nutmeg seed is used in the treatment of Acne. As hair follicles get blocked with dead skin cells and skin oil, acne vulgaris, a chronic skin condition, develops. Blackheads, whiteheads, pimples, and oily skin are signs of acne, which can also leave scars. It is unknown what function nutrition plays in this because neither sunshine nor cleanliness seems to help. Diet may have a role, but neither cleanliness nor sunshine seems to be contributors. If people are given a safe alternative to chemicalbased cosmetics, they will accept it like anything. By doing this, individuals can safeguard their attractiveness from being wrecked by hyper pigmentation and deformity.

Scientific classification

Kingdom: Planate Class: Magnolipsida Order: Magnoliales Family: Myristicaceae Subfamily: Myristicaceae Genus: Myristica Species: A. Vera

Pharmacological Property of Nutmeg

- Antioxidant Activity
- Anti-Inflammatory and Analgesic Activity
- Antimicrobial Activity

PROFILE OF VIGNA RADIATA SEED:-

In Asia, the mung bean (Vigna radiata) is grown for its sprouts and edible seeds. It is a warm-season legume with a rapid growth rate that may be seeded in the fall or summer and matures quickly in tropical and subtropical environments. Its chemical components include phenols, amino acids and flavonoids. It is believed to have been brought to southern and eastern Asia from the Indian subcontinent. Figure No.3 Vigna Radiata

Scientific classification

Kingdom: Planate Class: Tracheophytes Order: Fabales

Family: Fabaceae Subfamily: Faboideae Genus: Vigna Species: V.radiata Pharmacological Property of Vigna Radiata

- Antioxidant
- Anti-inflammatory
- Antimicrobial.



II. MATERIAL AND METHODS:

Extraction of Nutmeg & Vigna Radiata:-

Aloevera leaves were picked, split along the centre, and the gel was extracted 500g of fresh nutmegs were purchased at a nearby market air dried and then ground in an electric blender. The powder was heated with distilled water. After filtering through Whatmann No. 40 filter paper, the extract was gradually heated and continuously stirred in a water bath until dry. The left over

dark brown material was recovered and used for analysis. A conical flask that was five times as full of a 1:1 water-ethanol mixture was filled with the necessary amounts of herbal medicines one at a time after being metered out. The mixture was allowed to come to a boil on a water bath under reflux conditions for approximately 30 minutes. Filtered components were taken out, and the remaining ingredients went through a second five-Times-volume boil A 1:1 mixture of water and ethanol was heated in a Water bath with reflux for around 15 minutes. After content was removed through filtering, filters were combined. The filtrate was left to evaporate. In the evaporating pan until the required extract concentration was reached. The Vigna radiata seeds were collected and finely milled. About 500gm of the powdered Vigna radiata were extracted using a Soxhlet device and a hot extraction method using ethanol as the solvent. It was done several times until the



Figure No.4 Extraction of Nutmeg

Thimble's solution was completely clear. The extract was then vacuum desiccated to dry it.

Preparation of Gel:-

Gels are semi-rigid systems in which enough water has been employed to disperse the gelling agent by movement of the dispersing medium. Propylene glycol 400, a plasticizer and humectants, was present in the dispersion. Further excipients, such as methylparaben and propylparaben, were added while stirring continuously. With Triethanolamine, the pH of the vehicle was brought to a neutral state in Carbopol gels (Triethanolamine). The gel's final weight was altered to 50 gram using distilled water. After that the mixture was stirred for two hours at 500 rpm using a propeller. After being agitated, this homogeneous gel appeared bubble-free. The gel was kept at room temperature for 24 hours to evaluate its consistency and stability.

Development of Formulation

Several batches of formulations were produced in accordance with the required amount of gelling agents were precisely weighed, completely mixed in hot, filtered water (not more than 60°C, 50% of the batch size), with air entrapment prevented, and allowed to soak for the entire night. The remaining water was gently heated in order to dissolve the required amount of methyl paraben. The necessary quantity of polyethylene glycol 4000, propylene glycol, and herbal extracts were subsequently added to the aforementioned mixture. This was eventually mixed with the previously soaked gel mixture. Triethanolamine was lastly incorporated into the pH solution. Formulas were produced, put into the proper container, and given the necessary labels.

INGREDIENTS	F1	F2	F3
Aloe barbadensis gel	5ml	5ml	5ml
Nutmeg extract	3ml	3ml	3ml
Vigna radiata extract	1%	1.5%	2%
Carbopol	2%	2%	2%
Propylene glycol	5ml	5ml	5ml
Methyl Paraben	0.15gm	0.15gm	0.15gm
Propyl Paraben	0.30gm	0.30gm	0.30gm
Triethanolamine	5ml	5ml	5ml
Water	q. s	q. s	q. s

Evaluation of Formulations

Physical evaluation:

Physical parameters such as color, appearance and consistency were checked visually.

Wash ability:

Formulations were applied on the skin and then ease and extent of washing with water were checked manually.

PH:

PH of 1% aqueous solution of the formulation was measured by using a calibrated digital pH meter at constant Temperature.

Bloom Strength:

The bloom strength of the gel was determined by means of Texture Analyzer equipped with 5 kg load cell using a cylindrical probe of 0.5 diameters as fixture. The sample in the container was placed centrally on the platform beneath the cylindrical probe. After calibrating the height of the probe, the test was commenced. A trigger force of 10 g was used for the study.

Spread ability:

A sample of 0.5 g of each formula was pressed between two slides and left for about 5 minutes where no more spreading was expected Diameters of spreaded circles were measured in cm and were taken as comparative values for spread ability.

 $S = M \times L / T$

Viscosity:

The viscosity of Antiacne gel was determined by using digital Brook filed viscometer. Measured quantity of Antiacne gel was taken into a beaker and the tip of viscometer was immersed into the gel and viscosity was measured in triplicate

Antimicrobial testing of the prepared formulations:

The screening of antibacterial activity of the extracts against pathogens was performed using disc diffusion method. Nutrient agar media was prepared, sterilized and aseptically spread on three sets of Petri plates which were previously marked as formulation coding. Microorganisms used were Staphylococcus aureus, Escherichia coli. The plates were inoculated with microorganism suspension and incubated at 37°C for 24h. Next day filter paper discs loaded with alcohol based herbal gel and synthetic gel was placed in the respectively marked plates. It was taken care that the sterile discs completely absorb the formulation. After 24 h test results were observed to determine the efficacy of formulations in terms of zone of inhibition of microorganism. Higher the zone of inhibition, the more effective is the test formulation.

Composition of nutrient agar culture media

Ingredients	Quantity (gm/lit)	
Peptic digest of animal tissue	5.0	
Sodium chloride	5.0	
Beef extract	1.5	
Yeast extract	1.5	
Agar	15.0	
Final pH at	25^{0} c 7.4 \pm 0.2	

Preliminary phytochemical screening of Nutmeg & Vigna radiate: ≻

nstituents	Test	End point Of Vigna Radiata	End point of Nutmeg	Results
Flavonoids	Ferric chloride	Green colour	Red Colour	++
T lavonolas	Lead acetate	Yellow precipitate	Yellowish white color	++
Protein	Xanthoprotein	Yellow precipitate	Yellow precipitate	++
	Ninhydrin	Blue color	Blue colour	++
Amino acids	Ninhydrin	Purple colour	Purple colour	++
	Tyrosine	Dark red colour	Dark red colour	++
Dhanal	Ferric chloride	Blue colour	Blue colour	++
Phenol Organic acid	Phosphoric acid	Light yellow precipitate	Light yellow Colour	++

++ Presence of active constituents

≻ Zone of inhibition of the extract

Name of Organism								
	Staph	ylococcus	aureus	Mean	Esch	nerichia co	li	Mean
Vigna Radiata				(in				(in mm)
	1 (mm)	2 (mm)	3 (mm)	mm)	1 (mm)	2 (mm)	3 (mm)	
10µl/ml	13.2	13.4	13.2	13.3±	13.2	13.3	13.2	13.2±
				0.1				0.06
20µl/ml	13.5	13.4	13.4	13.4±	13.4	13.3	13.3	13.3±
				0.03				0.03
30µl/ml	13.5	13.6	13.4	13.5±	13.5	13.5	13.4	13.5±
				0.1				0.03
Nutmeg	2	Staphyloco	occus	Mean	Esch	erichia co	li	Mean
		aureus		(in				(in
				mm)				mm)
	1	2	3		1 (mm)	2	3 (mm)	
	(mm)	(mm)	(mm)			(mm)		
10µl/ml	14.2	14.4	14.2	14.3±	14.2	14.3	14.2	14.2±
				0.1				0.06
20µl/ml	14.5	14.4	14.4	14.4±	14.4	14.3	14.3	14.3±
				0.03				0.03
30µl/ml	14.5	14.6	14.4	14.5±	14.5	14.5	14.4	14.5±
				0.1				0.03

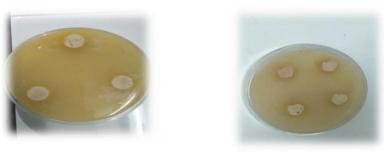


Figure No: 4 Antimicrobial study of Vigna Radiata & Nutmeg against Pathoge

OPTIMIZATION OF GELLING AGENT

Different concentrations of carbopol-940 such as 1, 1.5 and 2% were optimized to obtain gel with desired physical characteristics. Carbopol gel with 2% concentration shows good physicochemical properties for incorporating ethanol extracts of Vigna radiata and Aloe barbadensis.

Formulation of polyherbal gel containing Aloe barbadensis Nutmeg & Vigna Radiata

Polyherbal gel containing Aloe barbadensis Nutmeg & Vigna Radiata was incorporated into optimized 2% Carbopol gel base. Different concentrations of ethanol extract of Vigna radiata such as 1, 1.5 and 2% were incorporated in to Carbopol gel base. Aloe barbadensis concentration was kept constant [5 ml] in all the Carbopol gel base. The formulated polyherbal gel was shown below.



Figure No 5: Image of Formulated of polyherbal gel containing Aloe barbadensis ,Nutmeg &Vigna Radiata

EVALUATION OF POLYHERBAL GEL

Physical appearance - The formulated gel was checked visually for color, appearance and homogeneity.

Sr. No	Physical Appearance	Color	Homogeneity
F1	Transparent Yellow gel	Slightly Yellow	Absence of agglomerates
F2	Transparent Yellow gel	Slightly Yellow	Absence of agglomerates
F3	Transparent Yellow gel	Slightly Yellow	Slightly agglomerates

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Measurement of pH -The pH of all prepared formulation ranged from 5.7-5.9. The pH of the prepared gel formulation was considered to be acceptable to avoid the risk of irritation upon application to the skin.

FORMULATION CODE	рН
F1	5.9
F2	5.7
F3	5.8

Determination of Viscosity

Viscosity is an important property of fluids which describes a liquids resistance to flow and is related to the internal friction within the fluid. This rheological property helps in determining consistency and also the diffusion rate of drug from gel. The measurement of viscosity of the prepared gel was done with Brookfield viscometer with spindle no: 62. By keeping the viscosity below about 15,000 cps the advantages of more appealing cosmetic characteristics and ease of accurate application through improved flow and pour ability are achieved.

Measurement of viscosity

FORMULATION CODE	Viscosity [cps]
F1	1438±0.1
F2	1426±0.75
F3	1359±0.25

Spreadability

Spread ability denotes the extent of area to which the gel readily spreads on application to skin or the affected part. The spreading was expressed in terms of time in seconds taken by two slides to slip off from the gel, 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 dimensions were taken. The gel formulation was placed over one of the slides. Spread ability of different gel formulation was studied. The formulation F2 produced good spread ability than the other formulation.

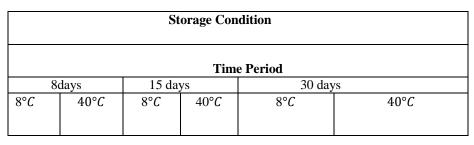
Measurement of spread ability

FORMULATION CODE	SPREADABILITY (gm.cm/sec)
F1	19.37
F2	21.35
F3	22.13

Stability studies

Stability study of different formulations was carried out at storage condition of 8° C and 40° C for a period of one month. Samples were withdrawn at the time interval of 7, 15 and 30 days and the results are tabulated in during the study period, all the formulations [kept at 8° C & 40° C] were found to be homogenous and free from microbial growth which may attribute to the presence of preservatives. There is a slight change of color in F1 formulation and formulation F3 shows bad smell when stored at 40° C at 30 day and pH of the gel was also changed in both F1 and F3 formulation.

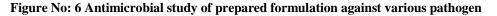
Measurement of stability studies



Antimicrobial activity by Cup plate method

The sterile Petri dishes were filled with Muller Hinton Agar medium which was then inoculated with a suitable dilution of a test organism (Staphylococcus aureus, Escherichia coli and four cylinder or cups were made in the medium with the sterile borer in each plate. The formulated polyherbal gel, standard disc and solvent control were prepared. A uniform amount of 0.2 ml solution was added to the cup and incubated at 37°C for 24 hrs. The well diffusion test was performed in triplicates and antimicrobial activity was expressed as the mean of inhibition in diameter (mm).





IV. DISCUSSION:

The formulation of herbal Antiacne gel was done by incorporating the extract of nutmeg & vigna radiata and Aloevera the gel was prepared. After completion of Antiacne gel it was evaluated for its physicochemical parameters like color, odor, pH, spreadiblity etc. The Antiacne gel was Slightly yellow in color and translucent in appearance and gave smooth on application which mass maintained after tested stability Study.pH also maintained throughout the studies 5.6 - 5.9. Spreadiblity was also measured and found to be less variation with the initially prepared formulations after perform the stability study. The formulation was evaluated for the antimicrobial activity properties against the specified microorganism by using cup plate method according to the zone of inhibition formed result from the herbal Antiacne gel (f2) against different bacterial isolates shows the maximum activity as compared to f1 & f3 respectively and after stability study there were not variation at different temperature. The Antiacne gel was non-irritant upon application on skin.

V. CONCLUSION:

Natural plants are more acceptable in the belief as they are safer which fewer side effects than synthetic ones. Medicinal plants produce a diverse range of bioactive molecules making rich source of different types of medicines. This study targets the chronic skin condition acne with the aim of formulating an effective and safe Polyherbal gel by using Aloe barbadensis. Vigna radiata and nutmeg. The ethanol extract of Aloe barbadensis. Vigna radiata and nutmeg collected Aloe barbadensis gel were incorporated in to optimized Carbopol gel base. The combination of these three herbal constituents may produce an effect to minimize the Acne problem. Antimicrobial study shows that there was no microbial contamination observed and it showed good zone of inhibition results showed that there was no skin lesions like defeating of skin, adverse skin reactions, and local systemic change. All over in this research here it can be concluded that the formulation of polyherbal gel may offer an effective and safe dosage form which leads to patient adherence and compliance to the therapy.

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