DEVELOPMENT AND EVALUATION OF HERBAL VANISHING CREAM

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ABSTRACT
The skin is the largest organ of the body. There are three structural layers for skin - epidermis, dermis, and subcutis. Hair, nails, sebaceous gland, sweat gland and apocrine glands are regarded as derivatives of skin. There are different types of skin problems and the surge for discovering natural products. Skin problem is one of the major problems in both men and women. It may be due to certain hereditary factors, stress, alcohol consumption, environmental factors etc. This study aims to determine the potent skin care activity of herbal plant material, their extraction and to select the safest and effective formulation for their related problem. Crude drugs were obtained from local vendors. plant Curcuma longa (turmeric), Azadirachta indica (Neem) was collected and were carried out and formulated. The present work aimed to formulate herbal vanishing cream having antibacterial properties. Herbal vanishing cream is an o/w type emulsion formulated by using neem and turmeric. Then it is evaluated by using various parameters like appearance, pH, irritancy, smear test, spreadability etc. Antibacterial property of herbal vanishing cream was proved by using microbial assay. Hence it is confirmed that the formulated herbal vanishing cream shows better antibacterial properties, skin moisturizer, sheen effect, daily day creams, moisture locking power etc.

KEYWORDS: polyherbal, crude drugs, ethanolic extract, emulsion, microbial assay, pH, dye test, vanishing cream.

INTRODUCTION
The skin is referred to as the largest part of the body organs and it contains 15% of the total
adult weights. Skin has a surface area of about 2m². Normally the skin is very smooth. However, due to aging and exposure to heat and cold, sunrays, pressure, and abrasion, dust and microbial infection, etc. the smoothness may be lost and skin becomes rougher and thicker. Skin is one of the most readily accessible organs of the human body. There are two kinds of human skin; one that is hair-less such as soles of foot and palms of hand, and the other kind which bears hair and sebaceous glands such as arms and face. It includes gland, hair, nails and they perform many vital functions, protection against physical, chemical, biological assailants, prevention of excess loss of water and thermoregulation. The skin is consisting of three layers that are epidermis, dermis and subcutaneous tissue. The epidermis consisting of constellation of cells known as keratinocytes. Its function is to synthesize keratin which have protective role. The middle layer dermis is made up of structural proteins called collagen and the dermis lying on the subcutaneous tissue or panniculus. It contains small lobes of fat cells.

STRUCTURE OF SKIN
The skin consisting of following layers
 a) Epidermis
 b) Dermal epidermal junction
 c) Epidermal appendages
 d) Dermis
 e) Subcutaneous fat

Figure no: 1 –structure of skin.
a) Epidermis
The epidermis is a stratified, squamous epithelium layer and it contains two types of cells, keratinocytes and dendritic cells. The main cell of the epidermis is the keratinocytes which make up 95% of the total cells present in the epidermis. Thickness of epidermis varies from 0.05 mm to 0.8-1.5 mm. The epidermis is further divided into four distinct layers according to keratinocytes, morphology and position.\(^7\)

1. Basal cell layer (stratum germinativum)
2. Squamous cell layer (stratum spinosum)
3. Granular cell layer (stratum granulosum)
4. Cornified or horny cell layer (stratum corneum)

![Layer of epidermis](image)

**Figure No: 2-layer of epidermis.**

1) basal cell layer
It is the deepest layer of the epidermis, just above the dermis and is composed of a single layer of basal cells. Keratinocytes are produced in this sublayer. Stratum basale forms the boundary to the dermis. It holds approximately 8% of the water in the epidermis. With aging, stratum basale becomes thinner and loses the ability to retain water. Keratinocytes undergo division following differentiation to form melanocytes, which produce the pigment called melanin.

2) Squamous cell layer
It is composed of variety of cells that differ in shape, structure and subcellular properties depending on their location. The spinosa cells are polyhedral in shape and rounded nucleus.
Basal cells, through the process of turn-over, make their shape to flatter and form these layers. These cells are hence called prickle cells which have little spines on the outside of their membrane. The thickness of this sublayer is from 50 to 150 μm.

3) **Granular cell layer**
The basal cells are nucleated, columnar and about 6 μm wide.
In this sublayer, cornification or keratinization of keratinocytes starts. By this process, organelles like nuclei and mitochondria start to resolve. Cells gradually fill with keratin fibers and contain less moisture as compared to basal and prickle cell layers. The shape of these cells becomes much flatter during this process.

4) **Horny layer**
The outermost layer of the skin, the stratum corneum, is responsible for the barrier function of the skin. The stratum corneum is 10-15 μm in thickness which is composed of flattened corneocytes and is surrounded by an extracellular matrix of lipids. Epidermal keratinocytes that undergo a mortaial differentiation to form corneocytes. It consists of different enzymes which helps in maintenance of health. It also helps to regulate the exchange of moisture and oxygen with the external environment.

Nonkeratinocyte cells of the epidermis.
1) Melanocytes
2) Merkel cells
3) Langerhans cells

1) **Melanocyte**
It is a dendritic, pigment synthesizing cells derived from neural crest. It’s functions are,
- Impart the color to the skin and hair
- Protect the skin from uv radiation
- Biochemical neutralization of toxic free radical oxygen derivatives

2) **Merkel cells**
Merkel cells are oval shaped, slow adapting. They are found in the digits, lips, regions of the oral cavity. The high concentration of the merkel cells in certain regions such as fingertips results in smaller and more densely packed receptive fields and thus higher tectile resolution and sensitivity.
3) Langerhans cells
Langerhans cells are involved in a variety of T-cell responses. It constitute 2-8% of the total epidermal cell population. It’s role are, immune process like allergic contact dermatitis, immune tolerance.

b) Dermal epidermal junction
The dermal epidermal junction represents the acellular zone present between dermis and epidermis. It supports the epidermis, establishes cell polarity, direction of growth and direct the organization of cytoskeleton in the basal cells and it provides developmental signals and functions as a semipermeable barrier between layers.

c) Epidermal appendages
The skin adnexa are grouping of ectodermally derived appendages, including eccrine, apocrine glands, ducts and pilosebaceous units that originate as down growth from the epidermis during development. After injury, the adnexal structure capable of re epithelialization.

a) Eccrine sweat gland
Eccrine glands are distributed on the surface of the body.
Principle function is heat control.
b) apocrine gland
They are 10times larger than acrine gland which secrete milky substance.
c) sebaceuos galnd
Its secrete sebum, it constitute most of the fatty layer.
d) hair
Hair has valuable biological function like protection from the elements and distribution of sweat gland products.

d) Dermis
It is a system of fibrous filamentous, amorphous connective tissue. The dermis provides pliability, elasticity, and tensile strength to skin. It protect the body from mechanical injury, binds water, thermoregulation and sensory stimulation.

e) Subcutaneous fat
Subcutaneous is an elastic layer which includes a large amount of fat cells that work as a
shock absorber for blood vessels and nerve endings. The thickness of this layer is 4 to 9 mm on average.

FUNCTIONS OF SKIN

- protection
- thermoregulation
- heat production
- heat loss
- control of body temperature
- activity of sweat glands
- regulation of blood flow through the skin
- formation of vitamin D
- cutaneous sensation
- absorption
- excretion

- **Protection**
  It acts as a barrier against invasion by microorganisms, chemical, physical agents, e.g. mid trauma, UV light, dehydration.
  The pigment melanin offers some protection against harmful UV rays.

- **Thermoregulation**
  Body remains constant at about 36.8 °C which is optimum for enzyme activity.

- **Heat production**
  The body temperature rises when metabolic rate increases and vice versa. The energy released during metabolic activity is in the form of heat and the most active organ produces the most heat. The organs are skeletal muscles, liver and digestive organs.

- **Heat loss**
  The small amount of heat in expired air, urine and faeces. Only the heat loss through the skin can be regulated.

- **Control of body temperature**
  The temperature regulating centre in the hypothalamus is sensitive to the temperature of circulating blood. This centre responds to decreasing the temperature by sending nerve
impulses.

- **Activity of the sweat glands**
  When the body temperature is increased, the sweat glands secrete the sweat onto the skin surface.

- **Regulation of blood flow to the skin**
  The amount of heat loss to the skin depends largely on blood flow through dermal capillaries.

- **Formation of vitamin D**
  7-dehydrocholesterol is a lipid-based substance in the skin. Ultraviolet rays convert it into vitamin D.

- **Cutaneous sensation**
  The dermis that are sensitive to touch, pressure, temperature or pain.

- **Absorption**
  The drugs in transdermal patches, eg: hormone replacement therapy in menopause, nicotine as an aid to stop smoking.

- **Excretion**
  The skin is a minor excretory organ.
  Eg: sodium chloride in sweat, urea when kidney function is impaired, aromatic substances like garlic and other spices.

**DISEASES OF SKIN**

1. **Vitiligo:**
   Vitiligo is a condition in which areas of skin lose their normal pigment and so become white. It is common, and affects about 1% of the world’s population. The pigment that gives your skin its normal colour is melanin, which is made by cells known as melanocytes.

2. **Scabies:**
   Scabies is a common and very itchy skin condition caused by human scabies mites. It can affect people of any age but is most common in the young and the elderly. The parasite that cause scabies are tiny parasites, smaller than a pinhead. The rash of scabies is a mixture of scratch marks and red scaly areas; later it can become infected and develop small pus spots.
3. **Rosacea**: Rosacea is a common rash, found on the central part of the face, usually of a middle aged person. A tendency to flush easily is followed by persistent redness on the cheeks, chin, forehead and nose. The cause of rosacea is not fully understood, but many think that the defect lies in the blood vessels in the skin of the face, which dilate too easily.

4. **Psoriasis**: Psoriasis is a common skin problem affecting about 2% of the population. It occurs equally in men and women, at any age, and tends to come and go unpredictably. It is not infectious, and does not scar the skin.

5. **Melanoma Cutaneous malignant**: melanoma is a cancer of the pigment cells of the skin. If it is treated early, the outlook is usually good. It is not contagious. The word ‘melanoma’ comes from the Greek word ‘melas’, meaning black. Melanin is the dark pigment that gives the skin its natural colour.

6. **Eczema (Atopic Eczema)**: Atopic eczema is an inflammatory condition of the skin. Atopic is the term used to describe conditions such as eczema, asthma, seasonal rhinitis and hay fever, which often have a genetic basis. Eczema is the term used to describe changes in the upper layer of the skin that include redness, blistering, oozing, crusting, scaling, thickening and sometimes pigmentation.

**CREAMS**

Creams are the topical preparations which can be applied on the skin. Creams are defined as “viscous liquid or semi-solid emulsions of either the oil-in-water or water-in-oil type” dosage forms which consistency varies by oil and water. Creams are used in cosmetic for further purposes like cleansing, beautifying, improving appearances, protective or for therapeutic function. These topical formulations are used for the localized effect for the delivery of the drug into the underlying layer of the skin or the mucous membrane. These products are designed to be used topically for the better site specific delivery of the drug into the skin for skin disorders. Creams are considered as a pharmaceutical product as they are prepared based on techniques developed in the pharmaceutical industry; unmedicated and medicated creams are highly used for the treatment of various skin conditions or dermatoses. Creams can be ayurvedic, herbal or allopathic which are used by people according to their needs for their skin conditions. They contain one or more drugs substances dissolved or dispersed in a suitable base. Creams may be classified as o/w or w/o type of emulsion on the basis of phases. The term ‘cream’ has been traditionally applied to semisolid formulated as either...
water-in-oil (e.g.: cold cream) or oil-in-water (e.g.: vanishing cream).[1]

**TYPES OF SKIN CREAMS**

They are divided into two types:

1. **Oil-in-Water (O/W)** creams which are composed of small droplets of oil dispersed in a continuous phase, and an emulsion in which the oil is dispersed as droplets throughout the aqueous phase is termed an oil-in-water (O/W) emulsion.

2. **Water-in-Oil (W/O)** creams which are composed of small droplets of water dispersed in a continuous oily phase. When water is the dispersed phase and an oil the dispersion medium, the emulsion is of the water-in-oil (W/O) type.

**CLASSIFICATION OF CREAMS**

All the skin creams can be classified on different basis:

1. According to function, e.g. cleansing, foundation, massage, etc.
2. According to characteristics properties, e.g. cold creams, vanishing creams, etc.
3. According to the nature or type of emulsion

Based on function

1. Make-up cream (o/w emulsion):
   - Vanishing creams.
   - Foundation creams.
2. Cleansing cream, Cleansing milk, Cleansing lotion (w/o emulsion)
3. Winter cream (w/o emulsion)
   - Cold cream or moisturizing creams.
4. All-purpose cream and general creams
5. Night cream and massage creams
6. Skin protective cream.

**1. Make-up cream:** These are mainly o/w type of emulsion. It is cream-based product which leaves a smooth hydrated finish (either stain matte or luminous) on the skin. It nourishes skin and is basically sweat-resistant and creates a dewy sheen.

   - Vanishing creams: They are called vanishing creams because they seem to disappear when rubbed onto the skin. These formulations are based on stearic acid. After
application, the cream leaves a dry but tacky residual film.

- which also has a drying effect on the skin. Because of this reason, these are used particularly in hot climates which cause perspiration on the skin.

- Foundation creams: These cream serve as a foundation base for make-up. It acts as an adherent base for application of make-up powders. They provide emollient action and a protective action against environment to the skin which is neither too greasy nor too dry. It is multi-coloured make up applied on the face to create an even, uniform colour similar to the complexion, to cover flaws and to change the skin tones.

2. **Cleansing creams**: These creams are used for body cleaning purposes and it is used for personal hygiene and beautification which is important for cosmetics. Cleansing creams or lotions can be used for the removal of make-up, surface grim, oil mainly from the face and neck.

3. **Winter creams**: These are w/o type of formulation and in this formulation oil content will be more than water content. These creams are mainly used for chapped and dry skin.
   - Cold cream: It is known as moisturizer or moisturizing cream. Cold cream must have an emollient action. It should produce a cooling sensation in use and the oil film on the skin should be non-occlusive.

4. **All purpose creams and general creams**: These creams are used more nowadays than before. These creams are oily but non-greasy type and can spread on the skin easily. This can also be used as a night creams, nourishing creams, protective creams for prevention or alleviation of sunburns or for the treatment of roughened skin areas.

5. **Night cream or massage creams**: These creams are mainly used for the nourishing the skin or as a treatment to dry skin. Creams which are generally applied on skin and left for few or several hours over night are mainly known as night creams. Creams which acts as an emollient by rubbing the cream on the skin with massage is known as massage cream.

6. **Skin protective creams**: These creams are smooth, thick bodied creams formulated to provide an invisible, uniform protective film barrier to the skin. It helps to maintain the barrier between the skin and contaminants that may irritate the skin (contact dermatitis and occupational dermatitis). Strengthens the natural properties of the skin and maintains the balance of normal to combination skin.
7. **Hand and body creams**:- Hands are one of the first places to show signs of aging. We tend to wash our hand several times a day, stripping off moisture. Applying cream softens and protects the skin and it keeps the skin looks younger. Since the skin on our palms and fingers needs oil to stay supple and to prevent it from chapping and cracking, it is sensible to use hand creams that puts plenty of oil back in. It is used on the hands more than other parts of the body.

**GENERAL INGREDIENTS USED IN SKIN CREAMS**

The raw materials which are used in a manufacturing of skin creams include:

1. **Water**: This is the most important and widely used raw material in any cream formulation. These are the cheapest and easily available. In skin creams, water is used as solvent to dissolve other ingredients of creams. Water, which is free of any toxins, pollutants, microbes, etc. is used in preparation of creams.

2. **Oil, fats and waxes**: Oil, fats and waxes and derivatives there form comprise an essential portion of creams. Waxes act as an emulsifier, fats act as a thickener and oil act as a perfuming agent, preservative, etc. according to its function. Oil may be two types’ mineral and glyceride.

3. **Mineral oil**: Mineral oil consists of hydrocarbons derived from petroleum oil. Mineral oil rarely causes allergic reactions and it cannot become solid and clog pores of the skin. It is light weight and inexpensive, it helps to reduce water loss from the body and keeps body moisturized. Examples: Light liquid paraffin, Heavy liquid paraffin, Liquid petroleum.

4. **Glyceride oil**: Glyceride oil is mostly vegetable oils. Examples of glyceride oils are almond oil, arachis oil, castor oil, coconut oil, olive oil etc.

5. **Vegetable oil**: Form a barrier on the surface of the skin and slow down the loss of water, helping to maintain plumpness of skin. Vegetable oils may also be used to increase the thickness of the lipid or oil portion of cream or personal care products. E.g. Almond oil, germ oil, avocado oil, sunflower oil etc.

6. **Waxes**: Which are used in preparation of cream includes beeswax, carnauba wax, cerasin, spermaceti, etc. Waxes are used in cosmetics because it helps to keep an emulsion from separation of oil and liquid components. also increase the thickness of the lipid portion and sticks on the surface of the skin.
**7. Fats:** Different types of fats are used in the preparation of creams. These materials can be obtained from animals, plants or mineral origin. Glyceride oils and fats may be of animals or vegetable origin. The most common of these fatty acid are lauric, margaric, plamitic, stearic etc.

**8. Lanolin:** It is derived from wool fat of a sheep. Lanolin are of two types- the hydrous lanolin contains between 25%- 30% water. Anhydrous lanolin has point of 38°C-42°C and has a slight odour. These ingredients act as a lubricant on the skin surface, which gives the skin soft and smooth appearance. Lanolin helps to form emulsion and blends well with other substances used in cosmetic and personal care products.

**9. Colours:** Before the development of the modern technology, colours primarily came from substances found in nature such as turmeric, saffron, indigo, etc.

**10. Emollients:** Emollients, also commonly referred to as moisturizers, are products that help to soften skin or to treat skin that has become dry. Most emollients are forms of oil or grease, such as mineral oil, squalene, and lanolin.

**11. Humectants:** These are important multi-functional ingredients found in most skin care formulations. Humectants are hydroscopic organic compounds. These are the materials that can absorb or retain moisture. These has many benefits such as moisturization, exfoliation, etc. Examples of humectant are glycerin, Hydroxyethyl urea, betaine, sodium PCA, Sodium-L-Lactate, etc.

**12. Perfumes:** Perfume is a substance that imparts a scent or order, including a sweet and pleasant smell. Examples of natural perfumes used in creams are- White Blossoms: Rosy Dreams Orange Blossom.

**13. Vitamins:** Vitamins plays an important role in maintaining the physiological function of whole body and the skin. Vitamin A, B, C, E etc. are generally used in formulation of the creams.

**14. Preservatives:** The use of preservatives in cosmetics is essential to prevent alteration caused by microorganism and contamination during formulation, shipment, storage and consumer use.
15. **Antioxidants**: It can be used to protect alteration caused by exposure to oxygen.[1]

**VANISHING CREAM**

**Definition**
They are oil in water type of emulsions. When applied on the surface of the skin, they spread as thin oil less film which is not visible to the naked eye. Hence, they are called as vanishing creams.

It is prepared by the process called emulsification of stearic acid and water by using alkalies like potassium hydroxide borax, sodium hydroxide etc. Stearic acid is one of the main ingredients in vanishing cream which provides a pearly white shine to the cream. Vanishing cream when formulated by using herbal extract of turmeric and neem gives more action and less side effects.

**Advantages**
- It is used as a skin moisturiser and cleanser
- It is helpful for skin softening and providing shiny texture to the skin
- It is used as a base on the skin before the application of any other cosmetics because it vanishes from skin surface once applied
- It is used to remove pimple and scares
- It is helpful to preventing skin chapping or roughening

**Disadvantages**
- Vanishing creams are not to be used all the time
- It does not remove scars, not meant to be used as complexing and Anita cream
- It should be removed or washed of when not intended to be kept on the skin
- These products have stability issues and hence should be stored at prescribed conditions.
- It might cause skin allergic reaction-itching, peeling, Irritation, reddening due to the presence of compound in the cream.

**Applications**
- Sheen effect
  One characteristic due to which these vanishing creams are proffered is the' sheen effect'. Rather than giving a caked look to the face, they give a natural attractive sheen to the skin.
- Daily day creams
Vanishing cream has the advantage of being non greasy which makes them suitable for use during the day and by women with oily skin. In addition to keeping powder on the face, they also protect the skin from the elements such as chapping winds and dry breezes.

- Moisture locking power.

The magical quality of vanishing cream that makes it a choice as daily cream is the moisture locking power. As many women know that moisture is the key element of healthy skin, the mystical power of vanishing cream still eludes some women. It is equally effective for normal and oily skin types.

REVIEW OF LITERATURE

1. Seema y mendhekar et al (2017)- formulation and evaluation of polyherbal vanishing cream. Herbal vanishing cream was prepared by using mixture of alcoholic extract of crude drugs. It was prepared by using oil in water type of emulsion. This formulation prepared by using crude drug including peppermint, green tea leaves, clove bud, ginger, Mustard, turmeric, cinnamon, cumin, nutmeg, drumstick bark. She concluded that the prepared herbal cream have best properties and having nutritional value. It also shows the antioxidant and antibacterial activity due to this it's retard aging signs and pimple formation on the face. By combining all the three ingredients it can be concluded that this cream can be used as a multipurpose cream and the ingredients mixed can produce synergistic effect of other.[3]

2. Shinde prayakta et al(2020)- formulation and evaluation of vanishing herbal Cream of crude drugs. The plant material is collected and identified and prepared using alcoholic extract of crude drugs including c offinale, turmeric, nutmeg, cinnamon. In this preparation steps include preparation of alcoholic extract of crude drugs, prepare the all phase, aqueous phase and addition of aqueous phase to oil phase. This vanishing cream are evaluated for physicochemical property and antibacterial activity and stability studies are also performed. The cream was used for Antibacterial and antioxidant property.[2]

3. Ravirajsinh Gohli et al(2021) The main of research poly herbal vanishing cream and evaluate basis various evaluation parameter polyherbal vanishing cream were formulate various medicinal properties. Poly herbal vanishing cream formulated natural ingredients like mentha, clove, linseed, liquourice green tea, orange peel along with synthetic ingredients. evaluation of formulation F1&F2 were done on different parameter pH, stability etc.[26]
4. Bhavana D Tambe et al (2021) Formulation and evaluation of vanishing herbal cream of crude drugs. o/w emulsion based cream was formulated using natural ingredients and was evaluated. By combining all these ingredients it can be concluded that this cream can be used as multipurpose cream and the ingredients mixed can produce synergistic effect of the other.

This work focuses on the potential of herbal extracts for cosmetic purposes and the use of bioactive ingredients. Cosmetics potentiate biological functions of the skin and provide nutrients essential for healthy skin or hair.\cite{4}

5. Chetana V Bhugadikattikar et al (2020) photochemical screening and preparation of vanishing cream from leaves of Acacia nilotica. Acacia leaves having antibacterial activity. The study was carried out on anti microbial activity for final formulation of prepared vanishing cream and photochemical screening of ethanolic extract, aqueous extract, acetone extract of Acacia nilotica. The study was focused on activity of acacia plant extract in the form of cream as anti microbial action on organism, although it shown good zone of inhibition by spreading extract formulation through media and inhibit organism activity that the acacia has a better anti microbial activity. Acacia nilotica contain some medicinal properties, such as antioxidant, antifungal, anti microbial and antiviral properties.\cite{5}


The prepared formulation is devoid of any phase separation activity, showed good Spreadability and consistency during the entire study period. These studies suggest that the polyherbal composition of the extract and base of vanishing cream are stable and safe without side effects due to the presence of many natural compounds.\cite{6}

7. Richard Lobo et al (2020) formulation and evaluation of antiseptic activity of the herbal cream containing Curcuma longa and Tea tree oil. The cream was prepared by using vanishing cream base incorporating the Curcuma longa 1% ethanolic extract and tea tree oil 5%. The prepared cream was subjected for anti microbial screening using various skin pathogens by punch well method. The prepared cream showed significant activity against all the pathogens used in the test measured by zone of inhibition.\cite{15}
8. Ashwini S Dhase et al (2014) formulation and evaluation of vanishing herbal cream of crude drugs. The purpose of the research work was to formulate and evaluate vanishing herbal cream. This cream shows fairness to skin. The prepared herbal cream has antioxidant and antibacterial activity. This cream have best properties and nutritional values.\textsuperscript{[31]}

9. R.E Ugandar et al (2016) formulation and evaluation of natural palm oil based vanishing cream. Vanishing cream prepared by using natural base from palm Oil and palm kern oil and standard vanishing cream using stearic acid were prepared. The cream were oil in water type of emulsion containing suitable combination of oil phase and aqueous phase along with preservative. This study concluded that the vanishing cream with natural palm oil base will be useful as skin moisturizer when compared with other preparation. From the result it concluded that natural palm oil base can be used as base for the preparation of vanishing cream. The prepared vanishing cream with natural palm oil base was please T, easily washable with good spreadability, and extrudability there by increase patient compliance.\textsuperscript{[13]}

10. L.V vigneshwaran et al (2022) Formulation and evaluation of herbal face cream with green tea extract. The purpose of the work are to formulate and evaluate the herbal face cream with green tea extract, turmeric, aloe Vera gel as a skin toner. The herbal face cream was prepared by oil in water type of emulsion using mixture of alcoholic extract of crude drugs.

11. Kamal shindae et al (2017) Formulation and evaluation of poly herbal vanishing plus fairnes expert cream. herbal vanishing creams offer several advantages over other creams majority cream are synthetic orgin have several side effect itching or several allergic reaction.

12. Pooja Giradhkar et al (2021). The poly herbal cream is ready bu utilisation following that are curcuma loga, solanum lyco perisum, carica papaya, rose oil, olive oil, almond oil, refined water.

13. Ashish aswal et al (2013). Prepare & evaluation of polyherbal cosmetic cream. The studies that composition of extracts and base of cream F6 & F7 are more stable and it more synergic action and safe to use for skin.\textsuperscript{[16]}

14. Suchith.S.Nair et al (2012) Formulation and evaluation herbal cream containing curcuma longa in this study cream were formulated on the antioxidant potential of herbal extract so it is evaluation.\textsuperscript{[53]}
15. R.Lobo.k.prashu et al (2011) Formulation cream showed excellent microbial activity against pathogen used in this test incling MRSA & styphylococcus aureus.

16. R.Ravinchandra et al (2013) Comparison of physical characteristerstics of vanishing cream base cow ghee and shata- dhauta ghrita as per pharmacopeial standarsds. It found that while cow ghee & vanishing cream base had comparable characteristics, SDG exhibit less degree of unsaturation suggesting better physico chemical stability & better constituency (and hence suitable for topical application) and it is suggested that SDG might be potential candidate as a base for topical preparation.\textsuperscript{[12]}

17. V sarovar reddy et al( 2021) Formulation and evaluation of analgesic vanishing cream. the purpse of research to formulate evaluate analgesic vanishing cream. Prepared analgesic cream was evaluated the physical parameter and in vitro study determined. Prepared analgesic vanishing cream was pleasant, easily washable and avoid patient compliance.\textsuperscript{[54]}

18. Chauhan Lalita et al(2020) Cream are consider an important part of cosmetic product as topical preparation. In this review focused on the use of topical drug delivery system pharmaceutical cream for wound healing with detailed discussion relating to the wound healing proc ss, suitable method of preparation of cream, their classification based on their function, their advantages and disadvantages, characteristics and various types of creams. ingredients used in the formulation of cream there various evaluation parameter.

19. Mamilappalli vani et al (2020) This study aims at formulation polyherbal vanishing cream and face wash, and determined the total flavonoid content of herbs used in the evaluation of formulation for various physical parameter followed by antibacterial and antifungal activity. The total flavonoid content of herbs used determined followed by evaluation of various physical parameters such as pH, viscosity, spreadability compare with marketed formulation. Further formulation where tested against gram positive S.aureus, E.coli, Albicans where common association of acne and cosmetic application.

20. Kuntal Das et al (2012) Herbal vanishing cream based the drug formulation (2.5% and 5.0%) where designed and prepared for skin moisturiser with Stevia white extract. stability studies of cre is carried out at different temperature for the period of 3months as per ICH guidelines and results. reveal the both formulation shows good stability over control
preparation. The formulation studied for primary skin irritation test in rabbit, 30 number of human volunteers for 24 hours. observed skin rashes, inflammation and redness portion.

21. Wegdain Aly Shehta et al(2020) The study performed to isolate curcumin from curcuma longa, formulating curcumine containing moisturizing condition creams. herbal cream was prepared using primary emulsion method to ensure the stability and prevent incompatibility. The result of the evaluation of different physicochemical property where found good and matching with standard value.

22. A premkumar et al (2014) A Novel Cream formulation consisting combination of miconazole nitrate, mupirocin, and hydrocortisone was prepared. The formulation was subjected to in vitro diffusion studies. Microbiological studies and invivo skin irritation studies were performed to find out the safety of materials used in the formulation. The development of cream consisting of miconazole nitrate, mupirocin, and hydrocortisone was found safe and effective for treatment of skin infection.

23. Hardik SK et al(2011) The purpose of phase 3 clinical trial was to evaluate the safety and efficacy of polyherbal fairness cream. He concluded that fairness cream improves complexion, nourishes and makes the skin soft, and ensures ease of application and faster absorption, which provides better nourishment to skin. Therefore, it may be concluded that the polyherbal fairness cream is safe and efficacious.

24. Rhaghavamma S.T.V et al(2014) In the present study the total antioxidant potential was determined. From above results, it was concluded that on combining the extracts of Punica granatum leaves and Daucus carota different components in differ ratio to get multipurpose effects such as whitening, antiwrinkle, antiaging and sunscreen effects on skin.

AIM AND OBJECTIVE
- The aim of the work is, formulation and evaluation of herbal vanishing cream.
- The neem and turmeric extract are naturally obtained, chances of side effects is less.
- Neem and turmeric are abundantly available in surrounding and it shows various medicinal properties.
- The dried neem and turmeric extract have high antibacterial property.
PLAN OF THE WORK
The present design, development and evaluation of antibacterial activity on herbal vanishing cream. The study was proposed to carry out in the following study.
1. Collection of neem and turmeric
2. Preparation of dry neem and turmeric extract
3. Preparation of aqueous and oily phase
4. Addition of aqueous phase to oil phase
5. Evaluation of herbal vanishing cream-pH, viscosity, spreadability, skin irritation test, homogeneity, smear test, dilution test, solubility test etc.

MATERIALS AND METHODS
Table no 1: General ingredients.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Uses</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Main Ingredient Eg:-stearic acid</td>
<td>It controls the cream consistency and imparts pearlescent property by forming crystals</td>
</tr>
<tr>
<td>2) Humectants Eg:- Glycerin, sorbitol,</td>
<td>It prevents excessive drying of the cream</td>
</tr>
<tr>
<td>3) Alkalis Eg:-a) Potassium hydroxide</td>
<td>It imparts fine texture and consistency without providing harshness</td>
</tr>
<tr>
<td>b) Sodium hydroxide</td>
<td>It is used with potassium hydroxide, since if used alone it makes the cream hard</td>
</tr>
<tr>
<td>c) Carbonates, ie potassium and sodium carbonate</td>
<td>They are used because they liberate carbon dioxide and makes the skin spongy</td>
</tr>
<tr>
<td>d) Ammonia</td>
<td>It is effective, but difficult to handle. Because of it’s odour and volatility. It is also makes cream yellow in color with age.</td>
</tr>
<tr>
<td>e) Borax</td>
<td>It is used with potassium hydroxide to produce white emulsion.</td>
</tr>
<tr>
<td>4) Emulsifying agents Eg:-Triethanolamine soap, amino glycol soap or glyceryl monostearate</td>
<td>These provide less luster.</td>
</tr>
</tbody>
</table>
5) Purified water (i.e, Distilled and Deionised)  

It provides stability to the cream. If hard water is used, it forms soap of lime and magnesium, which causes inversion of emulsion and hence reduces stability.

6) Preservatives  
Eg:- Methyl paraben, Propyl paraben  

They prevent deterioration caused by bacteria and fungi.

7) Perfume, i.e, Perfume solvent or Perfume dissolved in alcohol. They should be added when the cream attains 40 Celsius temperature.  
Eg:- Geranium, sandalwood, lavender oil, terpineol etc  

It provides odour to the cream and also has aesthetic value.

<table>
<thead>
<tr>
<th>SL.NO</th>
<th>HERB</th>
<th>BOTANICAL NAME</th>
<th>FAMILY</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Neem</td>
<td><em>Azadirachta indica</em></td>
<td><em>Meliaceae</em></td>
</tr>
<tr>
<td>2.</td>
<td>Turmeric</td>
<td><em>Curcuma longa</em></td>
<td><em>Zingiberaceae</em></td>
</tr>
</tbody>
</table>

**Table no. 2: List of herbs.**

**NEEM**

Figure No. 3-fresh neem leaves.  
Figure No. 4-Dry neem leaves.

**Botanical description**  
Botanical name of Neem, *Azadirachta Indica* also called Nim or Margosa belonging to the family *Meliaceae*.

**Geographical distribution**  
Neem species are probably native to India subcontinent and to dry areas throughout South Asia. It has been introduced to parts of Africa, the Caribbean and numerous countries in
South and Central America.

**Morphology**

Colour – vibrant green  
Taste-bitter  
Part used-whole plant  
Surface characteristics - smooth and glossy with sharp serrated.  
Shape-elongated to edges in shape

**Characterestics**

Neem is a large glabrous tree, 10-20 m high with a straight trunk and long spreading branches. Leaves are imparipinnate, alternate, 3-6cm long on long slender petioles; leaflets 7-17; alternate or opposite, very shortly stalked, 1-1.5cm long, ovate-lanceolate, attenuate at the apex, unequal at the base, the upper half much longer than the lower and the leaflet falcate, coarsely and bluntly serrate, smooth and dark green. Odour is typical and taste is bitter.

The fruit is an ovoid, bluntly pointed, smooth drupe, green when young and unripe, yellow to brown when mature and ripe, with a very scanty pulp and hard bony endocarp. The seed is solitary with a thick testa and embryo with foliaceous cotyledons in the axis of scanty endosperm.

The seeds contain fixed acrid bitter oil(23-31%), deep greenish-yellow to brown in colour, extracted from the seeds by pressure, sp. gr.0.91; soluble in ether, chloroform; practically insoluble in alcohol and water, odour of garlic, bitter taste.

**Uses**

antifungal, antiprotozoal, anti allergic, dermatological diseases, antibacterial, antiviral, dental diseases.[8,9]
TURMERIC

![Figure No. 5-Fresh turmeric.](image1) ![Figure No. 6-Dry turmeric.](image2)

**Botanical description**

Botanical name of turmeric is *curcuma longa* perennial herbaceous plant of ginger family *zingiberaceae*.

**Geographical distribution**

Curcumias are perennial plants native to Southern Asia. They grow in warm humid climate and thrive only in temperature above 60°F India, Sri Lanka, the east India, Fiji and Queensland all have climates that are conducive to growing turmeric.

**Morphology**

Young rhizomes - pale yellow to brown orange
Old rhizomes - scaly and brown
Taste-pungent bitter to taste
Part used-rhizome
Surface characteristics-soft and fine
Shape – cylindrical

**Characteristics**

The primary rhizomes are ovate or pear shaped, oblong or cylindrical and often short breached. The rhizomes are known as bulb or round turmeric. The secondary, more cylindrical, lateral breached, tapering on both ends, rhizomes are 4-7cm long and 1-1.5 cm wide and called as fingers. The bulbous and fingers shaped parts are separated and the long figures are broken in to convenient bits. They are freed from adhering dirt and fibrous root
and subjected to curing and polishing process. The curing consists of cooking the rhizomes along with few leaves in water until they become soft. The cooked rhizomes are cooled, dried in open air with intermittent turning over and rubbed on a rough surface. Colour is deep yellow to orange, with root scar of leaf base. Fracture is horny and the cut surface is waxy and resinous in appearance. Outer surface is deep yellow to brown and longitudally wrinkled. Taste is aromatic, pungent, and bitter odour is distinct.

**Uses**

Turmeric is used as a condiment or spice and colouring agent, especially for ointments and creams. Chemically, it is used for the detection of boric acid. It is antiseptic and anti-inflammatory too. Curcumin is also a powerful antioxidant.[9]

**Table No. 3: List of chemicals and manufactures.**

<table>
<thead>
<tr>
<th>SL. NO</th>
<th>CHEMICALS</th>
<th>MANUFACTURE/SUPPLY</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Stearic Acid</td>
<td>Isocom Laboratories, Kannur</td>
</tr>
<tr>
<td>2.</td>
<td>Potassium Hydroxide</td>
<td>Medilise Chemicals, Kannur</td>
</tr>
<tr>
<td>3.</td>
<td>Glycerine</td>
<td>Isocom Laboratories, Kochi</td>
</tr>
<tr>
<td>4.</td>
<td>Methylparaben</td>
<td>Himedia Laboratories pvt Ltd, Mumbai</td>
</tr>
<tr>
<td>5.</td>
<td>Amaranth (Dye)</td>
<td>Nice Chemicals pvt Ltd, Kochi</td>
</tr>
<tr>
<td>6.</td>
<td>Perfume</td>
<td>Dabur India Ltd, New Delhi</td>
</tr>
</tbody>
</table>

**FORMULATION**

**Table No. 4: Formula for 20g of herbal vanishing cream.**

<table>
<thead>
<tr>
<th>SL NO</th>
<th>INGREDIENT</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Stearic acid</td>
<td>3g</td>
<td>3g</td>
<td>3.2g</td>
<td>2.9g</td>
<td>3.4g</td>
</tr>
<tr>
<td>2</td>
<td>Sodium hydroxide</td>
<td>0.2g</td>
<td>0.27g</td>
<td>0.15g</td>
<td>0.25g</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Potassium hydroxide</td>
<td>-</td>
<td>-</td>
<td>0.17g</td>
<td>-</td>
<td>0.1g</td>
</tr>
<tr>
<td>4</td>
<td>Glycerine</td>
<td>2g</td>
<td>1g</td>
<td>2g</td>
<td>-</td>
<td>1.2g</td>
</tr>
<tr>
<td>5</td>
<td>Water</td>
<td>14ml</td>
<td>14ml</td>
<td>13ml</td>
<td>15ml</td>
<td>14.2ml</td>
</tr>
<tr>
<td>6</td>
<td>Methyl paraben</td>
<td>-</td>
<td>1g</td>
<td>0.1g</td>
<td>-</td>
<td>0.25g</td>
</tr>
<tr>
<td>7</td>
<td>Propyl paraben</td>
<td>-</td>
<td>0.23g</td>
<td>-</td>
<td>0.5g</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Ammonia</td>
<td>-</td>
<td>1g</td>
<td>1.2g</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>Sodium carbonate</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1g</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>Perfume</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>q.s</td>
<td>q.s</td>
</tr>
<tr>
<td>11</td>
<td>Neem extract</td>
<td>0.8g</td>
<td>-</td>
<td>0.18g</td>
<td>0.2g</td>
<td>0.48g</td>
</tr>
<tr>
<td>12</td>
<td>Turmeric extract</td>
<td>-</td>
<td>0.5g</td>
<td>-</td>
<td>0.15g</td>
<td>0.39g</td>
</tr>
</tbody>
</table>
PROCEDURE
Firstly collect fresh neem leaves and turmeric from local nearby. After collection, it is washed and dried in the sun shade. Then it is separately powdered and ethanolic extract was prepared from it.

PREPARATION OF ETHANOLIC EXTRACT OF NEEM AND TURMERIC
Put the powder in to iodine flask, macerate in ethanol for 24 hours with a shaking for 6 hours interval. After 24 hours, ethanolic solution filtered. The filtered solution is heated to water bath at 50°C till a concentrated solution was prepared.

FORMULATION OF VANISHING CREAM
1) Preparation of oil phase
Stearic acid was taken in to one porcelain dish and melt up to 70°.

2) Preparation of aqueous phase
Dissolve KOH, glycerine and preservatives in a porcelain dish and heated this mixture at 70°C.

3) Addition of aqueous phase to oil phase
The aqueous phase is added to the oil phase with continuous stirring at 70°C. At the same time boiled neem extract is added to the above preparation, perfume and preservatives are added to the preparation when it attains 40°C.¹³¹

STORAGE
- Stored in a well closed container at a temperature not exceeds 25°C
- Stored in a cool place

LABELLING
Label should indicate the following:
- Name of the ingredients
- Quantity
- Net content
- Batch
- Mfg date
- Exp date
- Manufactured by
• Marketed by
• Storage condition
• Direction (for external use only)
• Special instructions if any

MICROBIAL ASSAY
The antibacterial activity of herbal vanishing cream was evaluated against agar well diffusion method.\textsuperscript{[5,10]}

Steps
1) Preparation of bacterial suspension
2) Preparation of nutrient agar medium
3) Addition of bacterial suspension to agar media
4) Making well on agar medium and add prepared herbal vanishing cream
5) Kept it 37 degree celsius for 24 hours in an incubator
6) Observe the zone of inhibition

EVALUATION TEST FOR CREAMS
It is necessary to evaluate the effectiveness of the skin. Evaluation tests are following.\textsuperscript{[2,3,4]}

A) **pH TEST**
The most common method for determination of pH is done by using a pH meter.

Procedure
pH meter was calibrated and measured the pH by placing the beaker containing 20 mg of cream.

B) **SPREADABILITY TEST**
The cream should spread easily without too much drag and should not produce greater friction in the rubbing process. Spreadability was calculated using Spreadability apparatus.

Procedure
Take two slides and 500 mg of cream was placed in between them. On the upper side put 100g. Remove the weight and scrap out the extra formulation. Fix the lower side on board of the apparatus and the upper side with a non flexing string on which—g weight was applied. Note down the time taken by the upper slide to slip off.

\[ S = m \times l / t \]
Where,

S - Spreadability
m - Weight tied to upper slide
l - Length moved on a glass slide
t - Time taken

The determinations done in triplicate and record the average of three readings.

C) DYE TEST

It is done to find whether the emulsion is o/w type or w/o type.

In this test an emulsion is mixed with a water soluble dye (Amaranth and observed under the microscope.

If the dispersion phase appears in red coloured globules - the cream was o/w type of emulsion, if the continuous phase appears red colour the cream was w/o type of emulsion.

![Dye test](image)

**Figure No. 7-Dye test.**

D) APPEARANCE

Test was carried out by physical testing. Appearance of the cream was observed by identifying its colour and opacity.

E) IRRITANCY TEST (skin irritation test)

It is an in vitro non animal test designed to identify those chemicals and mixtures capable of inducing moderate skin irritation.

**Procedure**

Cream was applied on the left hand and dorsal side surface and observed at equal time intervals up to 24 hours for irritancy, redness, edema etc.
F) HOMOGENECITY
The test was done by physical touch with hands.

G) SMEAR TEST
Procedure
The test was conducted after the application of cream on the skin the smear formed was oily or aqueous in nature.

H) DILUTION TEST
Procedure
In this test emulsion is diluted either with oil or water
If the emulsion is oil in water type and it diluted with water it will remain stable as water is the dispersion medium but if it diluted with oil, the emulsion will break as oil and water are not miscible with each other. oil in water easily be diluted with aqueous solvent, whereas water in oil type can be diluted with only liquids.

I) REMOVAL
The removal of the cream applied on the skin was done by washing under tape water with minimal force to remove the cream.

J) MOISTURE ABSORPTION TEST
Procedure
About 50 mg of cream was taken on a watch glass.
A beaker was taken with full of water
kept in a desiccator without adsorbents.

Watch glass with cream was introduced into the desiccator.

left for 24 hrs

RESULT AND DISCUSSION
Herbal vanishing cream was prepared and evaluated, on the basis of general characteristics and physical parameters.

Table No. 5: General characteristics of herbal vanishing cream.

<table>
<thead>
<tr>
<th>FORMULATION</th>
<th>COLOUR</th>
<th>OBSERVATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>White</td>
<td>Make the cream hard, prone to deterioration</td>
</tr>
<tr>
<td>F2</td>
<td>Pale yellow</td>
<td>Bad odour</td>
</tr>
<tr>
<td>F3</td>
<td>Pale green</td>
<td>Bad odour</td>
</tr>
<tr>
<td>F4</td>
<td>Yellowish white</td>
<td>Drying of the cream</td>
</tr>
<tr>
<td>F5</td>
<td>Yellowish white</td>
<td>Good consistency and texture</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Imparts pearlescent property</td>
</tr>
</tbody>
</table>

This result shows that F5 has good properties like consistency, texture, appearance. So further studies of F5 are carried out. The following are,

Table No. 6: Physical parameters.

<table>
<thead>
<tr>
<th>SL.NO</th>
<th>PARAMETERS</th>
<th>RESULT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Appearance</td>
<td>yellowish white colour</td>
</tr>
<tr>
<td>2.</td>
<td>Homogeneity</td>
<td>Homogeneous Smooth and consistent</td>
</tr>
<tr>
<td></td>
<td>● By visual</td>
<td></td>
</tr>
<tr>
<td></td>
<td>● By touch</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>Irritancy</td>
<td>No redness and edema</td>
</tr>
<tr>
<td>4.</td>
<td>Dye Test</td>
<td>Oil in water type</td>
</tr>
<tr>
<td>5.</td>
<td>pH</td>
<td>6.7</td>
</tr>
<tr>
<td>6.</td>
<td>Smear Test</td>
<td>Non greasy</td>
</tr>
<tr>
<td>7.</td>
<td>Removal</td>
<td>Easily removed by water</td>
</tr>
<tr>
<td>8.</td>
<td>Emolliency</td>
<td>No residual effect</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>9.</td>
<td>Dilution Test</td>
<td>Oil in water type emulsion</td>
</tr>
<tr>
<td>10.</td>
<td>Spreadability</td>
<td>Uniform with a value of 42 g.cm/sec Easily spreadable</td>
</tr>
<tr>
<td>11</td>
<td>Consistency</td>
<td>Good</td>
</tr>
<tr>
<td>12</td>
<td>Moisture absorption test</td>
<td></td>
</tr>
</tbody>
</table>

The herbal vanishing cream was prepared using mixture of neem and turmeric and the ethanolic extract were used and formulated 5 different formulations named F1,F2,F3,F4 and F5. Among these formulations F5 shows best result and other test were carried out and evaluated the antibacterial activity and found the zone of inhibition.

**Figure No. 9-Herbal Vanishing Cream.**

**Figure No: 10-zone of inhibition.**

**SUMMARY AND CONCLUSION**

**Summary**
Skin care problems are considered the major difficulty these days. There are numerous products that are available in the market among which the majority are synthetic products.
Synthetic products are more harmful than natural products. It is necessary to change some herbal formulation which has good results and which reduces damage in the skin.

Many natural herbs prove effective against itching, Rashes, Acne, psoriasis, skin care problems in both male and females. So we have formulated herbal vanishing cream to reduce the dermatological disorder. In this formulation herb like neem and turmeric shows antimicrobial activity. The ethanolic extract of neem and turmeric have more power to fight against germs. The stearic acid governs the consistency of the cream and imparts the pearlescent property to the cream. Glycerin (humectant) - they help to keep the skin moistened, feels cool and gives emollient effect. Potassium hydroxide provide fine texture and consistency and methylparaben uses as preservative is to prevent deterioration cause by microorganism.

Where the herbal vanishing cream was formulated (o/w type emulsion) and evaluated by invitro methods like ph, spreadability, dye test, appearance, irritancy test, homogeneity, removal, smear test, and they shows good results.

**CONCLUSION**

Herbal vanishing cream was formulated using combination of Neem and Turmeric and evaluated. The prepared vanishing cream helps to keep skin moistened, when applied on the surface of the skin, they spread as thin oil less film which is not visible to the naked eye., and it shows better antibacterial properties due to the presence of neem and turmeric. From this study it is concluded that on combining the extract of neem leaves and turmeric with synthetic product and may produce synergic action without side effects as this cream comprising of natural substances. This prepared herbal vanishing cream will carried out further invivo studies.

**ACKNOWLEDGEMENT**

We are glad to stay that we have done this project at most sincere. It has been a great pleasure for us to express our gratitude to our teachers and well wishes who made this project possible. First of all, we thank god almighty, for his blessing through this project. First of all we express our sincere gratitude towards our honorable Principal, Prof. Dr. Anjana John, for lending us all the facilities required to proceed with our study.

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