

Formulation, development and validation of herbal based topical anti acne gel

Submitted in partial fulfillment of the requirements for the award of
Bachelors of technology degree in Biotechnology

By

L.JWALAA (38230017)



**DEPARTMENT OF BIOTECHNOLOGY
SCHOOL OF BIO AND CHEMICAL ENGINEERING**

SATHYABAMA

**INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)**

**Accredited with Grade “A” by NAAC
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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **L.JWALAA (38230017)** who carried out the project entitled “**Formulation, development and validation of herbal based topical anti acne gel**” under our supervision from September 2021 to April 2022.

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Submitted for Viva voice Examination held on _____ 2022

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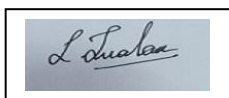
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DECLARATION

I, **L.JWALAA (38230017)** hereby declare that the Project Report entitled **“Formulation, development and validation of herbal based topical anti acne gel”** done by us under the guidance of **Dr. Jerrine Joseph** is submitted in partial fulfillment of the requirements for the award of Bachelors of Technology degree in Biotechnology.

DATE:21/04/2022

PLACE: CHENNAI

A rectangular box containing a handwritten signature in black ink. The signature appears to be 'L. Jwalaa' written in a cursive style.

SIGNATURE OF THE CANDIDATE

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ABSTRACT

Usage of herbal medicine has increased many folds on account of side effects observed with conventional drugs. Acne is a skin condition that arises due to over production of oil in sebaceous glands. It is rarely considered as disease and is neglected many a times. A non oily gel formulation for topical application suitable for treating this condition will be prepared with natural ingredients. The prepared gels will be evaluated for various physical parameters like pH, colour, odour, grittiness, viscosity, homogeneity, spreadability, skin irritancy etc. The plants chosen for preparing gels were *Taraxacum Asteraceae* and *Myristica Fragrans*. The gels were subjected to antimicrobial study against *P.acne*. The result of the zone of inhibition of prepared gels compared to an equivalent (standard) and thus can be a better alternative to chemical gels.

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CHAPTER 1

INTRODUCTION

1.1 Acne vulgaris:

Acne vulgaris is a chronic inflammatory disease of the pilosebaceous unit (comprising the hair follicle, hair shaft and sebaceous gland) (Moradi Tuchayi et al., 2015) Although often perceived as a self-limited disease of adolescence, its prevalence remains high into adulthood. Nearly 90% of teenagers have acne, and half of them continue to experience symptoms as adults.(Dawson & Dellavalle, 2013) globally acne ranks 8th in overall disease prevalence, with the highest rates reported in Western Europe, “high-income” North America, and southern Latin America. Acne is typically categorized according to age and includes neonatal and childhood manifestations (Cunliffe, 1970). It is caused by several factors including increased sebum production, follicular hypercornification, colonization with *Propionibacterium acnes*, and a lymphocytic and neutrophilic inflammatory response(Jacob et al., 2001) All body areas with high concentrations of pilosebaceous glands are involved, but in particular the face, back and chest. Inflammatory acne lesions can result in permanent scars, the severity of which may depend on delays in treating acne patients. The prevalence and severity of acne scarring in the population has not been well studied, although the available literature is usually correlated to the severity of acne (Fabbrocini et al., 2010). At puberty increased sebum production creates an environment that can sustain the colonization of *Propionibacterium acnes*. As *P. acnes* proliferates, inflammatory and chemotactic mediators are produced, which in turn drive inflammatory process (Bhate & Williams, 2013). Subsequent expansion of the propionibacterial skin flora (in the neck and then facial skin) occurred earlier in children who developed acne than in children of the same age and pubertal

status who did not, suggesting that postponement of sebum production or expansion of propionibacterial skin flora until after puberty could prevent acne or minimize disease severity. Predictors of acne severity include early onset of comedonal acne, and increasing number of family members with acne history(Williams et al., 2012) The earliest morphological change in the pilosebaceous unit in acne vulgaris is abnormal follicular epithelial differentiation. Desquamated cornified cells of the upper canal of the follicle become abnormally adherent; instead of undergoing the normal process of shedding and discharge through the follicular orifice, these cells form a retained, microscopic hyperkeratotic plug (the microcomedo) in the follicular canal. The mechanism of this process, also known as comedogenesis, is not known, but a follicular deficiency of linoleic acid may be involved (Brown & Shalita, 1998). This type of acne tends to be mild to moderate and inflammatory in nature with few comedones. It mainly affects the face, perioral area, chin, and jaw line, and is very resistant to treatment with conventional antibiotics. There are 2 main groups with distinct clinical characteristics persistent acne and late onset acne(Rivera & Guerra, 2009) Bacterial hypercolonization of the sebaceous follicle, especially with *Propionibacterium acnes*, has been considered as a major causative factor for acne vulgaris. In addition, it is widely accepted that inflammation in acne may be mainly mediated by an immunological reaction to extracellular products of *P. acnes*, such as sebum free fatty acids which are proposed to be products of triglyceride metabolism induced by follicular bacteria (Zouboulis, 2001).

1.2 Acne marks and inflammation:

Inflammatory lesions develop from comedones arising due to an abnormal pattern of keratinization in the sebaceous follicle. Comedogenesis was once thought to not be part of the inflammatory process, but detailed immunohistological studies that have followed the development of inflammatory acne lesions have shown this to be the first step toward formation

of an inflammatory lesion. The microcomedone is now believed to be the earliest type of subclinical acne lesion.(Farrar & Ingham, 2004) Acne is essentially a sub-clinical and clinical inflammatory disease of the PSU, involving abnormalities in sebum production, follicular keratinisation, bacterial proliferation, inflammation and delayed-type immune response. Despite much research, the aetiology and pathogenesis of acne are not completely understood. However, emerging evidence points to key factors in the formation of microcomedone and its progression to the inflammatory lesions of severe acne(Gollnick, 2003) It is a well established clinical fact that inadequate control of acne inflammation can later lead to scarring. Scarring is the most unfortunate and undesirable sequela of acne, because once formed, it is not amenable to pharmacological treatment and requires invasive procedures such as chemical peels, dermabrasion, or laser resurfacing to improve its appearance. These procedures are not only costly, but they themselves are associated with further risks of scarring and pigmentary abnormalities. Thus, preventing scar formation is a major goal in the treatment of inflammatory acne.(Kang et al., 2005)

Acne is characterized by comedones, papules, pustules, nodules and cysts, as follows.

- Comedones are the most basic acne lesion and can be open or closed. Closed comedones (whiteheads) are small plugged follicles whose contents are not exposed to the skin surface. Open comedones (blackheads) are small follicles with dilated openings onto the skin. The black colour results from oxidation of the debris within the follicle.
- Papules are small, usually red, raised elevations of the skin.
- Pustules resemble papules but have a central pocket of pus.
- Nodules and cysts are larger painful swellings usually more than 5 mm in size(Mahto, 2017)

inflammatory markers are present in microcomedones. Specifically, IL-1 was expressed in early inflamed lesions . Interestingly, IL-1 was found in comedonal contents both in the presence and absence of *P. acnes*; inflammatory components (CD4 T cells, macrophages, cytokines, and integrins) were also identified in the perifollicular area of uninvolved skin from acne patients. Dendritic cells pick up *P. acnes* antigenic material and T-cells primed at the local lymph node invade the follicular wall, leading to spongiosis which in turn allows influx and reflux of mediators .A recent hypothesis is that hypoxia due to increasing pressure from the ductal plug may serve as the link between a subclinical lesion and increased inflammatory responses at the pilosebaceous duct. Low oxygen levels allow *P. acnes* to grow in the infra-infundibulum and can stimulate keratinocytes to produce cytokines, which are released as the duct ruptures. Further, release of *P. acnes* into the dermis triggers an intense local reaction of the innate immune system. Collections of neutrophils and lymphocytes in the follicular epithelium also likely contribute to breakdown of the follicular wall(Dreno et al., 2015) Acne scarring is more likely after certain forms of acne vulgaris, with specific skin types, and in particular anatomic locations. Typically, acne scarring is commonly seen after acne that has been characterized by deep inflammation, nodules, or cysts. In these forms of acne, inflammation resulting from follicular rupture and expulsion of sebum, bacteria, and keratin into the dermis of the skin ultimately causes skin surface abnormalities. In some cases, inflammatory injury to the dermis may cause deposition of new collagen that creates uneven surface elevations. In other cases, resolution of cysts and nodules may cause tethering of the skin surface, resulting in small crypts or depressions. Inflammation may also cause injury to pigmented epithelia, causing melanin to be released into the dermis, which results in brown pigmentation. In some postacne individuals, blood vessels may become permanently dilated as part of a wound healing response at the sites of focal inflammation; for an observer, this is perceived as areas of

persistent redness.(Rao, 2011)

1.3 Propionibacterium Acne:

Propionibacterium acnes is considered an opportunistic pathogen, causing a range of infections as well as being associated with a number of inflammatory conditions. It is primarily recognized for its role in acne vulgaris where it is thought to contribute to the inflammatory phase of the condition. This association is supported by the correlation between antibiotic resistant P. acnes and treatment failure. Other conditions where an association with P. acnes has been suggested include synovitis, acne, pustulosis, hyperostosis and osteitis (SAPHO) (Schaeffer et al. 1998) and sarcoidosis, a systemic granulomatous disease of unknown aetiology.(A. L. Perry & Lambert, 2006)

1.3.1 Taxonomical classification:

- ❖ **Kingdom** –bacteria
- ❖ **Phylum** - Actinomycetota
- ❖ **Class** – Actinomycetia
- ❖ **Order** – propionibacteriales
- ❖ **Family** – propionibacteriaceae
- ❖ **Genus** – cutibacterium
- ❖ **Species** – acnes

The cutaneous propionibacteria (P. acnes, P. avidum, P. granulosum, P. propionicum, and P. lymphophilum) are found as commensals on human skin and other keratinized epithelia. Over the

years, they have been variously classified as *Bacillus* spp, *Corynebacterium* spp, anaerobic diphtheroids, and *Propionibacterium* spp, and this must be borne in mind when surveying the literature. They are gram-positive and nonmotile, and when first isolated exhibit a typical coryneform appearance under the microscope with irregular, short branching. Isolation is best performed at 35°C under anaerobic conditions on a growth medium supplemented with Tween 80, although propionobacteria are neither strictly anaerobic nor lipophilic. *P. acnes* and *P. granulosum* are commonly isolated from sebum-rich areas of the skin (eg, head, chest, and back) with reservoirs in the pilosebaceous follicle(Bojar & Holland, 2004) On the skin surface, the microbial community is mostly constituted by bacteria belonging to the three main genera of *Corynebacteria*, *Propionibacteria* and *Staphylococci*. Interplay between members of this cutaneous microbiota is essential for the maintenance of a healthy skin. While the commensal bacterium *Propionibacterium acnes* (*P. acnes*), predominant in sebaceous sites, is critical in the regulation of skin homeostasis and prevents colonisation from other harmful pathogens,it can also act as an opportunistic pathogen in acne vulgaris. New findings on *P. acnes* reveal that, contrary to what was previously thought, its proliferation is not the trigger of acne but instead, a tight equilibrium between members of the skin flora and among *P. acnes* phylotypes might play a more critical role in acne onset. Loss of microbial diversity can indeed lead to chronic inflammatory skin diseases(Dréno et al., 2018) The link between proliferation of *Propionibacterium acnes* in the environment of the microcomedo and the development of the inflammatory phase of acne has been well established. Over the past 25 years, antimicrobial therapy has been the major area of new drug development by the pharmaceutical industry. *P. acnes* is highly sensitive to a wide range of antibiotic classes (table 1) including the tetracycline and macrolide families. The challenge for antimicrobial therapy has been delivery into the lipid-rich environment of the microcomedo where *P. acnes* is proliferating in a cocoon of abnormally desquamated follicular

corneocytes.(Eady et al., 2003) *P. acnes*' role in the pathophysiological mechanisms of acne is well established; however, an increasing number of reports have implicated the organism as an opportunistic pathogen responsible for a wide range of inflammatory conditions, post-surgical and device-related infections. As such, it is becoming apparent that the traditional description of *P. acnes* as nonpathogenic should be disregarded and that its significance should be carefully considered following culture of the organism from clinical samples.(A. Perry & Lambert, 2011)

1.4 *Myristica fragrans*

1.4.1 TAXONOMICAL CLASSIFICATION

Kingdom –plantae

Subkingdom – viridiplantae

Class – mangoliopsida

Order – mangoliales

Family – myristicaceae

Genus – myristica

Species – fragrans

1.4.2 General information :

Myristica fragrans is commonly known as “nutmeg”, it produces two spices: mace and nutmeg. Nutmeg is the seed kernel inside the fruit and mace is the red lacy covering (aril) on the kernel.

M. fragrans belongs to Myristicaceae family in the order Magnoliales which contains about 150 genera and more than 3000 species. *M. fragrans* is a spreading aromatic evergreen tree usually growing to about 5 to 13 m high, occasionally 20 m. The bark contains watery pink or red sap. The pointed dark green leaves (5 to 15 cm × 2 to 7 cm) are arranged alternately along the branches and are borne on leaf stems about 1 cm long. Upper leaf surfaces are shiny. Flowers are usually single sexed; occasionally. Nutmeg is popular as a spice and also possesses various therapeutic properties. Nutmeg has a characteristic pleasant fragrance and a slightly warm taste. It is used to flavor many kinds of baked foods, confections, puddings, meats, sausages, sauces, vegetables and beverages. It is also used as components of curry powder, teas and soft drinks or mixed in milk and alcohol. (Jinous Asgarpanah, 2012)

1.4.3 Geographical distribution

The plant grows wild on rich volcanic soils in lowland tropical rain forests. Nutmeg needs a warm and humid tropical climate. *M. fragrans* is indigenous native to Indonesia, and is exotic to Grenada, India, Mauritius, Singapore, Sri Lanka, United States of America, South Africa, as well as the majority of African countries. World production of nutmegs is estimated to average between 10,000 and 12,000 tons per year with annual world demand estimated at 9000 tons; production of mace is estimated from 1500 to 2000 tons. Indonesia and Grenada are the first two producers and exporters of both products with a world market share of 75 and 20%, respectively (Kuate, 2017).

1.4.4 Medicinal benefits :

There are many medical benefits of *Myristica fragrans* some of them are

- ❖ Brain booster
- ❖ Tonic for the heart
- ❖ Detoxifier for kidney and liver
- ❖ Induces sleep
- ❖ Removes bad breath
- ❖ It's an aphrodisiac
- ❖ Treats stomach disorder
- ❖ Pain Reliever
- ❖ Used in cough syrup(Agbogidi & Azagbaekwe, 2013)

1.4.5 Nutritional composition:

Nutritionally, nutmeg is rich in energy, carbohydrates, proteins and dietary fibre. It is particularly rich in vitamins A, C. and E. It also contains electrolytes (Sodium and Potassium) minerals (calcium, copper, iron, magnesium, manganese, zinc and Phosphorus) and phytonutrients including carotene-B and crypo-xanthin B. Nutmeg is also rich in moisture (14.3%) ether extract (36.4%), fibre (11.6%) volatile oil(6-16%), starch (4.6-24.24.2%), pentosans (2.25%), furtural (1.5%) and pectin (0.6%). The flavour and therapeutic action and due to the volatile oil. The nutmeg oil is a mobile, colourless and pale yellow liquid, with a characteristic odour and the major constituents of the oil are d-prinene and d-camphene Nutmeg bitter produced from the plant is commercial and highly aromatic fat is ground and cooked or steamed before pressing. It is a soft solid, yellow or yellowish red in colour with an odour and taste of nutmeg (Gordon, 2004).(Agbogidi & Azagbaekwe, 2013).

1.5 Taraxacum ASTERACEAE

1.5.1 Taxonomical classification

Kingdom –plantae

Subkingdom – tracheophyta

Class – angiospermae

Order – asterales

Family – asteraceae

Genus – taraxacum

Species – Taraxacum ASTERACEAE

1.5.2 General information :

Plants of the genus *Taraxacum* have long been used as medicinal herbs. A first reference to its application is reflected in its name, which is derived from the Greek words “taraxis” for inflammation and “akeomai” for curative. In English speaking countries, dandelion, from the French word “dent-de-lion”, referring to the serrated leaves of the plant, is its common name. In French alluding to its diuretic action, the term “pissenlit” (bedwetter) is commonly used (Sch et al., 2006)

Dandelion root extracts contain sesquiterpenes such as the eudesmanolides tetrahydroidentin B and taraxacolide O-b-glucopyranoside,¹⁹ the guaianolides 11b,13-dihydrolactucin and ixerin D,²⁰ and three germacranolide esters, taraxinic acid b-glucopyranoside, its derivative 11,13-dihydrotaraxinic-acid b-D-glucopyranoside, and ainslioside.^{19,20} In addition to these five sesquiterpene lactones, dandelion contains

taraxacoside, an acylated γ -butyrolactone glycoside, *r*-hydroxyphenylacetic acid, benzyl glucoside, dihydroconiferin, syringin, and dihydrosyringin.²⁰ The root also contains several phenolic compounds. Thirteen benzenoids have been found, the predominant one being chicoric acid; others include hydroxycinnamic acid and dicaffeoyltartaric acid and their derivatives, particularly caffeic acid esters and several caffeoylquinic acid isomers such as monocaffeoyltartaric acid, 4- caffeoylquinic acid, chlorogenic acid, caffeic acid, coumaric acid, ferulic acid, *r*-hydroxybenzoic acid, protocatechuic acid, vanillic acid, syringic acid, and *r*-hydroxyphenylacetic acid as well as coumarins (umbelliferone, esculetin, scopoletin, cichoriin, and aesculin) and various flavonoids such as luteolin, isorhamnetin, apigenin, and quercetin derivatives (rutinosides and pentoside), among others.^{21–23} Others constituents of dandelion root include various triterpenes and phytosterols such as taraxasterol and γ -taraxasterol as well as their acetates and their 16-hydroxy derivatives arnidol and faradiol, α - and β -amyrin, β -sitosterol, and stigmasterol.^{10,19,24,25} Moreover, dandelion roots contain carbohydrates (e.g., inulin), carotenoids (e.g., lutein), fatty acids (e.g., myristic acid),^{21,22} minerals, sugars (e.g., fructose, glucose, sucrose), vitamins, choline, mucilage (approx. 1.1%), and pectin.(González-castejón et al., 2012)

1.5.3 Geographical distribution :

The genus *Taraxacum* is a member of the family Asteraceae, subfamily Cichorioideae, tribe Lactuceae and is found widely distributed in the warmer temperate zones of the Northern Hemisphere, inhabiting fields, roadsides and ruderal sites. The genus *Taraxacum* WIGG. Includes approximately 30–57 varieties with many microspecies, divided into nine sections. In Europe, plants of the species *Taraxacum officinale* WEBER ex WIGG. belonging to the section *Borealia*

are used for medicinal purposes, whereas plants of the species *Taraxacum platycarpum* DAHLST are utilized in Traditional Chinese Medicine(Sch et al., 2006)

1.5.4 Medicinal benefits:

Dandelion is traditionally used in the form of infusions and decoctions as aperitive, mild laxative, tonic, stimulant, and most importantly diuretic (Schu'tz et al. 2006). Although studies suggest only a minor effect for this action (Schu'tz et al. 2006), it has the advantage to contain high amounts of potassium that can hence be replaced if conventional diuretics are used (Hook et al. 1993). Commission E monographs approved the use of dandelion's aerial parts for the loss of appetite and dyspepsia, such as the feeling of fullness and flatulence with dosages of 4–10 g of herb, 3 times daily or 4–10 ml liquid extract 1:1 in 25% alcohol, 3 times daily. On the other hand, it was also specified that it is contraindicated in case of bile ducts obstruction, gall bladder and ileus empyema. It should also be avoided when the rare allergy to sesquiterpene-lactones present in the latex manifests, while one should consult with a physician in case of gallstones(Grauso et al., 2019)

1.6 Traditional treatment for acne:

Achyranthes aspera - This medicinal plant is traditionally used for the treatment of *Acne vulgaris*, eruptions of the skin, boils, scabies and other skin diseases. Saponin, alkaloid and non-alkaloid fractions obtained from the leaves of this plant have enormous inhibitory effect on the Epstein-Barr virus early antigen activation in Raji cells, with the most inhibitory activity (96.9%; 60% viability) observed for the non-alkaloid fraction, which contains non-polar compounds. . The plant has abortifacient properties in rodents and also has contraceptive activity, which might be due to its potent estrogenic activity (Hunt MJ et al., 1992).

Allium cepa - In another study, the antimicrobial and antifungal properties of a *A. cepa* and *A. sativum* were revealed against *Malassezia furfur*, *Candida albicans* and some other *Candida* sp, as well as some strains of dermatophytes and *Acne vulgaris* microbes. The results indicated that *A. cepa* and *A. sativum* might be promising in the treatment of bacterial and fungal-associated infections (Radiei Z et al., 2014)

Azadirachta indica in a study, conducted on an anti-acne formulation prepared from herbal extracts, it was revealed that ethanol extract of *Azadirachta indica*, *G. glabra*, *Andrographis paniculata*, *Ocimum sanctum*, and green tea possessed the potential for inhibiting acne. In this study the anti-acne formula successfully acted against *Propionibacterium* and *Staphylococcus epidermis* (Rahimian GA et al., 2013)

Cannabis sativus the seed oil of *Cannabis sativus* is useful for the treatment of acne rosacea, seborrheic dermatitis, eczema, dermatitis, psoriasis and lichen planus. The leaves powder of this plant is very useful as a wound and sore dressing. *Cannabis sativus* extract is externally useful to relieve pain in itchy skin. The seed oil strengthens the skin and makes it more resistant to bacterial, fungal and viral infections (Rabiei Z et al., 2014)

Rosmarinus officinalis oil has also been effective against *P. acnes*, a type of bacteria that causes acne. In a study, the antibacterial properties of *R. officinalis* essential oil was evaluated against *P. acnes* in which significant changes were reported in size and morphology of *P. acnes* in response to treatment (Rafieian-Kopaei M et al., 2014)

1.7 COMMERCIAL PRODUCTS AVAILABLE FOR ACNE TREATMENT:

1.7.1 SCRUBS

Abrasive scrubs came to fruition after the anecdotal observation that desquamation of the SC can

lead to younger, smoother-appearing skin. Scrubs may contain different types of abrasives, such as polyethylene beads, aluminum oxide and ground fruit pits, or sodium tetraborate decahydrate granules (Draelos ZD., 2005)

1.7.2 Cleansing cloth (nonwovens, towelettes):

Cleansing cloths offer a less abrasive cleansing alternative in addition to providing conditioning and exfoliation in a simple application process. The cloths come in the following two forms: 1) cloths that lather, requiring wetting before and rinsing afterwards and 2) moist cloths that do not requiring rinsing after use. Most wipes tend to be mild because of the low surfactant content and also have the additional benefit of increased deposition of active ingredients onto the skin (Abbas S et al., 2004)

1.7.3 Cosmetic adhesive pads:

Developed to remove adherent corneocytes, dirt, oil, or loose open comedones from the skin surface, adhesive pads can be used to remove keratotic plugs (comedones) from the follicular orifices. Biore® (Kao Brands Company, Cincinnati, Ohio) pore strip is a commercially available adhesive pad, onto which a cationic adhesive polymer is deposited. Comedones (follicular plugs) contain anionic amino acids that are attracted to the cationic adhesive polymer; the active agent polyquaternium 37 purportedly binds to comedonal plugs facilitating their extraction on removal of the adhesive pad (Elston DM et al., 2000)

1.7.4 Tea tree oil:

Australian tea-tree oil comes from trees of the *Melaleuca* genus; the most common species used is *Melaleuca alternifolia*. Tea tree oil has been used medicinally for approximately 70 years,

including for furunculosis and vaginal infections, due to its broad antimicrobial and antifungal properties. *Staphylococcus aureus* and most gram-negative bacteria are reported to be sensitive to tea tree oil. Terpinen-4-ol is considered the active ingredient of tea tree oil, but studies have shown alpha-terpineol and alpha-pinene also have intrinsic antibacterial properties (Raman A et al., 1995)

1.8 NATURAL GEL BASE IN COSMETICS:

1.8.1 Hydroxyethyl cellulose (HEC):

It is a cellulose-derived thickening and the gelling agent used in capsules containing hydrophobic drugs in order to improve dissolution of drugs within GI fluids in a method known as the hydrophilization method. This nonionic and water-soluble polymer is also used in cosmetics, cleaning solutions, and other household products. It will fabricate crystal-clear gel products and thicken the aqueous phase of cosmetic emulsions. Their tendency to lump or agglomerate, when first wetted with water, is a disadvantage associated with HECs and other water-soluble thickeners. The R-grade of HECs facilitates solution preparation without lumping when wetted with water, thus increasing dissolution and total processing times (Joshi and Petereit, 2013).

1.8.2 Xanthan gum:

It is a naturally obtained heteropolysaccharide and a commonly used biopolymer. The xanthan gum, later additionally popularized as polysaccharide 1459 that is widely obtained from the microorganism *Xanthomonas campestris* NRRL B-1459. The gum was immensely researched due to its excellent rheological properties, which would enhance other kinds of widely used natural

and synthetic soluble gums and different polysaccharides. Extensive analysis was conducted in different industrial laboratories throughout the 1960s, which caused the manufacturing of semicommercial product referred to as Kelzan¹ by Kelco¹ (Vuyst LD et al., 1987)

Xanthan gum (XG) is a naturally obtained extracellular polysaccharide of bacteria *X. campestris*. XG can also be obtained from other species of *Xanthomonas* such as *X. arboricola* and *X. axonopodis*. The primary structure of XG consists of pentasaccharide subunits and D-glucosyl, D-mannosyl, and D-glucuronyl acid residues in the ratios of 2:2:1 with different proportions of O-acetyl and pyruvyl residues (Candia JL et al., 2002)

1.8.3 Phenoxyethanol:

It is an ether and aromatic alcohol, which is also known as 2-phenoxyethanol, ethylene glycol monophenyl ether, phenoxytol, 1-hydroxy-2-phenoxyethane and (2-hydroxyethoxy) benzene (Puschmann J et al., 2018)

CHAPTER 2

REVIEW OF LITERATURE:

1. Antimicrobial testing is a time-consuming and costly method for evaluating newly developed pharmaceutical formulations for topical use. The correlation between free preservative concentration in emulsion gels measured by equilibrium dialysis and preservative effectiveness testing for *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Candida albicans*, and *Aspergillus brasiliensis* was investigated in this study. The higher the lipophilicity of the oil phase and the lower the aqueous phase content in terms of dissolved ingredients, the more phenoxyethanol is preferably distributed to the water phase and, as a result, the greater the efficacy against microbes. Micellar interactions reduced the free amount of the preservative as emulsifier concentrations increased. .(Puschmann J, Herbig ME, Muller-Goymann CC. Correlation of antimicrobial effects of phenoxyethanol with its free concentration in the water phase of o/w-emulsion gels. *Eur J Pharm Biopharm* 2018; 131: 152–161)

2. Over centuries, silver has been widely recognized for its antimicrobial activity among all metals. Silver has recently been discovered to be even more effective in antimicrobial activity when it is at the nanoscale. A novel protocol for green synthesis of silver nanoparticles was developed, and

their anti-bacterial activity was experimentally validated. This protocol differs from the prior art in that it allows for the first time the green synthesis of non-polar organic plant extracts. The raw material was a dichloromethane extract of *Plectranthus zeylanicus*, a medicinal plant widely used in Sri Lanka. SEM images of the formulation revealed that the synthesised objects were predominantly spherical in shape and approximately 100 nm in size. The presence of elemental silver was confirmed by an energy dispersive X-ray (EDX) spectrum. Standard qualitative phytochemical screening assays detected the presence of phytochemical classes that could be responsible for the green synthesis of nano silver. The compound's antibacterial activity was evaluated using the agar well diffusion method against Gram positive and Gram negative bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA). In comparison to aqueous silver ion solution and commercial disinfectants containing silver ions as active ingredients, these new formulations demonstrated significant antibacterial activity against all tested microorganisms. This is the first time the anti-MRSA potential of silver nanoparticles synthesized in an environmentally friendly manner has been documented. (S. G. De Soyza, W. M. D. G. B. Wijayaratne, M. Napagoda, and S. Witharana, "Antimicrobial potential in biogenic silver nanoparticles synthesized from *Plectranthus zeylanicus*," *Journal of Molecular Nanotechnology and Nanomedicine*, vol. 1, p. 105, 2017.)

3. A study was conducted to scientifically validate the plant's traditional use in the treatment of inflammatory conditions, gout, and microbial infections. The anti-inflammatory and anti-gout activity of *L. zeylanica* extracts was determined by inhibiting 5-lipoxygenase (5-LO), microsomal prostaglandin E2 synthase (mPGES)-1, and xanthine oxidase (XO). The antibacterial and antifungal activities of the bioactive extracts were also investigated, and the relevant constituents in the extracts were tentatively identified. The identification of different classes of phytochemicals

in the ethyl acetate extract of seeds of *N. 2 +e Scientific World Journal sativa*. All the tests were conducted along with a suitable positive control.(*M. Napagoda, J. Gerstmeier, H. Butschek et al., "Lipophilic extracts of Leucas zeylanica, a multi-purpose medicinal plant 6 +e Scientific World Journal in the tropics, inhibit key enzymes involved in inflammation and gout," Journal of Ethnopharmacology, vol. 224, pp. 474– 481, 2018)*

4. Phytochemical studies and antioxidant activities were performed on n-hexane, ethyl acetate, ethanol, and methanol extracts of *Lippia rugosa* A. Chev (Verbenaceae), a medicinal plant used traditionally in the Cameroonian savannah region to protect foodstuffs and cure degenerative diseases. These extracts were found to contain terpenoids, phenolic and flavonoid compounds, according to the results. Except for the n-hexane extract, all of the obtained extracts exhibit antioxidant activity, with the ethanol extract being the most effective, with an inhibition percentage of 85.668 percent 1.233 percent and an inhibition concentration (IC₅₀) of 58 g/ml (R² = 0.987, P 50) of 221 g/ml (R² = 0.966, P 50) of 221 g/ml (R² = 0.966, P 50). *L. rugosa* and its antioxidant activity were investigated. The microbial flora isolated from acne patients include *P. acnes*, *S. epidermidis*, *S. aureus*, *Klebsiella pneumonia*, and *Streptococcus* whose pathogenic mechanisms and the genes associated with virulence factors are believed to play a significant role in the development of acne (*G. E. Trease and W. C. Evans, A Textbook of Pharmacognosy , Bailliere Tindall Ltd, London, UK, 13th edition, 1989.*)

5. Acne vulgaris, a common skin disorder, has been linked to a 7.1% increase in suicidal ideation in acne patients. This raises the question of whether acne is a potentially fatal disease in humans. The primary cause of this multifactorial disease is microbial fluctuation of common resident microbes on the skin, each with their own purpose and style in protecting the human body. In

order to survive, the microbial population must get past the host skin's defence barriers while also being able to resist them. Their pathogenic lifecycle and associated virulence factors, which are coded within their pathogenic islands on a single circular chromosome, have resolved these issues. The analysis of correlation between the antimicrobial resistance of *P. acnes* and *S. epidermidis* had revealed that more than 80% of the patients who carried clindamycin-resistant *P. acnes* also carried clindamycin-resistant *S. epidermidis* (B. Kumar, R. Pathak, P. B. Mary, D. Jha, K. Sardana, and H. K. Gautam, "New insights into acne pathogenesis: exploring the role of acne-associated microbial populations," *Dermatologica Sinica*, vol. 34, no. 2, pp. 67–73, 2016)

6. Normal skin inhabitants *Propionibacterium acnes* and *Staphylococcus epidermidis* are frequently isolated from acne lesions, and these microorganisms are thought to contribute to acne inflammation. From 2009 to 2010, we investigated the antimicrobial susceptibilities and resistance mechanisms of *P. acnes* and *S. epidermidis* isolated from acne vulgaris patients at a Japanese university hospital. In addition, we investigated the relationship between *P. acnes* antimicrobial resistance and the severity of acne vulgaris. Clindamycin resistance was found in some *P. acnes* strains (18.8 percent; 13/69). Except for one strain that expressed *erm(X)*, which encodes a 23S rRNA methylase, all strains had a mutation in the 23S rRNA gene. Tetracycline-resistant *P. acnes* strains were found to account for 4.3 percent (3/69) of all strains. This resistance was brought about by a mutation in the 16S rRNA gene. Furthermore, three strains with reduced nadifloxacin susceptibility (MIC = 16 g ml⁻¹) were identified. When the antimicrobial resistance of *P. acnes* and *S. epidermidis* was compared, more than 80% of the patients who had clindamycin-resistant *P. acnes* also had clindamycin-resistant *S. epidermidis*. When the *P. acnes* strains were analysed using PFGE, no epidemic strains with antimicrobial resistance were found. As a result, our findings suggest that *P. acnes* antimicrobial resistance is closely related to antimicrobial therapy.

Furthermore, those *P. acnes* strains were more commonly found in severe acne patients than in mild acne patients. As a result, the data support a link between the use of antimicrobial agents. Apart from that, the adverse effects associated with benzoyl peroxide, retinoids, isotretinoids, azelaic acid, and salicylic acid and other widely used antiacne agents could not be neglected (K. Nakase, N. Noguchi, M. Kawashima, N. Hayashi, Y. Takenaka, and H. Nakaminami, "Relationship between the severity of acne vulgaris and antimicrobial resistance of bacteria isolated from acne lesions in a hospital in Japan," *Journal of Medical Microbiology*, vol. 63, no. 5, pp. 721–728, 2014.)

7. Acne vulgaris affects approximately 85 percent of teenagers and can last into adulthood. Teenagers visit doctors about two million times per year, and the direct cost of acne treatment in the United States exceeds \$1 billion per year.

Acne vulgaris can be treated with a wide range of medications, including benzoyl peroxide, retinoids, isotretinoids, keratolytic soaps, alpha hydroxy acids, azelaic acid, salicylic acid, and hormonal, anti-androgen, or antiseborrheic treatments. However, none of these methods are without side effects, and their precise role in therapy is unknown. Aside from presenting the possible causes of acne vulgaris and available drugs, this paper reviewed recently published papers on medicinal plants used in the treatment of acne vulgaris. MICs of 0.005–0.6 $\mu\text{L/mL}$ have been reported in essential oils of *Citrus obovoides*, *Citrus natsudaidai*, *Cryptomeria japonica*, and *Cymbopogon nardus*, while phytochemicals such as pulsaquinone, hydropulsaquinone, rhodomyrtone, and rhinacanthin-C were found to possess MIC in the range of 0.5–12.5 $\mu\text{g/mL}$ against *P. acnes* (H. Nasri, M. Bahmani, N. Shahinfard et al., "Medicinal plants for the treatment of acne vulgaris: a review of recent evidences," *Jundishapur Journal of Microbiology*, vol. 8, no. 11, article 25580, 2015.)

8. Acne is a common but serious skin disease that affects approximately 80% of adolescents and young adults aged 11–30. This disease affects 42.5 percent of men and 50.9 percent of women well into their twenties. Bacterial resistance has reached an alarming level as a result of irrational antibiotic use. As a result, the search for new lead molecules/bioactives, as well as rational delivery of existing drugs (for improved therapeutic effect), to the site of action, is critical. Plants and plant-derived products have always been an important part of the health-care system. As a result, the current review summarizes plants that are currently used for acne treatment as well as those that have a high potential. The most active plant extracts, specifically *P. granatum*, The minimum inhibitory concentration (MIC) of *M. alba*, *A. anomala*, and *M. aquifolium* against *P. acnes* is 4–50 g/mL, whereas aromatic oils of *C. obovoides*, *C. natsudaoidai*, *C. japonica*, and *C. nardus* have MICs of 0.005–0.6 L/mL and phytomolecules such as rhodomyrtone, pulsa In addition, novel drug delivery strategies for important plant leads in the treatment of acne have been discussed. the effect of topical gel formulations comprising the extracts on those microorganisms is yet to be documented(*P. Sinha, S. Srivastava, N. Mishra, and N. P. Yadav, "New perspectives on antiacne plant drugs: contribution to modern therapeutics," BioMed Research International, vol. 2014, Article ID 301304, 19 pages, 2014.)*

9. Thymoquinone is an active principle found in the *Nigella sativa* seed, also known as "Habbah Al-Sauda" in Arabic and "Sinouj" in Tunisia. During infections, bacterial biofilms show significant tolerance to antimicrobial drugs.

Thymoquinone (TQ) antibacterial activity and biofilm inhibition potencies were investigated in 11 human pathogenic bacteria. The crystal violet (CV) and the 2, 3-bis [2-methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxanilide (XTT) reduction assays were used to evaluate the

biofilm's growth and development. TQ exhibited significant bactericidal activity against the majority of the bacteria tested (MICs ranging from 8 to 32 g/ml), particularly Gram positive cocci (Staphylococcus aureus ATCC 25923 and Staphylococcus epidermidis CIP 106510). The crystal violet assay revealed that the minimum biofilm inhibition concentration (BIC50) for Staphylococcus aureus ATCC 25923 and Staphylococcus epidermidis CIP 106510 was 22 and 60 g/ml, respectively. Furthermore, TQ supplementation influenced cell oxidative activity, according to our findings. Similarly, TQ inhibited cell adhesion to the surface of glass slides.

TQ's ability to prevent biofilm formation calls for more research into its use as a bioactive substance with antibiofilm potential. the antibacterial potential of methanolic extract of seeds of N. sativa was evaluated against P. acnes in a recent study; however, the gel formulation prepared with the above extract was not subjected to the relevant antimicrobial assay(K. Chaieb, B. Kouidhi, H. Jrah, K. Mahdouani, and A. Bakhrouf, "Antibacterial activity of thymoquinone, an active principle of Nigella sativa and its potency to prevent bacterial biofilm formation," *BMC Complementary and Alternative Medicine*, vol. 11, no. 1, p. 29, 2011.)

10. The purpose of the study was to determine the efficacy of Nigella Sativa against the microbial species Propionibacterium Acnes, to observe the zone of inhibition, and to develop a topical Nigella Sativa formulation for the treatment of Acne Vulgaris. The topical drug delivery system is simple, effective, and extremely useful in the treatment of acne. Carbopol 940 was used to create the gel. As a result, the developed gel was evaluated for a variety of physicochemical parameters such as physical appearance, pH, viscosity, and homogeneity. Acne vulgaris is the most common chronic inflammatory skin disease in humans. Many patients do not improve with current anti-acne therapy due to the high cost of treatment, adverse effects that cause noncompliance, or a lack of therapeutic benefits from current antibiotics (resistance). Acne prevalence has been reported to

range from 35% to 90% of adolescents at some point in their lives. Acne is a multifactorial disease that involves hormonal influences, keratinization changes, inflammation, and immune changes. Because of its accessibility and larger surface area, skin has been considered a promising route of drug administration. A topical drug delivery system is intended to deliver a variety of drugs to the body via diffusion across the skin layers. The comparison of MIC values of the gel formulations and the positive control against *P. acnes* could have improved the outcome of this study(*U. Bhalani and K. Shah, "Preparation and evaluation of topical gel of Nigella sativa (kalonji)," International Journal of Research and Development in Pharmacy and Life Sciences Impact Factor, vol. 4, pp. 1669–1672, 2015.)*

CHAPTER 3

AIM

To Formulate, develop and validate herbal based topical anti acne ointment

OBJECTIVES

- ❖ To prepare a herbal formulation to cure acne
- ❖ To check the formulation on Acne Vulgaris caused by P.acne by invitro assay
- ❖ To standardize the formulation into an ointment
- ❖ To evaluate the shelf life of the ointment

CHAPTER 4

MATERIALS AND METHODS

4.1. SAMPLE COLLECTION

Sample are collected from the nearby Ayurveda shop and washed thoroughly with water and then it crushed into powder.

4.2. EXTRACT PREPARATION

The powdered samples were extracted by soaking them in methanol, ethyl acetate and dichloromethane separately in the ratio of 1:5(sample:solvent) and kept in shaker to soak for 24 hrs .Then the solvents are evaporated using rotary evaporator.

4.3. PHYTOCHEMICAL QUALITATIVE ANALYSIS

The different qualitative chemical tests were performed for establishing the profile of giveextract for its chemical composition. The following tests were performed on the extracts to detect various phyto constituents present in them.

1. Detection of alkaloids (Evans, 1997)

To the extract, few drops of con hydrochloric acid were added by the sides of the test tube, followed by few drops of saturated picric acid solution. Yellow precipitate indicates the test as positive for alkaloids

2. Detection of phenol

To the extract, 200µl of neutral ferric chloride solution was added and shaken well. Dark green or Violet or Purple or dark brown colour indicates the presence of phenol.

3. Detection of glycosides (Evans, 1997)

To the extract, Sodium nitroprusside solution few drops and 3-5 drops of Pyridine was added. Blood red colour indicates the presence of glycosides.

4. Detection of terpenoids (Salkowski test)

To the extract was added in 2 ml of chloroform. Concentrated H₂SO₄ (3ml) was carefully added. A reddish brown ring coloration of the interface indicates the presence of terpenoids.

5. Detection of flavonoids

To the extract, few drops of 1% NaOH solution, were added. A yellow colour formation within short period is positive for flavonoids.

6. Detection of tannins

To the extract, few drops of lead acetate solution was added and observed for brownish green or a blue-black coloration

7. Detection of saponins Foam test (Kokate, 1999)

The extract was diluted with 5mL distilled water. The suspension was shaken in a graduated cylinder for 15 min. A 2cm layer of foam indicated the presence of saponins

4.4. ANTIBACTERIAL ACTIVITY (preliminary screening):

Crude extracts that are prepared are tested for Antibacterial Activity against *S. aureus*. . agar well diffusion method was used to determine the antibacterial activity against *S. aureus*. Mueller Hinton Agar (MHA) plates were inoculated with a saline suspension of bacteria prepared using isolated colonies of one-day-old pure cultures of *S. aureus* (MTCC 1430) thereafter, wells (6 mm diameter and 5 mm depth) were prepared in these culture plates at equidistance using a sterilized cork borer. wells were filled with 50 μ L of each of the test separately. Thereafter, the plates were incubated at 37°C overnight, and the zone of inhibition solutions around each well was measured. Zones >6 mm were considered as inhibitions resulting from significant antibacterial activity. Experiments were performed in duplicate, and the diameter of the zone of inhibition was expressed as mean $\pm$ SD. Depending on the diameter of the zone of inhibition, the best solvent for the large-scale extraction was selected.

4.5 Formulation of the gel:

Gel was prepared by combining different ratios of the extracts to xanthum gum which is a gelling agent and teatree oil was added to get good odour

S.N	Gelling agent	Extract of Taraxacum Astracea in %	Extract of mrystrica fragans in %	Tea tree oil in drops
1	Xanthum gum	2	0	5
2	Xanthum gum	0	2	5
3	Xanthum gum	1.5	0.5	5
4	Xanthum gum	0.5	1.5	5
5	Xanthum gum	1	1	5

Table 1 : composition of gel formulation

4.6 Antimicrobial activity against Propionibacterium Acne:

The different ratios of the gel that are prepared are tested for Antibacterial Activity against P.acne. .Agar well diffusion method was used to determine the antibacterial activity against P.Acne. Brain Heart Infusion Agar (BHA) plates were inoculated with a saline suspension of bacteria prepared using isolated colonies of one-day-old pure cultures of P.Acne. (MTCC 1951) thereafter, wells (6 mm diameter and 5 mm depth) were prepared in these culture plates at equidistance using a sterilized cork borer. Wells were filled with 50 μ L of each of the test separately. Thereafter, the plates were incubated at 37°C in an anaerobic incubator for 48-72 hrs , and the zone of inhibition solutions around each well was measured. Zones >6 mm were considered as inhibitions resulting from significant antibacterial activity. Experiments were performed in duplicate, and the diameter of the zone of inhibition was expressed as mean $\pm$ SD. Depending on the diameter of the zone of inhibition, the best gel formulation was selected

4.7 Determination of MIC using Resazurin Microtitre Assay:

Test was carried out in a 96 well Plates under aseptic conditions. A sterile 96 well plate was labeled. A volume of 100 μ l of sample was pipetted into the first well of the plate. To all other wells 50 μ l of nutrient broth was added and serially diluted it. To each well 10 μ l of resazurin indicator solution was added. 10 μ l of bacterial suspension was added to each well. Each plate was wrapped loosely with cling film to ensure that bacteria did not become dehydrated. The plate was incubated at 37 °C for 18–24 h. The colour change was then assessed visually. Any colour changes from purple to pink or colourless were recorded as positive. The lowest concentration at which colour change occurred was taken as the MIC value.

The resazurin solution was prepared by dissolving 270 mg in 40 ml of sterile distilled water. A vortex mixer was used to ensure that it was a well dissolved and homogenous solution.

4.8 MTT assay

The cytotoxic activity of samples on VERO cells were determined by the MTT assay. Cells (1×10^5 /well) were plated in 0.2 ml of medium/well in 96-well plates. Incubate at 5 % CO₂ incubator for 72 hours. Then, added various concentrations of the samples in 0.1% DMSO for 24 hrs at 5 % CO₂ incubator. View the images under Inverted microscope 40X and take the photos. After removal of the sample solution and 20 μ l/well MTT reagent was added. Viable cells were determined by the absorbance at 540nm. 50% inhibition of cell viability (IC₅₀) value was determined graphically. The effect of the samples on the proliferation of VERO cells was expressed as the % cell viability, using the following formula:

Calculation

$$\% \text{ cell viability} = A_{540} \text{ of treated cells} / A_{540} \text{ of control cells} \times 100\%$$

4.9 ANTIOXIDANT ASSAY

The Antioxidant or the free radical scavenging capacity of the gel was determined by using 1,1-Diphenyl-2-Picryl Hydrazyl (DPPH) assay.

Stock DPPH solution (0.1mM) was prepared in Methanol. Different concentration of standard (Gallic acid) and test solution were prepared by serial dilutions make the final volume to 1ml with methanol. In 96 well plate, add 100 μ l of 0.1mM DPPH solution to 100 μ l of samples and 100 μ l of standards which are prepared with different concentration. 200 μ l of DPPH solution is added as control for antioxidant assay. The reaction mixture was mixed and allowed to react at Room Temperature in Dark Environment for about 30 minutes. Absorbance value of the samples was read at 517 nm using UV spectrophotometer. The inhibition of radical scavenging activity percentage is determined by using the equation:

$$\text{Radical Scavenging activity (\%)} = A_0 - A_1 / A_0 \times 100$$

A_0 $\rightarrow$ Absorbance of DPPH radicals with methanol

A_1 $\rightarrow$ Absorbance of DPPH radicals with sample extract or standard

The IC₅₀ Values of extract and standard were calculated. Experiments were performed in triplicate and the data were presented in the average value.

4.10 IN VITRO WOUND HEALING ACTIVITY

Materials and Requirements:

- Dulbecco's Modified Eagle's medium with supplements (serum, antibiotics)
- Phosphate buffered saline
- Bovine serum albumin
- Poly-L-lysine stock
- Tissue culture dishes with 60mm
- Razor or fine sharpie marker
- p200 pipette tips
- Hemocytometer
- Phase-contrast microscope
- Stage incubator
- CO₂ incubator
- Video camera and
- Image analysis software

PROCEDURE

Coating of cell culture dishes

Take 60-mm culture dish and coat with ECM substrates and incubate the dish overnight for 2 hours at 37°C without shaking. Then remove unbound ECM substrate and coat with 3 ml of

bovine serum albumin at 37°C for 1 hour. Wash it with PBS and add media into the dish before plating the cells

Cell culture

Re suspend the sub confluent growing cells by washing with PBS twice in culture dish. Add trypsin and mix the cell with serum containing medium. Disperse the cell equally by pipetting the solution and rock the dish gently. Using hemocytometer, cell counts of aliquot from cell suspension are determined. Add cells to 60mm culture dish to form confluent monolayer. Incubate them at 37°C for 6 hours to adhere to substrate and develop into monolayer.

In Vitro Scratch assay

Scrape monolayer of the cell horizontally using p200 pipette tip and debris were removed by washing with PBS. Wash the cell again with 1ml of growth medium and replace it with 5 ml of medium. Create a reference mark on the culture plate by using a razor blade or with fine tipped marker. Then the cells were treated with extract at 40µg/ml. Cells without treatment is considered as control and positive control will be cell treated with EGF (2ng/ml). Place the culture dish on the phase contrast microscope and record the first image of scratch at 0 hour. Keep the culture dish in CO₂ incubator, observe them at the interval of 24h using the microscope and record the second and third set of Image. Migration rate of the cells are analysed by using the software "ImageJ".

CHAPTER 5

RESULTS AND DISCUSSION :

5.1. SAMPLE COLLECTION:

Taraxacum astrecea and *mystrica fragans* were dried in shade and crushed into powder



Fig 1 powdered *mystrica fragans*



Fig 2 powdered *Taraxacum astrecea*

These plants are selected because their high anti-bacterial and anti-inflammatory property (Grauso et al., 2019) (Agbogidi & Azagbaekwe, 2013)

5.2. EXTRACT PREPARATION:

The powdered sample of *mystrica fragans* and *Taraxacum astrecea* are soaked in different solvents like methanol, ethylacetate and dichloro methane separately and kept shaker then the solvents are evaporated using rotary evaporator

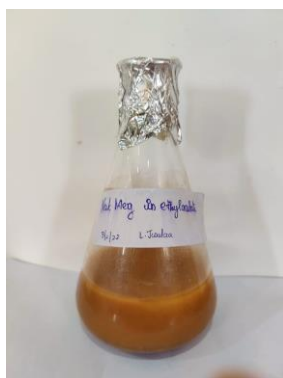


Fig 3 nutmeg in ethyl acetate

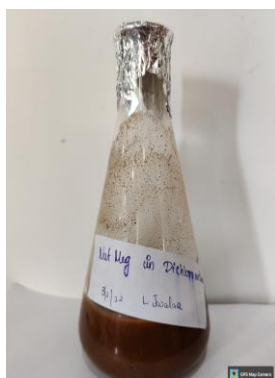


Fig 4 nutmeg in dichloromethane

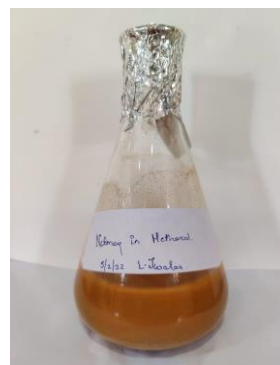


Fig 5 nutmeg in methanol

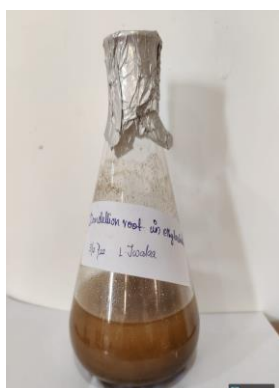


Fig 6 dandelion in ethyl acetate

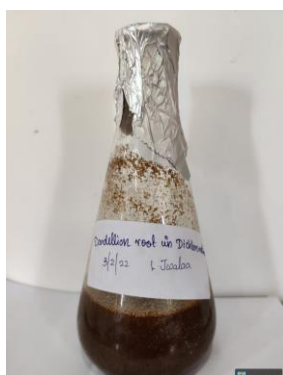


Fig 7 dandelion in dichloromethane

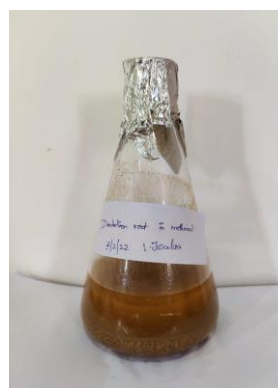


Fig 8 dandelion in methanol

5.3. Phytochemical analysis

The phytochemical screening of the extracts are listed in the table below. From the table +ve represent the presence of the phytochemical whereas –ve represents the absence of the phytochemical. alkaloids, terpenoids, phenols, flavonoids, tannins, glycosides are present in both the extracts whereas saponin is absent in both

S.no	Test	Taraxacum Astracea	mrystrica fragans
1	Alkaloids	+ve	+ ve
2	Terpenoids	+ ve	+ ve
3	Phenols	+ ve	+ ve
4	Flavonoids	+ ve	+ ve
5	Tannins	+ ve	+ ve
6	Glycosides	+ ve	+ ve
7	Saponins	- ve	- ve

Table 2:Represents the phytochemical analysis of the two plants.

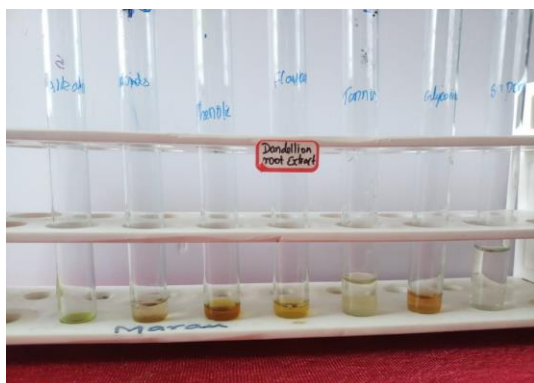


Fig 5.9 Represents the phytochemical
analysis of dandelion root



Fig 5.10 Represents the phytochemical
analysis of nut meg.

5.4 preliminary antimicrobial activity:

Sample	Zone of inhibition in mm
Nutmeg – Methylene (A)	-
Nutmeg – Dichloromethane (B)	-
Nutmeg – Ethyl acetate (C)	18mm
Dan – Methylene (D)	14mm
Dan – Dichloromethane (E)	-
Dan – Ethyl acetate (F)	24mm

Table 3 : zone of inhibition of preliminary antimicrobial activity

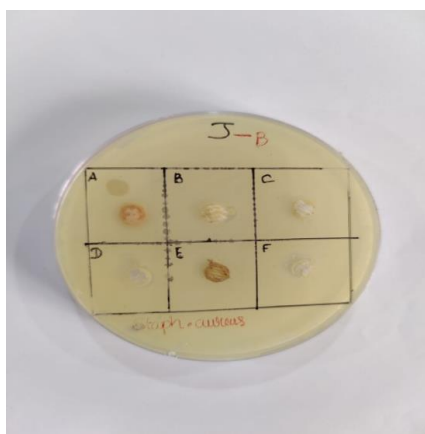


Fig 11 Antimicrobial activity against *s. aureus*

From the preliminary antimicrobial activity we conclude that the extracts of nutmeg and dandelion with ethyl acetate has maximum zone of inhibition 18 mm and 24 mm respectively

5.5 Gel Formulation:

In the preliminary antimicrobial activity we inferred that extract C and F (nutmeg and dandelion extract using solvent ethylacetate) has the maximum inhibition zone. Extract C and F has been mixed to the gel base in 6 different concentrations

S.No	Concentration
A	2:0
B	0:2
C	1.5:0.5
D	0.5:1.5
E	1:1
F	Commercial cream

Table 4 Ratios for gel formulation



Fig 12 anti acne gel

The gelling agent or base that is used in the formulation is Xanthum gum instead of commercially used carbopol to make the gel a completely natural formulation(Vuyst LD etal., 1987)

5.6 Antimicrobial activity against P.acne:

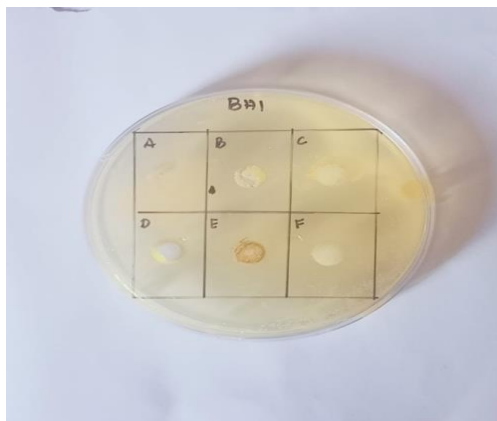


Fig 13 Antimicrobial activity against P. acne

S.No	Zone of inhibition in mm
A	10
B	11
C	10
D	14
E	16
F	25

Table 5 zone of inhibition of the gel formulation against P.acne

From the above table we infer that the zone of inhibition is maximum at concentration E(1:1)

5.7. Determination of Minimum inhibition concentration by using Resazurin Microtitre assay:

The minimum inhibition concentration was done for two types of organisms Staphylococcus aureus (gram positive) and Pseudomonas aeruginosa (gram negative)

microorganisms by keeping streptomycin as positive control and DMSO as negative control. Inhibition of growth seen at the ratio up to 1:7(gel formulation: DMSO), so the MIC value of gram negative microorganisms is found to be less than 1:7. For gram positive bacteria the growth inhibition is seen up to 1:2 after that there is an increase of growth of microorganism. From this the MIC value for *Pseudomonas aeruginosa* is found to be less than 1:7 and for *Staphylococcus aureus* it is found to be in the ratio 1:2.

S.No	Microorganisms/sample	Growth inhibition										
	Gel formulation	BLANK	1:1	1:2	1:3	1:4	1:5	1:6	1:7	STD (Streptomycin) 10µg	DMSO	Culture
1	<i>Pseudomonas aeruginosa</i> (-)	-	-	-	-	-	-	-	-	-	+	+
2	<i>Staphylococcus aureus</i> (+)	-	-	-	+	+	+	+	+	-	+	+

Table 6 represents the growth inhibition of the sample at different concentration against gram positive and gram negative

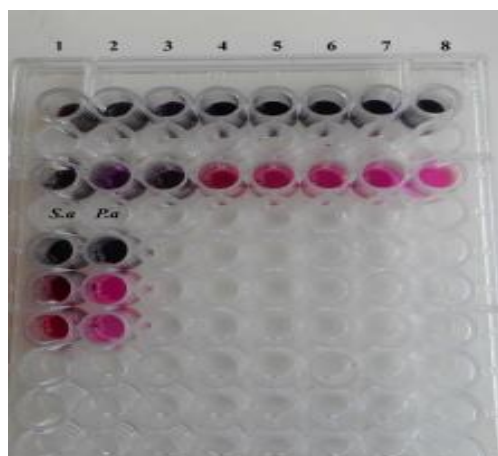


Figure 14. represents the MIC done by resazurin assay done in a 96 well plate.

5.8. Cytotoxicity using MTT assay

MTT assay is used to study about the level of toxicity of the compound to the cells and to find the lethal dose of the drug to human cells. MTT works on the principle of reduction of the yellow colored water soluble Tetrazolium dye 3-[4, 5-dimethylthiazol-2yl]-2, 5-diphenyl tetrazolium bromide (MTT) to formazan crystals. Mitochondrial lactate dehydrogenase produced by live cells reduces MTT to insoluble formazan crystals, which upon dissolution in an appropriate solvent exhibits purple color. The percentage of cell viability is given by the dividing the absorbance of treated cells and the absorbance of control cells. The sample is diluted with DMSO.

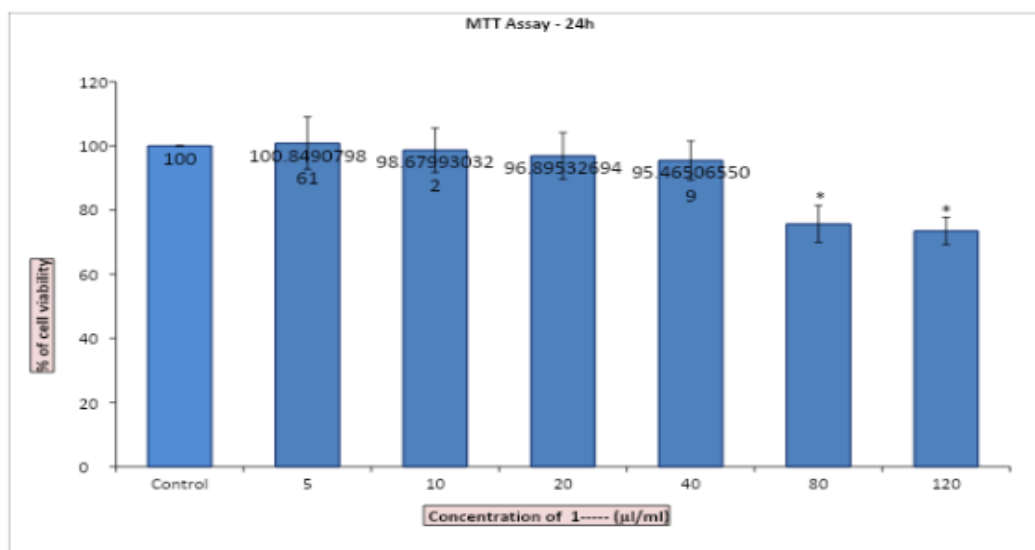


Table 7 represents the % of cell viability at different concentration of the gel

5.9 Antioxidant Activity the gel formulation:

The extracts were analyzed for Antioxidant activity by using the method, DPPH radical scavenging assay. The plant extract was allowed to react with violet colored DPPH solution which was reduced to yellow color product, diphenyl picryl hydrazine. The rate of conversion indicates the presence of antioxidant potential in the plant sample and the reduction reaction in plant sample occurs in the concentration dependent manner. The scavenging capacity of the sample were compared with the standard, Gallic acid which is a stronger DPPH scavenger

IC₅₀ value in Antioxidant activity denotes the concentration required to show 50% inhibition of the probe in antioxidant assay. The IC₅₀ value of gel was found to be 69.51 μ g/ml. Lower IC₅₀ values represent that antioxidant capacity will be high. Therefore, the result indicates that the gel have high radical scavenging activity and graphically represented in the figure

Sample	Concentration	OD value	% of Inhibition	IC 50 value
Anti acne gel	10	0.174	48.6	69.51
	100	0.160	52.8	
	250	0.135	60.1	
	500	0.111	67.2	

Table 8 percentage of inhibition and IC 50 value for anti-oxidant activity

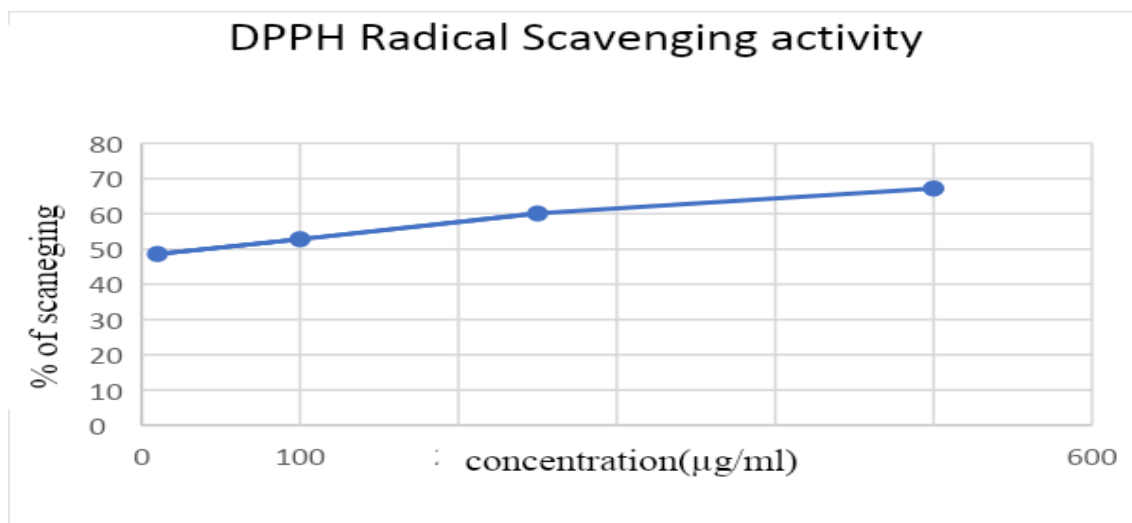
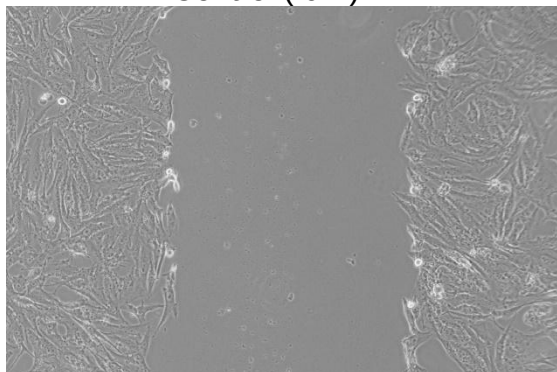


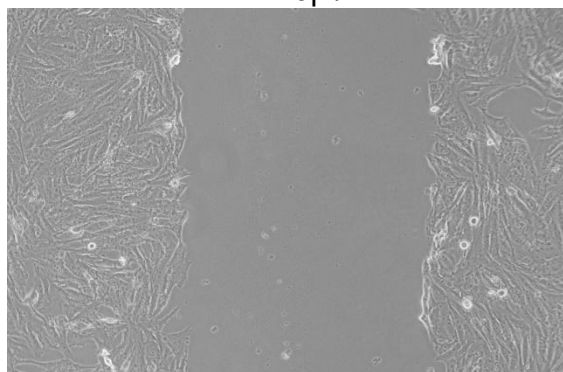
Table 9 DPPH radical scavenging activity of the gel

5.10 IN VITRO WOUND HEALING ACTIVITY

Control (0hr)

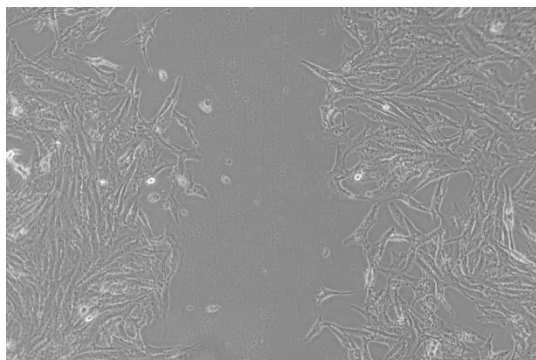


40 μ l/ml

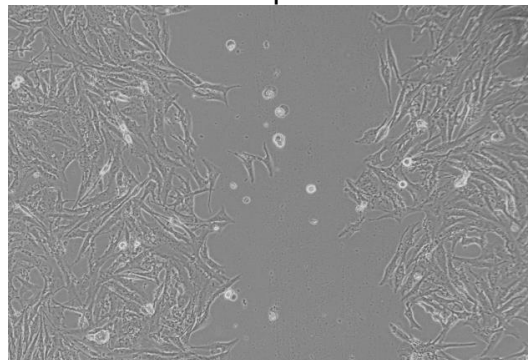


After 24 hrs. in 10 X microscope

Control



40 μ l/ml



CHAPTER 6

SUMMARY AND CONCLUSION :

The two medicinal plants were collected and their phytochemical study showed the presence of all the essential phytochemicals anti-microbial activity of the extracted sample was checked by using well diffusion assay against *S. aureus* the gel formulation was made with a completely natural gel base different concentrations of the extracts were mixed and the final composition was found by testing its anti microbial activity against *P. acne* which is the major cause of the acne vulgaris by well diffusion method . The study of the minimum inhibitory concentration of the Marukarrai kudineer against *Staphylococcus aureus* and *Pseudomonas aurogenosa* was reported. MTT assay showed their level of toxicity to the epithelial cells and the lethal dose was found. The antioxidant activity of the gel was found that The IC₅₀ value of gel was found to be 69.51µg/ml. Lower IC₅₀ values represent that antioxidant capacity will be high. The gel was also tested for its healing capacity by scratch analysis and found that it has a good healing capacity which is needed to treat damaged cells in the affected area. Thus the outcome of the study proved that the gel formulated was effective herbal, ecofriendly cosmeceutical application. It can be extrapolated as a face mask/ peel too .

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