

DEVELOPMENT OF VITAMIN RICH CELL REPAIR FACIAL SERUM USING NATURAL PLANT PRODUCTS

Submitted in partial fulfillment of the requirements for the award of Bachelor of
Technology degree in Biotechnology

By

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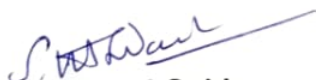
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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the Bonafide work of **Arthy M (40230004)** and **Aileen Aksharaa V Rayen (40230050)** who carried out the project entitled "**DEVELOPMENT OF VITAMIN RICH CELL REPAIR FACIAL SERUM USING NATURAL PLANT PRODUCTS**" under our supervision from **September 2023 to April 2024.**


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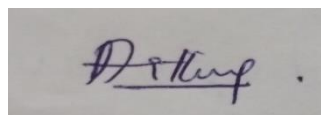

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DECLARATION

We, **Arthy M (40230004)** and **Aileen Aksharaa V Rayen J (40230050)** hereby declare that the Project Report entitled “**Development of vitamin rich cell repair facial serum using natural plant products**” done by us under the guidance **Dr. S. Usha Nandhini** (Internal) at **Sathyabama Institute of Science and Technology, Chennai, Tamil Nadu, India**, is submitted in partial fulfilment of the requirements for the award of **Bachelor of Technology degree in Biotechnology**.

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SIGNATURE OF THE CANDIDATE

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ABSTRACT

Facial wrinkles and skin aging are undesirable outcomes of photodamage and ultraviolet (UV) rays. Currently, no effective strategies are available to delay the skin aging process. Serum is a highly concentrated cosmetic product. Serum has the property of rapid absorption and the ability to penetrate the deeper layer of the skin. A cell repair facial serum also known as an anti-aging facial serum is a dual-phase serum, indicating its unique composition that incorporates both water-soluble and oil-soluble ingredients that are applied to the skin topically to reduce fine lines and wrinkles, protecting Against Environmental Damage, preventing premature aging, evening skin tone, promoting cell renewal and enhancing overall skin vitality. The cell repair facial serum is entirely formulated with botanical extracts and oils, including plant-based emulsifying agents and preservatives. The present preparation is made using plant extracts including gotu kola, amla, rose, orange peel, and green tea which works as an antiaging and removes dead cells as well as moisturizes, combined with seed oils such as pomegranate seed oil and Bakuchiol seed oil, in formulating an effective anti-aging facial serum. Pomegranate seed oil and Bakuchiol seed oil an important fat-soluble antioxidants. These two seed oils have been considered as an anti-inflammatory agent in the skin, as several studies have supported its prevention of inflammatory damage after UV exposure and it is considered a powerful antioxidant that may be effective in reducing UV damage in skin. The formulation is enriched with active ingredients like niacinamide and additionally, sunflower lecithin ensures oil and water binding, xanthan gum contributes to thickening, and glycerine acts as a humectant. In this Cell repair facial serum, the plant extracts for glowing skin and the humectant prevents drying of skin. Then the active ingredient Niacinamide helps for skin elasticity and acts as an antiaging removes dead cells and also fights dark spots and acne. Studies suggest that the niacinamide in the serum also manages oily skin and sebum production, which prevents acne. The face serum was evaluated for its physio-chemical parameters, pH, globule size, and consistency. The stability study result showed that there was no change in visual appearance, homogeneity, and globule size.

Keywords: Anti-ageing, Cell repair, Antioxidant, Anti-inflammatory, UV damage, Moisture.

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LIST OF ABBREVIATIONS

ACRONYM	EXPANSION
UV	Ultraviolet
pH	Potential of hydrogen
USD	United States dollar
EU	European Union
USA	United States of America
CAGR	Compound Annual Growth rate
NAD	Nicotinamide adenine Dinucleotide
AHA	Alpha hydroxy acid
NAG	N-acetyl glucosamine
COX	Cyclooxygenase
PG	Prostaglandin
PMFs	Polymethoxyflavonoids
DMSO	Dimethyl sulfoxide
DPPH	2,2-diphenyl-1-picrylhydrazyl
pH	Potential of Hydrogen

CHAPTER 1

INTRODUCTION

Skincare is an essential aspect of personal grooming and well-being, influencing both physical appearance and self-confidence. With an increasing emphasis on natural and sustainable solutions, there has been a growing interest in skincare products formulated with botanical extracts and vitamins. This aims to explore the development of a vitamin-rich cell repair facial serum using natural plant products. By investigating the efficacy of botanical extracts and vitamins in promoting skin health and repair, this research seeks to contribute to the understanding of natural skincare formulations. Through a combination of literature review, formulation development, and efficacy testing, this endeavors to provide valuable insights into the potential benefits and applications of natural ingredients in skincare products. Skincare routines have evolved significantly, with an increasing demand for products that not only nourish the skin but also harness the power of natural ingredients and advanced actives. This embarks on exploring the formulation of a vitamin-rich cell repair facial serum, integrating potent botanical extracts alongside active ingredients such as niacinamide. With an ever-growing appreciation for natural skincare solutions, this research endeavors to delve into the synergistic effects of these ingredients on skin health and rejuvenation. By examining the efficacy of niacinamide alongside botanical extracts, this study aims to contribute valuable insights into the development of cutting-edge skincare formulations that marry the best of nature and science. Through comprehensive literature review, formulation optimization, and efficacy testing, this seeks to pave the way for innovative skincare solutions that cater to the evolving needs of consumers seeking both efficacy and natural goodness in their skincare regimen.

1.1 COSMETICS AROUND THE WORLD

The cosmetic industry is a global, everchanging growing sector. Over the past decades, industry innovation has been immense, resulting in a wide range of new products and an increase in sales. In 2020 alone, the global cosmetic market was valued at USD 341.1 billion and is expected to hit USD 560.50 billion by 2030, with a compound annual growth rate of 5.1% from 2021 to 2030 (*Ferreira et al., 2022a*). The major cosmetic market consists of the European Union (EU), and the United States of America (USA). India, quickly catching up with the cosmetic market globally following its own regulations. The cosmetics market in India is growing at 15- 20% annually, twice as fast as that of the United States and European markets (*Yanaga et al., 2006*). In the US, cosmetics are regulated by the Federal Food, Drug, and Cosmetic Act. Figure 1: A comparison of cosmetic regulations between the USA, EU, and India. The growth of the market has shifted from the western hemisphere to developing regions in South America, Eastern Europe, and Asia, especially China: having a consumption of more than 10.4% CAGR (Compound Annual Growth Rate) per year during the past 5 years (*Kumar et al., 2018*). There are a couple of major cosmetic companies around the world that are competing against each other to capture a share of the multi-billion dollar cosmetic market. There are a couple of major cosmetic companies around the world that are competing against each other to capture a share of the multi-billion dollar cosmetic market (*Kumar et al., 2006*). L'Oréal SA is the global leader in cosmetics with a 16.8% market share and gross sales of roughly \$5 billion according to Euro Monitor International (*Kumar, 2005*). Estee Lauder Company is ranked second with 10.9 %, Procter and Gamble Company third with 9.3%, fourth is Revlon Inc. with 7.1 %, and Avon products with 4.7% rounds up the top five. Overall, the top twenty cosmetics producers accounted for roughly 73% of the global marketplace (*Kumar et al., 2006*). In China the regulation of cosmetics is complex, involving two separate government bodies. Cosmetics legislation in China is momentarily under evaluation and may be the focus of substantial alteration in the near future. The quickness of growth in the Chinese cosmetics industry is so steep that each product group is successful and has incredible prospects to expand its market share. (*Holt & McNeillis, 2007*)

1.2 COSMECEUTICALS

A *cosmeceutical* is a cosmetic product whose active ingredient is meant to have a beneficial physiologic effect resulting from an enhanced pharmacologic action when compared with an inert cosmetic (*Kligman, 2000*). Cosmeceuticals being cosmetic products having medicinal or drug-like benefits can affect the biological functioning of skin owing to the type of functional ingredients they contain (*Turkington & Dover, 2009*). Vitamin A Forms Several forms of vitamin A are used cosmetically, in particular retinol, retinyl esters (eg, the acetate, propionate, and palmitate esters of retinol), and retinaldehyde. Through endogenous enzymatic reactions, these are all ultimately converted to trans-retinoic acid, which is the functional form of vitamin A in the skin (*Bissett, 2009*).

Vitamin B3 Forms The primary forms of vitamin B3 that have been used in skin care products are niacinamide (nicotinamide), nicotinic acid, and nicotinate esters, such as tocopherol nicotinate, myristoyl nicotinate, and benzyl nicotinate. Vitamin B3 is an essential vitamin. It is a precursor to a family of endogenous enzyme cofactors, specifically nicotinamide adenine dinucleotide (NAD), its phosphorylated derivative NAD(P), and their reduced forms NAD(H) and NAD(PH), which have antioxidant properties. These cofactors participate in many enzymatic reactions in the skin and thus can potentially influence many skin processes (*Farage et al., 2009a*). Vitamin C Forms There are many forms of this vitamin. Some commonly used ones are ascorbic acid, ascorbic phosphate (magnesium or sodium salt), ascorbic palmitate, and ascorbic glucoside. Vitamin C has been used as a skin lightener because of its antioxidant activity and tyrosinase inhibition effect. It has also been described as an anti-inflammatory agent because it reduces the erythema associated with postoperative laser resurfacing. In addition, ascorbic acid serves as an essential cofactor for the enzymes lysyl hydroxylase and prolyl hydroxylase, both of which are required for posttranslational processing in collagen (types I and III) biosynthesis (*Suekawa et al., 2017*). Vitamin E the more commonly used forms of vitamin E, in particular tocopherol and tocopherol acetate, several other esters, for example, succinate, nicotinate, linoleate, and phosphate, are used in the cosmetic area. Natural tocopherol also has several isomers (alpha, beta, gamma, and delta) that differ in some of the side chains (*Lindgren et al., 2021*). Vitamin E has the potential to prevent and improve skin problems caused by these radicals, in particular, those caused by UV exposure such as sunburn, skin photoaging (wrinkling), and hyperpigmentation (*Draelos & Thaman, 2005*).

Peptides A few peptides with known sequences have been of particular interest to the cosmetic industry, such as palmitoyl-lysine-threonine threonine-lysine-serine (pal-KTTKS; Matrixial), acetyl-glutamate- glutamate-methionine-glutamine-arginine-arginine (Ac-EEMQRR; Aegirine), and the tripeptide copper glycine-histidine-lysine (Cu-GHK). The peptide pal-KTTKS is a fragment of human dermal collagen that stimulates new collagen production and has been proposed for wound healing. The peptide pal-KTTKS is a fragment of human dermal collagen that stimulates new collagen production and has been proposed for wound healing (*Katayama et al., 1993*).

Panthenol Form Panthenol, or provitamin B5, is also known as pantothenol and pantothenol alcohol. The D optical isomer of panthenol is termed Dexpanthenol. It is a precursor to pantothenic acid (vitamin B5). Pantothenic acid is a component of coenzyme A, which is critical in cellular metabolism, including in acyl group transfer during fatty acid biosynthesis and gluconeogenesis (*Ross et al., 2020*). panthenol is water soluble and hygroscopic, it has skin moisturization potential, especially when combined with the widely used moisturizing agent glycerol (*Proksch & Nissen, 2002*). Topical panthenol is extremely well tolerated by the skin and does not induce skin irritation responses (redness, dryness, burn, sting, or itch (*Ebner et al., 2002*).

Many compounds are included within this group, including α -hydroxy acids (AHA) such as glycolic, malic, tartaric, lactic, and citric acids; polyhydroxy acids such as gluconolactone and lacto bionic acid; and β -hydroxy acids such as salicylic acid and alkyl derivatives of it, including octanol salicylic acid (*Kornhauser, 2010*). A mechanism of these acids involves the exfoliation of stratum corneum, most likely by chelation of the calcium involved in cell-cell cohesion. This accelerated exfoliation then results in improvement effects on skin surface texture and color appearance (*Draelos & Thaman, 2005a*). Sugar amines Form Many materials fall within the broad category of sugar amines, with some widely known ones being the hexose amines glucosamine and N-acetyl glucosamine (NAG). Glucosamine and NAG are precursors to the biopolymer hyaluronic acid, which is an important water-binding structural component of skin in the epidermis and the dermis

(*Draelos et al., 2019*). Glucosamine has inhibited protein glycosylation in vitro, inhibiting activation of pro tyrosinase to the active enzyme tyrosinase and thus inhibiting the

production of melanin(*Imokawa & Mishima, 1985*).Ceramides Forms Ceramides are lipids essential for normal stratum corneum water barrier(*Mao-Qiang et al., 1996*).Externally supplied ceramides function by incorporating into the intercellular lipid of stratum corneum to replace the depletion that occurs with aging and environmental damage such as from surfactant exposure(*R. et al., 2023*). Metals Forms Of the many metals, cosmetic products typically contain only a few, among them zinc, copper, selenium, strontium, magnesium, and manganese, as salts and complexes with organic compounds. A few examples are zinc oxide, copper peptide, and selenomethionine (*Lindgren et al., 2021b*). Topical use of copper peptide complex (see Peptides section) can provide facial skin antiaging effects. Topical application of selenomethionine prevents UV-induced erythema and is discussed as providing facial anti-wrinkle effects (*Kaur & Rath, 2019b*)

1.3 FACIAL SERUM AND ITS IMPORTANCE

The study of human skin represents an important area of research and development in dermatology, toxicology, pharmacology, and cosmetology, to assess the effects of exogenous agents, their interaction, their absorption mechanism, and their toxicity toward the different cutaneous structures (*Kokate et al., 2021*). A skincare formulation must be able to deliver a powerful agent into the skin to fulfill the intended objective. Face serum is the answer to deliver the precious active ingredient into the skin thus eliminating the use of hazardous chemicals in giving instant results. Serum is a concentrated product that is widely used in Cosmetology (*Draelos & Thaman, 2005a*). Serum is a thin-viscosity topical product that contains a concentrated amount of active ingredients Serum is lighter and delivers active ingredients to the skin quickly it goes on first, after you've cleansed your skin (*Seol et al., 2012*). The name comes from itself in professional cosmetology. The cosmetic serum is as concentrated in water or oil as any other cream. Serums are defined as a concentrated product that contains ten times more organic matter than cream (*Amnuaikit et al., 2019*). Face serum is a highly concentrated emulsion that is available in water-based and oil-based serum and shows noticeable results within a month or less (*Cai et al., 2004*).

This is because face serums are made of very small molecules that help them to penetrate deep into the skin quickly.

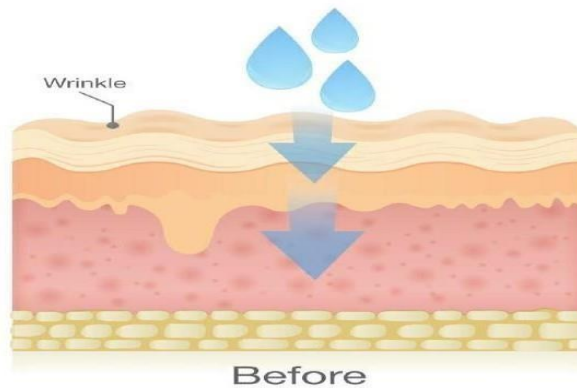


Fig:1.1 appearance of wrinkles

The appearance of wrinkles on the surface of the skin is actually caused by the loss of collagen, which leads to the collapse of the inner dermis of the skin.

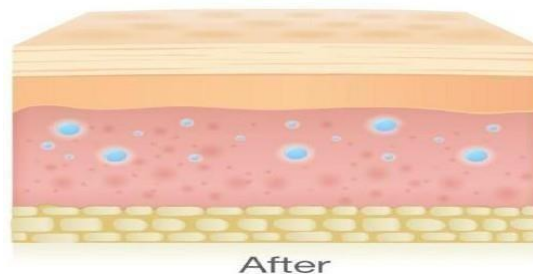


Fig:1.2 Smooth out wrinkles

Smooth out wrinkles and repair damaged skin. Elastic fiber tissue transports nutrients and locks in moisture

The serum is packed with a bunch of beneficiary active components and nutrients such as antioxidants, ceramides, amino acids, and others. This explains why face serum always being the costliest item in a skincare set (Glaser, 2003). Whether it is a moisturizer, anti-wrinkle or anti-aging product, or skin serum, all these products should contain antioxidants, cell-communicating ingredients, and skin-identical ingredients. All skin type needs these ingredients to be as healthy as possible. Gel and liquid

preparations are best for oily and combination skin, serums and light lotions are best for normal to dry skin, and more emollient lotions and moisturizing creams are best for dry to very dry skin (*Aburjai & Natsheh, 2003*). Texture is all about skin type- but the brilliant ingredients for healthy skin are the same for everyone, regardless of product, texture, or personal preference (*Karnelasatri et al., 2023*)

1.4 ROLE OF PLANT AND FRUIT EXTRACTS-PHYTOCHEMICALS

Plant-based bioactive extracts and phytochemicals serve two purposes: (1) as ingredients to improve the biological functioning of the skin and (2) as skin care essentials that supply nutrients for skin health (*Sumpio et al., 2006*). A variety of compounds and extracts from a broad range of plants and fruits are utilized as natural antioxidants in the cosmetics sector. These extracts can protect the items from oxidative destruction or reduce oxidative stress on the skin (*He et al., 2021*). A variety of phytochemicals found in fruits possess antioxidant qualities that protect against the damaging effects of free radicals, which in turn cause chronic diseases associated with aging (*Serra et al., 2011*). The topical application of vitamins and antioxidants found in skin care products is thought to help prevent and potentially even reverse the damage caused by free radicals. Additionally, the effects of several vitamins on the skin, such as their ability to reduce bruising and pigmentation, activate the creation of collagen, refine keratinization, and have anti-inflammatory properties, may be advantageous (*Lobo et al., 2010*). Astringent, ant-irritant, antioxidant, photoprotective, antiaging, moisturizing, and antibacterial are just a few of the attributes that make botanical extracts multifunctional in nature (*Saraf & Kaur, 2010*). Among many bioactive ingredients found in botanical products are vitamins, antioxidants, phenols, quinones, flavonoids, proteins, terpenoids, anthraquinones, etc. (*Trovato & Ballabio, 2017*). Plant-based antioxidants are increasingly common and could someday take the place of synthetic antioxidants in certain applications. An isolated or single pure chemical, a mixture of chemicals, or plant extracts can all be considered natural antioxidants, and they are frequently found in cosmetology (*Valko et al., 2007*). By means of an electron donation/transfer mechanism, vitamin C, a potent antioxidant, can counteract oxidative stress (*Caritá et al., 2020*). The most well-known function of flavonoids on the skin is linked to their antioxidant characteristics. The majority of

flavonoids that contain phenol are resonance-stabilized anion radicals with a comparatively high reduction potential. The structural and physicochemical characteristics of flavonoids have a major impact on their scavenging activity (*Arct & Pytkowska, 2008*). One of the flavonoids' many functions is their ability to reduce inflammation. Cosmetology is one area where this action is frequently utilized (*Kuiper et al., 1998*). Vitamins work well topically to improve dermal-epidermal junction cohesiveness, reduce skin photodamage, differentiate keratinocytes, and alleviate hyperpigmentation (*Hoang et al., 2021b*).

1.5 NEED FOR USE OF ORGANIC INGREDIENTS

Although increasing numbers of individuals are becoming aware of the long-term health advantages of natural products, the market for plant-based skincare has expanded dramatically on a global scale (*Nadeeshani Dilhara Gamage et al., 2022b*). The different dangerous allergens found in cosmetics can be categorized under metals, parabens, phthalates, dioxanes, and chlorofluorocarbon propellants. Over time, the body's bioaccumulation of these dangerous chemicals and metals has been linked to various health issues such as malignancies, allergies, skin diseases, lung damage, aging, hair loss, and abnormalities pertaining to reproduction and development (*J. N., 2015*). The Food and Drug Administration, however, states that goods that are made with a variety of approved cosmetic ingredients to serve as the foundation for the application of one or more herbal ingredients to provide specific cosmetic benefits—such as influencing skin functions or supplying nutrients needed for healthy skin or hair—are classified as “herbal cosmetics” (*Nadeeshani Dilhara Gamage et al., 2022c*). Among the functional benefits of organic components include their ability to scavenge free radicals, as well as their antibacterial, anti-inflammatory, antiaging, sun protection, and ability to reduce hyperpigmentation (*Aburjai & Natsheh, 2003b*). Essential oils, lipids, oils, plant extracts, waxes, and colorants are a few significant plant-based substances found in skincare. The final cosmetic goods contain these substances for a variety of purposes, including coloring, scenting, moisturizing, thickening, and stabilizing (*Aburjai & Natsheh, 2003c*).

CHAPTER 2

LITERATURE SURVEY

Moisturizers are complex formulations designed to maintain the water content of the skin between 10% and 30%. Water is essential to maintain skin elasticity and barrier integrity. Skin that is water deficient, such as the thickened skin commonly present over the heels, fissures readily with trauma and is rough to the touch. Water is necessary to allow skin distensibility, a mandatory requirement in an organ that is in constant motion. Skin hydration is important to maintain an intact skin barrier, tactilely perceived as smooth, soft skin.) The polysaccharide-rich composition of *Aloe vera* extracts (*Aloe barbadense* Miller), often used in cosmetic formulations, may impart moisturizing properties to the product. The aim of this study was to evaluate the effect of cosmetic formulations containing different concentrations of freeze-dried *Aloe vera* extract on skin hydration, after a single and a 1- and 2-week period of application, by using skin bioengineering techniques. (Ferreira et al.,2022)

Formulation and Characterization of Cosmetic Serum Containing Argan Oil as Moisturizing Agent Nowadays, cosmetics are becoming more high demand in daily life and it was used frequently by many people per year. The moisturizing serum is a bland of oleaginous substances that are applied to the skin by rubbing which is used to replace natural skin oil, to cover tiny fissures in the skin, and to provide a soothing protective film. Argan oil is a plant oil that is produced from the Argan tree (*Argania spinose*) which become one of the main roles in the dermo-cosmetic field due to its higher moisturizer contents. The main objective of the research is to evaluate the characteristics of cosmetic serum and to identify the best formulation of cosmetic serum containing Argan oil as a moisturizing agent. ("Corrigendum,"2017b)

The measurement of static physio-chemical properties of oil-water interfaces provides a useful method of indicating where problem emulsion stability areas arise for water-in-crude oil emulsions and the effectiveness of chemical demulsifiers in resolving these problems. The paper includes a discussion of interfacial tension, interfacial shear viscosity, and interfacial film compressibility, particularly as applied to complex crude oil systems, and deals with the modification of these properties with the addition of a chemical emulsifier.

With age, decreasing dermal levels of proteoglycans, collagen, and elastin leads to the appearance of aged skin. Oxidation, largely driven by environmental factors, plays a central role. The aim of this study was to assess the antiaging efficacy of a topical serum containing L-Ascorbic acid, soluble proteoglycans, low molecular weight hyaluronic acid, and a tripeptide in ex vivo and in vivo clinical studies. Human cell cultures are being developed to replace various body tissues. To assess the safety and efficacy of dermal regeneration with the injection of young autologous fibroblasts obtained from a culture containing serum from patients themselves. (*Draelos et al., 2019*)

Platelet-rich plasma (PRP) is traditionally used as an injectable material for enhanced healing, hair growth, and facial rejuvenation. This research examined the novel use of topical autologously sourced PRP added to a preservative cosmetic base and applied twice daily to the face following electroporation for 8 weeks. Alveolar epithelial type II cells are essential for regenerating an intact alveolar barrier after the destruction of type I cells in vivo. The first objective of these experimental studies was to develop an in vitro model to quantify alveolar epithelial cell wound repair. The second objective was to investigate mechanisms of alveolar epithelial cell wound healing by studying the effects of serum and transforming growth factor-alpha (TGF-alpha) on wound closure. Primary cultures of rat alveolar type II cells were prepared by standard methods and grown to form confluent monolayers. The repair of a craniofacial or nose deformity requires a large volume of reconstructive material. A conventional cartilage graft does not provide a sufficient volume of reconstructive material. Therefore, augmentation of the facial form to the defect shape is quite difficult. The authors developed a new treatment method that provides a sufficiently large volume of reconstructive material and enables an easier reconstruction of the original shape. (*Kheradmand et al., 1994*)

Collagen hydrolysate has been widely used as a nutraceutical agent against skin aging and has gained increasing attention. Previous research has suggested that oral administration of antioxidant collagen peptides (ACPs) exerted beneficial effects on the photoaging skin structure and collagen. However, the bioactive components in ACP metabolites that are responsible for the repair effects have not been elucidated. In this study, serum containing collagen peptides (CPS) after oral administration and collagen peptides isolated from serum metabolites (SCP) were collected, and their effects on cell proliferation, hyaluronic acid secretion, and the collagen synthesis pathway in UVA-

induced skin fibroblasts (ESF) were evaluated. Furthermore, hydroxyproline (Hyp)-containing collagen peptides from SCP were analyzed and their repair effects were examined (*Liu et al., 2019*)

The role of the monocyte/macrophage in wound repair has been investigated by studying the healing process in wounds depleted of this cell and/or its phagocytic activity. Hydrocortisone acetate (0.6 mg/g body weight) administered as a subcutaneous depot was used to induce prolonged monocytopenia in guinea pigs, and antimacrophage serum (AMS) was used for local elimination of tissue macrophages. In vitro, the presence of complement macrophages is rapidly lysed and killed by AMS. In the absence of complement, AMS is not cytotoxic but potently inhibits adherence to and phagocytosis of opsonized erythrocytes by macrophages. AMS titers were obtained by observation of adherence and phagocytosis of opsonized erythrocytes in serial dilutions of AMS. (*Watanabe et al., 2014*)

Autologous nerve grafting is the current procedure used for repairing facial nerve gaps. As an alternative to this method, tissue engineering cell-based therapy using induced pluripotent stem cells, Schwann cells, and bone marrow-derived mesenchymal stem cells has been proposed. However, these cells have major problems, including tumorigenesis in induced pluripotent stem cells and invasiveness and limited tissue associated with harvesting for the other cells. Here, we investigated the therapeutic potential of adipose-derived stem cells (ASCs), which can be harvested easily and repeatedly by a minimally invasive liposuction procedure. The ASCs had characteristics of mesenchymal tissue lineages and could differentiate into Schwann-like cells that were relatively simple to isolate and expand in culture. (*McCall-Perez et al., 2011*)

Dermatology visits for the prevention and treatment of aging skin are rapidly increasing. The clinical sequelae including wrinkling, pigmentary changes, roughness, laxity, and telangiectasia can all result in the appearance of aging skin, impacting the quality of life. A facial serum was developed with ingredients associated with an improvement in the appearance of fine lines and wrinkles and an increase in stratum corneum barrier function. Patients were instructed to use a gentle wash before applying the formulation and a moisturizer afterward. (*Sanz et al., 2015*)

Epithelial regeneration in the skin is achieved by the constant turnover and differentiation of keratinocytes. Epidermal and dermal stem cell compartments are fundamental for the continuous renewal of the skin. Adult stem cells are a unique source for skin tissue

renewal. Plants have stem cells and plant-derived stem cell extracts are now used in topical products for their potential anti-ageing and anti-wrinkle effects. A new dermo-cosmetic product containing apple stem cell extract, urea, creatine, and palmitoyl tripeptide-38 (Urea Din Fusion Serum Lift Antitragus, ISDIN S.A), has been recently developed for different aspects involved in skin aging. (*Theobroma Cacao L.*,” 2019)

The high polyphenol content of cocoa pod extract gives it the potential to be developed as an antioxidant and tyrosinase inhibitory agent in cosmetic preparations. Phyto some systems known could enhance skin penetration of phytoconstituent like polyphenol-rich extract. The objectives of this research were to formulate a Phyto-containing cocoa pod extract, develop a Phyto complex into face serum preparation, and determine the antioxidant and tyrosinase inhibitory activities of the extract and the formulated serum. The cocoa pod extract was developed into Phyto a system by thin-layer method using soy phosphatidylcholine. The Phyto some then develop into a face serum formulation using Viscofan MAC 10 as a gelling agent. The antioxidant activity was conducted by 2,2diphenyl- 1-picrylhydrazyl radical scavenging assay and tyrosinase inhibitory activity was conducted by colorimetric enzymatic assay. The cocoa pod extract has very strong antioxidant activity with an inhibitory concentration (IC₅₀) of 17.21 ppm. The extract also has tyrosinase inhibitory activity with IC₅₀ of 199.98 ppm (Savic *et al.*, 2021)

The growing consumers' preferences and concerns regarding healthy aging, youthful skin appearance, environmental protection, and sustainability have triggered an ever-increasing trend toward natural, eco-friendly, and ethically sourced anti-aging products. Accordingly, this paper describes the design and evaluation of novel, safe, effective, and high-quality emulsion serums, completely based on ingredients of natural origin, intended for improving facial fine lines and wrinkles. *Carica papaya L.* leaves are considered agricultural waste in Thailand but have been reported to possess natural antioxidants. This study aims to extract and quantify the phenolic and flavonoid contents in *C. papaya* leaves and evaluate their bioactivity for use in anti-aging cosmetics (Banov *et al.*, 2023).

Skin aging is a natural process that occurs because of oxidative stress. Facial skin aging is often concerning for individuals due to the exposure of the face. To assess and compare the effects of two anti-aging facial serums on the following characteristics associated with facial skin aging: fine lines/wrinkles, age spots, firmness, elasticity,

texture, radiance, tone, lifting, clarity, and complexion. The growth and demand for cosmeceuticals (cosmetic products that have medicinal or drug-like benefits) have been enhanced for the last few decades. Lately, the newly invented dosage form, i.e., the pharmaceutical-based cosmetic serum has been developed and widely employed in various non-invasive cosmetic procedures. Many pharmaceutical-based cosmetic serums contain natural active components that claim to have a medical or druglike effect on the skin, hair, and nails, including anti-aging, anti-wrinkle, anti-acne, hydrating, moisturizing, repairing, brightening and lightening skin, anti-hair fall, anti-fungal, and nail growth effect, etc. (*Khan et al., 2023*)

In comparison with other pharmaceutical-related cosmetic products (creams, gels, foams, lotions, etc.), pharmaceutical-based cosmetic serums produce more rapid and incredible effects on the skin. This chapter provides detailed knowledge about the different marketed pharmaceutical-based cosmetic serums and their several types such as facial serums, hair serums, nail serums, under-the-eye serums, lip serums, and hand, and foot serums, respectively. Skin redness is a common cosmetic concern affecting predominantly fair-skin individuals and often leading to rosacea. Based on the current scientific knowledge of the physiological mechanisms underlying the problem, a complex and integral skin care serum(100RXED2025) was developed and tested clinically for efficacy (*West et al., 2021*)

Face serum composed of a combination of biologically active compounds was evaluated for safety and efficacy in vitro, in a repeat insult patch test, and in a human clinical efficacy trial. The serum inhibited tyrosinase activity modestly, decreased collagenase activity, and exhibited notable free radical scavenging activity in vitro. It is gentle to the skin, as the serum did not irritate the skin or produce symptoms of allergic contact dermatitis in the 55 healthy adults who participated in the repeat insult patch test. In the efficacy trial, daily application of the face serum for 30 days significantly increased skin hydration, with all 35 volunteers experiencing improvement. Substantial improvements in skin elasticity, roughness (fine lines and wrinkles), and brightness also occurred during the trial. The assessment of cosmetic efficacy is rarely performed in studies comparing different concentrations of active compounds. The present study aimed to determine the skin hydrating and the skin-firming dose-response effects of cosmetic formulations enriched in

compounds derived from algae and fish collagen. A series of factors were studied including the type of formulation (cream or serum), the concentration of active ingredients, the effect of repetitive applications, as well as any residual effect of the formulations after stopping their applications. The serum enriched in marine compounds showed a better moisturizing effect in the short term. The cream appeared more active later, particularly following repeat applications. A sustained tensor (firming) effect was observed during treatment with both the lotion and the cream. However, no remnant firming effect was perceived after stopping treatment (*Xhauftaire-Uhoda et al., 2008*)

The goal of this work was the development and characterization of skin care formulations with anti-aging properties. The focus was the development of different face serums, aqueous and milky serums. The preparation of aqueous serums proved to be a faster and easier process than the milky serums. Six formulations were developed, three base formulations and three containing active ingredients. The stability of developed formulations was performed through the evaluation of different physical and chemical properties for three months. The properties included: the determination of pH, viscosity, stability at extreme temperatures, and evaluation of the organoleptic characteristics (odor, color, and appearance) at different temperatures (4C, room temperature, and 37C). It was also studied the influence of light and the packaging material on the formulation's stability. The results obtained showed that Formulation A, both the base and the active ingredients, have stability in all conditions. (*Caçador, 2022*)

A novel treatment serum formulated to target multiple pathways in the anti-aging cascade was tested both *in vitro* and in clinical settings. *In vitro*, testing was performed to assess the ability to stimulate key proteins and genes fundamental to the anti-aging cascade. The antioxidant potential of the formulation was studied in a UV-irradiation clinical study. A 12-week, open-label, single-center study was conducted to determine whether this uniquely formulated topical treatment serum could improve visible signs of facial photodamage. Clinical evaluations showed statistically significant reductions in fine wrinkles and coarse wrinkles and improvements in skin texture, tone, and radiance starting at week 4 with continued improvements at weeks 8 and 12. Subject self-assessments confirmed that the beneficial effects of the treatment serum were readily observed by the users (*McCall-Perez et al., 2011*)

This study aims to evaluate the *in vivo* efficacy in terms of elasticity, hydration, and antiwrinkle enhancement of a new cosmetic cream formulation: Apollonia anti-aging facial cream. The latter is an O/W (oil/water) emulsion with high volume percentages of snail secretion filtrate and donkey milk serum in its composition. The investigation has been performed on (twelve) healthy subjects: 8 (eight) females, and 4 (four) males, all presenting evident skin wrinkles in typical facial areas (e.g. forehead and eye contour). The subjects had to apply about 2 grams of Apollonia anti-aging facial cream every day. To evaluate the anti-wrinkles efficacy instead, we proceeded with digital camera imaging and image analysis processed through ImageJ software. The new Apollonia facial cream cosmetic formulation, based on donkey milk whey and snail secretion filtrate from *Helix Asperse Müller*, showed significant enhancement effects on skin elasticity and hydration as well as important skinwrinkles-reduction (*Mazzulla et al., 2018*).

The high demand for anti-aging skin care encourages the improvement and development of more formulations with additional benefits for general skin health and age-associated skin signs. The skin aging physical and biological phenotypes manifest differently between diverse ethnic populations. A highly polluted environment can be viewed as an extrinsic factor accelerating the skin aging process. To develop a unique formula with active complexes, having multifunctional effects for anti-pollution, brightening, and antiaging/barrier strengthening purposes with confirmed activities *in vitro* and *ex vivo* skin models, suitable for polluted skin. In the mammalian system, the hair follicle is known to be the most significant organ that determines, appearance, and gender distinction, provides intense temperature protection, and plays a role in self-defense. The younger generations have begun to suffer extreme hair loss problems due to many reasons. The hair loss is not temporary in most cases. but it results in alopecia. Many people suffering from hair loss are in search of multiple treatments due to extreme anxiety and tension. To improve hair growth and prevent hair loss, hair root activation is required. *Citrus sinensis* is used for antidandruff protection and hair care. It has antibacterial and anti-inflammatory properties. *Nigella sativa* improves the shine, strength, volume, and texture of hair (*Khmaladze et al., 2019*)

Flax seed is full of fatty acids and antioxidants that tend to remove pollutants and dead cells from the scalp. Coconut oil may help to moisturize and seal hair. They can help to prevent dry, flaky scalp and dandruff. This review article focuses on the materials required

for the formulation of hair serum and various evaluation tests to evaluate hair serum.

Allegations on the benefits of incorporating vitamin C, vitamin E, and combinations thereof in topical skincare formulations are mostly based on in vitro and ex vivo experiments and/or limited protocols of specific stress conditions (pollution, UV exposure, laser irradiation,...). To evaluate the instrumentally measurable effects and quantitative consumer perceptions of a protective and reparative serum on a panel of volunteers under normal nonstressed conditions of use, employing FOITS technology and innovative self-assessment methods (Lintner et al., 2020)

In recent years, interest in the health effects of natural antioxidants has increased due to their safety and applicability in cosmetic formulation. Nevertheless, the efficacy of natural antioxidants in vivo is less documented than their prooxidant properties in vivo. Plant extracts rich in vitamins, flavonoids, and phenolic compounds can induce oxidative damage by reacting with various biomolecules while also providing antioxidant properties. Because the biological activities of natural antioxidants differ, their effectiveness for slowing the aging process remains unclear. This review article focuses on the use of natural antioxidants in skincare and the possible mechanisms underlying their desired effect, along with recent applications in skincare formulation and their limitations (Hoang et al., 2021)

Food chemistry and cosmetic chemistry come together to promote both inside and outside well-being. Nutricosmetic optimizes the intake of nutritional microelements to meet the needs of the skin and skin appendages, improving their conditions and delaying aging, thus helping to protect the skin from the aging action of environmental factors. Numerous studies in the literature show a significant correlation between the adequate intake of these supplements, improved skin quality (both aesthetic and histological), and the acceleration of wound healing. This review revised the main foods and bioactive molecules used in nutricosmetic formulations, their cosmetic effects, and the analytical techniques that allow the dosage of the active ingredients in the food. The hybrid of cosmetic and pharmaceutical compounds, known as cosmeceuticals, possesses therapeutic as well as beautification potential based on its key ingredients. Natural products are well regarded as a rich source of cosmeceuticals. d other natural sources open up a big market in the cosmetic industry (Dini & Laneri, 2021)

CHAPTER 3

AIM AND OBJECTIVE

AIM

To analyze the chemical properties and to standardize the composition of the ingredient for the “Development of Vitamin rich cell repair facial serum using natural plant products”

OBJECTIVES

- To optimize the ingredients including natural extracts, thickeners, and emulsifiers official serum formulation concerning the viscosity and pH value.
- To determine the physiochemical properties of the final serum formulation.
- To determine the antioxidant and antibacterial activity of the final formulation.
- To characterize the face serum formulation in terms of its flow behavior and sensory profile, stability, and half-life.

CHAPTER 4

MATERIALS AND METHODS

- *Centella asiatica* (Gotu Kola)
- *Phyllanthus emblica* (Amla)
- *Citrus aurantium* (Orange Peel)
- *Camellia sinensis* (Green Tea)
- *Rosa rubiginosa* (Rose Flower)
- *Raphanus sativus* (Radish Root)
- *Psoralea corylifolia* seed oil (Bckuchiol seed oil)
- *Punica granatum* seed oil (Pomegranate seed oil)
- Lecithin
- Xanthu Gum
- Glycerine
- Niacinamide

4.1 PLANT MATERIALS

4.1.1 *Centella asiatica*

Gotu Kola is an herbaceous plant known as *Centella Asiatica* (L) and belongs to the family Apiaceae(Cu et al., 2020). Gotu Kola is one of the plants used as a skin care product since it consists of triterpenoid, asiaticosida, madekakosida, and flavonoids, which have antioxidant, anti-inflammatory activities and help in premature aging(*R. et al., 2023*). Gotu Kola is rich in fatty acids and has moisturizing property that helps to improve skin hydration. It is primarily found in anti-aging because it promotes the production of collagen, repairs scars, and increases the levels of antioxidants in newly formed tissues(*Bojja, 2022*). The cosmetic ingredient review (CIR) Expert Panel reviewed the safety of gotu kola-

derived ingredients, which function primarily as skin conditioning agents in cosmetic products, and concluded that they are safe in present uses and concentration levels in cosmetics when formulated to be non-sensitizing (Bylka *et al.*, 2013).



Fig 4.1 *Centella asiatica*

Table 4.1 Bioactive compounds isolated from *Centella Asiatica*

CLASS	COMPOUND	REFERENCE
Triterpene acid	asiatic acid, asiaticoside, madecassic acid, madecassoside, terminolic, centic, centellic, centoic acid, indocentoic acid, isobrahmic, brahmic, betulic, and madasiatic acid	(Singh & Rastogi, 1969b)
Polyphenolic compounds	quercetin, quercitrin, kaempferol, luteolin, chlorogenic acid	(Cherry, 1998)
Alkaloids	hydrocotylin	(Belwal <i>et al.</i> , 2019)
Volatiles and fatty acids glycerides of oleic, palmitic, stearic, lignoceric, linoleic, and linolenic acids	glycerides of oleic, palmitic, stearic, lignoceric, linoleic, and linolenic acids	(Belwal <i>et al.</i> , 2019)

Glycosides asiaticoside A, asiaticoside B, madecassoside, centelloside, brahmoside, brahminoside, thankuniside, glycoside D, and glycoside E	asiaticoside A, asiaticoside B, madecassoside, centelloside, brahmoside, brahminoside, thankuniside, glycoside D, and glycoside E	(Alnassrallah et al., 2022)
Other oligosaccharides centellose, stigmasterol, sitosterol, campesterol, polyacetylenes, carotenoids, vitamins B and C, Bellarine, pectic acid, tannins, sugars, inorganic acid, and resins	oligosaccharide centellose, stigmasterol, sitosterol, campesterol, polyacetylenes, carotenoids, vitamins B and C, Bellarine, pectic acid, tannins, sugars, inorganic acid, and resins	(Kapoor, 2018)

4.1.2 *Phyllanthus emblica*

Phyllanthus emblica (syn. *Emblica officinalis*), commonly known as the Indian gooseberry in English, belongs to the family Euphorbiaceae (Mathai et al., 2015). Amla is a rich source of vitamin C, and reports indicate that levels of this crucial vitamin in amla are greater than in oranges, tangerines, or lemons (Tierney et al., 2013). Emblica extracts have been reported to possess a special property of promoting pro-collagen content while inhibiting the MMP, thus effectively inhibiting ultraviolet B (UVB)-induced photoaging in human skin fibroblasts (Adil et al., 2010). *Emblica* has been proven to protect the skin from the damaging effects of free radicals, non-radicals, and transition metal-induced oxidative stress, and may be used as an effective sunscreen and anti-aging skin product (Chaudhuri, 2002).



Fig 4.2 *Phyllanthus emblica*

Table 4.2 Chemical constituents of *Phyllanthus emblica*

TYPE	CHEMICAL CONSTITUENTS
Hydrolysable Tannins	Emblicanin A and B, Punigluconin, Pedunculagin, Chebulinic acid (Ellagitannin), Chebulagic acid (Benzopyran tannin), Corilagin (Ellagitannin), Geraniin (Dehydroellagitannin), Ellagotannin
Alkaloids	Phyllantine, Phyllembein, Phyllantidine
Phenolic compounds	Gallic acid, Methyl gallate, Ellagic acid, Trigallayl glucose
Amino acids	Glutamic acid, Proline, Aspartic acid, Alanine, Cystine, Lysine
Carbohydrates	Pectin
Vitamins	Ascorbic acid
Flavonoids	Quercetin, Kaempferol
Organic acids	Citric acid

4.1.3 *Citrus aurantium*



Fig 4.3 Citrus aurantium (Orange)

The orange peel is the fresh or dried outer part of the pericarp of *Citrus aurantium* Linn which belongs to the family Rutaceae. Ultraviolet light (UV) induces an inflammatory response in the skin by cyclooxygenase (COX)-2 expression and prostaglandin (PG) E2 production. Citrus peel has been used as a natural medicine. It contains polymethoxyflavonoids (PMFs) as a major ingredient, which have anti-inflammatory activity (Yoshizaki et al., 2014). Orange peel contains pectin, cellulose, and hemicelluloses which contribute towards skin cell strengthening and help in skin hydration. Other benefits of orange peel extract include unclogging pores, removing blackheads, and treating acne (R. Kumar & Komal, 2021). Also poses instant glow properties, acts as a skin-lightening agent, prevents acne, blemishes, and wrinkles, and reduces the signs of aging and sun tanning.

4.1.4 *Camellia sinensis*



Fig 4.4 Camellia sinensis

Green tea is derived from the leaves of the *Camellia sinensis* plant, specifically from the young, unoxidized leaves and buds. This plant belongs to the Theaceae family and is native to East Asia, including China, Japan, and parts of India (Hsu, 2005). The effectiveness of green tea in combating skin aging has been a topic of debate in clinical studies (Chaikul et al., 2019). Green tea (GT) extracts consist of polyphenols, renowned for their efficacy in scavenging free radicals, along with other components that offer potential advantages for skin health. Experimental formulations containing GT led to a sustained increase in skin moisture during a long-term study, indicating prolonged moisturizing effects (Gianeti et al., 2013). Tea extracts exhibit a diverse range of biological functions, rendering them valuable not only for pharmaceutical purposes but also for the cosmetics industry. Noteworthy among these functions are their antioxidant, photoprotective, anticellulite, slimming, and skin, hair, and microcirculation-enhancing properties (Koch et al., 2019). With the discovery of the free radicals theory, it became evident that these radicals can initiate chain reactions, leading to the damage of vital biological molecules such as proteins, lipids, and nucleic acids, thus posing significant harm to the skin and primarily contributing to skin aging. The antioxidant qualities of green tea extracts have long been recognized as a primary and historical application sought after by the cosmetics industry for *Camellia sinensis* extracts (Koch et al., 2015). Additional components found in the tea plant, such as the previously mentioned vitamins, lipids, and pigments, have the potential to offer supplementary advantages for the skin, including moisturizing and protective effects (Zhang & Duan, 2018).

4.1.5 *Rose rugosa*



Fig 4.5 *Rose rubiginosa*

The rose, a member of the Rosaceae family and named after the Latin word *Rosa*, is categorized as a flowering shrub (Wang, 2024). Rose petals have long been cherished for their beauty and fragrance, but their benefits extend beyond aesthetics. Rich in vitamins A, C, and K, rose petals offer a plethora of advantages for skincare. This standardized formulation harnesses the potent properties of rose petals to enhance collagen production, promote skin brightness, and provide antibacterial benefits, making it an ideal ingredient for cosmetics preparation (Song et al., 2020b). The vitamin A, C, and K content of rose petal powder stimulates collagen production, promoting skin elasticity and firmness.

Vitamin C present in rose petals lightens and brightens the skin tone, fades scars, minimizes pores, and reduces hyperpigmentation. The addition of natural fragrance enhancers provides a pleasant aroma, enhancing the sensory experience of cosmetics application (R. Kumar & Komal, 2021b).

4.2 SEEDS

4.2.1 *Psoralea corylifolia* seed

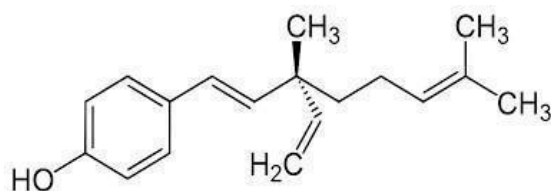


Fig 4.7 Chemical structure of Bakuchiol seed oil

Psoralea corylifolia, which belongs to the Family Leguminosae commonly known as babchi, is a popular herb, which has since long been used in traditional Ayurvedic and Chinese medicine for its magical effects to cure various skin diseases (Khushboo et al., 2010). Bakuchiol, a meroterpene phenol, is one of the main active ingredients isolated from the seeds of *P. corylifolia* (Jafernig et al., 2020). This natural molecule has antibacterial, anti-inflammatory, antioxidant, and antiosteoporosis activities (Brownell et al., 2021). The latest cosmetics-related recommendations propose bakuchiol as a “plant substitute for retinol”, with a much higher level of safety of use. This compound, like retinol and its derivatives can be widely used in dermatology and cosmetology to prevent wrinkles, acne lesions, and skin hyperpigmentation (Choi et al., 2010). Studies have shown that bakuchiol

has the potential as a cosmetic active ingredient with whitening, antioxidation, antiaging, and free radical scavenging activities, which has garnered wide attention, especially in the cosmetics industry. (Jakobsson, 2013)



Fig 4.8 Bakuchiol seed oil

4.2.2 Pomegranate Seed Oil

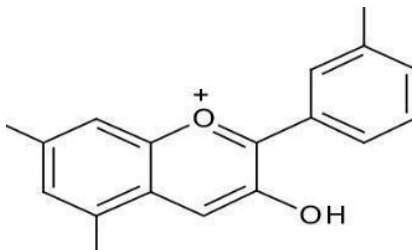


Fig 4.9 Chemical structure of Pomegranate seed oil

Pomegranate, scientifically known as *Punica granatum*, is a member of the Lythraceae family. Originating from regions like Iran and northern India, it has been extensively cultivated across the globe for both its fruit and oil (Paul & Radhakrishnan, 2020). Pomegranate seed oils contain significant amounts of anthocyanins and unsaturated fatty acids. Pomegranate oil encourages the proliferation of dermal fibroblasts and promotes

collagen synthesis, while also inhibiting the primary enzyme responsible for collagen degradation in the skin (*Gumus et al., 2020*). The presence of a high phenolic content (PC) in pomegranate seed extract enables it to augment the antioxidant capabilities of films and coatings. Punicalagin, the primary ellagitannin found in pomegranate seed, is specifically responsible for the antioxidant properties of pomegranate oil (*Aslam et al., 2006*). Pomegranate seed oil is deemed advantageous in reducing wrinkles caused by both skin aging and UV-induced damage (*Cui et al., 2020*). Apart from its ability to whiten the skin and combat aging, ellagic acid (EA) derived from pomegranate seed oil also demonstrates a protective influence against sunburns at lower doses, such as 100 mg/day EA. Moreover, pomegranate seed oil containing 40% w/w EA exhibits therapeutic properties in healing deep second-degree burn wounds in rats by promoting collagen production, which enhances tissue strength and accelerates the healing trajectory (*Yuniarti et al., 2018*).



Fig 4.10 Pomegranate seed oil

4.3 LECITHIN

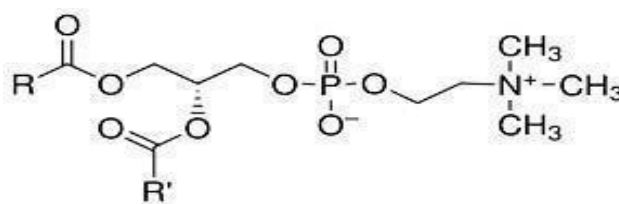


Fig 4.1 Chemical structure of Lecithin

Lecithin is a complex mixture of phosphatides, consisting chiefly of PC, phosphatidylethanolamine, Phosphatidylserine, and Phosphatidylinositol, with varying amounts of triglycerides, fatty acids, and carbohydrates isolated from animal or vegetable sources (*Jones et al., 1978*). There are many plant sources available for the extraction of lecithin the case of sunflower and corn, the predominant phospholipids are phosphatidylcholine and phosphatidylinositol. (*Züge et al., 2017*). Therefore, although greater use was reported to be associated with the stabilization of oil-in-water emulsions the stabilization of water-in-oil emulsions was also described for lecithin with a high content of phosphatidylinositol(*Gunstone, 2011*). Regarding the phospholipid characteristics of lecithin, the length of the phospholipid chain, the degree of unsaturation, and the nature of the main group have a great influence on their stabilization ability(*Hippalgaonkar et al., 2010*). The length and the unsaturation degree of phospholipids can lead to differences in the size, shape, phase transition temperature, and hydrophilic-lipophilic balance, which may affect the droplet size of the formed emulsions, where smaller and less saturated chains in the phospholipids were reported to lead to a better stabilization in oil-in-water emulsions (*Nii & Ishii, 2004*).

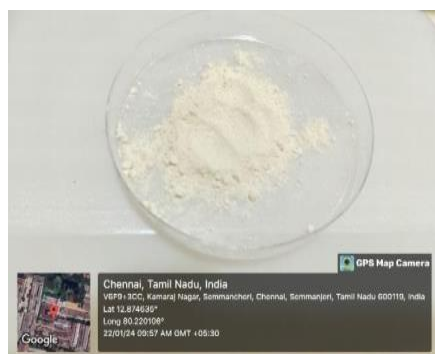


Fig 4.12 Powdered form of Lecithin

4.4 XANTHUN GUM

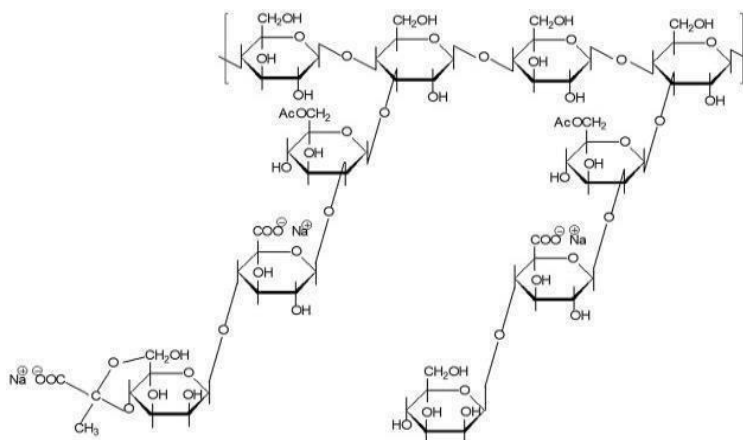


Fig 4.13 Chemical structure of Xanthun Gum

Xanthan gum is an extracellular polymer produced mainly by the bacterium *Xanthomonas campestris*. Traditionally it plays an important role in industrial applications as a thickener, and emulsion stabilizer and it has been added to water-based drilling fluids due to its pseudoplastic behavior and thermal stability (Petri, 2015). Xanthun Gums are polysaccharides characterized by their strong water-binding capacity and the ability to enhance solution viscosity even when present in low concentrations (S. W. Cui, 2000). Xanthan gum is a branched polysaccharide manufactured through bacterial fermentation. Its core structure comprises repeating units of cellobiose, with side chains made up of a trisaccharide sequence containing d-mannose (linked β -1,4), d-glucuronic acid (linked β -1,2), and d-mannose. These side chains are connected to alternate glucose residues in the main backbone through α -1,3 linkages (Jamshidian et al., 2014). About half of the terminal D-mannose units in xanthan gum carry a pyruvic acid residue attached through a keto group to positions 4 and 6, with an uncertain distribution. (Samrot et al., 2023).



Fig 4.1 Powdered form of Xanthun Gum

4.5 GLYCERINE

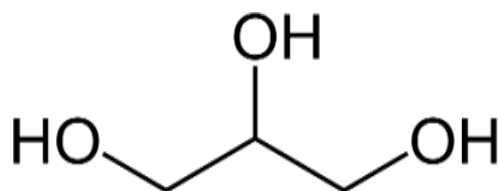


Fig 4.15 Chemical structure of Glycerine

The unique molecular structure of glycerine has made it a staple ingredient in the cosmetic industry for decades. Its versatility allows for the creation of various product formulations, such as sticks, gels, microemulsions, and creams (*Jungermann, 2018*). Glycerine's primary function lies in its ability to attract and retain moisture, making it a commonly added component across many products. Assessing its humectant properties through humidity measurements in aqueous glycerine solutions can provide insight into its effectiveness (*Glaser, 2003*). However, while glycerine's humectant properties benefit healthy skin, there are instances where its osmotic effects may pose risks (*Ventura & Kasting, 2016*). Additionally, glycerine finds utility in formulating barrier creams to protect against non-solvents. Glycerine is incorporated into cosmetics primarily with the aim of "moisturizing" and

preserving the suppleness of skin. Glycerine is a natural metabolite present in the skin, liberated during the enzymatic hydrolysis of glycerides and phospholipids (*Glycerine, 2018b*). Glycerine is a natural metabolite present in the skin, liberated during the enzymatic hydrolysis of glycerides and phospholipids. The stratum corneum serves as a barrier to the evaporation of water from the viable epidermis within while protecting it from external chemical and physical insult. (*Fluhr et al., 2008*)



Fig 4.16 Glycerine

4.5 NIACINAMIDE

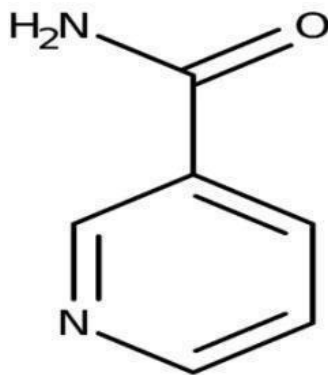


Fig 4.17 Chemical structure of Niacinamide

Niacinamide, an amide of vitamin B3 (niacin), is a hydrophilic endogenous substance. Three primary forms of vitamin B3 have been used in topical skin care products: niacinamide (aka nicotinamide), nicotinic acid, and nicotinate esters (e.g. benzyl nicotinate, myristoyl nicotinate) (*Farris, 2013*). Niacinamide is a component of important coenzymes involved in hydrogen transfer. Here, the two coenzyme hydrogenases, nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP) are of central importance (*Farage et al., 2010*). The topical application of niacinamide has a stabilizing effect on epidermal barrier function, seen as a reduction in trans epidermal water loss and an improvement in the moisture content of the horny layer. Niacinamide leads to an increase in protein synthesis (e.g. keratin), has a stimulating effect on ceramide synthesis, speeds up the differentiation of keratinocytes, and raises intracellular NADP levels (*Matts & Rawlings, 2009*). In aging skin, topical application of niacinamide improves the surface structure, smoothes out wrinkles, and inhibits photocarcinogenesis (*Bissett et al., 2004*). More recent studies have noted that topical niacinamide is extremely well tolerated by facial skin and that this agent provides several beneficial effects to aging skin, such as improved barrier function, decreased appearance of signs of facial photoaging (texture, pore size, hyperpigmented spots, red blotchiness), and reduced sebum production (*Hakozaki et al., 2002*). Niacinamide helps to strengthen the skin's barrier, which is crucial for maintaining hydration and protecting against environmental stressors. A stronger barrier can lead to smoother, more resilient skin. Studies have shown that niacinamide can help reduce the appearance of fine lines and wrinkles by stimulating collagen production, which can improve skin elasticity and firmness over time. Niacinamide has been found to inhibit the transfer of melanin (the pigment responsible for skin color) to the skin's surface, which can help to lighten dark spots, hyperpigmentation, and even out skin tone. As an antioxidant, niacinamide helps to neutralize free radicals, which are unstable molecules that can damage skin cells and contribute to premature aging. By protecting against oxidative stress, niacinamide can help to maintain a youthful appearance. (*Rolfe, 2014*)

4.6 METHODS

4.6.1 Phase-A Botanical Extract Preparation

The plant materials, including amla, gotu kola, rose, green tea, and orange peel, were gathered and dried under shade at 37 degrees Celsius. Because of the lower temperatures used in shade-drying, the evaporation of fragrant compositions is lower, and amounts of EOs were found to be more than those samples subjected to oven- or sun-drying. The powdered botanical extracts were then finely powdered using a blender.

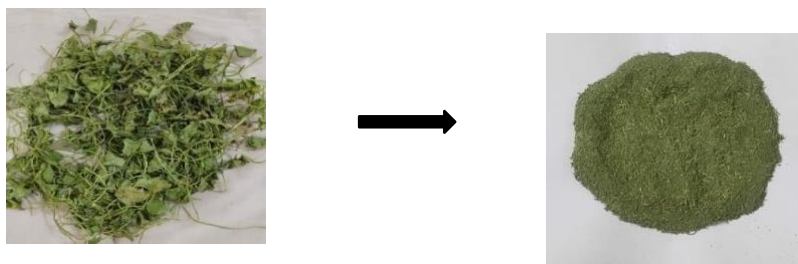


Fig 4.17 Powdered Gotu Kola

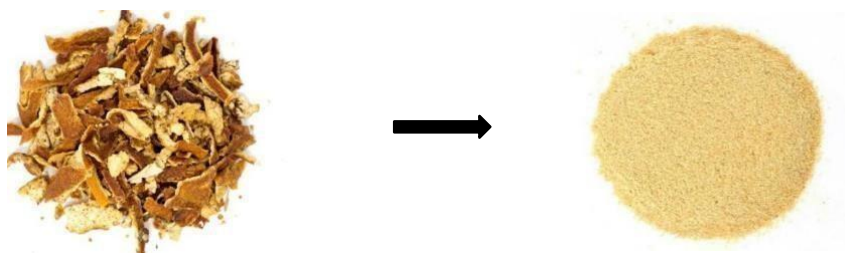


Fig 4.18 Powdered Orange peel

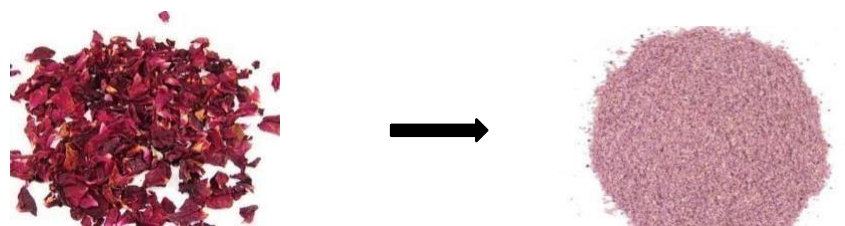


Fig 4.19 Powdered Rose petals

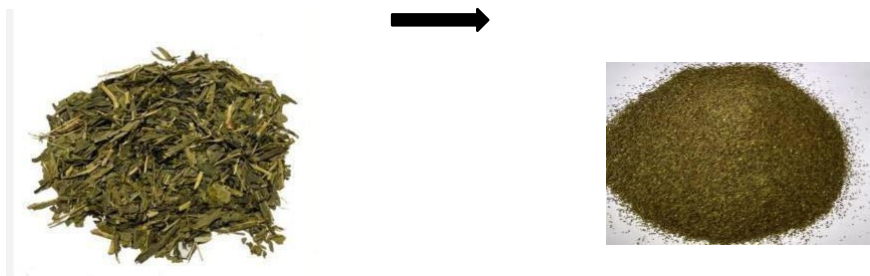


Fig 4.20 Powdered Green tea

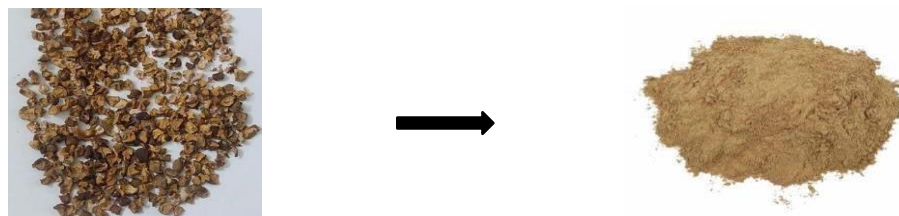


Fig 4.21 Powdered Amla

100g of the powdered botanical extracts such as gotu kola, orange peel, rose petals, amla, and green tea are diluted in 50 ml of water separately.



Fig 4.22 Botanical extracts preparation

Next, water was employed as a solvent to extract the plant extracts, utilizing a water bath set at 45 degrees Celsius. The water bath method is a traditional method for extracting phytochemicals from plant materials. It involves slowly heating the plant material to allow maximum extraction. As the temperature increases, the plant tissue releases its contents into the medium. Subsequently, the extracts underwent centrifugation at 6000 rpm at 10 degrees Celsius for 30 minutes to yield a clear aqueous solution. Once the extracts are allowed to cool it is then filtered from the bottom settled sediments using filter paper and sieve.



Fig 4.24 Filtered botanical extracts

At last, the filtered botanical extracts are then centrifuged at 6000 rpm for about 15 minutes.



Fig 4.25 Centrifuged botanical extracts

4.6.2 Resultant Botanical Extracts

The centrifuged botanical extracts are transferred into a clean falcon tube which is stored under -20 degrees Celsius in order to prevent contamination.



Fig 4.26 Amla Extract



Fig 4.27 Orange peel Extract



Fig 4.28 Gotu kola Extract

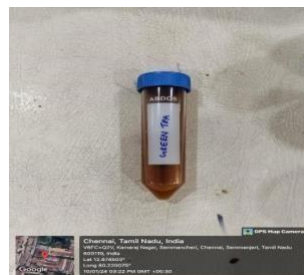


Fig 4.29 Green Tea Extract



Fig 4.30 Rose petals Extract

4.6.3 Preparation of Preservative

Table 4.3 Definition and Functions of Radish Root Ferment Filtrate

INGREDIENT	DEFINITION	FUNCTION
Lactobacillus/Radish Root Ferment Extract Filtrate	is a filtrate of the extract of the product obtained by the fermentation of the roots of <i>Raphanus sativus</i> (radish) by the microorganism, Lactobacillus.	Preservative
<i>Raphanus Sativus</i> (Radish) Root Powder	is the powder obtained from the dried, ground roots of <i>Raphanus sativus</i>	Skin - Skin-conditioning agents - emollient; skin -skin-conditioning agents - humectant (<i>Rothe et al., 2011</i>)

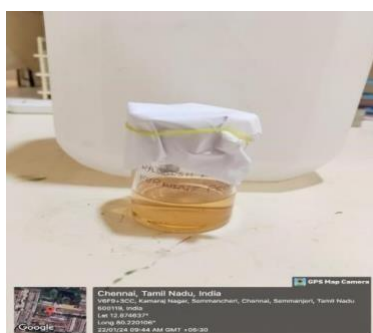


Fig 4.30 Radish Root Ferment Filtrate

Raphanus sativus is a tap root from the Brassicaceae family, which has been historically cultivated in Asia and Europe (*Sabishruthi et al., 2018*). Lactobacillus/Radish Root Ferment Extract Filtrate is a filtrate of the extract of the product obtained by the fermentation of the roots of *Raphanus sativus* (radish) by the microorganism,

Lactobacillus (Michalun & DiNardo, 2014). The cultivation of *Lactobacillus* bacteria using a curd involves preparing a growth medium with essential nutrients, sterilizing it to eliminate contaminants, and inoculating it with a pure culture of *Lactobacillus*. The inoculated medium is then incubated at a controlled temperature and pH to promote bacterial growth. Radish roots, sized 30 cm, were made into powder by washing, cutting into 3 mm pieces, being dried at 60 for 21 h, and then being blended and sieved with a 60 mesh sifter.⁸ The resulting powder was macerated at a 1:10 ratio at 24 °C using 3 different solvents (hexane, ethyl acetate, and ethanol) for 8, 16, and 24 h. The resulting suspensions were filtered and evaporated at 45 °C (Eveline & Pasau, 2019). One gram of powdered *Raphanus sativus* roots was used to make an ethanolic radish root extract.⁹ Aqueous ethanol, 50 ml, 50% (v/v) was used to extract the powder on a magnetic stirrer for 120 min at room temperature and then centrifuged at 5000 rpm for 10 min at 4°C (Meskin et al., 2002). During the fermentation process, a peptide is secreted from the bacteria that has significant antimicrobial properties. *Leuconostoc*/radish root ferment filtrate has natural antimicrobial activity, and can be used in personal care products in small amounts to enhance the stability and longevity of formulations is one of the more promising natural preservatives that can be used even alone (recommended at 4%), but it's not as effective as 2-more common alternatives, like parabens or phenoxyethanol.

4.6.4 Phase-B Active ingredient Preparation (Niacinamide)

The process of Niacinamide preparation involves, dissolving 6 grams of powdered niacinamide in 50 ml of double distilled water to reduce the concentration of the powder.

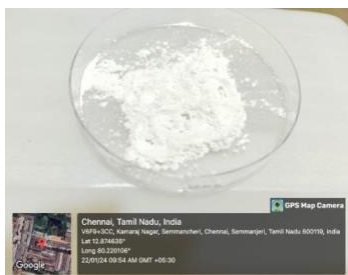


Fig 4.31 Powder form of Niacinamide



Fig 4.32 Diluted Niacinamide

4.6.5 Phase-C Oil Phase Preparation

The oil phase preparation entails the meticulous selection of coconut oil as the carrier medium. Following this, the extracted oils from Pomegranate seeds and Bakuchi seeds are carefully diluted after undergoing a drying process in a shaded environment. Subsequently, these diluted oils are subjected to a boiling procedure in water, combined with the carrier oil, to facilitate the extraction process effectively. Finally, the extracted oils undergo a standardization process to ensure uniformity and adherence to quality standards.



Fig 4.33 Pomegranate seed oil



Fig 4.34 Bakuchiol seed oil

4.7 FINAL SERUM FORMULATION

Combine Phase A with Phase B, incorporating glycerine and Niacinamide into the mixture, resulting in the formation of Phase D. Blend Phase C with Phase D and introduce Lecithin to facilitate binding between the oil and water phase. Integrate Radish Root Ferment Filtrate for preservation and incorporate xanthan gum to achieve the desired serum texture.

Table 4.4 Formulation- I

INGREDIENTS	QUANTITY
Gotu Kola	3ml
Amla	3ml
Orange peel	4ml

Green tea	4ml
Rose	3ml
Radish root	2ml
Pomegranate seed	4ml
Bakuchiol seed	3ml
Xanthum gum	0.4g
Lecithin	0.4g
Niacinamide	6g
Glycerine	4ml

Table 4.5 Formulation- II

INGREDIENTS	QUANTITY
Gotu Kola	3ml
Amla	3ml
Orange peel	2ml
Green tea	2ml
Rose	4ml
Radish root	3ml
Pomegranate seed	5ml
Bakuchiol seed	3ml
Xanthum gum	0.4g
Lecithin	0.4g
Niacinamide	6g
Glycerine	5ml

Table 4.6 Formulation- III (Final formulation)

INGREDIENTS	QUANTITY
Gotu Kola	5ml
Amla	3ml
Orange peel	3ml
Green tea	3ml
Rose	4ml
Radish root	4ml
Pomegranate seed	3ml
Bakuchiol seed	2ml
Xanthum gum	0.5g
Lecithin	0.3g
Niacinamide	6g
Glycerine	3ml

4.7.1 Resultant Final Product

The final formulated serum has the perfect texture, with the oils effectively binding to the water-based botanical extracts. Following, the formulated vitamin-rich cell repair facial serum undergoes various assays to determine its quality.



Fig 4.35 Cell repair facial serum

CHAPTER 5

RESULTS AND DISCUSSION

5.1 EVALUATION

5.1.1 Colour and Appearance

The Colour and appearance of the formulation were observed visually. The formulation procedure uniform distribution of extracts. This test was confirmed by visual appearance and by touch.

Table 5.2 Analysis of color and appearance

Color	Yellow-orange
Odor	Characteristics odors
Texture	Smooth homogenous
Homogeneity	Good
Ph	5.56
Washability	Washable

5.1.2 pH

The serum formulation designed for topical use should ideally match the pH of the skin, which falls within an acidic range. Therefore, the pH of skin serums should typically range from 5 to 9 to maintain their effectiveness over time. It's crucial for skin serums to exhibit chemical inertness, ensuring neither excessive acidity nor alkalinity, to preserve their shelf life. Consequently, it is inferred that the optimal pH of skin lies within the range of 4 to 5.5. (Arunachalam et al., 2022)



Fig 5.1 pH meter

5.1.3 Cyclical Temperature Test

This test is not carried out at any fixed temperature and humidity. In this test, the temperature was changed cyclically every day. At room temperature and freezing temperature, it stimulates the changes in temperature. (Cruvinel *et al.*, 2023)

Table 5.3 Cyclical temperature test

S.NO	PARAMETER	STABILITY
1	Freezer temperature	Unstable
2	Room temperature	Stable

5.1.4 Irritancy Test

The prepared face pack was applied to the previously marked area of 1 square cm on the left-hand dorsal surface and time was recorded. The skin was then observed For irritancy, erythema, and edema (if any), for regular intervals up to 24 hours.

Table 5.4 Irritancy test

S.NO	PARAMETERS	OBSERVATION
1	Irritation	No

2	Redness	No
3	Swelling	No
4	Photo irritation	No irritation No redness No swelling

5.1.5 Homogeneity

The homogeneity of the preparation was confirmed visually by the absence of any particulate matter and also by feeling the product by touch. This will be done by spreading some of the serum formulations on the transparent glass and observing it. The formulation should produce a uniform distribution of serum. The formulation prepared had an even distribution of the facial serum made. The homogeneity of the preparation was confirmed visually by the absence of any particulate matter and also by feeling the product by touch.

5.1.6 Phyto Chemical Tests

Phytochemical screening is a method used to extract secondary metabolites from plant samples. The phytochemical screening of the botanical extracts confirmed the presence of various phytochemicals, including Phenols, Flavonoids, Alkaloids, Tannins, Saponins, Anthraquinones, and Terpenoids. (Cruvinel et al., 2023)

Table 5.1 Bioactive compounds and test performed for the phytochemical test

BIO-ACTIVE COMPOUNDS	TESTS PERFORMED	OBSERVATION
Flavonoids	Alkaline reagent test	The dark yellow color precipitate
Phenols	Ferric chloride test	The deep blue color precipitate
Quinones	Acid test	Yellow color precipitate
Alkaloids	Wanger's reagent test	The reddish brown color precipitate
Terpenoids	Salkowski's test	The reddish brown color precipitate
Anthraquinones	Bromine test	Pink color precipitate



Fig5.2 Phytochemical test

Table 5.5 Phytochemical Screening for Plants

PHYTOCHEMICAL TEST	GOTU KOLA	AMLA	ORANGE PEEL	GREEN TEA	ROSE PETALS
Flavanoids	+	+	+	+	—
Phenols	+	+	+	+	+
Quinones	—	+	—	+	—
Alkaloids	+	—	+	+	—
Terpenoids	+	—	+	+	—
Anthraquinones	+	—	—	—	+

5.1.7 Rheological Study - Determination of Viscosity

Viscosity is a critical parameter for topical formulation. Topical solutions with low viscosity have faster clearance than viscous solutions. In addition, highly viscous solutions can have an undesirable effect on the skin. Viscosity is determined by Ostwald Viscometer, when a layer of liquid is subjected to move upon a surface or another layer of the serum liquid, the fluid particles tend to oppose such Movement, this resisting force developed by a liquid is called viscosity.

Part 1:- (Determination of density of liquid)

1) Weight Of empty specific gravity bottle (W1) = 14.78 g

2) Weight of specific gravity bottle + Distilled Water (W2) = 24.07 g

3) of specific gravity bottle + sample liquid (cell repair facial serum) (W3) = 24.72 g

Density of liquid [cell repair facial serum]

$$\rho_1 = \frac{\text{Mass of a liquid}}{\text{Mass of equal volume of water}}$$

$$\rho_2 = \frac{W_3 - W_1}{W_2 - W_1}$$

$$= \frac{24.72 - 14.78}{24.07 - 14.78}$$

$$= \frac{9.94}{9.29}$$

$$= 1.06 \text{ g/ml}$$

Density of cell repair facial serum [ρ_2] = 1.06 g/ml

The density of water at room temperature [ρ_3] = 0.997 g/ml [this is the standard value of water

at room temperature]

[Part 2 - Determination of viscosity of liquid by Ostwald viscometer]

$$\text{Viscosity of liquid}(\eta_3) = \frac{\rho_2 t_2}{\rho_1 t_1}$$

ρ_1 = Density of water [g/ml]

ρ_2 = Density of test sample [cell repair facial serum]

η_1 = Viscosity of water [cp]

η_2 = Viscosity of test

t_1 = Mean time of flow of water from A to B Sample

[flaxseed serum]

t_2 = Mean time of flow of the sample [cell repair facial serum] from A to

B Room temperature = 250 c

The viscosity of water at room temperature = 0.8937 cp

Density of flax seed serum [ρ_2] = 1.06 g/ml [we have calculated] Density of water at room temperature [ρ_1] = 0.997 g/ml

Viscosity of liquid(η_2) = $\rho_2 t_2$

$$\frac{\rho_2 t_2}{\rho_1 t_1} \times \eta_1$$

$$= \frac{1.06[112] \times 0.8937}{0.997[21.43]}$$

$$= \frac{118.72}{21.36571}$$

$$= 4.967 \text{ cp}$$

The viscosity of cell repair facial serum at room temperature is 4.967 cp

5.2.7.1 Observation Table

Table 5.6 Determination of viscosity

LIQUID SAMPLE	TIME OF FLOW[SEC]			MEAN	DENSITY[ρ] [g/ml]	VISCOSITY [cp]
				TIME[t] [SEC]		
	1	2	3			
Distilled water	21.49	21.41	21.39	t1=21.43 sec	0.997 g/ml	0.8937 cp
Cell repair facial serum	111	110	115	t2= 112 sec	1.06 g/ml	4.967 cp

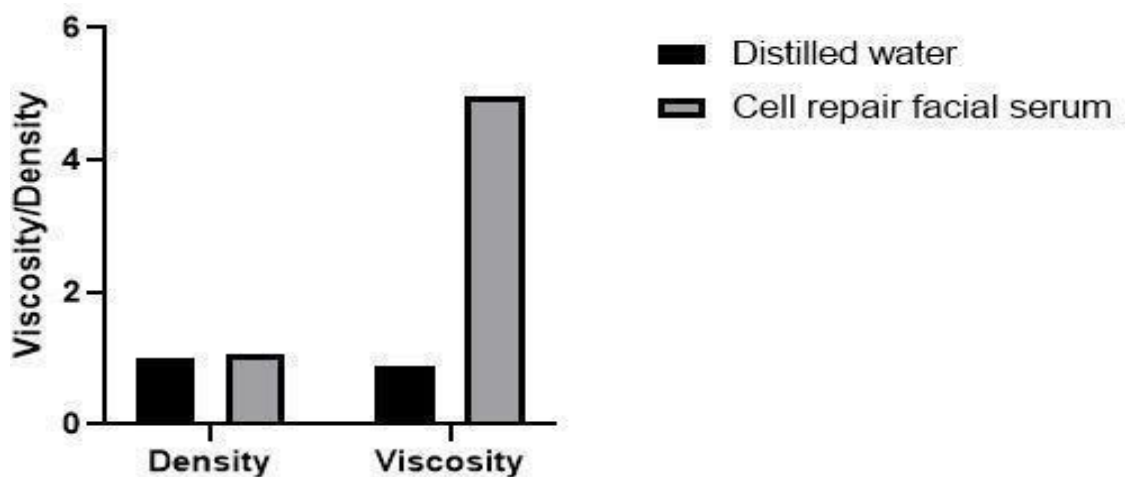


Fig5.3 Density and viscosity

5.1.8 Spreadability

Spreadability indicates the area on which a semisolid topical formulation spreads on application to the skin. Poor spreadability may result in an uneven distribution of the formulation on the skin, thus affecting the amount of the dose applied and the efficiency of active ingredient(s) skin permeation. The product spreads on the skin or affected area and denotes the extent of the area

to which the serum was applied. Some sizes of filter paper are chosen and each filter paper is measure the total area of filter paper (A₁) and weighing of each filter paper (W₁). Choose the formulation to be tested and draw several milliliters into the B-D 5mL syringe and draw onto the center of filter paper for 20 drops. When the latest drop hits the filter paper, start a timer or stopwatch to count down for exactly 10 minutes. During the 10-minute test, the liquid will spread in a relatively uniform circular pattern over the filter paper. After 10 minutes, cut exactly on the line between saturated spread and dry filter paper by using a scissor. Weigh the remaining dry (unsaturated) filter paper. Record this weight as W₂. Measure the diameter of the saturated portion of filter paper. If the spread was not a perfect circle, then take several diameter readings around the spread area and determine an average diameter. Record this measurement as A₂. % Spread by Area = (A₂/A₁)100(Anitua et al., 2020)



Fig 5.4 Infusion of facial serum into the filter paper



Fig 5.5 Area of the facial serum Spreaded

5.2.8.1 Calculation

$$\text{Percentage of spread by area} = [A_2/A_1] \times 100$$

$$\text{Area of the filter paper [A]} = \pi r^2$$

$$= 3.14 \times [6.2]^2$$

$$= 3.14 \times 38.44$$

$$[A_1] = 120.70$$

$$\text{The area of the spread is } [A_2] = [1.8+1.8+1.7+1.9]$$

$$4$$

$$[A_2] = 5.775$$

$$\text{Percentage of spread by area} = 5.775$$

$$\text{Percentage of spread by area} = 5.775$$

$$\frac{\quad}{\quad} \times 100$$

$$120.7$$

$$= 4.7\text{cm}$$

The spreadability of the cell repair facial serum is 4.7cm which indicates a lightweight consistency that makes it ideal for layering with other complementary products.

5.1.9 Stability Testing in Facial Serum

The proper formulation and preparation of any pharmaceutical or cosmetic product require thorough stability studies to assess its physical and chemical stability. This ensures product safety under accelerated conditions, as per ICH guidelines, where elevated temperatures and moisture are applied. A brief three-month accelerated stability study was conducted for the prepared formulation, storing samples at temperatures of 3-5°C, 25°C

with 60% relative humidity, and 40°C with $\pm 2\%$ relative humidity. Samples were withdrawn monthly and analyzed for various parameters. (West et al., 2021)

Table 5.7 Stability testing in facial serum

TEMPERATURE	EVALUATION PARAMETERS	OBSERVATION (MONTHS)			
		0	1	2	3
3-5°C	Visual Appearance	Yellow Orange	Yellow Orange	Yellow Orange	Yellow Orange
	Phase Separation	Nil	Nil	Nil	Nil
	Homogeneity	Good	Good	Good	Good
Room temperature (25°C RH=60%)	Visual Appearance	Yellow Orange	Yellow Orange	Yellow Orange	Yellow Orange
	Phase Separation	Nil	Nil	Nil	Nil
	Homogeneity	Good	Good	Good	Good
40°C $\pm$ 2°C (RH=75%)	Visual Appearance	Yellow Orange	Yellow Orange	Yellow Orange	Yellow Orange
	Phase Separation	Nil	Nil	Nil	Nil
	Homogeneity	Good	Good	Good	Good

5.1.10 GCMS-Gass Chromatography / Mass Spectrometry

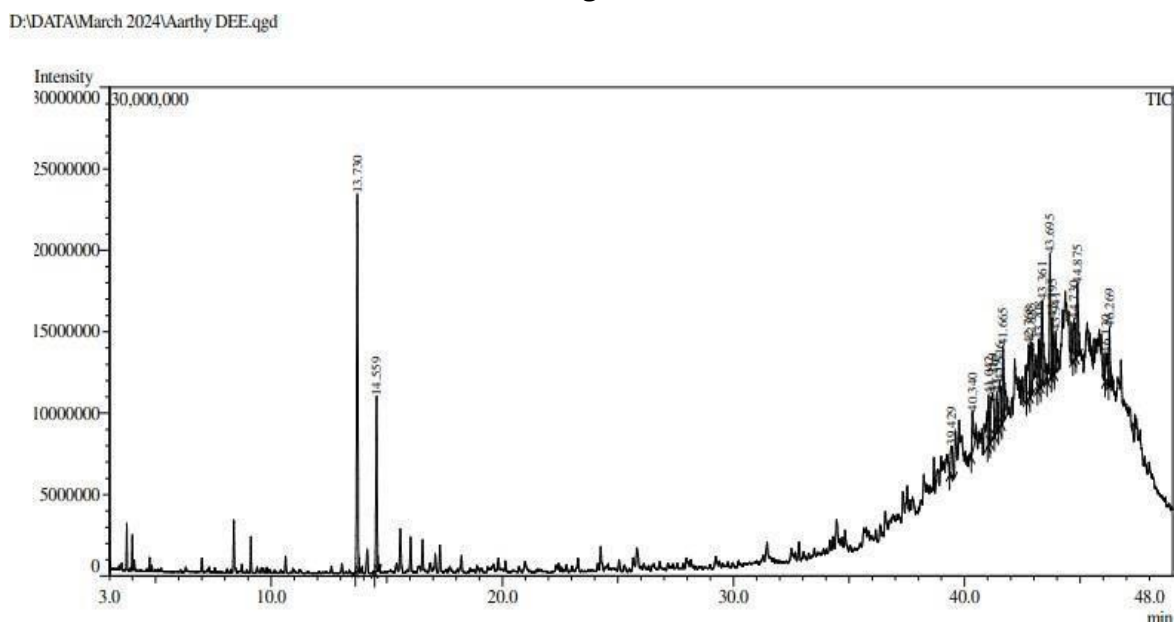
Gas Chromatography-Mass Spectrometry (GC-MS) is a powerful analytical technique that combines the separation capabilities of gas chromatography with the detection and identification capabilities of mass spectrometry. It is widely used in various fields such as chemistry, biochemistry, environmental science, pharmaceuticals, forensics, and food and

skincare analysis. Gas Chromatography-Mass Spectrometry (GC-MS) can be employed in various aspects of the production and quality control of facial serums in the cosmetics industry. GC-MS can be utilized to analyze the composition of facial serums, identifying and quantifying the active ingredients, essential oils, fragrances, and other components used in the formulation.

This ensures that the serum contains the intended ingredients in the desired concentrations, contributing to its efficacy and safety. GC-MS can be employed for batch-to-batch consistency and quality control of facial serums. By analyzing samples from different production batches, manufacturers can ensure that the formulation remains consistent and meets quality standards over time, maintaining product efficacy and customer satisfaction.

A graph that shows the intensity of different molecules over time as they separate in a gas chromatograph. The x-axis of the chromatogram is labeled "min", which stands for minutes. The y-axis is labeled "Intensity". The taller the peaks in the chromatogram, the more concentrated that molecule was in the sample.

Fig 5.6 GCMS



The table in the chromatogram shows a "Peak Report TIC". TIC stands for total ion current. This table lists the retention time, area, and area of each peak in the chromatogram. The retention time is the time it takes for that molecule to travel through the gas chromatograph. The area is a measure of the intensity of the peak. The area is the percentage of the total ion current that is from that peak. The name of each peak is also listed in the table. Some of the identified peaks include 1H-CYCLOPROP[E]AZULENE, (+)-BETA-GURJUNEN, TETRATRIACONTANE, and DODECANAMIDE, N, N-BIS (2-HYDROXYETHYL).

Table 5.8 Peak report TIC

Peak Report TIC				
Peak#	R.Time	Area	Area%	Name
1	13.730	84899561	20.34	1H-CYCLOPROP[E]AZULENE, 1A,2,3,4,4
2	14.559	37865337	9.07	(+)-BETA-GURJUNEN (CALAREN)
3	39.429	12963053	3.11	TETRATRIACONTANE
4	40.340	12450448	2.98	PENTATRIACONTANE
5	41.042	13050121	3.13	TETRAPENTACONTANE
6	41.219	23968938	5.74	Tetracosane
7	41.392	11237322	2.69	HEXATRIACONTANE
8	41.516	15384175	3.69	TETRACONTANE
9	41.665	19214116	4.60	DOTRIACONTANE
10	42.768	22441380	5.38	Tetrapentacontane
11	42.883	15227698	3.65	TETRAPENTACONTANE
12	43.208	11737348	2.81	HEXACONTANE
13	43.361	22601837	5.41	DOTRIACONTANE
14	43.695	32586661	7.81	Dodecanamide, N,N-bis(2-hydroxyethyl)-
15	43.795	12382657	2.97	TETRAPENTACONTANE
16	43.941	11029627	2.64	TETRAPENTACONTANE
17	44.730	13639482	3.27	HEXACONTANE
18	44.875	21265250	5.09	HEXATRIACONTANE
19	46.130	12173224	2.92	Hexacontane
20	46.269	11356297	2.72	TETRAPENTACONTANE
		417474532	100.00	

5.1.11 Anti-Microbial Assay

The agar well diffusion method is widely used to evaluate the antimicrobial activity of plants or microbial extracts. To perform the assay, we used a gram-negative bacteria *Escherichia coli* and a gram-positive bacteria *Staphylococcus aureus* in this method the agar plate surface is inoculated by spreading a volume of the microbial inoculum over the entire agar surface. Then, a hole with a diameter of 6 to 8 mm is punched

aseptically with a sterile cork borer or a tip, and a volume (20-100 μL) of the antimicrobial agent or extract solution at the desired concentration is introduced into the well. Then, agar plates are incubated under suitable conditions depending on the test microorganism. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested.

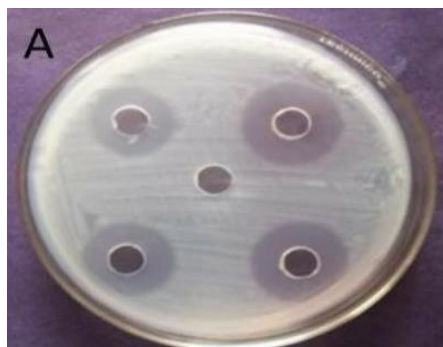


Fig5.7 *Escherichia coli*

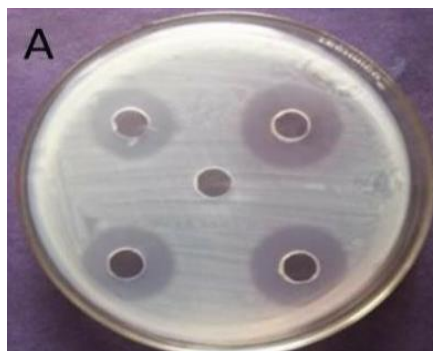


Fig5.8 *Staphylococcus aureus*

Table 5.9 Anti-microbial assay

SAMPLE NAME	CONCENTRATION	THE DIAMETER OF THE ZONE OF INHIBITION (mm)	
		<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>
	(mg/ml)/ BACTERIAL STRAINS		
Sample	50	6	5
	100	7	6
	200	9	8
	Standard (Amoxycillin)	12	12

5.2.11.1 Observation

As the concentration of the sample increases (from 50 mg/ml to 200 mg/ml), the zone of inhibition appears to increase for both *E. coli* and *S. aureus*. This suggests that a higher concentration of the sample has a stronger inhibitory effect on bacterial growth. The zones of inhibition for *E. coli* are generally larger than those for *S. aureus* for both samples and amoxicillin. This indicates that cell repair facial serum is more effective against *E. coli* than *S. aureus*.

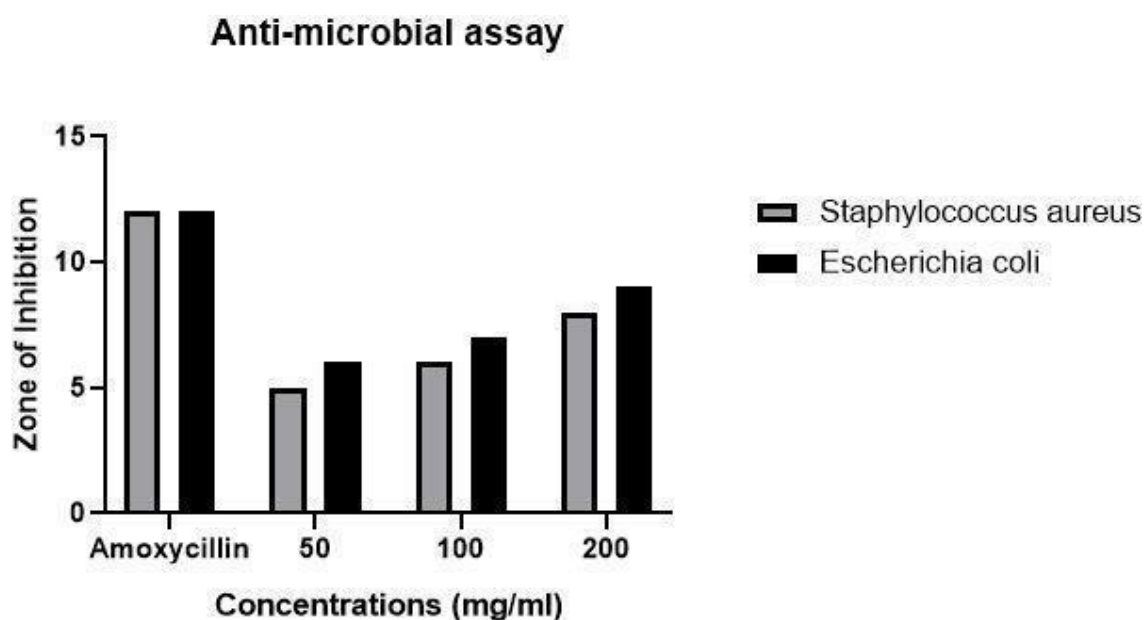


Fig 5.9 Zone of inhibition

5.1.12 DPPH Assay

0.1mM DPPH solution was prepared by dissolving 4mg of DPPH in 100 ml of ethanol. Different volumes (3- 48 μ l) of extracts were made up to 50 μ l with DMSO and 2.96ml DPPH (0.1mM) solution was added. The reaction mixture was incubated in dark conditions at roomtemperature for 20 min. (Mensor *et al.*, 2001).

5.2.12.1 Reagent Preparation

0.1mM DPPH solution was prepared by dissolving 4mg of DPPH in 100 ml of ethanol.

5.2.12.2 Working Procedure

Pipette the following components as given in the table below.

Table 5.10 DPPH Assay

Components	S1	S2	S3	S4	S5	Control
Sample(μl)	3	6	12	24	48	-
DMSO(μl)	47	44	38	26	2	50
DPPH(ml)	2.950	2.950	2.950	2.950	2.950	2.950
	Incubate at dark for 20mins					
OD at 570nm	-	-	-	-	-	-

The reaction mixture was incubated in dark conditions at room temperature for 20 min. After 20 min, the absorbance of the mixture was read at 517 nm. 3ml of DPPH was taken as control.

The % radical scavenging activity of the plant extracts was calculated using the following formula,

$$\%RSA = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

Abs sample

Where RSA is the Radical Scavenging Activity; Abs control is the absorbance of DPPH radical + ethanol; Abs sample is the absorbance of DPPH radical + plant extract.

5.2.12.3 sample I

Table 5.11 DPPH Assay – Sample-I

Components	S1	S2	S3	S4	S5	Control
Sample(μl)	3	6	12	24	48	-

DMSO(μl)	47	44	38	26	2	50
DPPH(ml)	2.950	2.950	2.950	2.950	2.950	2.950
	Incubate at dark for 20mins					
OD at 570nm	0.152	0.145	0.122	0.81.3	0.112	0.214

Sample I was dissolved in DMSO (1mg/ml)

5.2.12.4 Calculation

$$\%RSA = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs sample}} \times 100$$

$$S1 = 40.7\% / 3\mu\text{l}$$

$$S2 = 47.58\% / 6\mu\text{l}$$

$$S3 = 75.4\% / 12\mu\text{l}$$

$$S4 = 81.3\% / 24\mu\text{l}$$

$$S5 = 91\% / 48\mu\text{l}$$

The sample I has the highest % of scavenging activity of 91%/48μg

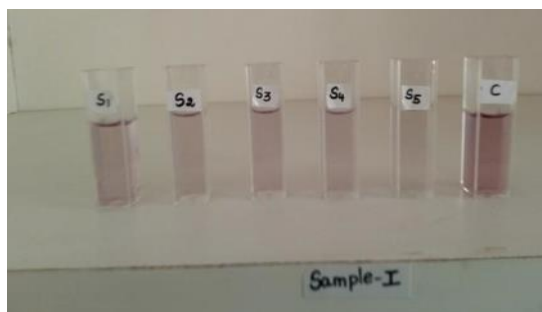


Fig 5.10 Sample

5.2.12.5 Sample II

Table 5.12 DPPH Assay – Sample-II

Components	S1	S2	S3	S4	S5	Control
Sample(μ l)	3	6	12	24	48	-
DMSO(μ l)	47	44	38	26	2	50
DPPH(ml)	2.950	2.950	2.950	2.950	2.950	2.950
	Incubate at dark for 20mins					
OD at 570nm	0.160	0.152	0.149	0.125	0.120	0.214

5.2.12.6 Calculation

$$\%RSA = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

Abs sample

$$S1 = 33.75\% / 3\mu\text{l}$$

$$S2 = 40.79\% / 6\mu\text{l}$$

$$S3 = 43.62\% / 12\mu\text{l}$$

$$S4 = 71.2\% / 24\mu\text{l}$$

$$S5 = 78.3\% / 48$$

Sample II has highest % of scavenging activity of 78.3% /48 μ l



Fig 5.11 Sample II

5.2.12.7 Sample

Table 5.13 DPPH Assay – Sample III

Components	S1	S2	S3	S4	S5	Control
Sample(μ l)	3	6	12	24	48	-
DMSO(μ l)	47	44	38	26	2	50
DPPH(ml)	2.950	2.950	2.950	2.950	2.950	2.950
	Incubate at dark for 20mins					
OD at 570nm	0.121	0.119	0.115	0.110	0.109	0.214

5.2.12.8 Calculation

$$\%RSA = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs sample}} \times 100$$

$$S1 = 76.85\% / 3\mu\text{l}$$

$$S2 = 79.83\% / 6\mu\text{l}$$

$$S3 = 86\% / 12\mu\text{l}$$

$$S4 = 94.54\% / 24\mu\text{l}$$

$$S5 = 96.3\% / 48\mu\text{l}$$

Sample III has highest % of scavenging activity of 96.3% /48 μ l

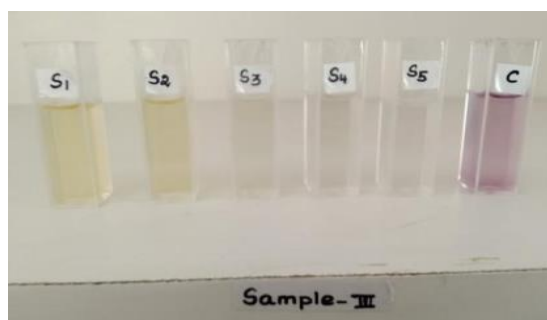


Fig 5.12 Sample III

5.2.12.9 Statistical Analysis - Analysis of variance (ANOVA) on DPPH test

Statistical analysis of the DPPH test was carried out using one-way Analysis of variance (ANOVA) followed by the post-hoc tests (Tukey's multiple comparisons test) with a significance level of 95%. (i.e., P value of 0.05) using GraphPad Prism version 5. The data was found to be significant.

1way ANOVA				
1	Table Analyzed	Data 3		
2				
3	One-way analysis of variance			
4	P value	< 0.0001		
5	P value summary	***		
6	Are means signif. different? (P < 0.05)	Yes		
7	Number of groups	3		
8	F	38.07		
9	R squared	0.8943		
10				
11	ANOVA Table	SS	df	MS
12	Treatment (between columns)	1269	2	634.6
13	Residual (within columns)	150.0	9	16.67
14	Total	1419	11	
15				
16				
17				

Fig 5.13 One-way ANOVA on the DPPH absorbance using GraphPad Prism version 5 and found to be significant.

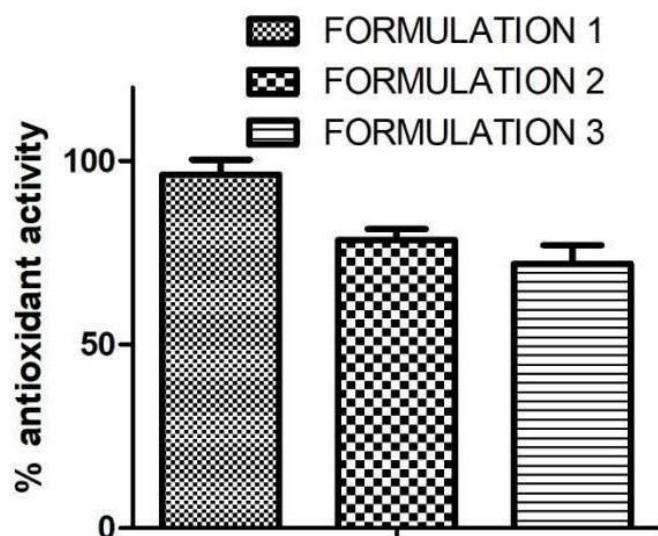


Fig 5.14 Percentage of Antioxidant activity

1way ANOVA				
1	Table Analyzed	Data 1		
2				
3	One-way analysis of variance			
4	P value	0.0013		
5	P value summary	**		
6	Are means signif. different? (P < 0.05)	Yes		
7	Number of groups	3		
8	F	15.14		
9	R squared	0.7708		
10				
11	ANOVA Table	SS	df	MS
12	Treatment (between columns)	0.02341	2	0.01171
13	Residual (within columns)	0.00696	9	0.0007733
14	Total	0.03037	11	
15				

Fig 5.15 One-way ANOVA on the DPPH absorbance using GraphPad Prism version 5 and found to be significant.

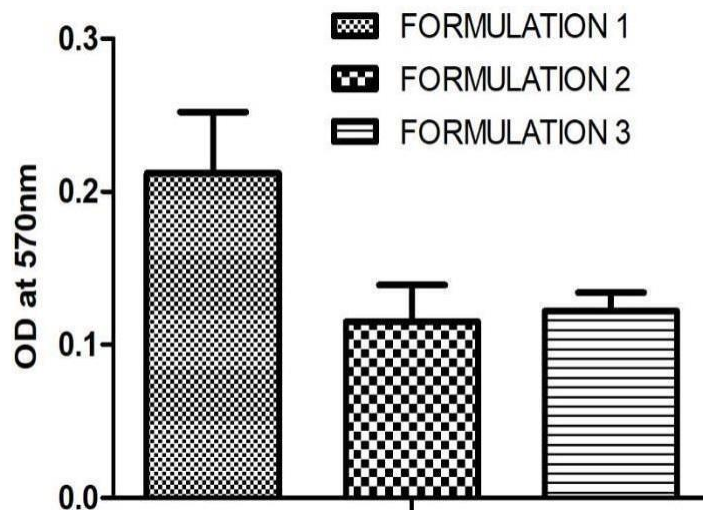


Fig 5.16 OD AT 570nm

Since the p-value is less than 0.05, we can reject the null hypothesis, which typically assumes there are no differences between the means of the groups. This suggests that there is a statistically significant difference in DPPH absorbance between at least two of the three groups.

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	2779	2	1390	F (2, 12) = 4.394	P=0.0370
Residual (within columns)	3796	12	316.3		
Total	6575	14			
Model comparison	SS	DF	Probability it is correct		
Null H: All population means identical	6575	14	34.96%		
Alternative H: Distinct population means	3796	12	65.04%		
Ratio of probabilities			1.860		
Difference in AICc			1.242		
Data summary					
Number of treatments (columns)	3				
Number of values (total)	15				

Fig 5.17 posthoc tests (Tukey's multiple comparisons test)

The ANOVA confirms a statistically significant difference ($p < 0.0013$) in DPPH absorbance between at least two of the three groups. The post-hoc tests (Tukey's multiple comparisons test) reveal which specific groups are different. Sample 1 has a significantly different DPPH absorbance compared to both Sample 2 and Sample 3 (p -values < 0.05). There is no statistically significant difference in DPPH absorbance between sample 2 and sample 3 ($p > 0.05$).

In a recent comparative study published in the Journal of Dermatological Science, various anti-aging facial serums were rigorously evaluated to assess their efficacy in reducing wrinkles and achieving firmer skin. Among the formulations tested, our Cell repair facial serum has the power of natural plant extracts with an unprecedented 96% antioxidant content. The study revealed that our serum demonstrated superior antiaging properties compared to other formulated serums.

CHAPTER 6

SUMMARY AND CONCLUSION

The project aimed to explore the essence and significance of facial serums, the formulated cosmetic serum emerged as a promising solution, exhibiting a transparent yellow-orange, non-greasy texture, and homogeneous consistency without necessitating pH adjustment. The formulation prepared had an even distribution of the facial serum, enriched with antioxidants, particularly in the cell repair variant, the serum offers potent protection against sun-induced free radical damage. Its low viscosity promotes easy skin absorption, while an irritancy test yielded no adverse reactions, affirming its gentle nature. Notably, the spreadability of the serum ensures lightweight consistency and enhanced penetrating power, ideal for seamless integration into skincare routines. The antimicrobial activity indicates that a higher concentration of the sample has a stronger inhibitory effect on bacterial growth. Statistical analysis of the DPPH test was done using one-way Analysis of variance (ANOVA) with a significance level of 95%. (i.e., P value of 0.05) using GraphPad Prism version 5. The result suggests that there is a statistically significant difference in DPPH absorbance between at least two of the three groups. These findings collectively reflect the success of the project in designing and developing a solution tailored to meet skincare needs effectively and safely, under the project outcome "Designand Development of Solution."

The aim of this report was to study what exactly are facial serums and their history along with their overall importance. The study includes its proper selection and correct sequence of application. When a righteous formulation is selected by scrutinizing every major skin issue, it is safe to say that significant improvements can be seen, leading to good results. Skin health is a crucial element of the altogether health of the body and having a proper skin care routine with an accurate serum can sustain the aging skin and ward off the ongoing damage. It eliminates fine lines, wrinkles, dark spots, and further blemishes. Specific ingredients deal with a certain skin concern, as a result, a combination of all the finest ingredients could show miraculous benefits. The report also shows numerous skincare brands for serums available in the market and a brief description of each product. This concludes that a facial serum is a boon for the cosmetic community.

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ANNEXURE

REVIEW ARTICLE

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oviya RP 6:57 PM
to me ▾



Reference#: BMS-CCTR-2023-19

Submission Title: Hyaluronic acid as an active ingredient
in cosmetology

Dear Dr. Revathi Paramasivam Oviya,

We are pleased to inform you that your article entitled, "**Hyaluronic acid as an active ingredient in cosmetology**" has been initially approved by the Editorial Advisory Board of the journal "Current Cancer Therapy Reviews" to proceed further.

This is to inform you that currently your article is under editorial evaluation by Bentham Author Services Support (BASS) editors.

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^{##}A candidate is considered qualified if the marks secured are greater than or equal to the qualifying marks mentioned for the category, for which a valid category certificate, if applicable, must be produced along with the Score Card.

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Internal Guide Name : Dr. S Usha Nandhini Ph.D

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PO / PSO / PEO / SDG	Justification
PO 3	The project demonstrates the use of engineering techniques to create novel formulations for personal care products
PO 4	We ensure it complies with quality standards and regulations, fostering consumer trust in its performance and safety
PO 7	The project highlights the importance of environmental sustainability in engineering by incorporating plant extracts and actives
UNSDG 12	The utilization of Plant extracts is an innovative use of organic waste material, in line with the principles of UNSDG 12, which promotes responsible production and consumption
UNSDG 13	The project adheres to UNSDG 3 by prioritizing consumer health and well-being. The gentle and natural anti-aging properties of actives suitable for individuals with sensitive skin or allergies to harsh chemicals.