

INFUSION OF BIOENZYMES FROM FRUIT PEELS FOR FABRICATING BODY WASH

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

BY

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**INSTITUTE OF SCIENCE AND TECHNOLOGY
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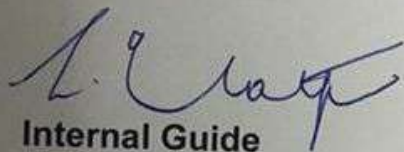
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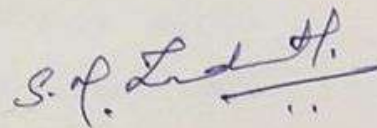
BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **Natishah AJ (40230030)**, **Showparnickaa SR (40230042)** who have done the Project work entitled **"Infusion of Bioenzymes from Fruit Peels for Fabricating Body Wash"** under our supervision from September 2023 to April 2024.



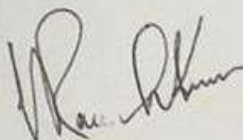
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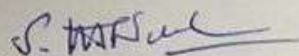
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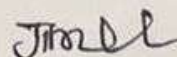
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We, **Natishah AJ (40230030)**, **Showparnickaa SR (40230042)** hereby declare that the Project Report entitled “**Infusion of Bioenzymes from Fruit Peels for Fabricating Body Wash**” done by us under the guidance of **Dr.L.Inbathamizh** (Internal) and **Dr.Indumathi SM** (External) at the Department of Biotechnology, Sathyabama Institute of Science and Technology, Chennai, is submitted in partial fulfillment of the requirements for the award of Bachelor of Technology degree in Biotechnology.

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ABSTRACT

The personal care business is looking for more environmentally friendly alternatives as environmental sustainability becomes more and more of a concern. In order to formulate body washes, this study explores the feasibility of using bioenzymes produced from fruit peels. Compared to traditional body wash ingredients, bioenzymes have many benefits, including biodegradability, less of an adverse effect on the environment, and may lower production costs. The study investigates efficient techniques for obtaining bioenzymes from different fruit peels, including orange, lemon, and mango peels. After the enzymes are isolated, their characteristics and enzymatic activity will be fully investigated. In order to characterize them and ascertain how well they break down common components of body soils such as proteins, carbohydrates, or lipids, methods such as enzyme assays may be used. The goal of the research is to formulate body washes with the inclusion of these bioenzymes. To make a stable, efficient, and enjoyable body wash, this may need experimenting with various enzyme concentrations, kinds of surfactants (cleaning agents), and other essential components. The pH, cleansing power, lathering ability, and general consumer acceptability of the bio enzyme-based body washes are evaluated. This project will be a major step forward in the creation of environmentally friendly body washes. Body washes enriched with bioenzymes may provide cleaning efficacy on par with traditional solutions, but they may be gentler on human skin and more environmentally friendly. This study opens the door for the development of body wash formulas that satisfy consumers' increasing need for environmentally friendly personal care products.

Keywords: Bioenzymes, body wash, environment friendly, sustainability

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LIST OF ABBREVIATIONS

GCMS	Gas Chromatography Mass Spectrometry
TDS	Total Dissolved Salts
DPPH	2,2-Diphenyl-1-picrylhydrazyl
HEK293	Human Embryonic Kidney Cell line
EPA	Enivironmental Protection Agency
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
FBS	Fetal Bovine Serum
EDTA	Ethylenediaminetetraacetic acid
DMEM	Dulbecco's modified Eagle's medium

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CHAPTER-1

INTRODUCTION

The regular consumption of harsh chemicals and food waste are two major issues in the modern era that must be addressed immediately (Lakra *et al.*, 2022). In recent decades, environmental concerns have grown in popularity and have inspired people to buy eco-friendly goods (Chin *et al.*, 2018). As consumers grow more aware of the need to purchase eco-friendly and green products, green consumerism is gaining popularity. The world of plants is one of the main sources of materials used in the food, pharmaceutical, and cosmetics industries. Plant materials, many of which have been used in traditional or folk medicine for a very long time, form the basis for many supplements, nutricosmetics, and cosmetic products. Herbal tea is one example of a product of plant origin that is enjoyed in many cultures for both medical and gastronomic reasons.

Plant products, including extracts, can also be topically used for skin care and the treatment of a variety of skin conditions (Michalak *et al.*, 2022). The primary advantages listed for plant extracts used in skin care products include tyrosinase inhibition, antioxidant, and antibacterial properties (Ribeiro *et al.*, 2015). Bioenzymes are fully natural liquids and are not toxic, dangerous, corrosive, or environmentally unfriendly, thus they can be prepared to help effectively combat these issues that question the sustainability of manufacturing personal care products (Lakra *et al.*, 2022). The safety profile of enzymes included in cleaning solutions is excellent, and they rarely have negative effects on people. Enzymes don't stand out when it comes to acute, genotoxic, subacute, or repeated dosage toxicity (Basketter *et al.*, 2012). Natural components are more common in modern formulations, despite the fact that they have been utilized for millennia for skin care. Herbs, fruits, flowers, leaves, minerals, water, and soil are examples of natural ingredient sources. The effectiveness of natural components in skin care products is determined by the sort of dermatological base they are incorporated into and by their performance in both *in vitro* and *in vivo* investigations (Ribeiro *et al.*, 2015).

The usage of plant-based personal care products is an important step in achieving environmental sustainability and reaching one's own well-being in a conscious and nature-inspired way. The globe is shifting towards the traditional use of safer, nontoxic, and natural products. In the cosmetics industry, plants are safe to utilize (Shahi *et al.*, 2017). In this review, the ill effects of hazardous synthetic chemicals that are used in most of the commercial personal care products that are available in the market today are discussed. These chemicals have various side effects that are unaware of. They are let out into water bodies, causing pollution and ecological imbalance. Plant products as better alternatives to existing synthetic products are elaborated. They are made from agricultural wastes, thereby promoting sustainability. Skin care and hair care products which are prepared from extracts of plants by eco-friendly processes are also explained. This study emphasizes the significance of ongoing research and innovation in this industry in order to fulfill the growing demand for sustainable, secure, and effective personal care products.

1.1 ECO-FRIENDLY BODY WASH

Surfactants find extensive application in various household commodities. Following their utilization, surfactants and their derivatives are primarily released into sewage treatment facilities and subsequently distributed into the environment via sludge disposal on terrestrial areas and effluent discharge into surface waters, thereby possessing the potential to exert an adverse influence on the ecosystem due to their detrimental effects on organisms residing within the environment. Aquatic toxicity data are readily accessible and have been compiled to provide an overview of the chronic toxicities of surfactants on aquatic organisms. Sewage sludges have been discovered to contain elevated levels of surfactants, along with other contaminants, and are increasingly being utilized as fertilizers for agricultural crops. Consequently, the application of sewage sludges has transformed the terrestrial environment into a significant repository for surfactants. Excessive concentrations of surfactants and the breakdown products of these substances can be harmful to living things in the environment. The current study focuses on bodywash, which is a popular surfactant used by people. Its pH values are higher than the normal range

for skin, which causes an increase in skin pH upon usage. This, in turn, leads to an increase in the dehydrating effect, irritability, and modification of the skin's bacterial flora (Kingsley *et al.*, 2020). Therefore, the goal of the current project is to create an eco-friendly and natural body wash, where natural extracts are used in formulations because of their qualities, which include antibacterial, antiseptic, anti-inflammatory, and antioxidant capabilities to replace the one that is currently on the market (Fatima *et al.*, 2013).

1.2 BENEFICIAL OUTCOMES OF PLANT PRODUCTS

Plant extracts have been utilized in cosmetics for their aromatic qualities and medicinal properties since ancient times. In place of synthetic chemicals, they offer a plentiful supply of bioactive components for the creation of personal care products. Total extracts and selective extracts are the two categories of extracts. Plants as a whole or individual parts, such as leaves, stems, bark, roots, flowers, and/or fruits, can be used to make extracts (Darbre *et al.*, 2023). Natural oils, fats, butters, or waxes, are some of the ingredients that are considered as a replacement of synthetic emollients (Bom *et al.*, 2020). Essential oils and plant-derived phytoestrogens are employed as natural alternatives to synthetic chemicals, are used as colorants, preservatives, soaps, and in wound healing, and skin whitening (Darbre *et al.*, 2023). In addition to providing nutrients for healthy skin, the utilization of bioactive extracts or phytochemicals from a variety of botanicals in cosmetics serves two purposes: body care and the effect on the biological activities of the skin (Ribeiro *et al.*, 2015).

Bio-enzymes are organic substances created through the fermentation of waste from fresh fruit and vegetables in the presence of water and jaggery. It demonstrates the bio-enzyme's superiority as a domestic and agricultural remedy. It is a natural liquid enzyme formulation made from fermented vegetable extracts that is non-toxic, non-flammable, non-corrosive, and improves the engineering properties of soil by enabling higher soil compaction densities and increasing stability. The fermented fruit peel extraction is to increase economic value, optimize agricultural product application, and reduce environmental waste (Khang *et al.*, 2021). In addition to preventing erosion and dust formation, stabilizing soils can increase their strength and resilience. Non-

conventional chemical stabilizers are being used in soil improvement on a daily basis. Organic waste from fruits and vegetables contributes significantly to soil, water, and air pollution. Body washes made with enzymes are organic products that contain enzymes and are made by simply fermenting fresh vegetable and fruit wastes with water, brown sugar, and yeast or bacteria (Dhavale *et al.*, 2020). Purifying this solution can help in obtaining a body wash that is found to be sustainable. It assists in reducing some waste and transforms it into a resource that is economical, readily available, and potentially entirely valuable for society (Dhavale *et al.*, 2020).

Choosing raw ingredients that satisfy all sustainability requirements, however, is still a difficult task for the processes involved in formulating new products. Only if a raw material satisfies ethical, social, and economic obligations while exhibiting environmentally favorable qualities can it be said to be sustainable. Therefore, when choosing sustainable raw materials, the following aspects should be taken into account: (i) the raw material's source, which is just as crucial as the process used to synthesize, extract, and/or purify it; (ii) the pattern of biodegradability and the ingredient's biobased content; and (iii) sustainability affects not just the environment but also the economic and social spheres. The final product's quality must be taken into account in light of the previous essential point because it will significantly affect the economic and social spheres (Bom *et al.*, 2020). These sustainable natural goods are less expensive than those made of synthetic materials. They are provided at affordable costs and are sold during sales for less money than synthetic cosmetics. Because natural goods have fewer side effects and are more affordable than synthetic medicines, according to estimations and research from the WHO, about 80% of the world's population relies on them for their healthcare.

Synthetic cosmetics can irritate our skin, resulting in breakouts, acne, and other conditions that can block skin pores and make the skin dry or greasy. However, utilizing natural cosmetics has no negative side effects and can be used anytime, anywhere (Palanivelu *et al.*, 2022). The skincare and cosmetics industry will benefit from their usage by having a more affordable, environmentally friendly, and long-lasting substitute for synthetic antioxidants (Adeel *et al.*, 2022). Green or sustainable cosmetics are those that use natural, renewable ingredients. Due to the use of synthetic materials in

cosmetics, our bodies may be exposed to a wide range of hazardous agents and deadly heavy metals. Therefore, employing natural ingredients produced from plants rather than synthetic chemicals yields safe, long-lasting cosmetic products. Fruit peel extracts are a fantastic, long-lasting source of the natural elements required for anti-acne, anti-aging, and dermo protective skincare formulas, which help to improve the general health of the skin (Adeel *et al.*, 2022). Eco-Enzyme satisfies a pressing need for trustworthy and environmentally friendly products today (Vama *et al.*, 2020).

Plant extracts can be utilized in cosmetic science to enhance and maintain the physiological balance of human skin because of the inherent economic potential of using natural resources found in ecosystems (Ribeiro *et al.*, 2015). Fruit peels are an important source of important phenolic compounds that are wasted by the food industry. Their use will assist the skincare and cosmetics sector by providing a more cost-effective, ecologically friendly, and durable alternative to synthetic antioxidants (Adeel *et al.*, 2022). These elements make it easier to create intricate formulas that enhance human existence by preventing illness, maintaining health, beautifying the environment, and fostering self-esteem. (Zulaikha *et al.*, 2015). Bio-enzymes are crucial nowadays because they aid in waste reduction and transform it into a useful product that is both economically feasible for people and environmentally sustainable, thereby raising everyone's quality of life (Johnson *et al.*, 2022). Eco-Enzyme satisfies a pressing need for trustworthy and environmentally friendly products today (Vama *et al.*, 2020).

1.3 NEGATIVE EFFECTS OF SYNTHETIC PRODUCTS

Many people use personal care items, which are a substantial source of chemical exposure. The biological effects of these compounds include their genotoxicity, cytotoxicity, neurotoxicity and mutagenicity on aquatic life and human health (Bilal *et al.*, 2020). The chemicals used in most of the products have been associated with a variety of negative health impacts, including cancer and disturbance of the endocrine and reproductive systems (Johnson *et al.*, 2022). To improve the quality, performance, and shelf life of cosmetic, aesthetic, and personal care products, a great range of chemical compounds are used as additives, perfumes, preservatives,

stabilizers, surfactants, stains, and shine (Bilal *et al.*, 2020). In addition to being exposed indirectly through indoor environmental contamination, people may be directly exposed to certain chemicals contained in personal care products through skin absorption, inhalation, and ingestion (Johnson *et al.*, 2022).

Because of the chemicals used in these cosmetics industries, the waste that gets eliminated gets mixed up with waterbodies, which are located nearby. Aquatic organisms like fish and plants get polluted. This leads to severe diseases in aquatic animals, and humans consume those aquatic animals, such as fish, crabs, etc., get affected by diseases caused by those chemicals, like food poisoning, liver disease, and skin allergies. Aerosol sprays and other personal care products that release volatile organic compounds can contribute to indoor and outdoor air pollution. These substances have the ability to combine with other air pollutants to create ground-level ozone, which is bad for the environment and human health. Researchers have also found a dangerous level of mercury present in anti-aging creams and hair dyes (Pramanik *et al.*, 2021).

The harmful chemicals, like sulfates, can cause eye and skin irritation. They have the potential to prematurely remove color from hair. Preservatives such as parabens are makeup fresh and germ-free, and are used in skin care. These parabens imitate estrogen by penetrating the epidermis and inducing excessive breast cell division, which eventually results in breast cancer. In order to increase a product's spread ability, phthalates are used. These are salts or plasticizing agents. They are reproductive and developmental poisons that are present in nail paints, cosmetics, and perfumes. Nail polish, shampoos, hair gels, nail hardeners, hair straightening products, lotions, deodorants and cosmetics all contain formaldehyde. This chemical is linked to neurotoxicity, asthma, scalp burns, hair loss and developmental toxicity. Formaldehyde may cause fainting or choking when breathed in. Cosmetics like shampoos, conditioners, and perfumes all contain fragrances. They contain substances that have been connected to reproductive system adverse effects, dermatitis, skin allergies, and respiratory issues. Many personal care products, including exfoliating scrubs and toothpastes, contain microplastics, tiny plastic particles that can contribute to plastic

pollution when they are washed down the drain. These microplastics can harm aquatic life and ecosystems. Synthetic colors can cause skin rashes, cancer and acne breakouts. They are made from petroleum or coal tar.

Natural elements are currently showing up more frequently in formulas due to customer worries about artificial additives and chemicals. Bioactive extracts derived from different botanicals are used in cosmetics to provide two functions: they nourish the skin and support the body's natural activities (Ribeiro *et al.*, 2015). Plant extracts are sources of vitamins, antioxidants, essential oils, proteins, hydrocolloids, terpenoids, and other bioactive substances. All parties involved including consumers, regulatory bodies, and producers, all share the same goal: They want personal care products and their ingredients to be safe, which means they should pose no or very little health risk to the user during routine use (Nohynek *et al.*, 2010). Due to consumer concerns about synthetic ingredients and chemical substances, natural ingredients are currently becoming more common in formulations. This is because the side effects of the hazardous chemicals used in personal care products not only affect human life but also the environment. Tyrosinase inhibition, antioxidant capabilities, and antibacterial qualities are the main benefits mentioned for plant extracts utilized in skin care products. (Ribeiro *et al.*, 2015). In order to determine whether a specific chemical used in a product is suitable for skin, it is important to examine the harmful and protective mechanisms of that substance using scientific methodologies (Liang 2020). Some of the hazardous chemicals found in commonly used personal care products are listed in (Fig.1.1) To mitigate these adverse effects, consumers are encouraged to choose personal care products with natural and safe ingredients, to be mindful of the chemicals listed on product labels, and to minimize excessive use. Additionally, regulatory agencies like the Food and Drug Administration (FDA) and industry standards are evolving to address concerns related to chemical ingredients in personal care products, with a focus on safety for both consumers and the environment.

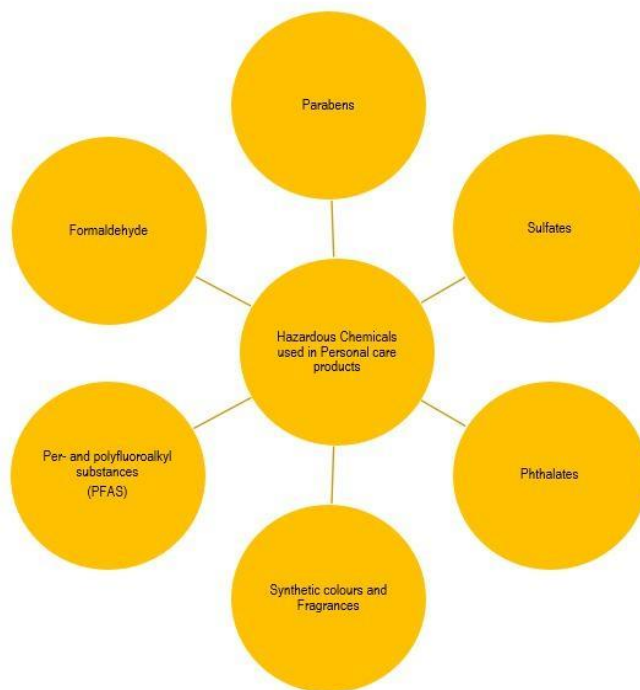


Fig 1.1: Hazardous Chemicals used in Personal Care Products

1.4 BIOENZYMES

Bio-enzyme is a natural substance that is typically extracted from waste materials and citrus fruit peels, among other sources. It is a sophisticated mixture made from fermenting fresh kitchen trash, including fruit and vegetable peels. This kind of vinegar is created by fermenting food scraps and sugar to produce alcohol. Eco-enzyme is a fermented solution mostly composed of water, fruit peels, and sugar in a 1:3:10 ratio (Lakra *et al.*, 2022). Because of the phenolic profile and antioxidant qualities of citrus fruit extracts and flavonoids, this project focuses specifically on the citrus eco-enzyme. These compounds may have intriguing biological activities. A thorough discussion of the various inventive methods employed to create eco-enzymes from various raw materials has been held. These methods have several uses, are inexpensive, and are environmentally benign. The synthesis of eco-enzyme reduces the accumulation of organic waste and offers an alternative to synthetic chemicals that are detrimental to the environment and human health. But the main uses, which include gardening, cleaning floors and kitchenware, and other

creative ways to recycle and reuse natural waste, will help cut down on fruit waste (Benny *et al.*, 2023).



Fig 1.2: Preparation of Bioenzymes

CHAPTER-2

LITERATURE SURVEY

Plant extracts in cosmetics provide natural healing and fragrance, replacing artificial ingredients. Their diverse bioactive compounds, sourced from various plant parts, offer benefits in sunscreens and essential oils. However, ensuring their efficacy and safety for prolonged use necessitates further research amid growing demand for natural products (Darbre PD., 2023). Fruit peels, like banana and pomelo, are highly valuable for diverse applications such as food, fertilizer, biofuel, enzymes, and cosmetics. Rich in phenolics, fibres, coumarins, flavonoids, and vitamins, they offer medicinal benefits including treating anaemia, diabetes, inflammation, and cancer, beyond their nutritional value (Khang *et al.*, 2021). Anaerobic fermentation of organic waste yields bio-enzymes, offering eco-friendly alternatives. Fruit and vegetable peel samples exhibited lipids, proteins, carbs, and antimicrobial properties. Bio-compost from solid waste serves as a natural manure substitute. Bio-enzymes have diverse applications, from domestic cleaning to soil stabilization, potentially replacing harsh chemicals, enhancing quality of life, and promoting sustainability (Lakra *et al.*, 2022). Cosmetic products globally utilize various chemical compounds to enhance quality and shelf life, but their bioactivity poses risks to human health and the environment. This review examines harmful impacts of compounds like parabens, triclosan, and microbeads on neurotoxicity, mutagenicity, and estrogenicity, emphasizing urgent need for mitigation and future research directions (Bilal *et al.*, 2020).

Natural ingredients, increasingly favoured over synthetics, offer antioxidant, antimicrobial, and tyrosinase inhibition benefits in skincare. This review highlights Portuguese plant extracts with skin care potential. Despite recognized properties, few studies focus on formulations. Further research is needed to meet consumer demand for natural skincare products (Ribeiro *et al.*, 2015). For ages, natural ingredients have been integral to skincare. Current focus is on finding novel bio-actives shielding skin from UV rays and free radicals while enhancing skin health. Plants offer antioxidants combating free radicals and age-related damage. This review highlights aromatic and medicinal plants as sources of anti-aging polysaccharides, peptides, and antioxidants.

(Michalak *et al.*, 2022). Cosmetics and pharmaceutical industries face mounting pressure to adopt sustainable practices, shifting towards natural, organic, and eco-friendly formulations. Emphasis lies on ethical sourcing, fair trade, waste reduction, recyclable packaging, biodegradable content, and pollution control. Short-term goals include minimizing environmental impact while enhancing market presence through holistic sustainability efforts (Bom *et al.*, 2020). Ancient practices of incorporating plants into cosmetics continue today, with ongoing scientific research revealing intricate interactions between plants and skin. Ethnobotanical studies and physico-chemical analyses identify numerous plants with potential to enhance modern beauty products, harnessing their diverse Phyto molecules for skin health and appearance (Faccio *et al.*, 2020).

Skin, crucial for immune defence, responds to infections or wounds, aiding recovery. Herbal skincare, rich in plant compounds, offers moisture, nutrition, and sun protection, mitigating sensitivities. This review underscores historical use of medicinal herbs globally, highlighting essential oils like sea buckthorn, olive, and lavender, whose lipid and antioxidant content enhance skincare efficacy (Selwyn *et al.*, 2023). Nature offers essential resources for human survival, with plant-based skincare and therapeutic products gaining global popularity. Concerns over synthetic sensitivities and changing social norms drive the shift towards natural ingredients in personal care. This article explores medicinal and skincare benefits of various plant extracts, meeting rising demand for natural solutions (Jahan *et al.*, 2022). Plant-derived phenolic compounds act as potent antioxidants, scavenging reactive oxygen species in cells like fibroblasts crucial for collagen synthesis and tissue integrity. This review assesses phenolics' impact on fibroblast function amid oxidative stress, highlighting their potential in skincare to mitigate age-related and wound-induced skin damage (Merecz-Sadowska *et al.*, 2021). Cosmeceuticals utilize phytochemicals for antiaging and sunscreen benefits, but face challenges of low penetration and instability. Nanotechnology resolves these issues, enhancing delivery and effectiveness of phyto-derived bio-actives *via* ethosomes, solid lipid nanoparticles, and nanostructured lipid carriers. Nano-sizing improves skin protection and product texture, employing ingredients like aloe vera and vitamins C and E. Safety research on delivery agents like

carbon nanotubes is needed. This review explores phytocompound use, nanotechnology transport, and nano cosmeceutical development for advanced skin therapy (Ganesan *et al.*, 2016). This review focuses on herbal cosmeceuticals for skin health and beauty. Aloe vera, coconut, olive, sunflower, and jojoba oils are researched for dry skin, while green tea, calendula, and turmeric offer skin protection. Carrot, ginkgo, and *Rhodiola rosea* show anti-aging potential. Shikakai, neem, and henna address dandruff. Rich in vitamins and antioxidants, herbal cosmetics provide anti-aging, anti-inflammatory, and wound-healing benefits, surpassing synthetic options with minimal side effects (Palanivelu *et al.*, 2022).

Bioenzymes, comprising proteins, lipids, carbohydrates, and metabolites, were employed in a shampoo formulation using *Hibiscus rosasinensis* and *Sapindus trifoliata*. Physicochemical analysis showed safer components compared to synthetic shampoos, but further research is needed to enhance conditioning and anti-dandruff efficacy of the bio enzyme shampoo (Hemalatha *et al.*, 2023). Green cosmetics, utilizing renewable sources like fruit peels, gain popularity. Ethanol, methanol, and acetone extracts polyphenols from apple and banana peels with high antioxidant activity and sun protection factor. Cream formulas incorporating these extracts offer photostable, antioxidant, UV-protective skincare, combating aging signs while responsibly repurposing fruit peel waste for environmental sustainability (Adeel *et al.*, 2022). Eco-enzymes, fermented from fruit waste in a 3:10:1 ratio with water and brown sugar, yield a dark brown solution with citrus fragrance. Metabolites like flavonoids, alkaloids, and enzymes are present, offering applications in gardening, utensil washing, and floor cleaning. This eco-friendly method reduces fruit waste, proving cost-effective and versatile (Vama *et al.*, 2020). Cleaning product enzymes generally pose minimal health risks, although some proteases may irritate the respiratory system at high concentrations. Airborne exposure limits and health monitoring ensure occupational safety, while understanding enzyme behavior on skin and materials ensures long-term consumer safety (Basketter *et al.*, 2012).

Environmentally friendly methods like ultrasound, subcritical water, supercritical fluid, and microwave-assisted extraction are critiqued alongside traditional techniques. The aim is to provide an overview of current extraction methods for isolating bioactive

substances from fruits (Moreira *et al.*, 2017). Personal care product (PCP) safety is governed by US, EU, and Japanese regulations, managing systemic exposure risks. Hair color allergens pose minimal cancer risk. UV filter safety is rigorously tested, while natural plant ingredient safety raises concerns. Threshold of Toxicological Concern aids trace drug assessment. Harmonized safety standards are crucial for consumer health (Nohynek *et al.*, 2010). The intersection of Ayurveda, anti-aging, and cosmeceuticals is increasingly significant in beauty and wellness. Ayurvedic cosmetic history dates back to the Indus Civilization. Contemporary research emphasizes Ayurvedic anti-aging principles like Vayasthapana, Varnya, and Sandhaniya, utilizing rasayana herbs like *Centella asiatica* and *Embolica officinalis* (Datta *et al.*, 2021).

Herbal extracts, prized for their antibacterial, antiseptic, anti-inflammatory, and antioxidant properties, remain popular in cosmetic formulations. Studies confirm their efficacy in dental, hair, and skincare. Herbal cosmetics combat aging and skin damage safely and effectively, offering environmental friendliness and holistic skincare benefits over synthetic alternatives amidst concerns about industrialization's environmental impact (Fathima *et al.*, 2013). The cosmetics sector prioritizes environmental, social, and economic sustainability, integrating it from design to post-consumer stages. Strategies include waterless products, green chemistry, upcycling agro-food wastes, ethical raw material sourcing, eco-friendly factories, consumer education on sustainable product use, and post-consumer operations focusing on recycling, reusing, or refilling packaging to minimize landfill waste (Martins *et al.*, 2023). Environmental Protection Agency (EPA) labels various cleaning product chemicals as volatile organic compounds, prompting interest in safer alternatives like bio-enzymes from Project Nesara. These enzymes, derived from fermenting fruit waste with sugar, offer effective home surface cleaning while reducing environmental contaminants. Aligned with global chemical-free and sustainability movements, Nesara also collaborates with Non-Governmental Organisations (NGO) to create job opportunities for the impoverished (Vrishti *et al.*, 2023).

CHAPTER-3

AIM AND OBJECTIVES

3.1 AIM

To investigate the potential of incorporating enzymes derived from fruit peels into the formulation of body wash, aiming to create a sustainable, biodegradable, and skin-friendly product that not only enhances cleansing efficacy but also aligns with eco-conscious principles in the realm of personal care.

3.2 OBJECTIVES

- To develop a shower gel formulation with bioenzymes.
- To investigate the potential of bio enzymes to enhance the cleansing efficacy of body wash.
- To evaluate shower gel's performance and sustainability.

CHAPTER-4

MATERIALS AND METHODS

4.1 SAMPLE COLLECTION

Fresh mosambi peels (*Citrus limetta*) were purchased at local market in Sholinganallur. The fruit peels then were washed with tap water and rinse with distilled water, then cut into smaller sizes (about 3–5 mm) to prepare for next experiments.



Fig 4.1 Mosambi Peels

4.2 MATERIALS

Our body wash is crafted with a meticulous selection of ingredients, each chosen for their safety and efficacy in providing a gentle yet effective cleansing experience. Among these ingredients are bioenzyme solution, soap nut solution, coco glucoside, and other carefully curated components.

4.2.1 Bioenzyme

Bioenzyme solution, derived from natural sources, offers powerful cleaning properties while being gentle on the skin. It aids in breaking down organic matter and impurities without causing irritation, making it suitable for even the most sensitive skin types.

4.2.1.1 Ingredients

- Mosambi fruit peels
- Jaggery
- Water

- Yeast Granules(little amount)

Jaggery, citrus peels and water in the ratio 1:3:10

Process: 30 days

To prepare a fermented citrus peel bioenzyme, begin by acquiring an airtight jar of suitable size. Ensuring precision, measure the ingredients in the correct ratio: citrus peels, water, jaggery, and yeast. Start by dissolving the jaggery in water within the jar. Once dissolved, add the citrus peels and yeast to the mixture. Seal the jar tightly to maintain an anaerobic environment necessary for fermentation. Place the jar in a shaded area, away from direct sunlight, and leave it undisturbed. Regularly opening the lid, gently stir the mixture once a day for the initial week. As fermentation progresses, reduce the frequency to stirring once every 2-3 days to allow flavors to develop. After a duration of 30 days, the fermentation process should be complete.



Day-1

Week-1

Week-3

Fig 4.2: Process of Making Bioenzyme

Note: Yeast is added to accelerate the production of bioenzyme within 30 days or else it would take 90 days.

4.2.2 Cocoglucoside

Coco glucoside is a natural, non-ionic surfactant derived from coconut oil and glucose. It is commonly used in personal care and household products such as shampoos, body washes, facial cleansers, and dishwashing detergents. Coco glucoside is valued for its ability to cleanse effectively while being gentle on the skin and environmentally friendly. It is biodegradable and considered safe

for both humans and the environment, making it a popular choice in natural and eco-friendly formulations.

4.2.3 Soapnuts

Soap nuts, also known as soapberries or reetha, are the fruits of the Sapindus genus trees. They contain saponins, natural surfactants that produce a lather when in contact with water. This property makes soap nuts a popular natural alternative to conventional laundry detergents and cleaning products.

4.2.4 Xanthum gum

Xanthan gum has moisturising and conditioning properties in addition to thickening and stabilising effects on the skin. It creates a shield-like layer on the skin's surface to help it retain moisture and stop transepidermal water loss (TEWL), which can result in dryness and dehydration.

4.2.5 Citric acid

Citric Acid works by exfoliating the upper layer of dead skin cells to help clean pores, even skin tone and soften and smooth the skin. It is an AHA (Alpha Hydroxy Acid) and can be used in exfoliating formulations to help remove dead skin cells and dirt to reveal fresh, softer, smoother skin. AHAs are commonly used in cosmetic chemical peels and acne therapies for this reason.

4.2.6 Coconut oil

Coconut oil may be a very helpful addition to your skincare regimen since it gives your skin the vital nourishment and protection it needs. Because of the abundance of fatty acids it contains, especially lauric acid, the skin is left feeling soft and supple and is well moisturized. In addition to its moisturizing qualities, coconut oil provides the skin with several other advantages. Its antibacterial and anti-inflammatory qualities can help reduce inflammation, relieve irritation, and fight germs that cause acne. Because of this, coconut oil is especially beneficial for people with sensitive or acne-prone skin since it can lessen outbreaks and encourage a smoother complexion.

4.2.7 Sandalwood essential oil

Sandalwood is a much-loved fragrance across the world, often evoking soft breezes, restful spaces, and a sense of peace and calm. It's commonly used in aromatherapy and meditation practice around the world. Sandalwood oil has multiple beneficial qualities for the skin like anti-inflammatory, antimicrobial, antiproliferative, antiviral, antiseptic etc

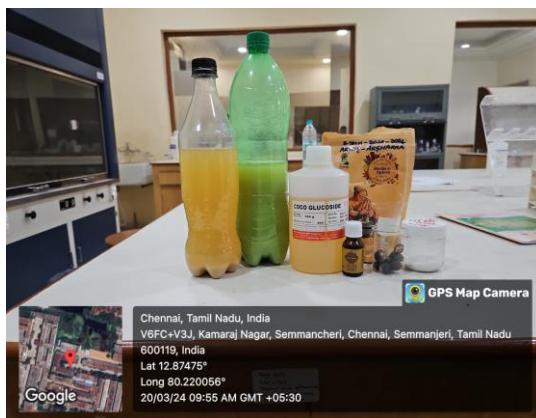


Fig 4.3: Ingredients Used for Formulating Bodywash

4.3 BIOCHEMICAL TESTS

Initial assays were conducted to qualitatively analyze the presence of various biochemical components, particularly bio-enzymes, as part of a study on the fermentation of fruit and vegetable peels. This investigation aimed to detect the organic substances generated during the fermentation process, as indicated by the production of bio-enzymes. Given the nature of fermentation, the samples inherently contained alcohol and carboxylic groups. Subsequently, additional tests were performed to identify the specific metabolites resulting from this process:

4.3.1. Test for Flavanoids- Alkaline reagent test

To the 2ml of bio enzymes sample, few drops of freshly prepared 10% lead acetate (2g of lead acetate in 20 ml of distilled water) was added and observed for the formation of dark yellow or orange colour (Kumar *et al.*,2014)

4.3.2. Test for Phenols-Ferric chloride test

To the 1 ml of bioenzyme sample, 1ml of freshly prepared 5% ferric chloride solution was added and observed for the formation of blackish green colour (Gonfa *et al.*, 2020).

4.3.3. Test for Quinones-Acid test

To the 2ml of bioenzyme sample, 2 ml of concentrated HCl was added drop wise and observed for the formation of yellow colour (Ugochukwu *et al.*, 2013).

4.3.4. Test for Alkaloids-Wanger's reagent test

To the 2 ml of bioenzyme sample, 1ml of freshly prepared Wagner's reagent (0.25g of iodine and 0.4g of potassium iodide in 20 ml of distilled water) was added and observed for the formation of reddish/brown precipitate (Vama *et al.*, 2020).

4.3.5. Test for saponins-Foam test

To the 2 ml of bioenzyme sample, 3 ml of distilled water was added and shaken vigorously and observed for the formation of foam (Vama *et al.*, 2020).

4.3.6. Test for cardenolides-Keller test

Few drops of acetic acid were added to 2ml of sample in a test tube and few drops of dilute 5% Ferric chloride solution was added to it. Then concentrated sulfuric acid was added to the walls of the test tube and formation of brown ring was observed. (Lakra *et al.*, 2022).

4.3.7. Identification of Proteins:

Yeast is used to make bio-enzyme solutions. It creates enzymes to carry out growth-promoting metabolic processes and eventually perishes from a lack of nutrition, leaving the enzymes in the solution. Since complex proteins make up enzymes, proteins are also present in the bio-enzyme solution. *Ninhydrin test*- 1 ml of Ninhydrin solution was added to 1 ml of sample and was shaken and was kept on the water bath for 5-10 minutes till boiling. Then the dark purple to light purple colour observed (Lakra *et al.*, 2022).

4.4 BODY WASH FORMULATIONS

Table 4.4.1: Bodywash Formulation -1

Ingredients	Quantity(50 gl)
Water	35ml
Cocoglucoside	8ml
Bioenzyme	5ml
Glycerin	1.75g
Xanthum gum	0.25g

Table 4.4.2: Bodywash Formulation -2

Ingredients	Quantity(10 g)`
Water	7g
Bioenzyme	1.2g
Cocoglucoside	1.5g
Glycerine	0.25g
Xanthum gum	0.5g

Table 4.4.3: Bodywash Formulation -3

Ingredients	Quantity(10 gl)
Water	6g
Cocoglucoside	1.6g
Bioenzyme	1g
Glycerin	0.35g
Xanthum gum	0.05g
Citric acid	0.05g
Sugar	0.5g
Rose water	0.5g

Table 4.4.4: Bodywash Formulation -4

Ingredients	Quantity (10 g)
Soap nuts	4g
Bioenzyme	3g
Cocoglucoside	1.5g
Coconut oil	0.3g
Xanthum gum	0.1g
Citric acid	0.05g

Table 4.4.5: Bodywash Formulation -5

Ingredients	Quantity (10 g)
Soap nuts	4g
Bioenzyme	3g
Cocoglucoside	1.5g
Coconut oil	0.3g
Xanthum gum	0.05g
Citric acid	0.05g
Sandalwood essential oil	1-2 drops

Table 4.4.6: Bodywash Final Formulation

Ingredients	Quantity(30 g)
Soap nuts	12g
Bioenzyme	9g
Cocoglucoside	6g
Coconut oil	1.5g
Xanthum gum	0.15g
Citric acid	0.15g
Sandalwood essential oil	3-4 drops



Fig 4.4: Various formulations of body wash

4.5 ANTIBACTERIAL SUSCEPTIBILITY TEST

4.5.1 Suspension of *S.aureus* culture

Weigh the suitable powder medium and combine it with water in a sterile flask to prepare LB medium. Cool the broth to room temperature after autoclaving it. Transfer about 1 mL of the overnight *Staphylococcus aureus* culture to the flask in a laminar flow chamber. Use sterile cotton plugs that are not absorbent to close the flask's mouth; make sure the flask is not securely sealed. Overnight, incubate at 37 °C while shaking constantly.

4.5.2 Preparation of nutrient agar

In 100 ml of distilled water, weigh and suspend 0.75 g of nutrient agar and 1.3g of nutrient broth media. Stir until the suspension is well-composed, then bring the mixture to a boil to fully dissolve the medium. Autoclave at 121°C for 15 minutes to sterilise. Gently stir the media and transfer it into sterile petri dishes.

4.5.3 Disk diffusion

The antibacterial assay was performed using a disk diffusion assay. A small amount of *Staphylococcus aureus* was taken and spread onto the nutrient agar surface. Then, 25 µL of samples with different concentration was loaded onto sterile blank disk. Then the discs were impregnated with inoculated nutrient

agar. Water and streptomycin served as the negative and positive control, respectively. The plates were left for 15 min to allow the diffusion of samples, before being incubated at 37 °C for 24 h. Antibacterial activity was observed when the inhibition zone was measured

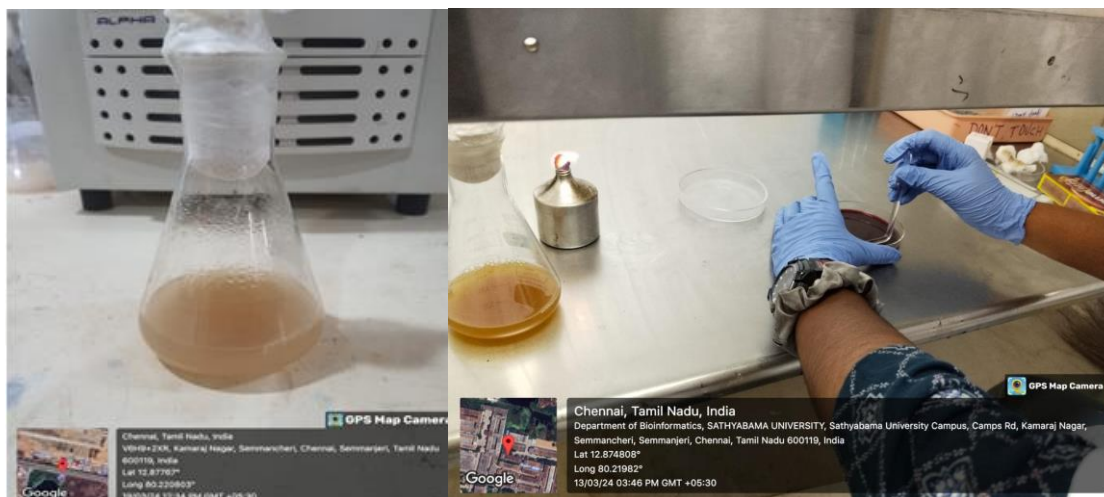


Fig 4.5: Antibacterial Susceptibility Test

4.6 ANTIOXIDANT ACTIVITY

In molecular biology, degenerative processes are associated with an excess of free radicals, which fuels oxidative reactions that are detrimental to the organism. Interest in employing plants in preventive and therapeutic phytotherapy has increased due to their high concentration of substances with antioxidant qualities that can absorb free radicals, such as carotenoidic, phenolic, flavonoid, and anthocyanic derivatives, unsaturated fatty acids, vitamins, enzymes, and cofactors. Antioxidants play a crucial role in biological cells by neutralizing free radicals, which can negatively affect living creatures. The superoxide dismutase (SOD) enzyme has a unique function in mitigating the consequences of oxidative stress caused by the presence of free radicals. It is a subunitary structurally organized metalloenzyme that serves as the primary modulator of oxidation processes in living cells. The oxygen radicals' recombination reaction is catalyzed by this enzyme.

DPPH (2,2-Diphenyl-1-picrylhydrazyl) is a π -radical that exists in both solid and solution states in its monomer form. According to the first structural

data, extensive conjugation had less of an impact on the molecule's distinct low reactivity than the efficient screening of the hydrazyl structure by the surrounding parts. It was established that eliminating the group in charge of safeguarding the o-nitro groups resulted in a notable increase in reactivity, even though the elimination of the p-nitro group had only a little impact. Moreover, conjugation stability is negatively impacted by the twisting of both phenyl groups. This test's application facilitates understanding of a variety of chemical processes and has several clear benefits, including low cost, ease of experimentation, reproducibility, room temperature applicability, and automation opportunities. It clarifies why, in order to get meaningful and comparable data, the scientific community continues to use the DPPH• test and is attempting to optimize and standardize methods.

A stock solution of DPPH is made by dissolving it to a concentration of 0.1 mM in an appropriate solvent (often methanol or ethanol). Get a new DPPH solution ready for every experiment. Using the same solvent that was used to dissolve DPPH, dilute the antioxidant samples to the appropriate concentration. Depending on the anticipated antioxidant activity of the samples, you might need to adjust their concentration if needed. Pour the DPPH solution into each test tube or microplate well to the proper volume. Fill each tube or well holding the DPPH solution with the same volume of the diluted antioxidant samples. As a control, include a blank that simply contains the solvent. Use a vortex mixer to completely mix the contents of each tube.

The reaction mixture should be incubated for a predetermined amount of time (typically 30 to 1 hour) at room temperature in the dark. The samples' antioxidants will react with the DPPH radical during this time, resulting in a reduction in the intensity of the purple color. Using a spectrophotometer, determine each reaction mixture's absorbance at the proper wavelength—typically 517 nm—after the incubation period.

Utilizing the following formula, determine the percentage inhibition of DPPH radical scavenging activity:

$$[(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}) / \text{Abs}_{\text{control}}] \% \text{ Inhibition} \times 100$$

To calculate the antioxidant activity, plot the percentage inhibition against the antioxidant sample concentration on a graph. The IC₅₀ value, or the concentration of the sample needed to scavenge 50% of the DPPH radicals, can also be computed.



Fig 4.6: Antioxidant Activity

4.7 GCMS ANALYSIS

Gas Chromatography Mass Spectrometry (GCMS) combines the separation capabilities of gas chromatography with the detection and identification abilities of mass spectrometry. Chromatographic separation and analysis of volatile chemicals in a mixture is accomplished by the use of GC. It is predicated on the separation of substances into a mobile phase (an inert gas such as nitrogen or helium) and a stationary phase (usually a coated capillary column). The sample passes through the column after being vaporized and injected into the GC system.

4.7.1 Conditions

- Column oven temperature - 100°C
- Injection temperature -250°C.
- Initial temperature is 100°C and 2 minutes hold time is maintained.
- After that 3 degree rate is increased till 200 temperature and 6 degree is increased till 270 temperature and 2 minutes hold time is maintained.
- Column flow - 1ml/minute
- Running time - 49 minutes



Fig 4.7: GCMS

4.8 ALAMAR BLUE ASSAY

Alamar Blue is a cell viability assay reagent which contains the cell permeable, non-toxic, and weakly fluorescent blue indicator dye resazurin. It has been extensively referenced and used in a wide range of research areas. Alamar Blue quantitatively measures proliferation in human, animal, bacterial, fungal, and mycobacterial cells. It is useful for cytokine bioassays, cell viability assays, and *in vitro* cytotoxicity determinations as well as cell growth monitoring. Popular Alamar Blue is a preferred alternative to other cell proliferation assays such as 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (MTT), Thymidine assay, or neutral red.

The prepared body wash was diluted 50 times with nuclease free water, and sterile filtered using a 0.22um syringe filter. This solution was further diluted in Dulbecco's modified Eagle's medium (DMEM) with 1% Fetal Bovine Serum (FBS), ranging from 2% to 20% of the total volume. Twelve hours before the treatment, the HEK293 (Human Embryonic Kidney) cells were disassociated using 0.25% trypsin-EDTA (Ethylenediaminetetraacetic acid) solution, and plated on a 96 well plate (20,000 cells/well). After 24 hours of incubation with different concentrations of body wash solution, the cells were incubated with Alamar blue solution. After 4 hours of incubation, the absorbance was recorded at a wavelength of 570 and 600nm, and graph was plotted. Hydrogen Peroxide was used as a negative control to assess the cellular viability.

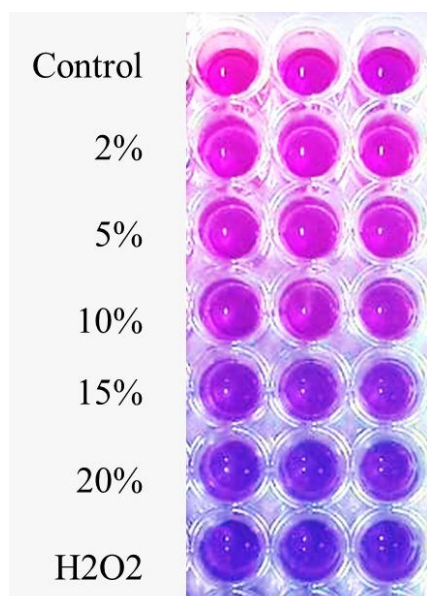


Fig 4.8: Alamar blue assay plate

CHAPTER-5

RESULTS AND DISCUSSION

The tests carried out on the body wash validated its efficacy, safety, and appropriateness for daily application. Findings indicated minimal skin irritation, optimal pH balance, efficient cleansing, and microbial safety, validating its quality.

5.1 PHYSICO CHEMICAL PROPERTIES

Body wash formulations are developed not solely for skin cleansing purposes but also to offer a delightful sensory encounter while being utilized. Comprehending the physicochemical characteristics of these formulations is essential for evaluating their efficacy and quality in skincare regimens. In this discourse, we explore the fundamental attributes of a body wash formulation:

pH (6.9): The pH level of a body wash holds significance as it dictates its compatibility with the skin's inherent pH. A pH of 6.9 falls within the mildly acidic spectrum, closely aligning with the skin's natural pH of approximately 5.5. Sustaining a pH akin to that of the skin aids in preserving the skin barrier's functionality and mitigating potential irritations or dryness.

Total Dissolved Solids (TDS - 350): The total dissolved solids present in a body wash indicate the concentration of dissolved substances within the formulation, encompassing salts, minerals, and other constituents. A TDS value of 350 signifies the cumulative concentration of dissolved substances, which can contribute to the cleansing and conditioning attributes of the body wash.

Color (Sandal): The hue of a body wash appears as sandal owing to the inclusion of soap nut extract and bioenzyme.

Fragrance (Sandalwood): Fragrance assumes a pivotal role in augmenting the user's experience with a body wash. The sandalwood fragrance imparts a serene and woody aroma, fostering a sense of tranquility and wellness during application.

Texture (Viscous): The consistency of a body wash impacts its spread ability and application on the skin surface. A viscous texture denotes a denser composition that may facilitate adherence to the skin, thereby enabling a more comprehensive cleansing encounter.

Lather (Mildly Lathering): A body wash with mild lathering capabilities generates a moderate amount of lather, aiding in uniform product distribution across the skin and assisting in the elimination of impurities and dirt. The physico-chemical properties of the body wash are shown in Tab. 5.1

Table 5.1- Physico-chemical properties of body wash

pH	6.9
TDS	350
Colour	Sandal/Mustard
Fragrance	Sandalwood
Texture	Viscous
Lather	Mild lathering

5.2 PHYTOCHEMICAL ANALYSIS

Phytochemical analyses are carried out on body wash formulations to detect the existence of bioactive plant compounds that could potentially contribute to their therapeutic effects or functional characteristics (Dhavale *et al.*, 2020).

Flavonoids, categorized as polyphenolic compounds, are recognized for their antioxidative and anti-inflammatory attributes. These examinations are capable of inducing alterations in color or fluorescence in the presence of flavonoids through the application of the lead acetate test.

Phenolic compounds, known for their antioxidative and antimicrobial features, are subjected to assessments such as the ferric chloride test, which triggers changes in color upon the detection of phenolic compounds.

Alkaloids, nitrogen-containing compounds with diverse physiological impacts, are commonly assessed using the Wagner's reagent test. These assessments are designed to generate precipitates or modifications in color when alkaloids are identified.

The confirmation of quinones is achieved through the observation of a yellow precipitate following treatment with concentrated hydrochloric acid.

The existence of Cardenolides is corroborated by the formation of a brown ring during the execution of Keller's test.

Saponins, known for their foaming and emulsifying attributes, are evaluated through methods such as the foam test, where stable foam indicates presence of saponins.

Phytochemical tests were carried out with diluted samples of body wash and results are shown in Tab.5.2

Table 5.2- Results of Phytochemical tests done for body wash

Phytochemicals	Test done	Result
Flavonoids	Lead acetate test	+
Phenols	Ferric chloride test	+++
Quinones	Acid test	+++
Alkaloids	Wagner's reagent test	++
Cardenolides	Keller test	++
Saponins	Foam test	++
Protein	Ninhydrin test	++++



Fig 5.2: Phytochemical tests done for body wash

5.3 ANTIBACTERIAL SUSCEPTIBILITY

Sterile paper discs infused with individual body wash formulations were placed onto agar plates that had been previously inoculated with strains of *S. aureus*. Following a period of incubation, the diameters of inhibition zones surrounding the discs were gauged in order to evaluate the antibacterial efficacy of the body washes.

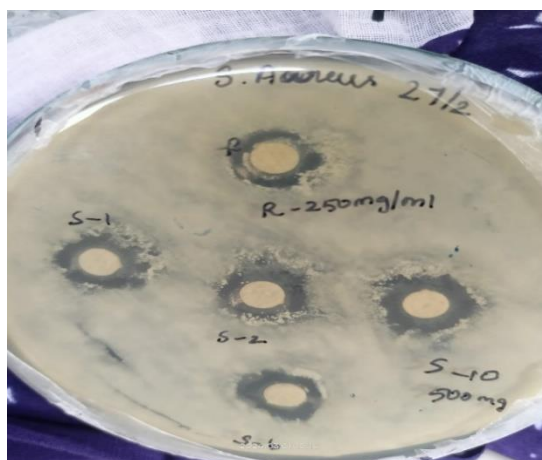


Fig 5.3.1: Appearance of zone of Inhibition on Nutrient agar

In order to statistically interpret the data derived from the agar diffusion tests, a chi-square analysis was performed. The chi-square examination enabled the determination of whether a notable disparity existed in the antibacterial effectiveness between the formulated body wash and the commercially accessible body wash (Dove body wash).

H0: There is no statistical difference between the zone of inhibition of sample and commercially available bodywash.

H1: There is statistical difference between the zone of inhibition of sample and commercially available bodywash.

Therefore Antibacterial susceptibility test conforms there is a statistical difference between the zone of inhibition of sample and commercially available body wash.

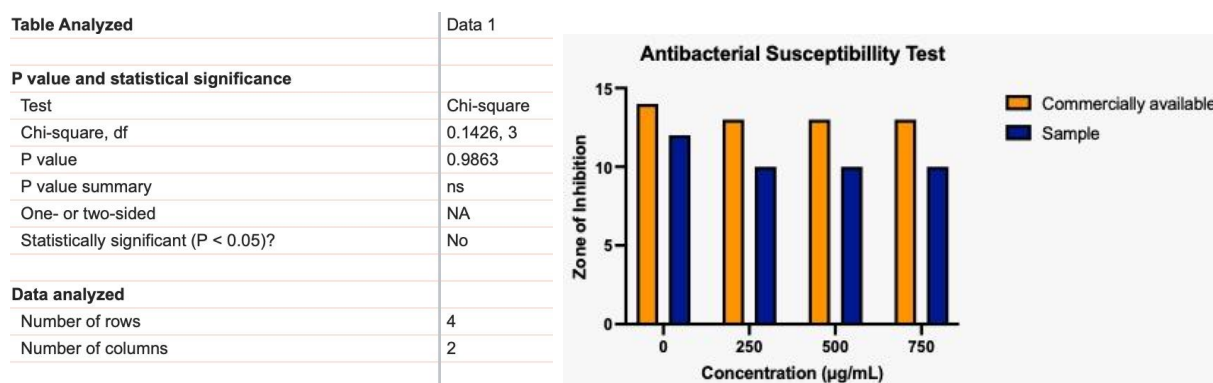


Fig 5.3.2 Chi square test results and graph

5.4 ANTIOXIDANT ASSAY

DPPH radical scavenging capacity assay was done to check the antioxidant potential of bodywash. Ascorbic acid (Vitamin C) was taken as standard. In order to evaluate the antioxidative potential of body wash specimens, solutions at concentrations of 250, 500, and 750 µg/mL were meticulously formulated. After the incubation period, the absorbance was measured at 517 nm in UV Spectrophotometer. DPPH assay indicates the antioxidant capacity of bodywash to be 69%. IC50 value for sample is found to be 251.42ug/ml. This comparative analysis facilitates comprehension of the capacity of body wash products to mitigate oxidative stress and enhance skin well-being, thus facilitating informed development of products and satisfaction among consumers.

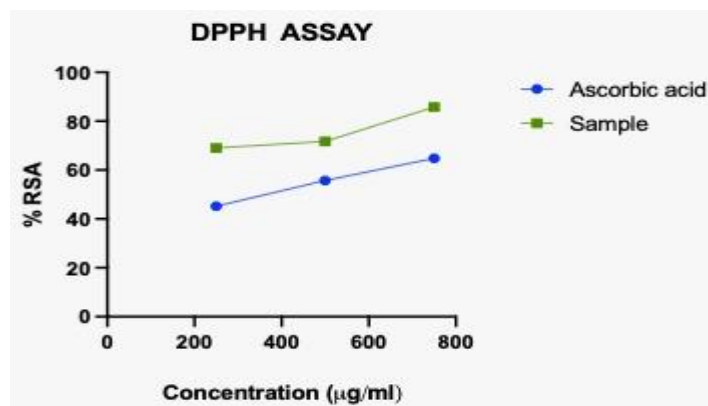


Fig 5.4. Antioxidant assay graph

5.5 GCMS ANALYSIS

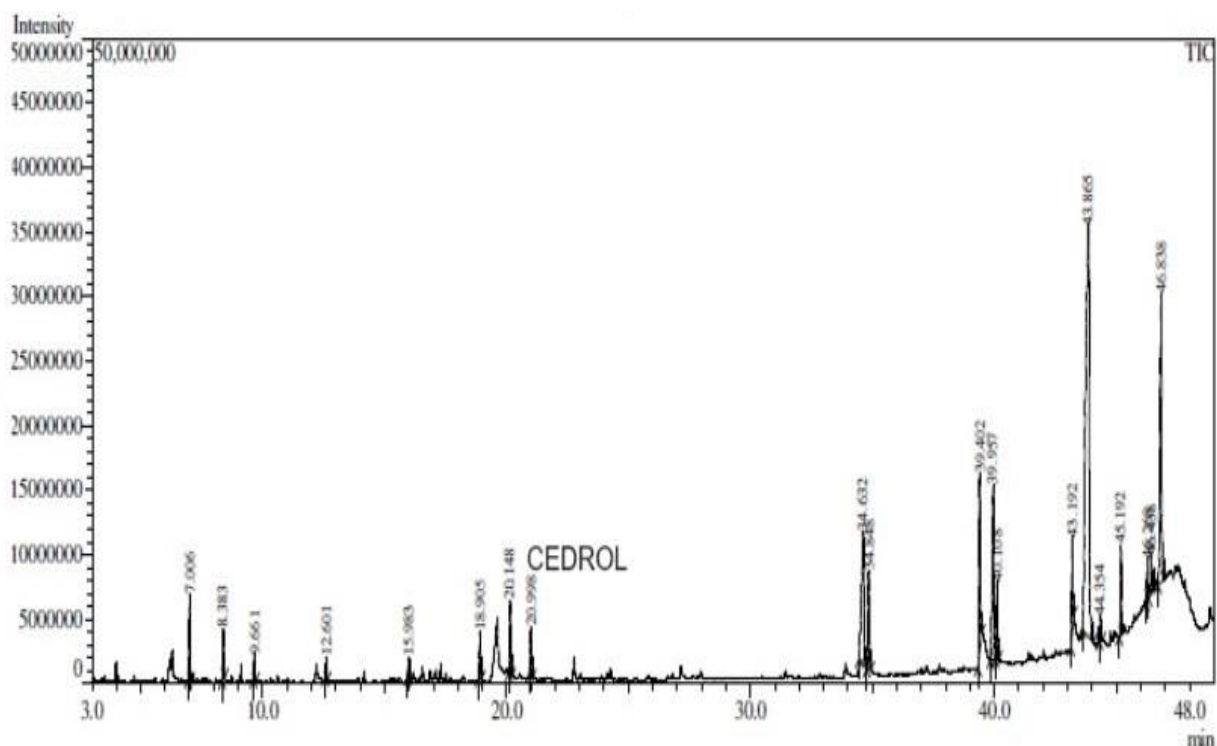


Fig 5.5.1: GCMS Result graph

Cedrol was identified as the primary component utilized in the body wash formulation, as revealed by GCMS analysis. GCMS analysis of a body wash showing a peak at cedrol indicates the presence of this sesquiterpene alcohol in the product. Cedrol has been studied for its various properties, including antibacterial potential in C.

junce (Visveshwari *et al.*, 2018). Therefore, the presence of cedrol in the body wash may offer benefits such as antibacterial properties, potential anticancer effects, or even relaxation benefits, depending on the concentration and formulation.

Peak Report TIC				
Peak#	R.Time	Area	Area%	Name
1	7.006	17889962	2.20	3-CYCLOHEXENE-1-METHANOL, .ALP
2	8.383	10952078	1.34	BENZENE, 1,3-BIS(1,1-DIMETHYLETHY
3	9.661	5492505	0.67	4-tert-Butylcyclohexyl acetate
4	12.601	4542167	0.56	.alfa.-Copaene
5	15.983	4934567	0.61	(3E)-3-METHYL-4-(2,6,6-TRIMETHYL-2-
6	18.905	12498882	1.53	Cyclohexane-1-methanol, 3,3-dimethyl-2-(3-
7	20.148	20509071	2.52	Diethyl Phthalate
8	20.998	12902804	1.58	Cedrol
9	34.632	73807672	9.06	N,N-BIS(2-HYDROXYETHYL)DODECAN
10	34.848	32072373	3.94	Glutarimide, N-(2-octyl)-
11	39.402	45808721	5.63	N,N-BIS(2-HYDROXYETHYL)DODECAN
12	39.957	62940031	7.73	Dodecanamide, N,N-bis(2-hydroxyethyl)-
13	40.108	18355742	2.25	Glutarimide, N-(2-octyl)-
14	43.192	19709112	2.42	N,N-BIS(2-HYDROXYETHYL)DODECAN
15	43.865	321346847	39.46	N,N-BIS(2-HYDROXYETHYL)DODECAN
16	44.354	5528758	0.68	9-Octadecenoic acid, 1,2,3-propanetriyl ester
17	45.192	16974235	2.08	Bis(2-ethylhexyl) phthalate
18	46.268	10109976	1.24	N,N-BIS(2-HYDROXYETHYL)DODECAN
19	46.438	13102085	1.61	4-METHYL-4-[1-METHYL-2-(4-MORPHC
20	46.838	104854921	12.88	Dodecanamide, N,N-bis(2-hydroxyethyl)-
		814332509	100.00	

Fig 5.5.2: Peak report obtained from GC-MS Analysis

5.6 ALAMAR BLUE ASSAY:

As observed in the fig 5.6, we could not find any significant cell death of HEK cells exposed to body wash up to 10%. Almost 23% cell death was observed on HEK cells exposed to 15%, and almost 60% death was observed at HEKs exposed to 20% body wash. Further, hydrogen peroxide exposure induced death in HEK cells by 65%. From this experiment, we can conclude that the body wash used at 50-fold dilution was non-toxic HEK293 cells, followed by gradual decrease in its viability.

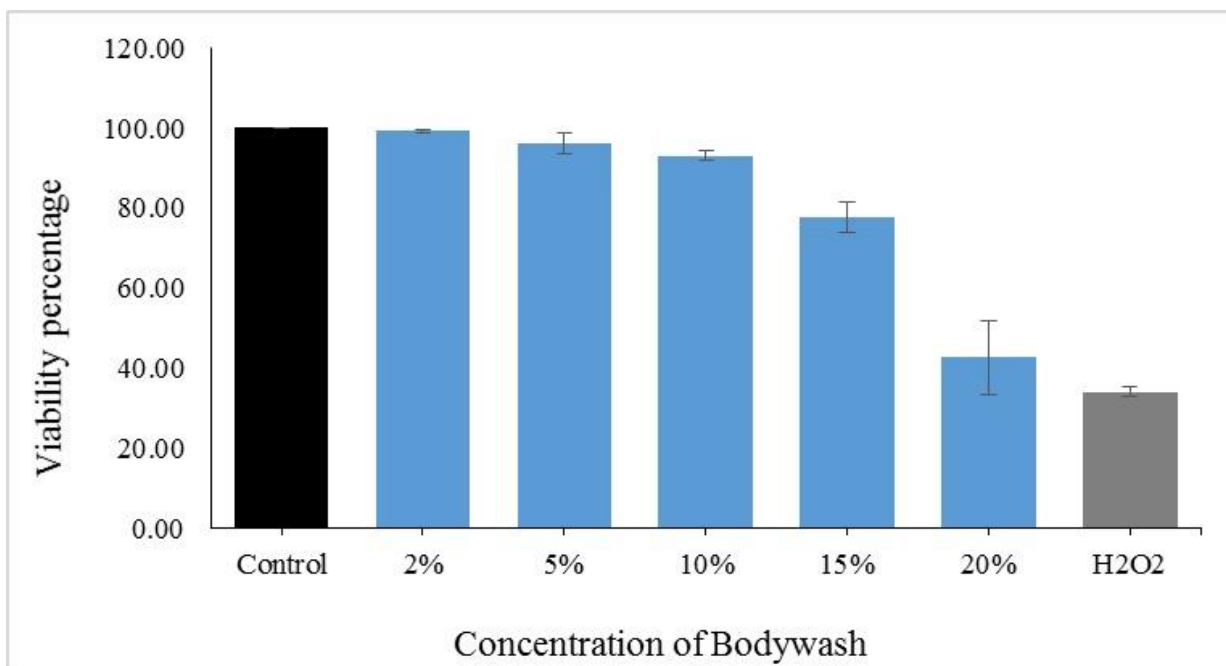


Fig 5.6 Alamar blue assay result graph

This project introduces a novel method for developing an environmentally friendly body wash product that is sustainable. Through the combination of bioenzymes derived from fruit peels and soapnut extract, the aim is to utilize the natural cleaning properties of these components while reducing environmental impact and encouraging responsible consumption habits. The utilization of bioenzymes extracted from fruit peels is an innovative use of organic waste material, in line with the principles of UNSDG 12, which promotes responsible production and consumption. Fruit peels are typically discarded, leading to environmental pollution and landfill buildup. However, by repurposing these peels to extract bioenzymes, the project helps in waste reduction and lowers the carbon footprint associated with traditional body wash production. Moreover, including soapnut extract enhances the sustainability aspect of the body wash formula. Soapnuts, originating from the *Sapindus* tree genus, contain natural saponins with cleansing properties. By using soapnuts as a key ingredient, the project avoids synthetic surfactants commonly present in commercial body washes, thereby decreasing environmental pollution and supporting eco-friendly options.

Furthermore, the project adheres to UNSDG 3 by prioritizing consumer health and well-being. The gentle and natural cleansing properties of bioenzymes and soapnut

extract make the body wash suitable for individuals with sensitive skin or allergies to harsh chemicals. By creating a product that enhances skin health and cleanliness while ensuring safety, the project contributes to overall well-being. Technologically, the project showcases the application of engineering solutions in developing innovative formulations for personal care items. The design process focuses on optimizing the extraction of bioenzymes from fruit peels and synergistically combining them with soapnut extract for effective cleansing. Additionally, examining the efficacy and safety of the body wash formula guarantees that it meets quality standards and regulations, building consumer confidence in its performance and safety. Lastly, by integrating bioenzymes from fruit peels and soapnut extract, the project emphasizes the significance of environmental sustainability in engineering practices. Through reducing dependence on synthetic chemicals and minimizing waste production, the project sets a sustainable approach to product development that emphasizes environmental responsibility and long-term ecological harmony.

This project represents a groundbreaking endeavor that merges innovation, sustainability, and consumer well-being. By leveraging natural components and engineering knowledge, the project provides a compelling resolution to the challenges of traditional body wash production, paving the way for a more sustainable and environmentally conscious future in personal care product development.

CHAPTER-6

SUMMARY AND CONCLUSION

In conclusion, a promising direction for all-natural, environmentally friendly personal care products is the infusion of bioenzymes derived from fruit peels and soap nut liquid into body wash formulations. This mixture offers various possible benefits by utilizing the cleaning capabilities of soap nuts and the enzymatic qualities of fruit peels. First off, adding fruit peel bioenzymes can act as a mild exfoliant, assisting in the removal of dead skin cells and encouraging a clearer complexion. These enzymes may also help to improve the overall texture and brightness of the skin. Second, soap nut liquid which has natural saponins offers powerful cleaning capabilities without using harsh chemicals. Because of this, the body wash may be appropriate for people with sensitive skin or those looking for more natural substitutes for conventional cleansers. When using soap nut liquid and fruit peel bioenzymes to make body wash, a synergistic strategy is presented that not only promotes sustainability and eco-consciousness but also provides cleansing, exfoliating, and maybe skin-beneficial characteristics. Customers may preserve their personal hygiene and well-being while simultaneously helping to preserve the health of the earth by selecting goods that highlight natural, eco-friendly components.

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ANNEXURE

PLAIGARISM REPORT

ORIGINALITY REPORT

10%

SIMILARITY INDEX

PRIMARY SOURCES

1	www.ncbi.nlm.nih.gov Internet	158 words — 3%
2	encyclopedia.pub Internet	55 words — 1%
3	Ribeiro, Ana, Marilene Estanqueiro, M. Oliveira, and José Sousa Lobo. "Main Benefits and Applicability of Plant Extracts in Skin Care Products", Cosmetics, 2015. Crossref	54 words — 1%
4	www.emergenresearch.com Internet	46 words — 1%
5	Nattaya Lourith, Mayuree Kanlayavattanakul. "Passion fruit seed: Its antioxidative extracts and potency in protection of skin aging", Elsevier BV, 2020 Crossref	44 words — 1%
6	Philippa D. Darbre. "Plant-based ingredients in personal care products", Elsevier BV, 2023 Crossref	42 words — 1%
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PUBLICATION

The review work has been presented at the “**International Conference on Innovations in Eco-Friendly Management of Toxic Pollutants (micro, macro, nano dimensional)-ICIEMTP-2023**” and received best presentation award.



REVIEW ARTICLE

Fwd: Editor decision - accepted [inbox](#)



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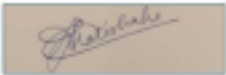
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FOR FABRICATING BODY WASH

Internal Guide Name : Dr.L.Inbathamizh

External Guide Name : Dr.Indumathi SM

PO / PSO / PEO / SDG	Justification
UNSDG 12	The utilization of bioenzymes extracted from fruit peels 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 cleansing properties of bioenzymes and soapnut extract make the body wash suitable for individuals with sensitive skin or allergies to harsh chemicals.
PO 3	The project showcases the application of engineering solutions in developing innovative formulations for personal care items
PO 4	Examining the efficacy and safety of the body wash formula guarantees that it meets quality standards and regulations, building consumer confidence in its performance and safety.
PO 7	By integrating bioenzymes from fruit peels and soapnut extract, the project emphasizes the significance of environmental sustainability in engineering practices.

INFUSION OF BIOENZYMES FROM FRUIT PEELS FOR FABRICATING BODY WASH:



This body wash transcends traditional cleansing methods by providing a revitalizing and environmentally friendly solution for daily bathing. Derived from natural sources, bioenzymes exhibit exceptional cleansing capabilities that efficiently disintegrate oils, dirt, and impurities present on the skin's surface without inducing any irritation. These bioenzymes operate in synergy with the skin's innate biome, fostering a well-balanced and healthy dermal milieu. Through the utilization of bioenzymes, this body wash guarantees thorough cleansing while preserving the skin's fragile pH equilibrium. Complementing the bioenzyme function is the mild yet powerful essence of soap nuts, esteemed for centuries due to their cleansing and nourishing attributes. Ethically sourced, these soap nuts provide a natural substitute for harsh chemicals, rendering the body wash particularly suitable for individuals with sensitive skin. Abundant in saponins, soap nut extract generates a lavish foam that deeply purifies without depleting essential moisture, resulting in skin that is smooth, flexible, and rejuvenated. Free from parabens, sulfates, and synthetic fragrances, this Body Wash represents a mindful option for both your skin and the ecosystem. It not only purifies but also nurtures and safeguards the skin. Embrace the potency of nature's premium components with each shower, and experience the transformative impact of our cutting-edge body wash.