"Preparation Of Herbal Face Pack Cum Scrub Using Papaya and Neem Extracts"

Project report submitted in partial fulfilment of the requirement for the degree of Bachelor of Technology

In

Biotechnology

By

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UNDER THE SUPERVISION OF

Dr. Udayabanu M, Associate Professor

To

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DECLARATION

We hereby declare that the work presented in this report entitled **"Preparation of Herbal Face Pack cum Scrub Using Papaya and Neem Extracts"** in partial fulfilment of the requirements for the award of the degree of Bachelor of Technology in Biotechnology/Bioinformatics submitted in the Department of Biotechnology & Bioinformatics, Jaypee University of Information Technology Waknaghat is an authentic record of my own work carried out over a period from January 2023 to May 2023 under the supervision of Dr. Udayabanu M, Associate Professor, Department of BT&BI. We also authenticate that the matter embodied in the report has not been submitted for the award of any other degree or diploma.

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CERTIFICATE

This is to certify that the work being presented in the major project entitled "Preparation of Herbal Face pack cum scrub using papaya and neem extracts" in partial fulfilment for the award of degree of B.Tech in biotechnology and submitted to the department of Biotechnology and bioinformatics, Jaypee university of information technology, Waknaghat is an authentic record of work carried out by Mrinal Sharma and Sanchit Dua during the time period from January 2023 to May 2023 under the supervision of Dr. Udayabanu M, Associate Professor, Jaypee university of Information technology, Waknaghat.

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The above statements made are true to the best of our knowledge.

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ACKNOWLEDGEMENT

Major Project completion and submission is an integral part of the engineering curriculum and it provides the students with the required knowledge and skill set for a successful career. The completion of this report would not have been possible without the continuous support and guidance of our supervisor Dr. Udayabanu M, Associate Professor, JUIT Solan.

We express our deepest gratitude to Dr. Udayabanu M, Associate Professor, JUIT Solan for helping and supporting us throughout the project period. His expertise and knowledge enabled us to get a good hold on different aspects of biotechnology and research. We would also like to thank the training and laboratory staff of our university for helping through the entire process.

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ABSTRACT

More than 85% of youngsters suffer from acne, one of the most common skin problems. Acne normally begins around adolescence and gradually goes away by the time a person is 20 years old, while some people continue to suffer from acne well into their 40s and 50s. It is frequently written off as a self-limiting condition even though it is rarely life threatening. It receives little consideration in undergraduate or graduate education. Despite appearing to be cosmetic, its effects can penetrate far deeper than the skin's surface and can cause patients to experience severe emotional and psychological distress that may be even worse than the physical symptoms.

The alteration in the look of the skin could result in a modified body image, which is known to provoke emotions such as rage, fear, humiliation, anxiety, melancholy, embarrassment, and bullying and stigmatisation among peers. Acne's side effects have also been linked to low self-esteem, social retreat, feelings of inadequacy and inferiority, less job chances, functional and interpersonal issues at work, and suicidal thoughts. According to estimates, the decline in quality of life is comparable to that caused by epilepsy, asthma, diabetes, or arthritis.

This project aims to create and assess a cosmetic herbal face pack for healthy-looking skin utilising natural elements. Papaya fruit extracts and Neem leaf extracts will be used as the two main ingredients in the face pack. Both ingredients are well renowned for greatly detoxifying the skin, which makes them both effective for treating acne. The extracts from the papaya pulp were prepared using a number of different techniques that were finally used. The procedure for producing the extraction that turned out to be the smoothest and most efficient was lyophilization.

This report concludes with a study that covers the processes for turning extracts into a face pack, and the conclusion is followed by a description of a number of important uses, advantages, and applications of herbal treatment for acne.

CHAPTER 1

INTRODUCTION

1.1 GENERAL – ACNE AND ITS ORIGIN

A skin disease that develops when oil and dead skin cells clog hair follicles[1]. Since antibiotics have a direct antibacterial as well as an anti-inflammatory action, their involvement in pathogenesis is uncertain. Around 20% of adolescents suffer from moderate-to-severe acne, and the severity increases with pubertal maturation. Due to premature puberty, acne may be manifesting earlier in life.Despite the fact that acne affects nearly everyone younger than 30, surprisingly little is known about its epidemiology.

Open and closed comedones and noninflammatory or inflammatory acne lesions, respectively (papules and pustules). Another hallmark is seborrhea, or the production of grease. Following inflammation, scarring frequently occurs . Acne's pathophysiological processes have also been investigated to a fair extent. A microcomedone is formed when keratinocytes that line the hair follicle desquamate to form lesions. Propionibacterium acnes can colonise in an environment that is supported by increased sebum production throughout puberty. Inflammatory and chemotactic mediators are created as P. acnes multiplies, and these mediators in turn fuel inflammatory processes.

Uncertainty exists regarding the genes and risk factors linked to the prognosis of acne. Acne is 78% heritable among first-degree relatives, according to a sizable survey of Chinese undergraduates, and similar findings from other population-based research. 34 People with a positive family history of acne experience it early and in a more severe manner. Numerous retrospective twin investigations have discovered familial clustering to have a potential genetic basis. The severity of acne was recently discovered to be genetically determined in a prospective twin research comparing monozygotic and dizygotic twins, while the study's limited power did not completely rule out the impact of additional environmental factors.

Skin can get dry from exposure to the sun, and even can cause skin to become oily —which tends to produce excessive amounts of oil and is acne-prone. Skin needs moisture. Sebaceous glands, which create the sebum that provides skin with the oils it needs, work overtime when skin is overexposed to the sun, and this excessive sebum production, known as seborrhea, is one of the crucial steps in the development of blemishes and other forms of acne.

1.2 TOPICAL AND ORAL TREATMENTS FOR ACNE

1.2.1 ***Benzoyl peroxide*** has been an essential part of therapy for more than 60 years now. It is an organic acid of the peroxide family. In addition, BP is employed for a number of different processes, including the whitening of teeth and hair, the manufacture of flour, and polymerization reactions. Due to its keratolytic, moderate comedolytic, and antibacterial capabilities, which include the decrease of P. acnes and Staphylococcus aureus on skin. [4]

Clinical trials have demonstrated that BP and erythromycin or BP and clindamycin combination gel formulations are superior to either active drug used alone in reducing acne lesions, particularly inflammatory lesions. The modest comedolytic action of BP, the separate antibacterial effects of the topical antibiotic and BP, and possibly the anti-inflammatory qualities of erythromycin or clindamycin, although these are less well defined, may all contribute to the success of these treatments.

1.2.2 ***a-Hydroxy acids*** (AHA) belong to one of the major groups of hydroxy acids . AHA acids are utilised for cosmetic purposes in dermatology, Glycolic, lactic, and citric acids are among the AHAs, a class of hydroxy acids. The stratum corneum is thinned, epidermolysis is encouraged, basal layer melanin is dispersed, and collagen synthesis in the dermis is increased, all of which have some impact. According to a study by Ditre et al., patients who used 25% glycolic, lactic, or citric acid for six months experienced an increase in epidermal and dermal thickness of about 25%. **β-*hydroxy acids*** are the sole -hydroxy acids utilised in dermatological treatment, salicylic acid, is lipophilic and a widely used active component in a variety of over-the-counter (OTC) acne cleansers, astringents, and lotions. Salicylic acid has mild comedolytic activity which mimics natural exfoliation, all thanks to its desmolytic qualities. Salicylic acid has concentration-dependent desmolytic and comedolytic effects. Mild visible peeling may develop as a result, and some products that include salicylic acid may cause skin irritation while others (such as multivesicular emulsion and emollient foam) are not.

1.3 DRAWBACKS OF TOPICAL AND ORAL TREATMENTS

Depending on the approach you use and the medication's potency, acne treatments may result in a variety of side effects. The most frequent adverse effects of topical acne medications are dryness and irritation of the skin [5]. Thankfully, these sensations are just transient. They frequently become better as your body adjusts to the drug. For oral drugs, there may be more severe adverse effects. You can have stomach trouble or feel faint and dizzy after taking antibiotics. Use a backup birth control technique if you're also taking birth control tablets. Some antibiotics lessen the effectiveness of birth control tablets' pregnancy prevention.

The majority of the time, benzoyl peroxide's cutaneous side effects are irritant in nature, may change depending on the surroundings, and are frequently mild. These side effects include signs like dryness, erythema, and fine scaling. Some people could get allergic contact dermatitis. Although it is available over-the-counter, Benzoyl Peroxide is a category C pregnancy medicine, which means it may not be advisable to use it while pregnant.

1.4 RESEARCH OBJECTIVES

The entire research was carried out keeping in mind the applications and practical usage of the product with no side effects on any skin type, with no added preservatives, the face pack cum scrub was developed.

1.4.1 Objective I -- Identification of Multifarious plants with therapeutic and remedial properties

1.4.2 Objective II – Preparation of extracts of selected plant materials and formulation of the face pack

1.4.3 Objective III – Quantitative analysis and evaluation of physicochemical activities of the formulation

Papaya cross section

Scientific classification	
Kingdom:	Plantae
(unranked):	Angiosperms
(unranked):	Eudicots
(unranked):	Rosids
Order:	Brassicales
Family:	Caricaceae
Genus:	*Carica*
Species:	***C. papaya***

Binomial name
Carica papaya

Neem

Azadirachta indica, flowers and leaves

Scientific classification	
Kingdom:	Plantae
(unranked):	Angiosperms
(unranked):	Eudicots
(unranked):	Rosids
Order:	Sapindales
Family:	Meliaceae
Genus:	*Azadirachta*
Species:	***A. indica***

Binomial name
Azadirachta indica

Fig 1(A) – Tables representing necessary information about the herbal plants under consideration

CHAPTER 2

LITERATURE REVIEW

This chapter includes critical analysis of various articles, research papers, journal articles and discussions about the use of medicinal plants or herbs utilised for the cure and management of acne, which helped us in understanding the different techniques , methods and formulations employed for the management of acne vulgaris. Brief summary of some research papers have been included in this chapter.

2.1 VITAMINS AND THEIR ANALOGUES AS A CURE FOR ACNE

2.1.1 ***Retinol*** or ***Retinoids*** are a class of physiologically active chemicals generated from vitamin A that can be found in both natural and artificial forms[6]. These substances are crucial for biological and physiological processes such as hematopoiesis, tissue maintenance and differentiation, growth, and vision. Retinoids promote epithelial differentiation, which paradoxically normalises hyperproliferative epithelium while increasing cell proliferation.
Retinol, unlike tretinoin, has not been well investigated for the treatment of acne vulgaris despite being less strong pharmacologically than tretinoin. It also causes less overall skin irritation and erythema.

2.1.2 ***Zinc*** is a vital trace element for animals, plants, and microorganisms to survive. However, topical acne treatments have regularly used these salts since the 1970s. It is well known that zinc salts in acne patients have an anti-inflammatory effect that is mediated through the suppression of chemotaxis[7]. Additionally, zinc salts may modify the expression of integrins, boost superoxide dismutase activity, reduce the production of inflammatory cytokines, and have a sebo suppressive impact.

2.1.3 ***Nicotinamide*** is applied both orally and topically. It is a water-soluble amide derivative of vitamin B3, and is used to treat acne vulgaris and other inflammatory skin disorders. It has been shown to reduce the expression of the interleukin (IL)-8 gene and the generation of IL-8 protein, which is a key factor in the stimulation of inflammation. It has also been found to limit cytokine release by keratinocytes. One study found that topical nicotinamide gel 4% was equally effective at treating AV as clindamycin gel 1% without leading to the development of antibiotic resistance, which is crucial for patients receiving treatment for an extended period of time.

2.1.4 ***Sulphur*** is coupled with additional topical medications such as BP, salicylic acid, and resorcinol. Sulphur is typically coupled with resorcinol in over-the-counter acne medications, whereas it is combined with sodium sulfacetamide in 10% concentration in prescription formulations. Although it is assumed that resorcinol has inherent antibacterial, antifungal, and keratolytic activity, it is not thought to be useful when used alone [8]. Sensitization and mild irritation are side effects of using sulphur and resorcinol.

Fig 2(A) – An Image representing different stages of Acne Vulgaris

2.2 SOME COMMON DRUGS UTILISED FOR ACNE

2.2.1 ***Macrolides*** - a class of drugs employed to manage and treat several bacterial infections, are applied topically to treat acne. For severe acne and acne that is resistant to topical treatment, oral administration is indicated. Because of their antioxidant and anti-inflammatory properties, the macrolides erythromycin and clindamycin are employed. However, after the use of macrolides, P. acne resistance, gastrointestinal discomfort, vaginal candidiasis, particularly photosensitivity, and medication interactions may be seen. Azithromycin, a drug with less gastrointestinal adverse effects, underwent structural change [13]. It was discovered to build up in breast milk.

2.2.2 ***Salicylic acid*** was used to dissolve follicular blockage in a variety of formulations, most notably an alcoholic cleaning solution. Salicylic acid is a moderate keratolytic and anti-inflammatory drug. When compared to benzoyl peroxide, this composition was more effective. Comparing salicylic acid to retinoids, it is a gentler substance. As their mechanisms are different, combining salicylic acid and benzoyl peroxide would improve therapy outcomes. Salicylic acid skin peeling, in addition to being a purifying agent, was discovered to dramatically lessen comedowns.

2.3 HERBAL TREATMENTS FOR ACNE

Due to its benefits, which include higher patient tolerance, a lengthy history of usage, fewer side effects, and being comparatively less expensive, herbal medications are becoming more and more popular. Additionally, they have offered solid support for the treatment of a wide range of incurable disorders. To cure acne, these herbs are used either alone or in conjunction with synthetic medications [17]. Without exception, herbal medications are also utilised to treat acne vulgaris, either alone or in conjunction with other techniques. Acne and other infectious diseases are treated in various ways with a variety of medicinal herbs that have anti-inflammation and antibacterial properties.

2.3.1 ***Aloe Vera*** Ayurvedic medicines often contain aloe vera extract. Lesions of acne were greatly lessened. This Asian dermatological treatment was compatible with South Africa's therapeutic usage of Aloe species. Aloe vera, however, had no effect on the proinflammatory cytokines and ROS(reactive oxygen species) produced by P. acnes. *Azadirachta indica*, *Curcuma longa*, and *Hemidesmus indicus* were utilised in the same ayurvedic composition to cure acne. These herbs considerably reduced the ROS production that P. acnes caused [14]. Their potential for treating inflammatory lesions is highlighted by the fact that their anti-inflammatory action should be stronger than A. vera's.

2.3.2 ***Allium Cepa*** (onion) , a common spice, has been used for acne traditionally because of its mild keratolytic, anti-fungal, and bacteriostatic qualities due to its sulphur content and anti-inflammatory flavonoids. However, its offensive smell restricts usage and raises the risk of discomfort.

2.3.3 ***Centella Asiatica*** (gotu kola), Other continents besides Asia also use conventional herbs to treat acne vulgaris. In Africa, *Centella asiatica* was used as a general tonic for acne, wounds, and leprosy [21]. In Asia, C. asiatica-containing skin care products are widely sold despite the fact that its exact mechanism is yet unknown.

2.3.4 ***Eucalyptus –*** Acne treatment has made substantial use of the essential oils of *Eucalyptus radiate* and *Melaleuca alternifolia*, also known as Australian eucalyptus and tea tree, respectively . When compared to benzoyl peroxide at the same dose, *M. alternifolia* oil gel efficiently reduced acne lesions with fewer side effects.

2.3.5 ***Glycyrrhiza Glabra*** (liquorice) Due to its anti-inflammatory properties, also known as the plant licorice, was topically administered in the treatment of acne. However, it had only moderate antioxidant activity. Additionally, due to its biologically active terpenoids, *Gossypium barbadense*, an antimicrobial and antioxidant herb, was utilised as a traditional cure for acne in Yemen.

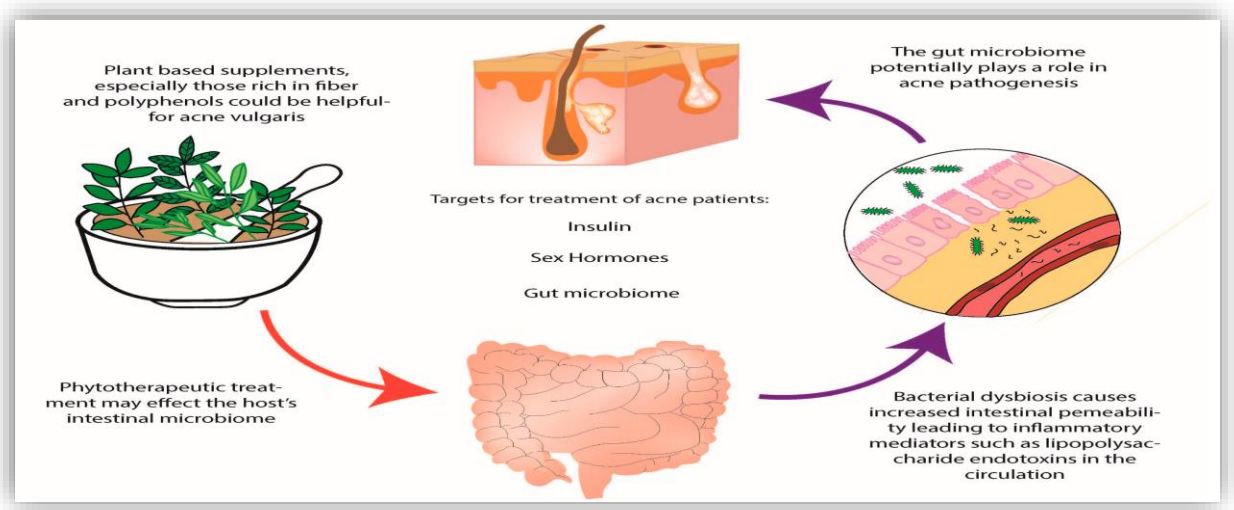


Fig 2(B) – Influence of Plant based supplements on gut microbiome and acne

2.3.6 ***Rosa Damascene*** (Damak rose) is mainly utilised as a scent, and was discovered to successfully inhibit P. acnes with regard to its anti-inflammatory function. Rose oil has also been used to treat acne in the past. As a result, rose was used as a versatile element in cosmetic goods. Because of its anti-inflammatory flavonoids, red clover, also known as *Trifolium pretence*, was used as a treatment for acne.

2.3.7 ***Phyllanthus emblica*** Because of its active ingredients, which mostly included ascorbic acid, gallic acid, skin-lightening agents, quercetins, and ellagic acid, emblica or Phyllanthus emblica was shown to be suitable for treating acne. The extracted geraniin in particular demonstrated the highest DPPH and lipid peroxidation testing activities as well as NO scavenging activities.

2.3.8 ***Cannabis Sativus*** seed oil of this plant is effective in treating psoriasis, eczema, dermatitis, seborrheic dermatitis, rosacea acne, and lichen planus. This plant's leaf powder is excellent for treating cuts and sores. Extract from the *cannabis sativus* plant can be used topically to soothe itching skin. The seed oil fortifies the skin and increases its resistance to viral, bacterial, and fungal illnesses.

2.3.9 ***Aspera achyranthes*** Traditionally used for the treatment of boils, scabies, boils, acne vulgaris, and other skin conditions. This plant's leaf extract were beneficial antitumor promoters in carcinogenesis. The plant possesses strong antioxidative effects very beneficial for cure and management of mild to severe acne conditions.

Fig 2(C)–Neem extracts based herbal formulation **Fig 2(D)–Aloe vera extracts for acne**

Fig 2(E) – Gel based Solution for Acne

2.4 POTENTIAL HEALTH BENEFITS OF PAPAYA

Papaws and pawpaws are other names for papayas, which are tropical fruits. They are a well-liked fruit because of their sweet flavour, vivid colour, and myriad health advantages.

Previously an uncommon and exotic fruit, papayas are now available throughout most of the year. Consuming papaya may provide health advantages such as a decreased risk of heart disease, diabetes, and cancer, as well as benefits for digestion, blood glucose control in diabetics, blood pressure reduction, and wound healing.

Numerous health advantages are attributed to the nutrients in papaya. They might offer some degree of health problem protection. Alkaloids, phenolics, flavonoids, and amino acids were found to be pharmacologically active phytochemicals in the young leaves of the Carica papaya. Additional research can be done on these constituents to determine and isolate the most potent bio constituents of papaya

1. Anticancer action
2. **Anti-acne activity**
3. Reducing menstruation pain
4. Nausea relief
5. Asthma prevention
6. Improved bone health
7. Aids in digestion
8. Reduces Inflammation
9. Improves hair health

2.5 POTENTIAL HEALTH BENEFITS OF NEEM

An Indian native tree called neem (*Azadirachta indica*) is used in complementary medicine to treat head lice and prevent gum disease. The neem tree, an evergreen tree with rapid growth that is a member of the Meliaceae family, sometimes known as the mahogany family, is renowned for its ability to withstand drought. The neem tree grows to be a substantial shade tree with a thick, rounded canopy and has a lifespan of 150 to 200 years.

Neem is known for its potent, disagreeable taste, yet its blooms have an enticing, honey-like aroma that can be detected for kilometres. Neem is also a particularly renewable resource because it is a fast-growing tree that uses minimal water. Because every portion of the neem plant has at least one health-improving quality, it is known as the "Village Dispensary" in several regions of the world.

It is offered as a pill, powder, oil, tincture, cream, and mouthwash. The bark, blossoms, or fruit of the neem tree are used in several traditional medicines. Numerous alleged health benefits of neem include –

1. Antioxidant activity
2. Antifungal activity
3. Antibacterial activity
4. Utilised for pest control purposes.
5. Inflammation is also reduced
6. Immune system is strengthened
7. Improved dental health
8. Healthy lungs and lowered blood sugar levels
9. Helps in the purification of blood

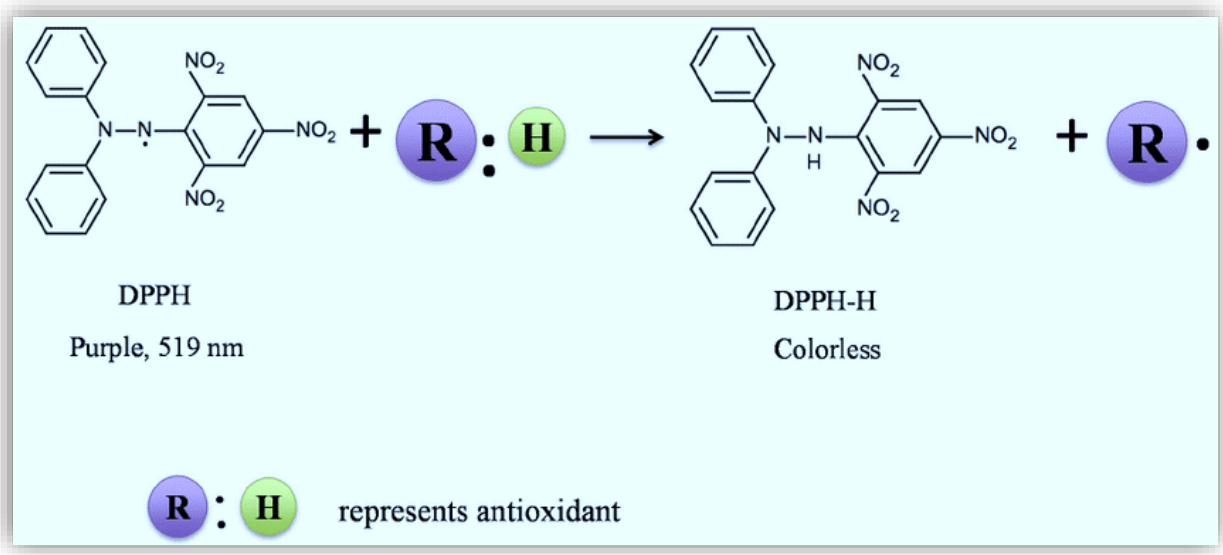
2.6 Anti-Microbial Importance of Plants

Phytochemicals, which can be found in grains, vegetables, fruits, and other plant products, are bioactive organic chemical substances that are found in medicinal plants. They act as a protective mechanism against major chronic diseases in both infectious disease and host-metabolic or genetic dysfunctional disease.

Primary metabolites, including the lipids and sugars present in all plants, conduct intermediary metabolic processes, whereas secondary metabolites are present in a narrower variety of plants and serve more specialised purposes. Examination of the chemical composition of medicinal plants showed that they contain a variety of bioactive substances, such as saponins, tannins, and alkaloids. Antioxidants, agents that fight cancer, immune system-stimulating substances, agents that help with detoxification, and neuropharmacological substances are the primary functional types of phytochemicals with therapeutic promise.

Many plants were found to possess anti-oxidative and anti-inflammatory properties due to the presence of several types of enzymes. Science has exploited this field of study vastly and methods and technologies are being used to extract these important chemicals from plants. To extract antioxidants from food and medicinal plants, a variety of extraction techniques, including traditional extraction methods and non-conventional extraction methods, can be used. Most often used traditional extraction techniques are hot water bath, maceration, and Soxhlet extraction, which are time-consuming, need a significant amount of organic solvents, and have low extraction yields.

Non-conventional techniques such as ultrasound, microwave, pressurised liquid, enzyme hydrolysis, supercritical fluids, high hydrostatic pressure, pulsed electric field, and high voltage electrical discharges have been investigated to obtain antioxidants from plants in an energy-efficient and economically sustainable manner.



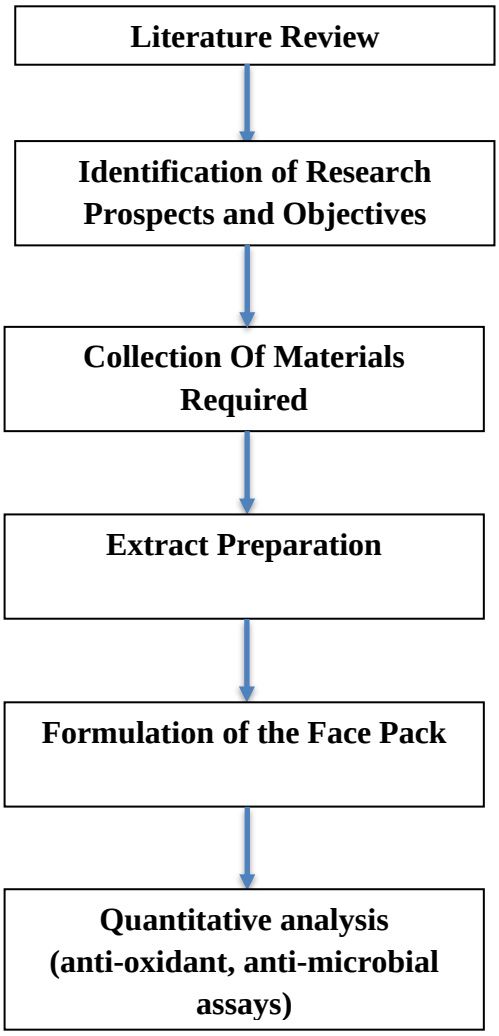
Reaction mechanism of 2,2-diphenyl-1-picrylhydrazyl (DPPH) with antioxidant. R : H = antioxidant radical scavenger; R = antioxidant radical. [27]

CHAPTER 3

MATERIALS AND METHODOLOGIES

3.1 GENERAL

A standardised approach was followed from obtaining the materials required for the formulation till the extract preparation. A series of steps were involved in the extract preparation for both - papaya and neem extracts.



3.2 COLLECTION OF MATERIALS REQUIRED

Each material and their roles are described using a table given below

Components used	Used as	Role/Benefits
Carica Papaya (Papaya)	Pulp Extracts & Seed Extracts	Pulp extracts - The enzymes present decreases inflammation.Removes dead skin and reduces acne. Seed extracts - Anti-oxidant
Azadirachta indica (Neem Tree)	Neem leaf extracts	Useful in detoxification, Anti-oxidant, Anti-bacterial.
Coconut(*cocos nucifera*)milk	Powdered form	Used to lock in moisture and hydrate skin and helps to maintain skin elasticity.
Aloe-barbadensis (Aloe-vera)	Aloevera extracts	Prevents skin ageing, lightens blemishes of skin, prevents sunburn and has healing effect.
Oryza Longistaminata(Red rice)	Rice extracts	High starch content, used as a binding agent for face pack and helps in reducing blemishes.

Table 3.1: Roles and benefits of selected components

3.3 PREPARATION OF PAPAYA EXTRACTS

The following protocols were followed for the preparation of papaya fruit extracts :

1. Papaya obtained was peeled and cut into small pieces.
2. The cut pieces were mashed to form a pulp-like appearance.
3. A clean muslin cloth was used as a filter membrane to filter out the juice from the cut pieces. The obtained juice was then stored in a clean reagent bottle in a refrigerator for further use. (approx 300ml juice obtained)
4. The drained pulp was discarded.
5. The obtained juice was poured into two separate clean beakers, each containing roughly 150 ml of the content, the beakers were covered with foil paper and stored overnight at -80°C
6. The next morning, the samples were carefully removed from the storage , the foil paper was replaced with paraffin paper.
7. Several holes were made in the paraffin paper, and the beakers containing the samples were kept in a lyophiliser for overnight freeze drying.
8. The next day, the samples were taken out of the lyophiliser and the dried extract was transferred to a fresh falcon tube for further processes.

Fig 3(A) – Fresh Papaya

Fig 3(B) – Papaya diced in small pieces

Fig 3(C) – Lyophiliser utilised for freeze drying

Fig 3(D) – Soxhlet apparatus

Fig 3(E) – Dried Papaya samples after lyophilisation

3.4 PREPARATION OF NEEM EXTRACTS

The leaves of ***Azadirachta Indica*** commonly known as neem were obtained from a neem tree. The plant parts obtained were free from any deformities or any visible diseases.the collected plant materials were brought to the laboratory for further processing.

The protocol followed was as follows –

1. The leaves of neem were cleaned under running water to remove any contamination.
2. After drying, the leaves were cut into small pieces.
3. A mixture grinder was used to convert the leaves into powdered form.
4. The powder obtained was then subjected to filtration through sieving to get a fine powder of the extracts.
5. The extracts obtained are then stored in a moisture free aseptic air tight container for further use.

Fig 3(F) – Dried Papaya leaves

Fig 3(G) – Leaves cut into small pieces

Fig 3(H) – Neem extracts isolated from the neem leaves

3.5 PREPARATION OF PAPAYA SEED POWDER

Papaya seeds were isolated from fresh fruit obtained from the local market. The protocol followed for powder preparation was as follows –

1. Seeds were isolated from a fresh papaya obtained from the local fruit market.
2. The isolated seeds were then washed thoroughly and were oven dried for 24 hours at 40 degree Celsius.
3. The dried seeds were then ground using a grinder to make fine powder.

Fig 3(I) – Dried Papaya seeds

Fig 3(J) – Papaya seed powder

3.6 PREPARATION OF COCONUT MILK POWDER

Cocos nucifera, commonly known as coconut has been used in various formulations fue to its moisturizing properties and is used to hydrate skin and helps in maintaining skin elasticity.

1. Coconut milk (2 x 250ml) were purchased from the local market.
2. 250ml of the milk was carefully poured into three different open mouthed beakers and stored at -80 degree Celsius overnight.
3. The frozen samples were then lyophilised the next day, the samples were removed after completion and the powdered form was stored in a falcon tube until further analysis.

Fig 3(K) –Coconut milk tetra pack

Fig 3(L)- Coconut milk powder

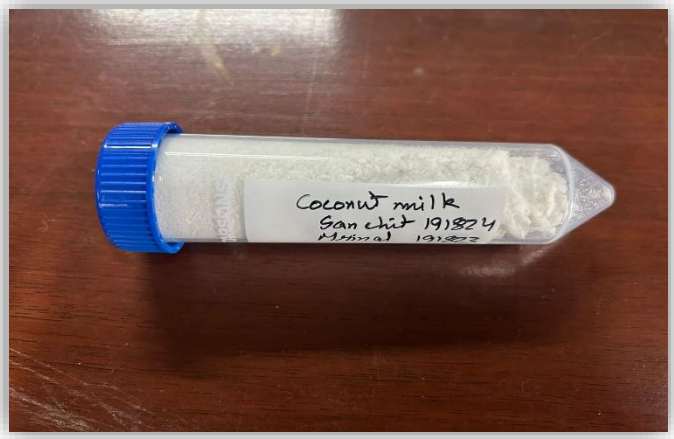


Fig 3(M) – Final stored milk powder

3.7 PREPARATION OF ALOE VERA EXTRACTS

Aloe barbadensis, more commonly known as aloe vera, can be easily grown in fields and has several benefits and uses for various purposes, the raw pulp of aloe vera contains more than 98% water content, other components of the aloe vera gel includes glycoproteins and polysaccharides.

1. 7-8 Fresh aloe vera was plucked from the garden at JUIT solan.
2. The leaves were washed under running water to remove any dirt.
3. A sterilised surgical blade was used to remove the top layer of the leaf to expose the inner content i.e the gel.
4. Using a clean spatula, the gel was scooped from the base and collected in a beaker.
5. The gel was freezed overnight at -80 degree Celsius, the next day, the gel sample was lyophilised.
6. The dried sample was stored to use as a whole.

Fig 3(N) – Aloe vera in field

3.8 PREPARATION OF RED RICE EXTRACTS

Oryza longistaminata, or red rice has a high starch content (about 25%) when compared to other types of rice. Rice is used as a binding agent for the face pack formulation. Due to high starch content, red rice is the most suitable type for binding purposes.

1. Red rice was obtained from the local market (250-300gms).
2. Rice was washed and shade dried.
3. The dried rice was ground using a grinder to produce a powdered form (not ground to 100%)
4. The samples were subjected to extraction using a Soxhlet apparatus. After the process completion, the samples were lyophilized to obtain the final product.

Fig 3(O) – Red rice

Fig 3(P) – Coarsely ground rice

Fig 3(Q) – Soxhlet apparatus

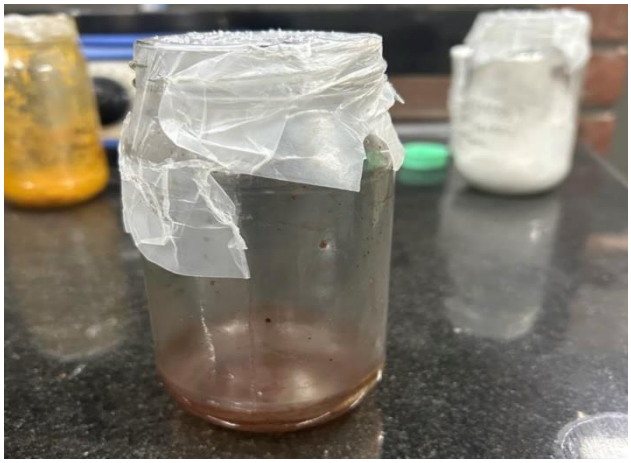


Fig 3(R) – Red rice extracts

3.9 FORMULATION OF ANTI-ACNE HERBAL FACE PACK

Each component is to be obtained in a dry and powdered form.

3.9.1 Weighing

Using a digital balance, each of the necessary herbal powders for the face pack preparation was precisely weighed.

3.9.2 Mixing

To create a uniform, fine powder, all of these fine ingredients— papaya pulp extracts, papaya seed powder, coconut milk powder, aloe vera gel powder, red rice extracts and neem extracts —were carefully combined together in a mixer.

3.9.3 Gathering and Keeping

The powder mixture was gathered, stored in an appropriate plastic container, and utilised for evaluation criteria.

3.9.4 FORMULATION STUDIES FOR ANTI-ACNE FACE PACKS

People who use the face packs indicated in ayurveda can get rid of their wrinkles, dark circles, pimples, and acne. Herbal face masks make skin smoother and fairer. Using herbal face packs in accordance with our skin type will allow us to get the most out of them. The finest ayurvedic method for boosting fairness is using ayurvedic face packs, which make the skin glow. In order to prevent, promote, and treat any skin issues, face packs are beneficial.

Skin is nourished. Fruit face packs give skin vital nutrients. Some of the benefits of herbal face packs include–

1. Depending on the herbal ingredients, helps to lessen scars, blemishes, and acne.
2. Skin dead cells are typically removed by face packs.
3. The soothing and calming effects of these face masks are felt by the skin.
4. In a short amount of time, they aid in restoring the skin's lost radiance and glow.
5. Regular usage of natural face masks enhances skin's radiance and improves the texture and complexion of the skin.
6. When used properly, face packs can effectively counteract the negative effects of pollutants and extreme weather.
7. They support the prevention of early skin ageing.
8. Natural face-care products can successfully prevent the development of wrinkles, fine lines, and skin sagging.

3.9.5 Ideal Qualities of a face pack

1. It should be non-toxic and non-irritating.
2. It must be chemically and physically stable.
3. There shouldn't be any grit in it.
4. It ought to smell good.

Acne, pimple, and blackhead-recommendation face packs The overproduction of sebum from the sebaceous glands is typically controlled with heads, which also eliminate the dangerous germs inside acne lesions. By combining a fine sandal, rose petal, and orange lentil powder with an acne face pack, the skin's scars and markings can be diminished9. Natural facelifts are an efficient way to reduce the appearance of wrinkles, fine lines, and sagging skin.

3.9.6 Precautions while using face packs

Face packs recommended for use against acne, and other skin problems need to be used with caution –

1. Depending on your skin type, choose a face pack.
2. Before using a face pack, consult a natural therapist or skin care professional.
3. A maximum of 15 to 20 minutes should be spent wearing the face pack. Keeping for a very long period may cause the skin to sag, develop wrinkles, and develop open pores that are larger.
4. Utilise a face pack once every week. The dry face pack should not be peeled or scratched. This can damage the skin underneath.
5. Before removing the dry face pack, spritz the skin with room-temperature water. Roll an ice cube over your face after taking off the mask. This helps skin become tighter and seal pores that are open. The skin is also soothed and toned by it.
6. Avoid forceful face washing. Pimples and black patches could appear as a result of this. After applying a face pack, stay out of the heat.

Fig 3(S) – Traditionally available face packs with anti-acne activity

Fig 3(T) – General Ingredients of a Face pack

3.10 PHYSICOCHEMICAL EVALUATION

3.10.1 Total Phenolic compounds estimation using Folin-ciocalteu Assay

To estimate the total amount of phenolic contents in the face pack cum scrub using a standard plot. Fresh solution of Gallic acid (10mg/ml) was prepared by dissolving in methanol. Different concentrations of gallic acid were prepared (0,25,50,75,100 µl/ml). the face pack sample was also prepared(10mg/ml) by dissolving in distilled water. Other required components for the test like F-C reagent, 14% NaCO3 were also prepared for the analysis. 100µl of the test sample was added along with 500µl of distilled water and 100µl of F-C reagent were all added to a 12 well plate. Six minutes incubation was given to the plate. Later 1ml of 14% NaCO3 was added along with 500µl of distilled water, after proper mixing and thirty minutes of incubation at room temperature, the absorbance was measured at 760nm.

3.10.2 Total Flavonoids Content

To estimate the amount of flavonoids, present in the sample, this assay is done, 1% Quercetin solution (10mg/ml) was prepared. Different concentrations of gallic acid were prepared (0,25,50,75,100 µl/ml) in methanol to prepare a standard plot. All the necessary components for the test like 5% sodium nitrite, 10% aluminium chloride, 1M NaOH were also freshly prepared. 100µl of the test sample was added along with 500µl of distilled water and 100µl of 5% sodium nitrite into a 12 well plate. After six minutes of incubation time, 150µl of 10% aluminium chloride was added to the mix and incubated for five minutes. Later 200µl of 1M NaOH was added to each test sample. Absorbance was measured immediately at 510nm.

3.10.3 DPPH Assay for anti-oxidant activity

Fresh solution of 0.002% (2,2-diphenyl-1-picryl-hydrazyl-hydrate) was prepared by dissolving in methanol for standard plot preparation. Different concentrations of gallic acid were prepared (0,10,20,30,40 µl/ml). 3.6ml of DPPH was added to each test sample and a 30 minutes incubation was given in dark at room temperature. The absorbance was measured at 517nm. Percentage inhibition was then calculated using the given formula –

$$\% \text{ Inhibition} = \frac{A-B}{A} * 100$$

Here, A – absorbance of control (pure 2,2-diphenyl-1-picryl-hydrazyl-hydrate)
B – absorbance of sample taken after 30 minutes of incubation

3.10.4 Anti-microbial Susceptibility Test

Agar well diffusion method:

This method is employed to identify if or not the extracts or the formulation prepared has anti-microbial properties. MHB (muller Hilton broth media) was prepared by dissolving 2.3 gms in 100ml distilled water and adding 2gms of agar agar to the media, this was then autoclaved before use. Bacteria was grown and maintained in Muller Hilton Agar broth. It was then inoculated and the plates were allowed to dry and wells were punched by using sterile cork borers. 20µl of the sample (10mg/ml) was added to the wells of different concentrations. Positive control was taken to be ampicillin (2mg/ml) and the negative control as water. The plates were kept in an incubator at 37 degrees Celsius for 24hrs and zone of clearance was allowed to form. The zone of clearance was measured by measuring the diameter of the ring formed.

CHAPTER 4

RESULTS AND DISCUSSIONS

The extracts and powders required were prepared and stored in an aseptic airtight container with zero moisture environment. The formulation was then prepared and stored in a similar air tight container.

Fig 4(a) – Papaya extracts isolated from a fresh papaya

Fig 4(b) – Neem extracts isolated from the neem leaves

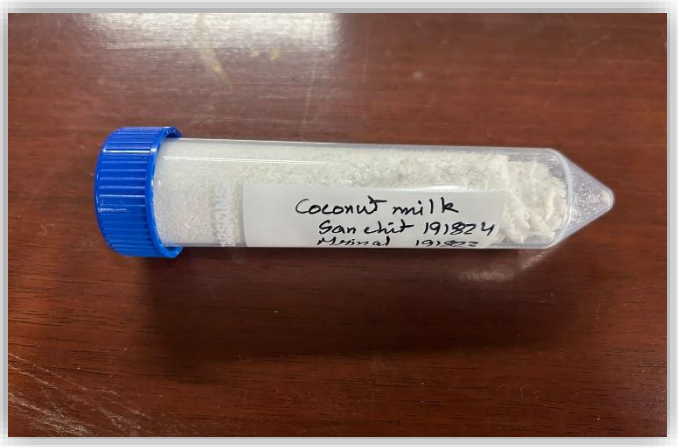


Fig 4(c) – Coconut milk powder

Fig 4(d) – Red Rice Extracts

Fig 4(e) – Papaya Seed Powder

The above extracts were mixed and a formulation was prepared according to **Table 4.1**

Component used	Weight (gms)	Percentage (%)
Papaya extracts	3	12
Red rice extracts	7	28
Papaya seed powder	2.5	10
Coconut milk powder	7	28
Aloe vera gel powder	5	20
Neem extracts	0.5	2
Face pack cum scrub	**25**	**100**

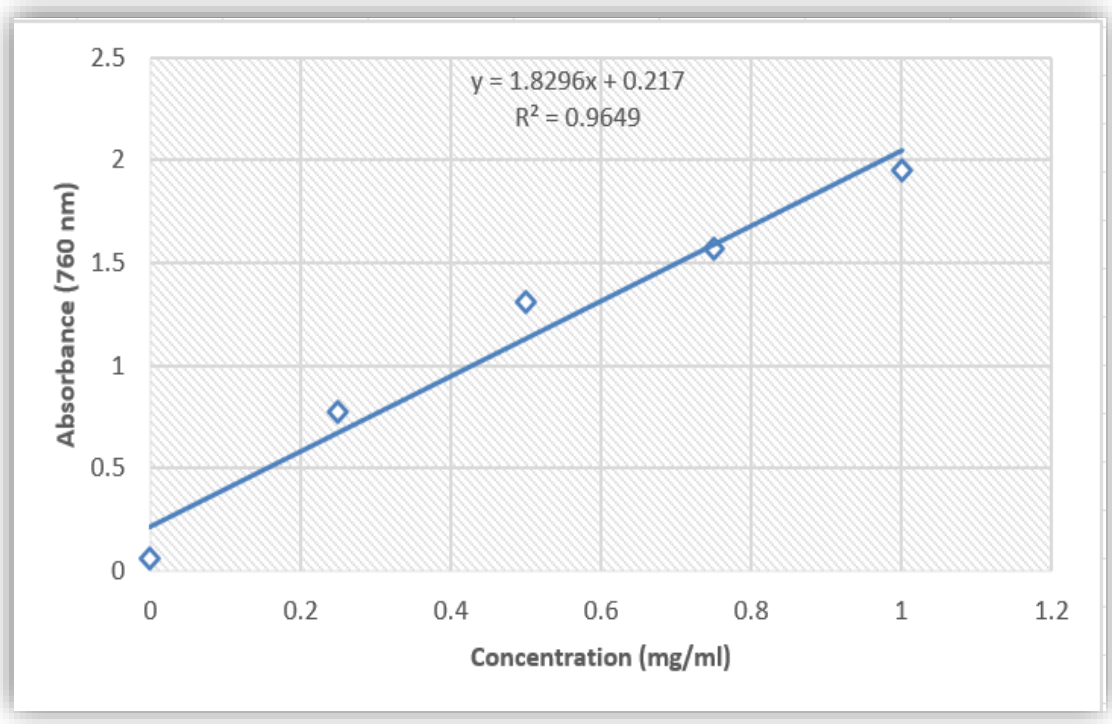
Table 4.1: Composition of the face pack

Fig 4(f) – Herbal face pack cum Scrub

4.1 RESULTS OF TOTAL PHENOLIC CONTENTS USING FOLIN-CIOCALTEU ASSAY

Concentration (mg/ml)	Absorbance (at 760nm)
0	0.06
0.25	0.772
0.5	1.311
0.75	1.566
1	1.95

Table 4.2: Presence of phenolic content in the face pack formulation



- The absorbance of the test sample i.e. the face pack sample came out to **be 0.547**
- The calculation of the total phenolic content in the sample was then calculated using the given equation –

$$y = 1.8296x + 0.217$$

Here, y = absorbance of face pack = 0.547, x = total phenolic content

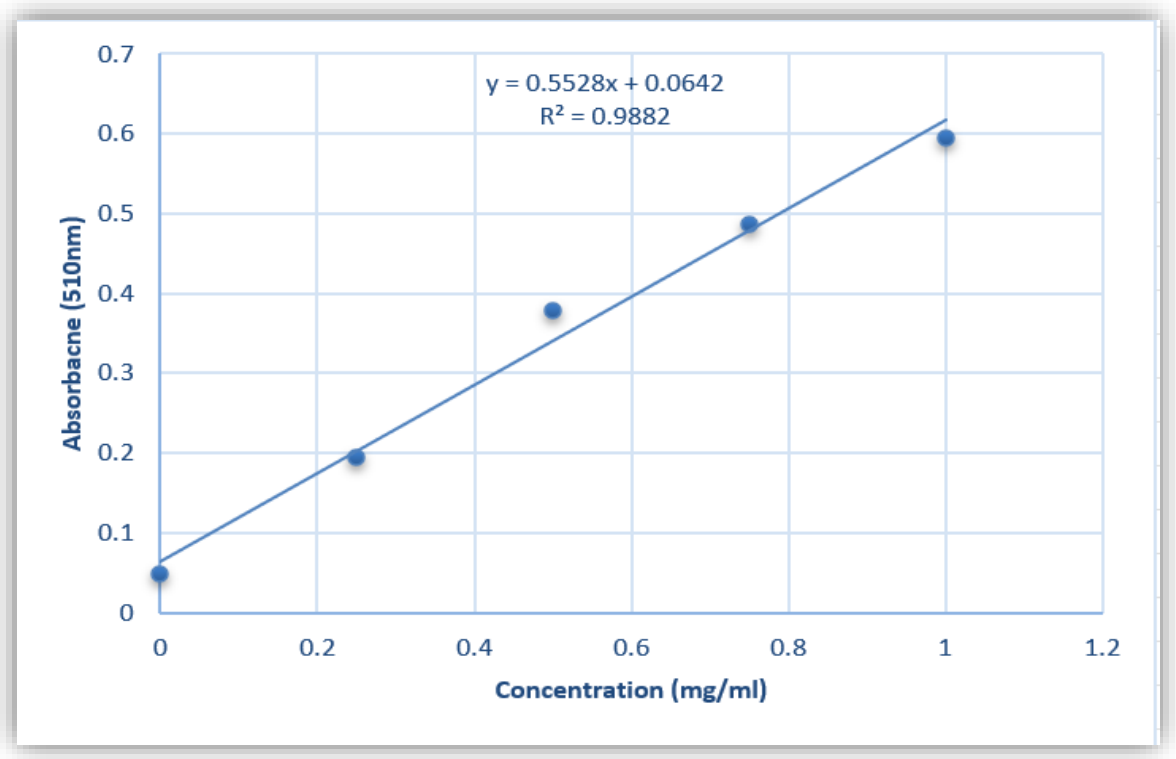
X = 0.180

Therefore, <u>180µg</u> GAE/mg of DFP was observed. (gallic acid equivalent of the dried face pack)

4.2 RESULTS OF TOTAL FLAVONOIDS CONTENTS

Concentration (mg/ml)	Absorbance (at 510nm)
0	0.049
0.25	0.195
0.5	0.378
0.75	0.487
1`	0.594

<u>Table 4.3:</u> Flavonoid content analysis



- The absorbance of the test sample i.e. the face pack came out to be **0.241**

- The calculation of the total phenolic content in the sample was then calculated using the given equation –

$$y = 0.5528x + 0.0642$$

Here, y = absorbance of face pack = 0.241, x = total phenolic content

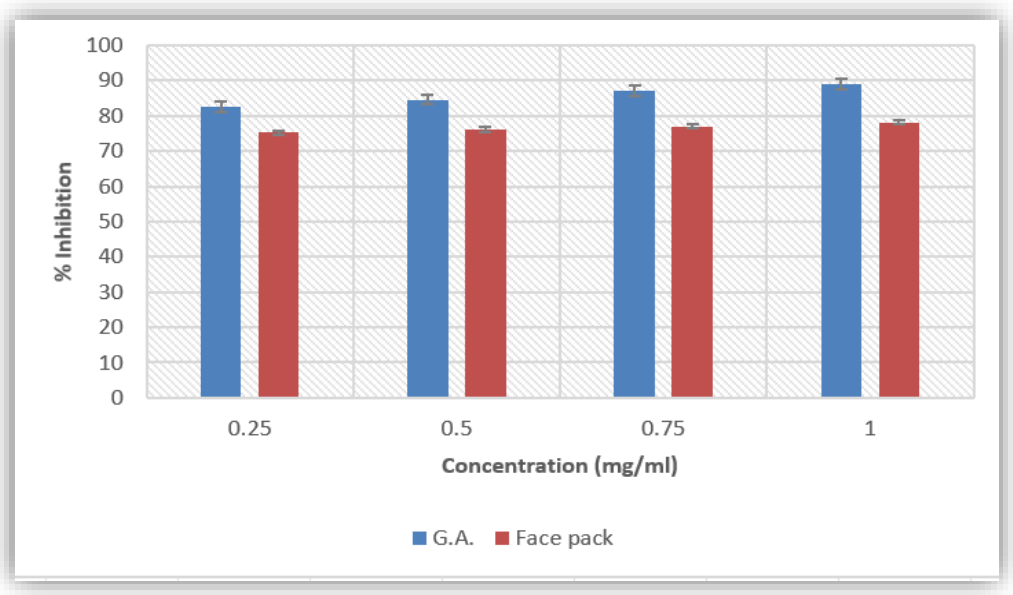
X = 0.319

Therefore, <u>319 µg</u> QE/mg of DFP was observed (quercetin equivalent of dried face pack)

4.3 RESULTS OF ANTI OXIDANT ACTIVITY USING DPPH ASSAY

Concentration (mg/ml)	% Inhibition	
	Gallic Acid	Face Pack
0		
0.25	82.52	75.28
0.5	84.59	76.09
0.75	87.12	77.01
1	89.07	78.16

Table 4.4: DPPH scavenging activity of face pack formulation



- Percentage inhibition was then calculated using the given formula –

$$\% \text{ Inhibition} = \frac{A-B}{A} * 100$$

A = absorbance of DPPH = 0.87, using this value, the % inhibition of all the concentrations were calculated and the desired table was obtained.

4.4 RESULTS OF ANTI-MICROBIAL SUSCEPTIBILITY TEST

Sample	*S. Typhii*	*E.Coli*
Positive control (Ampicillin)	22mm	20mm
Face pack	17.5mm	16.6mm
Negative control (H2O)	Not detected	Not detected

Table 4.5: Anti-microbial susceptibility test for *S.typhii* and *E.coli*

- The strain used for ***S. Typhii*** was – **MTCC98**
- The strain used for ***E. Coli*** was – **ATCC25922**

The inhibition rings formed can be observed through the following images –

Fig 4(g) – Inhibitory rings for *S. Typhii*

Fig 4(h) – Inhibitory rings for *E. Coli*

CHAPTER 5

CONCLUSION

The research objectives for this study were to identify prospective plants with various medicinal and remedial properties, the plants were carefully selected based on the availability and requirements, each component had a key role in the formulation. The formulation was completely herbal and free from any chemicals and preservatives. The herbal face pack cum scrub produced had many positive effects like anti acne activity, moisturising nature and also had a soothing effect with a pleasant fragrance. The key idea behind creating a face pack cum scrub with these components was to identify the use cases and the effects and benefits of each component separately as well as combined.

CHAPTER 6

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