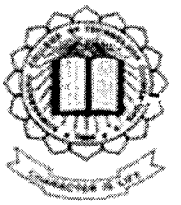

CREAM COMPOSITION FOR SKIN CARE USING CATECHIN AS THE PRIME ANTI MICROBIAL AGENT

A PROJECT REPORT

Submitted by

MANIKANDAN C. **71206214015**

In partial fulfillment for the award of the degree

Of

BACHELOR OF TECHNOLOGY

IN

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KUMARAGURU COLLEGE OF TECHNOLOGY, COIMBATORE

ANNA UNIVERSITY::CHENNAI 600 025

APRIL 2010

BIOTOR

Formerly known as Jayanth Oils, Mumbai

2nd April, 2010

TO WHOM SO EVER IT MAY CONCERN

This is to certify that the project report **"CREAM COMPOSITION FOR SKIN CARE USING CATECHIN AS THE PRIME ANTI MICROBIAL AGENT"** is the bonafide work of **MANIKANDAN C.,** who carried out this project work under my supervision.

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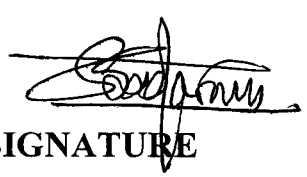

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The Report of the project work submitted by the above students in partial fulfillment for the award of Bachelor of Technology degree in Biotechnology of Anna University was evaluated and confirmed to be their original work. It was submitted for evaluation and viva – voce held on 20- 04-2010.

(INTERNAL EXAMINER)

(EXTERNAL EXAMINER)

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MANIKANDAN C.

Abstract

Plant tannins are considered as recalcitrants to microorganisms. Tannins bind with proteins and polysaccharides and inhibit microbial growth. Catechin, a tannin compound, is a polyphenol antioxidant obtained from green tea *Camellia sinensis* and *Coffee arabica.* Catechin was isolated from its crude extract and the yield was 18%. The isolated catechin was dried over phosphorus pentoxide. Alkylation of catechin gave protocatechuic acid. Confirmation of catechin was done on TLC and the spots were developed in the iodine chamber. The R_f value of the sample and the authentic standards were at a single spot at 0.27. This protocatechuic acid was used as the prime antimicrobial agent in the cream formulation. Cream was composed of blended oil phase consisting of castor and papaya seed oil mixed with lye consisting of 1% egg yolk as emulsifier in W/O emulsion at 67°C as optimized blending temperature and the other parameters are optimized. Protocatechuic acid and other additives were added to the cream mixture in various proportions and the cream is tested for its acid value, ester value, pH, saponification value, anti microbial activity, total fatty substance content. Based on these analyses, the suitability of this formulation as a skin care cream was determined.

Keywords: Recalcitrant, antioxidant, chemo attractants, TLC, R_f Value W/O emulsion, saponification value.

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Abbrevations

°C	- Degree Centigrade
DMDN	- Dimethylol Dimethyl
FDA	- Food and Drug Adminstartion
g	- gram
HPLC	- High Performance Liquid Chromatography
Kg	- Kilogram
KOH	- Potassium Hydroxide
Max.	- Maximum
ml	- Milliliter
mol/ l	- Mole per liter
NaOH	- Sodium Hydroxide
R_f	- Rentention Factor
SAP	- Saponification Number
TLC	- Thin Layer Chromatograpghy
US Pat.	- United States Patent
UV	- Ultraviolet
W/O	- Water in Oil emulsion
µ	- Micron
%	- Percentage

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1. INTRODUCTION

1. Introduction

Human Skin is a soft outer covering of a human being. Skin care thus plays an essential role in protecting the human skin. Man's urge to research on various subjects is a psychological propensity. Liquid soap is being said to have its origin in the 2nd century. Soap-like material found in clay cylinders during the excavation of ancient Babylon is evidence that soapmaking was known as early as 2800 B.C. Many creams for skin care have been formulated using natural and synthetic materials for protecting the human skin.

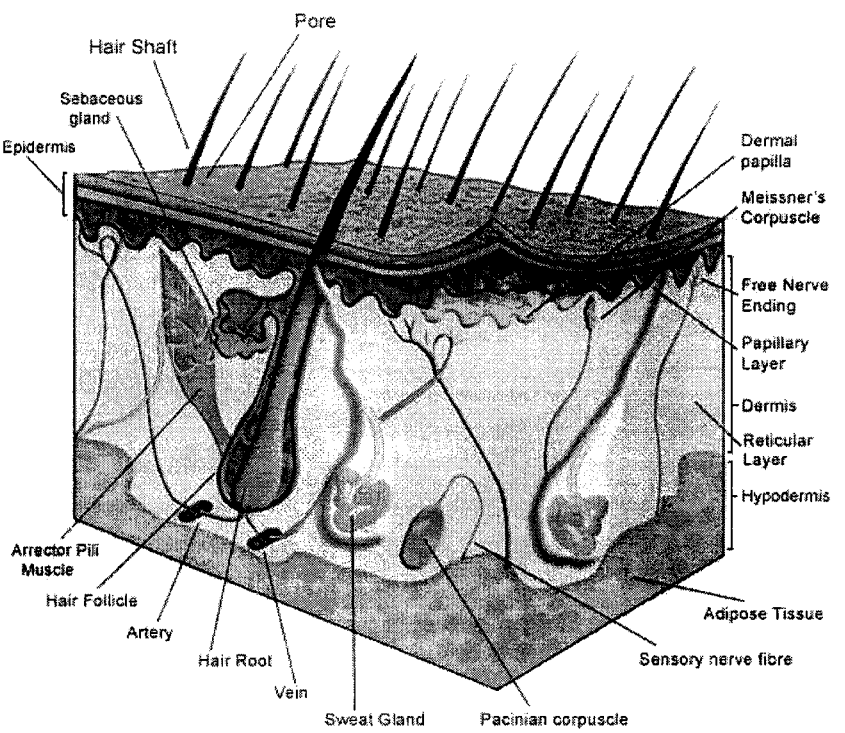


Fig. 1.1 Anatomy of human skin

1.1 History of cream/ soap making

Early soapmakers probably used ashes and animal fats. Simple wood or plant ashes containing potassium carbonate were dispersed in water, and fat was added to the solution.

This mixture was then boiled; ashes were added again and again as the water evaporated. During this process a slow chemical splitting of the neutral fat took place; the fatty acids could then react with the alkali carbonates of the plant ash to form soap (this reaction is called saponification). Skin cream evolved from soap and it gained importance only in the later stage of modern civilization.

In modern times, the use of soap/ cream has become universal in industrialized nations due to a better understanding of the role of hygiene in reducing the population size of pathogenic microorganisms. Synthetic cream first became available in the late nineteenth century, and advertising campaigns in Europe and the United States helped to increase popular awareness of the relationship between cleanliness and health. By the 1950s, soap had gained public acceptance as an instrument of personal hygiene.

1.2 Materials used for Cream/ Soap

- Synthetic Compounds
- Natural compounds

1.2.1 Synthetic Compounds for Cream

Various chemicals have been used for skin care cream production. Some of these chemicals have adverse effects on the human skin and body when used in proportions higher than the desired quantities. Some of the well known chemicals used in cosmetic industry are hydrogenated lecithin, glycerin, formaldehyde, ethylene glycol, disodium EDTA, coal tar, etc.

1.2.1.1 Advantages in Market Use

Synthetic compounds have varied advantages when compared to the natural raw materials used in cosmetic industry as,

- They are cheaper in production

- They can be easily isolated from their crude form
- Their medicinal usage in better known
- They can be preserved and used over a longer period of time

1.2.1.2 Harmful effects

In order to reduce the cost factor and to bring in desirable qualities in a skin care cream, synthetic compounds have been put into use in the near present. But the harmful effects of certain synthetic compounds lead to cancer or nervous system defects. Some of these ingredients include synthetic fragrances, FD& C color pigments, sodium lauryl sulfate, triclosan, butylenes glycol, DMDN hydanthion. People in recent times have realized the need of natural & herbal creams in order to protect the skin from the harmful effects of synthetic creams. Many formulations on natural cream for skin care have been identified and patented. This process has been in constant experimentation in order to get natural skin care creams suitable for specific skin types and of finer quality at affordable prices.

1.2.2 Natural Compounds for Cream

Natural compounds, in recent times, are used extensively in the cosmetic industry. These compounds are used for their minimal side effects. People feel assured as these raw materials are plant products and hence there is less damage to the skin. Some of the prominent natural compounds are mineral oil, collagen, fruit peels, aloe vera, etc.

1.2.2.1Advantages using Natural Compounds

Natural compounds are,

- Safer with minimal side effects
- Suitable for mostly all types of skin
- Do not possess carcinogens

1.2.2.2 Disadvantages in Market Use

Though the natural compounds are known to be better than the synthetic compounds, they are,

- Costlier owing to their isolation procedures
- Difficult to preserve over longer durations of time

1.3 Base Composition of a cream

A skin care cream is composed of an oil phase blended with an aqueous medium in a W/O emulsion containing a small percentage of emulsifier to add to the stability of the matrix. To this essential skin care actives and other additives like perfume, colorants which adds up to the external appearance of the cream are added in optimal quantities to serve the purpose of an effective skin care cream with minimal side effects. A skin care cream formulation should contain an oil phase, lye and an emulsifier as the primary base formation. Blending temperature for a skin care cream varies with respect to the oil phase and the emulsifier used. Higher the quantity of emulsifier, higher the rate of blending at lower temperatures. Blending temperature varies from 55°C to 72°C based on the proportions of oil, lye and emulsifier.

1.3.1 Additives to be added

- Anti microbial agent
- Antioxidant
- Emulsifier
- Emollient
- Skin actives

- Preservative
- Perfume
- Colorant &
- Others

Natural cream crafters today have many different ingredients to select from to produce wonderful and varied skin cream. These ingredients consist of:

- base oils available in today's market such as coconut oil, jojoba oil, avocado oil, castor oil, cottonseed oil, olive oil, palm oil, palm kernel oil, peanut oil and soybean oil
- various butters like shea butter, mango butter, and cocoa butter for extra moisturizing capabilities
- other nutrients such as sweet almond oil, avocado oil, aloe vera, calendula oil, carrot root oil, various clays, and seaweed
- essential oils including peppermint, eucalyptus, spearmint, chamomile, geranium, rosemary, lavender, etc for scenting and therapeutic effects
- and various herbs and spices for color

1.3.2 Advantages of natural / herbal cream

Natural soap/ cream are preferred owing largely to their following advantages:

- They are suitable for all skin types including sensitive skin.
- They do not contain chemical additives like commercial soap bars which may include animal fats, alcohols, esters (known carcinogenics), low grade oils, wax and fillers.
- One of the many benefits of using a natural soap bar is that since they do not contain animal fats they cut down on the soap scum.
- Natural cream doesn't strip the skin of its natural oils which means, the skin is left feeling softer and smoother compared to dry and itchy when using synthetic cream.

1.4 Importance of an Anti Microbial Agent

An anti microbial agent is either microbicidal or microbiastic. Anti microbial agents are classified as antibiotics, antifungals, antivirals and antiparasitics. A wide range of chemical and natural compounds are used as antimicrobials. Organic acids are used widely as antimicrobials in food products, e.g. lactic acid, citric acid, acetic acid, and their salts, either as ingredients, or as disinfectants. For example, beef carcasses often are sprayed with acids, and then rinsed or steamed, to reduce the prevalence of E. coli O157:H7. Many essential oils are included in pharmacopoeias as having antimicrobial activity. Flavanoids are greatly known for their antioxidant and recalcitrant properties.

1.4.1 Catechin

An antimicrobial agent plays a key role in the making of a skincare cream. A wide range of chemical and natural compounds are used as antimicrobials. Organic acids are used widely as antimicrobials in food products and, essential oils in cosmetic industry. Plant tannins are largely known for their recalcitrant properties. Catechin is a polyphenolic antioxidant plant secondary metabolite. The term catechin is also commonly used to refer to the related family of flavonoids and the subgroup flavan-3-ols (flavanols).

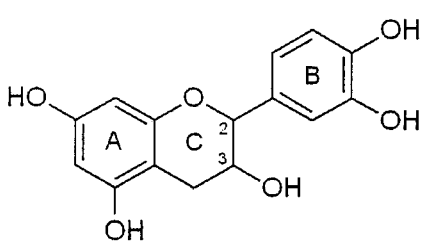


Fig. 1.4.1.1 Structure of Catechin

1.4.1.1 Structure of Catechin

Catechin possesses two benzene rings (A- and B-rings) and a pyran ring (called the C-ring). It has twochiral centers on carbons 2 and 3. It has therefore four diastereoisomers. Two of the isomers are in trans configuration and are called catechin and the other two are in cis configuration and are called epicatechin.

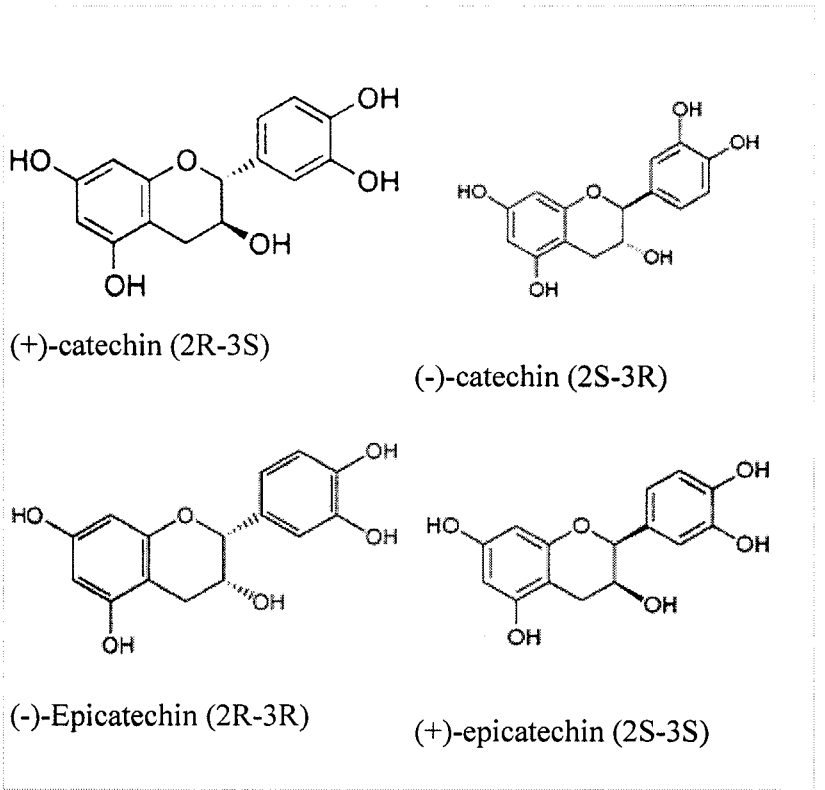


Fig. 1.4.1.1.1 Diastereoisomers of Catechin

Catechin is found in green tea and to a smaller proportion in cacao and coffee beans. It has very good phytotoxic and antimicrobial properties. Catechin acts as a carbon source for *Bradyrhizobium japonicum* ATCC 10324 and it cleaves catechin using catechin oxygenase to give protocatechuic acid. Protocatechuic acid is used as the prime antimicrobial agent in the following formulation apart from essential oils in suitable proportions.

1.4.2 Other Anti Microbial agents in Market Today

- Essential oils
- Chlorine
- Hypochlorites
- Halogenated hydantoins
- Peroxy compounds,
- Phenol compounds
- QAC/Quats & Polyquats
- Organic acids, salts, etc.

1.4.3 Advantages of Catechin over others

- It is a natural product
- It has good antioxidant properties
- It is highly used for anti aging and anti wrinkle cream formulations.
- It can be easily isolated
- It provides skin elasticity through astringent and soothing actions
- It prevents skin aging by eliminating harmful oxygen which is the biggest enemy to aging skin.
- It contains 5-8 times more Vitamin C than lemons and tocopherol preventing the collection of melanin pigments and inhibits the formation of freckles and dark spots.
- It contains acne treatment ingredients vitamin A and vitamin B2. One of the best acne treatment ingredients.
- It contains tannins contracting pores and flavonoid with strong detergency.

2. REVIEW OF LITERATURE

2. Review of Literature

2.1 Human Skin

Skin represents the major epithelium of man and provides a barrier to the external environment. Given that the skin is permanently threatened by various potential pathogenic micro-organisms, it is very plausible that the epidermis would express chemical substances to prevent microorganism penetration, in addition to the physical barrier of the lipid-rich stratum corneum. Human skin is permanently exposed to a wide variety of potential harmful microorganisms. Despite these microbial threats, skin is surprisingly highly resistant against infections (Harder et al., 2005).

2.1.1Factors affecting human skin

Earth's atmosphere is known to team with airborne microorganisms, though the high light intensities, extreme temperature variations, low concentrations of organic matter, and a scarcity of water, make the atmosphere as unsuitable environment habitat for microbial growth (Atlas, 1984). Biological material may contribute about 20%, 22% and 10% to the total airborne particulate by volume in remote continental, populated continental and remote maritime environments respectively (Matthais et al., 2000). Most of them originate from natural sources such as soil, lakes, animals and humans (Lindemann et al., 1985). Moreover, agricultural practices, healthcare units and industrial operations such as sewage treatment, animal rendering, fermentation processes, and food processing plants also emit viable microorganisms into the air (Cullinan et al., 2001). The main function of the human skin is to protect the tissue beneath it against the environment (Feingold, 1985).The skin forms an effective barrier between the organism and the environment preventing invasion of pathogens and fending off chemical and physical assaults, as well as the unregulated loss of water and solutes (Proksch et al., 2008). The human skin is populated by a large variety of microorganisms that mainly live as commensals in a relatively stable composition on the surface of the skin (Roth and James, 1988).

2.1.2 External & Internal factors

Skin is subjected to insults by many extrinsic and intrinsic factors. Extrinsic factors include ultraviolet radiation, environmental pollution, wind, heat, low humidity, harsh surfactants, abrasives, and the like. Intrinsic factors include chronological aging and other chemical changes from within the skin. Whether intrinsic or extrinsic, these factors result in visible signs of skin aging and environmental damage, such as wrinkling and other forms of damage. To many people, skin wrinkles are a reminder of the disappearance of youth. As a result, the elimination of wrinkles has become a booming business in youth-conscious societies. Treatments range from cosmetic creams and moisturizers to various forms of cosmetic surgery (Robinson et al., 2000).

2.2 Cosmetics

Cosmetics are defined as "articles with mild action on the human body, which are intended to be applied to the human body through rubbing, sprinkling or other methods, aiming to clean, beautify and increase the attractiveness, alter the appearance or to keep the skin or hair in good condition (The Pharmaceutical Affairs Law: Article 2)." The U.S. Food and Drug Administration (FDA) which regulates cosmetics in the United States defines cosmetics as: "intended to be applied to the human body for cleansing, beautifying, promoting attractiveness, or altering the appearance without affecting the body's structure or functions" (Reed, 2004). The worldwide annual expenditures for cosmetics today are estimated at U.S. $19 billion (National Geographic, 2007). Malhotra (2003) describes the main reasons for boom in cosmetic industry as increasing fashion and beauty consciousness coupled with rising incomes and focus on health and fitness. To complement this, beauty culture or cosmetology has emerged as a major occupational avenue with significant commercial potential. New scientific developments, techniques, products and media hype, has contributed the Indian fashion industry in generating mega revenues and this has in turn added to the growth of cosmetic industry. Rising hygiene and beauty consciousness due to changing demographics and lifestyles, deeper consumer pockets, rising media exposure, greater product choice, growth in retail segment and wider availability are the reasons reported by (Euromonitor International, 2006).

2.2.1 Cosmetics for Skin Care

A skin care cream should possess an oil phase, an aqueous phase, thickening agents, emulsifiers and other additives like skin care actives, whitening agents, flavonoids, sugar amines, sunscreen agents, perfumes, colorants and particulate materials (Mingua Chen et al., 2007). The International Cosmetic Ingredient Dictionary and Handbook (CTFA, Ninth Ed., 2002) describes a wide variety of nonlimiting cosmetic and skin care ingredients commonly used in the skin care industry.

2.3 Base of a cream

A cream is usually an emulsion that exhibits a certain degree of body or apparent viscosity sufficient to form a heavy fluid or a soft easily deformed gel. An emulsion is a heterogeneous system consisting of at least one immiscible liquid intimately dispersed in another (external or continuous) phase in the form of droplets (internal or dispersed) phase whose diameters in general, exceed 0.1μ. The British Pharmaceutical Codex (1973) further stipulates that the term creams should be restricted to preparations for external use. In total, there are 236 different types of cream based on the differences in oil phase,

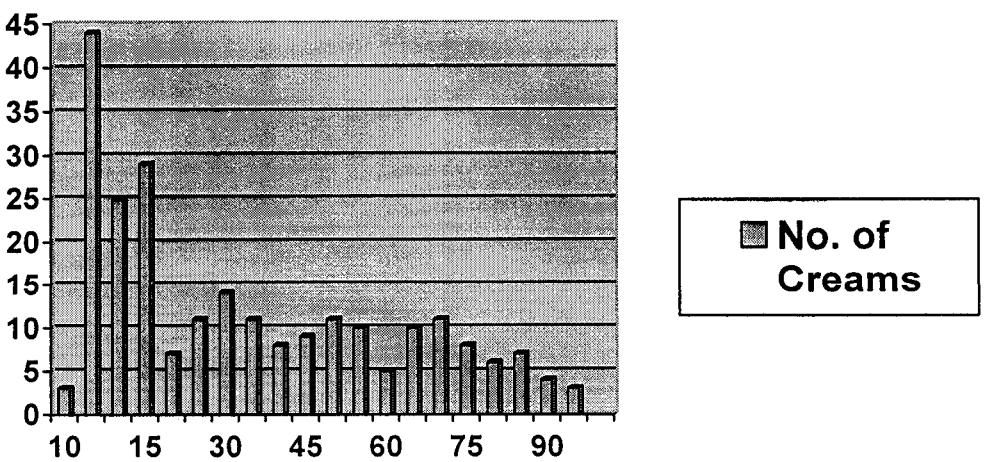


Fig. 2.3.1: Classification of the 236 types of cream based on Varied Oil Phase composition

2.3.1 Natural Compounds for skin care

Castor oil and its derivatives are found in many cosmetics as it is "non-comedogenic" (International Castor Oil Association, 2007). Flavonoids are largely known for their antimicrobial activity as mentioned in the International Journal of Antimicrobial agents (2005). Catechin is a flavonoid containing a ketone group, two benzene rings and a pyran ring (Balentine et al., 1997). Structurally tea catechins are, primarily, flavonols and form 20–30% of the dry weight of green tea (Balentine et al., 1997; Sanderson, 1972).Skin care creams should possess active agents with good antimicrobial properties. A body cream should be a W/O emulsion with high inhibitory percentage against epidermal microorganisms (Kapoor, 2005).

2.3.2 Cream base as W/O emulsion

U.S. Pat. No. 5,538,737 discloses a method of making a W/O emulsion containing a pharmaceutically acceptable salt/ peptide. The steps include dissolving the pharmaceutically acceptable salt/ peptide in an aqueous medium to form a water portion; combining the water portion with an oil portion, comprising an edible oil comprising an ester or mixed ester of glycerol and an emulsifying agent to form a water portion and oil portion matrix; then emulsifying the matrix to form the water-in-oil emulsion.

2.3.3 Ingredients added

A skin care cream comprises of thickening agents, high internal phase emollient in water emulsion, thickening agents, primary emulsifier, non- emulsifying silicone elastomer, active agent, anti- acne actives, anti wrinkle actives, anti oxidants/ radical scavengers, emollient, self tanning actives, skin lightening/ whitening agents, sun screen agent, humectant & solute, colorant, preservatives, fillers, coated silicone elastomers and other additives (Fares et al., 2008).

2.3.4 Anti microbial agent for a skin cream

Catechin serves the purpose of an antimicrobial agent as it is a polyphenol antioxidant which serves as chemo attractants for Rhizobia and Bradyrhizobia (Parke et al., 1985). Catechin also inhibits bacterial DNA gyrase by binding to its ATP binding site (Gradisar et al., 2007). It has been known that catechin, a unit of condensed tannin was degraded by *Bradyrhizobium japonicum* ATCC 10324 through catechin oxygenase giving protocatechuic acid (Waheeta et al., 1984). Protocatechuic acid is a potent natural antimicrobial agent against enterobacteria and epidermal microorganisms (Ana et al., 2006).

2.4 Polyphenols for skin care

The epicatechin derivatives of green tea, which are commonly called "polyphenols", are the active ingredients in green tea that possess antioxidant, anti-inflammatory and anti-carcinogenic properties. Many studies conducted on human and animal skin have demonstrated that topical green tea polyphenols prevent ultraviolet-B induced immune suppression and skin cancer induction (Katiyar et al., 2001). In these studies, the most potent chemopreventive constituent appears to be (-)-epigallocatechin-3-gallate (EGCg) (Komatsu et al., 1997, Lu et al., 2002). Prior to, or immediately after exposure to UVB, EGCg protects against sun induced local skin damage. Therefore, green tea extract cream is photoprotective, and can be used as a topical agent for the prevention of solar UV-B induced skin damage, which can eventually lead to skin cancers. However, for a topical cream based on tea extracts to be an effective cancer fighter, the tea catechins must be active and intact. This may be the most important factor, since a number of studies have shown that catechins break down over time and in the presence of water (Dvarakova et al, 1999, Chen et al., 2001, Proniul et al., 2002). Only topical creams based on formulations that preserve the effectiveness of the catechins are likely to be effective. The natural antioxidant properties of catechins in green tea extracts can improve the marketing potential of cereals, cakes and biscuits as well as traditional health food products and dietary supplements. Green tea extracts have been reported to be potent antioxidants in pork (Shahidi *et al.*, 1992), chicken meat (Tang *et al.*, 2001, 2002), vegetable oil (Chen

and Chan, 1996), fish oil (Wanasundara and Shahidi, 1996) and fish flesh (Lin and Lin, 2000), food emulsions (Huang and Frankel, 1997) and animal fat (Wang and Zhao, 1997). They can also be employed to give dairy products, instant noodles (Yang *et al.*, 1995), confectionery, ice-cream (Jiang *et al.*, 1995). The anti-bacterial and deodorizing effect of catechins slows tooth decay (anti-caries) and improves breath freshness, so providing natural added value for toothpastes, mouthwashes, chewing gums (Miki *et al.*, 1991; Yoshinori *et al.*, 1987) and breath-fresheners (Yasuda, 1992), with the potential for other body, skin and hair product applications. For example, many shampoos, moisturising creams, perfumes and sunscreens contain tea extracts as they are believed to have a soothing effect on the skin as well as acting as antioxidants to protect the skin from free radicals (Alexis *et al.*, 1999; Masao *et al.*, 1994). In recent years the analysis of polyphenolic compounds in raw and processed food became more important with regard to their numerous physiological properties (Arts et al., 2000). Due to their various effects, the flavan-3-ols group is of major interest. In plants they can act as chemical signals to attract or deter insects and provide a defense against pathogens and environmental stress (Truetter, 2006). Regarding their health benefits in humans, they show positive cardiovascular, anticancer or antiviral effects (Friedman, 2006).

2.4.1 Isolation of Catechin and protocatechuic acid

The conventional method (Ali et al., 1993) for the isolation of (+)-catechin from catechu is labourious, costly and low yielding (14.2 - 17.2%). Protocatechuic acid is isolated using improved technique of acetone extraction process (Karl et al., 1933) and this crystalline compound obtained is used as a prime antimicrobial ingredient for the cream formulation. The compound is confirmed at UV 276 nm (Harnley et al., 2006). Apart from this, a colorant, perfume, emollient, emulsifier and a preservative are used as additives (Hooper et al., 2006). The cream base consists of the oil phase and the aqueous phase blended in suitable proportions with 1% emulsifier (Chen et al., 2007).

2.4.2 Activity of Catechin

The tanning property of catechin in human skin may be supposed to be the active ingredient for the treatment of leucoderma (shiti) (Meyer, 1960). Catechin has antihormone activity. Further its activity has also been correlated with those of vitamin P (Higby, 1943).

2.5 Analytical methods

Thin layer chromatographic (Stahl, 1969) behavior of the isolated (+)-catechin was in good agreement with the authentic (+)-catechin. The compound shows absorption bands at 220 and 277 nm in the ultraviolet (Clark et al., 1990). Anthocyanins gives an absorption band at 275-280 nm regions. This ultraviolet behavior indicates the possibility of the isolated compound belonging to the flavonoid group The molecular mass of (+)-catechin is 290. In the spectra, the maxima m/z ratio is showed at 290. The IR spectra of (+)-catechin has a broad band around 3400-2600 cm-1 region corresponding to aliphatic and aromatic C-H, phenolic and alcoholic.

2.6 Product Formulation and market

A large number of research papers (Bagajewicz, 2007; Street, Woody, Ardila, & Bagajewicz, 2008; Wibowo & Ng, 2002; among others), reviewarticles (Gani, 2004; Hill, 2004;Wintermantel, 1999), textbooks (Cussler & Moggridge, 2001) were devoted to the understanding of product development, and formulation of the relevant techniques. The wide variety of activities in product developmentwas summarized in a table by Ulrich and Eppinger (2004), a version of which is presented in Fig.2.6.1. These activities span three phases in time – product conceptualization, detail design and prototyping, and product manufacturing and launch – and can be classified by job function in terms of management, sales and marketing, research and design, manufacturing, and finance and economics.

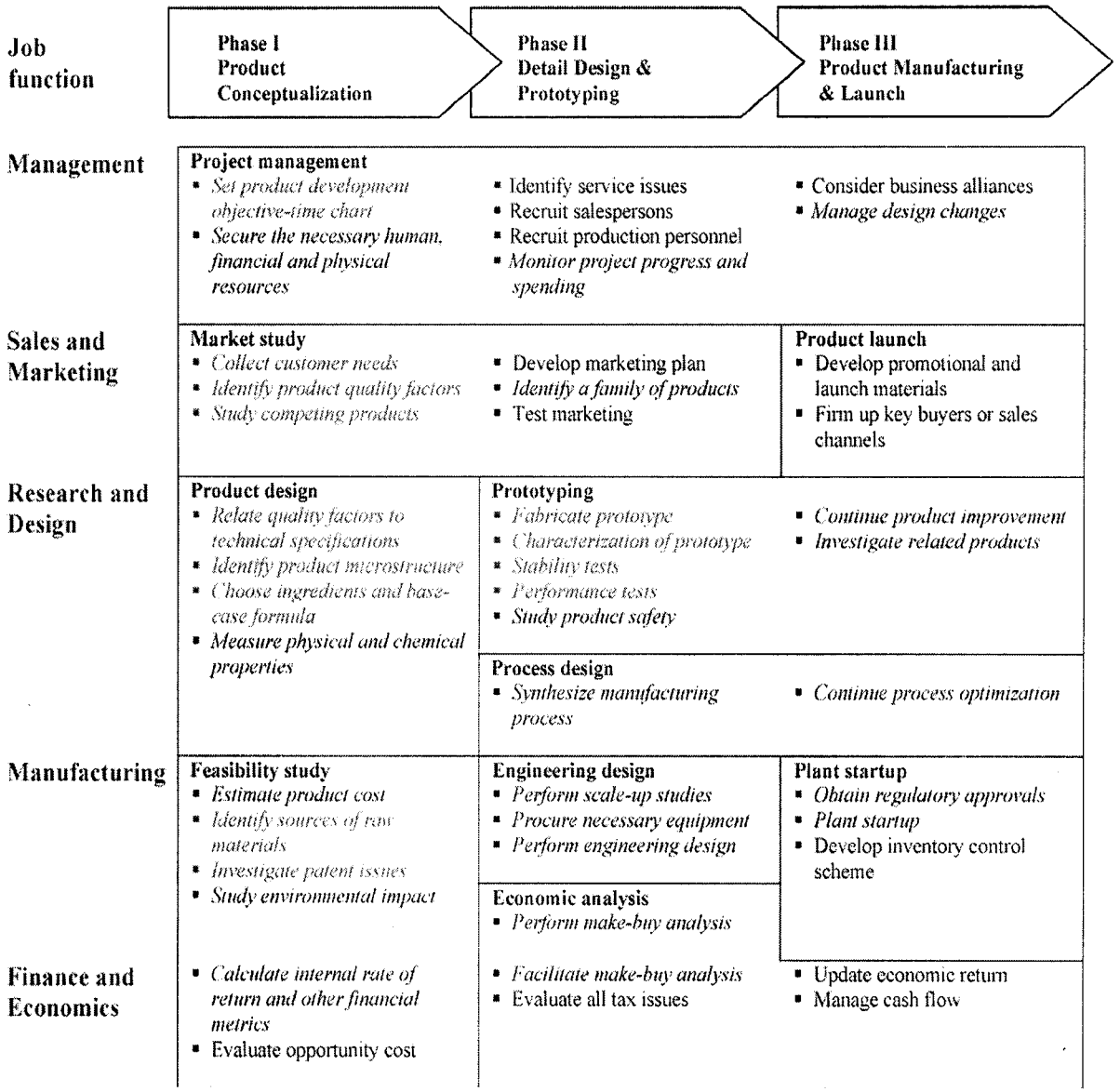


Fig. 2.6.1: Product formulation and marketing

3. Materials & Methods

3. Materials & Methods

3.1 Isolation of Crude extract of Catechin

- One kg of the dried beans (powdered) of *Coffee Arabica* (Coffee)/ *dried leaves of Camellia sinensis* (Green tea) was taken in an aluminium pot to which ten litres of water were added so that the chips were completely immersed under water.
- It was boiled over an open fire for 4 hours and allowed to stand for 4, 8, 12, 16, 20, 24, 28, 32 hours so that more catechin might diffuse into the water.
- The extract was decanted off in a pot and was filtered through a fine muslin cloth to remove suspended materials.
- The filtrate was evaporated and the residue obtained was air dried and weighed.
- Yield was calculated.

3.1.1 Isolation of Catechin

- Isolated catechin (150g) was taken in a five-liter stainless steel beaker containing one liter distilled water.
- It was boiled with constant stirring for complete dissolution and filtered through a filter paper.
- It was then evaporated to 500 ml and allowed to stand for 24 hours.
- The obtained precipitate was filtered using a filter paper.
- The aqueous filtrate was rejected and the residue was dissolve in ethanol and filtered.
- The ethanol solution was evaporated to dryness and the residue was dissolved into hot water (500 ml). It was allowed to stand for 24 hours.
- The precipitate was filtered and dried in air.
- The process of re-crystallization from water was repeated thrice.
- On drying over phosphorus pentoxide, the melting point of isolated catechin raised.
- Mixed melting point with an authentic sample did not show lowering.

3.1.2 Confirmatory test on TLC

- The isolated catechin and authentic samples (Catechin HPLC 99% pure from Sigma Aldrich) were applied on a silica gel 60G plate using alcohol and developed with toluene, ethyl acetate, formic acid (10:8:1).
- Spots were developed in an iodine chamber.
- Both the compounds showed single spot (*Rf*=0.27).

3.2 Preparation of Cream Base

3.2.1 Oil Phase Preparation

- 100 ml of castor oil and 15 ml of papaya seed oil were taken in a 250 ml beaker.
- 3 ml of egg yolk was added to the mixture.
- The resulting mixture was heated to temperatures ranging from 45°- 70°C in an electric oven.
- The time taken for homogenization at the respective temperatures was noted in table.
- The temperature optimization chart denoted the optimized temperature.

3.2.2 Lye/ Water phase Preparation

- 176 g of lye dissolved in 500 ml of distilled water was taken in a 1000 ml beaker.
- The solution heated up and the temperature was raised from 60°- 70° C in an electric oven.
- The time taken for homogenization at the respective temperatures was noted in table.
- The temperature optimization chart denoted the optimized temperature.

3.2.3 Cream base Preparation

- Once the lye/ water phase and the oil phase reached their required temperatures, the oil phase was poured in a 1000 ml beaker using a spatula.

- To this solution of oil phase, the lye/ water phase was added completely and the temperature was set at 67°C (Optimized temperature) for 1 hour.
- To this, the isolated catechin and other additives were added in quantities as shown in table.
- The temperature was then brought down to 45°C and kept untouched at that temperature for a mould time of 48 hours.
- After 48 hours, 10 ml of vegetable glycerin and 2 ml of grape seed extract was added for preservation over longer periods of time.

3.3 Analysis

3.3.1 Determination of acid value, ester value & saponification value

- In a 150 ml flat-bottomed flask with a ground-glass neck, the sample containing 1.5-2.5 g saponifiable matter was accurately weighed.
- To this, 25 ml of ethanol was added and swirled to dissolve. Mass of the sample is determined as W_1.
- The sample was heated if necessary but cooled before titration
- To the sample, 1 ml of phenolphthalein indicator was added and titrated with 0.5 mol/ l alcoholic potassium hydroxide solution. The value of V_1 was obtained.
- 50 ml of 0.5 mol/1 potassium hydroxide solution was added to the burette.
- A reflux condenser was attached to the flask and petroleum jelly was used for slightly lubricating the glass joint.
- This mixture was boiled under reflux for 1 hour and cooled.
- This solution was titrated with 0.5 mol/1 alcoholic hydrochloric acid solution. The value of V_2 was obtained.
- A blank experiment was carried out by titrating without the sample to get the value of V3.

V_1- volume of 0.5 mol/1 alcoholic potassium hydroxide solution

V_2- volume of the same solution (After titration)

V_3- volume of 0.5 mol/l alcoholic hydrochloric acid solution

W_1- Mass of sample

C_1- Exact conc. of alcoholic hydrochloric acid solution

C_2- Exact conc. of alcoholic potassium hydroxide solution

$C_2 = V_3C_1 / 50$;

$$\left\{ \begin{array}{l} \text{Acid Value} = 56.1\ V_1C_2\ I\ W_1\ mg\ \text{KOH/g} \\ \text{Ester value} - 56.1\ (V_3 - V_2)C_1\ I\ W_1\ \text{mg KOH/g} \end{array} \right\}$$

3.3.2 Determination of unsaponifiable matter

- The procedure mentioned in 'Determination of acid value, ester value and saponification value' was followed (steps 1, 3 and 4). Mass of the sample, W_1 was noted.
- Quantitatively, the solution was transferred to a 250 ml separating funnel, using 75 ml water. The flask was rinsed with 50 ml petroleum ether.
- The petroleum ether was poured into the separating funnel and the stopper was inserted.
- The mixture was shaken thoroughly occasionally releasing the pressure by inverting the separating funnel and cautiously opening the stopcock. The layers were allowed to separate.
- The lower aqueous layer was run into another separating funnel.
- 50 ml of petroleum ether was added and the extraction process was repeated. The layers were allowed to separate. This time, the aqueous layer was discarded.
- The three petroleum ether extracts were washed with 50 ml water. If an emulsion formed, few ml of ethanol were added.
- The petroleum extracts were in turn transferred to a tarred 250 ml beaker and evaporated to dryness on a steam bath. The separating funnels were rinsed with two

25 ml portions of petroleum ether. This was added to the beaker and evaporated to dryness.

- A few ml ethanol was added to the beaker and evaporated to dryness.
- The beaker was dried in an oven at 1000C for 20min, cooled to room temperature and weighed (W_2 g).

$$\% \text{ Unsaponifiable matter} = 100\ W_2 / W_1$$

3.3.3 Determination of acids and bases

- In a conical flask, a sample containing 1-3mmol of the acid(s) or base(s) to be determined accurately weighed. To this, 50 ml ethanol is added and swirled to dissolve (heated if necessary). Then cooled. Mass of sample is determined as *W g.*
- A few drops of phenolphthalein indicator is added and titrated with 0.1 mol/l alcoholic sodium hydroxide solution to the first permanent pale pink color to measure acids. Volume of alkali is determined as V_1 ml.
- A few drops of bromophenol blue indicator is added and titrated with 0.1 mol/l alcoholic hydrochloric acid solution to a clear green colour to measure bases. Volume of acid required is determined as V_2 ml.

$$\% \text{ Free acid, or acid combined with a weak base} = V_1 * Ma * C_1 * 100 / 1000\ Na * W$$

Where, V_1 is the volume of 0.1 mol/1 sodium hydroxide solution (step 2);

C_1 is the exact concentration of this solution in mol/1;

Ma is the molecular weight of the acid titrated;

N is the number of titratable hydrogen ions per molecule;

and *W* is the mass of sample (step 1).

Where, V_2 is the volume of 0.1 mol/1 hydrochloric acid solution (step 3);

$$\% \text{ Free acid, or acid combined with a weak base} = V_1 * Ma * C_1 * 100 / 1000\ Na * W$$

C_2 is the exact concentration of this solution in mol/1;

M_b is the molecular weight of the acid titrated;

N_b is the number of hydrogen ions absorbed per molecule of base;

W is the mass of sample (step 1).

3.3.4 Determination of neutral fatty matter and total fatty acids

- In a 250 ml beaker, a sample containing 0.4-0.6 g of either neutral fatty matter or total fatty acid, whichever is less, was accurately weighed. Mass of sample was determined as W_1 g.
- 25 ml of ethanol was added and stirred until the sample was completely dissolved, heated gently.
- Quantitatively, the sample was transferred to a 500 ml separating funnel, using several portions of ethanol.
- A volume of water equal to the total volume of ethanol was added. To this, 10 ml 1 mol/1 sodium hydroxide was added.
- 100 ml petroleum ether was added into the separating funnel and the stopper was inserted.
- The mixture was shaken thoroughly occasionally releasing the pressure by inverting the separating funnel and cautiously opening the stopcock. The layers were allowed to separate.
- The layers were allowed to separate. The lower aqueous layer was run into another separating funnel.
- Steps 5 and 6 were repeated twice more. The aqueous layer was kept .
- The three petroleum ether extracts were washed with 50 ml water. If an emulsion formed, few ml of ethanol were added.
- The petroleum extracts were in turn transferred to a tarred 250 ml beaker and evaporated to dryness on a steam bath. The separating funnels were rinsed with two 25 ml portions of petroleum ether. This was added to the beaker and evaporated to dryness. Weight of residue was determined as W_2 g.
- The aqueous layer was acidified by adding 20 ml 1 mol/1 hydrochloric acid solution.

- Steps 5 and 6 were repeated three more times.
- Step 8 was repeated.
- The petroleum ether extracts contained the total fatty acid. They were evaporated to dryness. Weight of residue was determined as W_3 g.
- Some neutral fatty matter remained unextracted. To the aqueous layer, an equal volume of water and extract, with three successive 100 ml portions of chloroform, were added.
- Each chloroform extract was washed in a second separating funnel with 50 ml water, then ran it into a tarred 250 ml beaker and evaporated to dryness. When all the chloroform was evaporated, it was dried to constant weight. Weight of residue was determined as W_4 g.

$$\begin{bmatrix} \% \text{ Neutral fatty matter} = 100\ (W_2 - W_4)/W_1 \\ \% \text{ Total fatty acid} = 100\ W_3/W_1 \end{bmatrix}$$

Where, W2 is the mass of dried residue;
W3 is the mass of dried residue;
W4 is the mass of dried residue;
W1 is the mass of sample.

3.3.5 Determination of pH

The samples were kept on the pH meter and the values were determined.

3.3.6 Determination of Anti microbial activity

- The sample was applied as a patch on one side of the face and left untouched for 2 hours.
- After two hours, an examination was made on the microorganism distribution on either side of the face.

- Each side of the subject's face was scraped with two cotton balls respectively, and each of the cotton balls was put in sterile distilled water contained in a beaker.
- It was vigorously stirred for 3 minutes.
- 100 µl of each solution was smeared over tryptic soy agar medium that was then incubated at 37°C for 24 hours.
- The number of colonies formed was measured using digital colony counter (Intech I- 37 Model).

4. Results & Discussion

4. Results & Discussion

This work focuses on the use of catechin as an anti microbial agent in a skin care cream. Catechin was preferred for its potent antioxidant properties which soothes the skin and acts as an anti aging skin active. Catechin, being a polyphenol antioxidant, is also a flavonoid showing good anti microbial properties. The ingredients used for the cream base were natural compounds and the additives added to the base were isolated from natural compounds in view of preventing the use of synthetic chemicals which have high side effects on the skin. A skin care cream of a specific composition using natural components from coffee bean and green tea leaves were used and analysed for its acid value, ester value, SAP number verification, pH,

4.1 Yield of catechin

Table 4.1.1: Yield of Crude Catechin from *Coffee arabica* (Coffee Beans)

S. No.	Allowed to stand for (Hours)	Yield of catechin (grams)	Yield percentage (%)
1.	4	81	8.1 %
2.	8	99	9.9 %
3.	12	104	10.4 %
4.	16	123	12.3 %
5.	20	147	14.7%
6.	24	187	18.7%
7.	28	189	18.9%
8.	32	189	18.9%

From table 4. 1. 1, the percentage yield of crude catechin was determined. The yield of crude catechin was max. after 28 hours at 18.9 % of the solution. Yield obtained was 189 g.

Table 4. 1. 2: Yield of Crude Catechin from *Camelia sinensis* (Green tea)

S. No.	Allowed to stand for	Yield of catechin	Yield percentage
1.	4	111	11.1 %
2.	8	126	12.6 %
3.	12	145	14.5 %
4.	16	169	15.9 %
5.	20	188	16.8 %
6.	24	201	20.1 %
7.	28	202	20.2 %
8.	32	202	20.2 %

From table 4. 1. 2, the yield of catechin from *Camellia sinensis* was determined. The yield of crude catechin was max. after 24 hours at 20.2 % of the solution. Yield obtained was 202 g.

The following graph shows the comparative yield of catechin from the two sources- coffee & green tea. From the graph, it was evident that isolation of catechin from *Camellia sinensis* provided better yield. It was 2 % higher than the yield obtained from *Coffee arabica.*

Graph (fig. 4.1.1) representing the yield values of catechin from *Coffee arabica* & *Camellia sinensis*

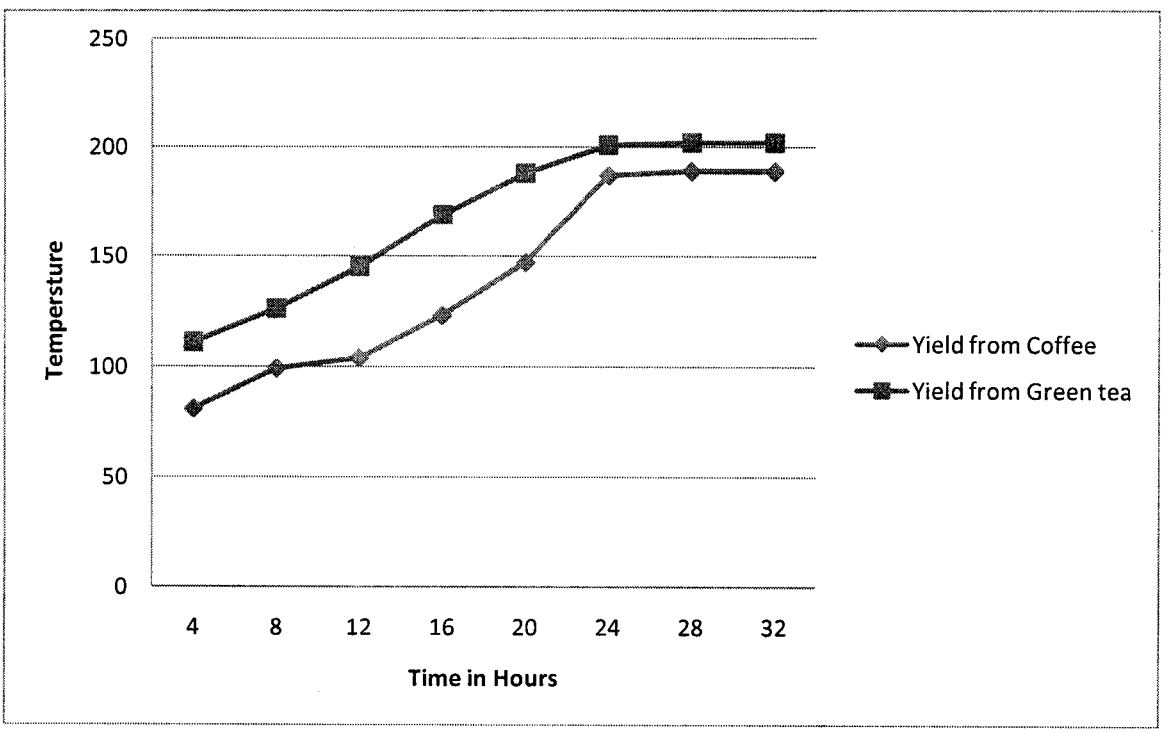


Fig. 4.1.1: Comparative Yield of Catechin

Isolation of pure catechin:

Pure catechin was extracted using ethanol and phosphorus pentoxide and the yield obtained was 37.5 g.

4.2 Confirmation of presence of catechin

4.2.1 Determination of melting point

Table 4.2.1.1: Melting Point determination using Opti Melt 3.0 (Melting Point Apparatus)

Sample taken	Isolated sample	Authentic sample (C1251 from Aldrich)	Mixed sample (1:1 ratio)
Clear point	175.6°C	174.7°C	174.9°C
Melting point	174.2°C	173.6°C	174°C

Fig. 4.2.1.1 denotes the graphical analysis and fig. 4.2.1.2 denotes the m.p determined.

Graph indicating the melting and clear points of the respective samples,

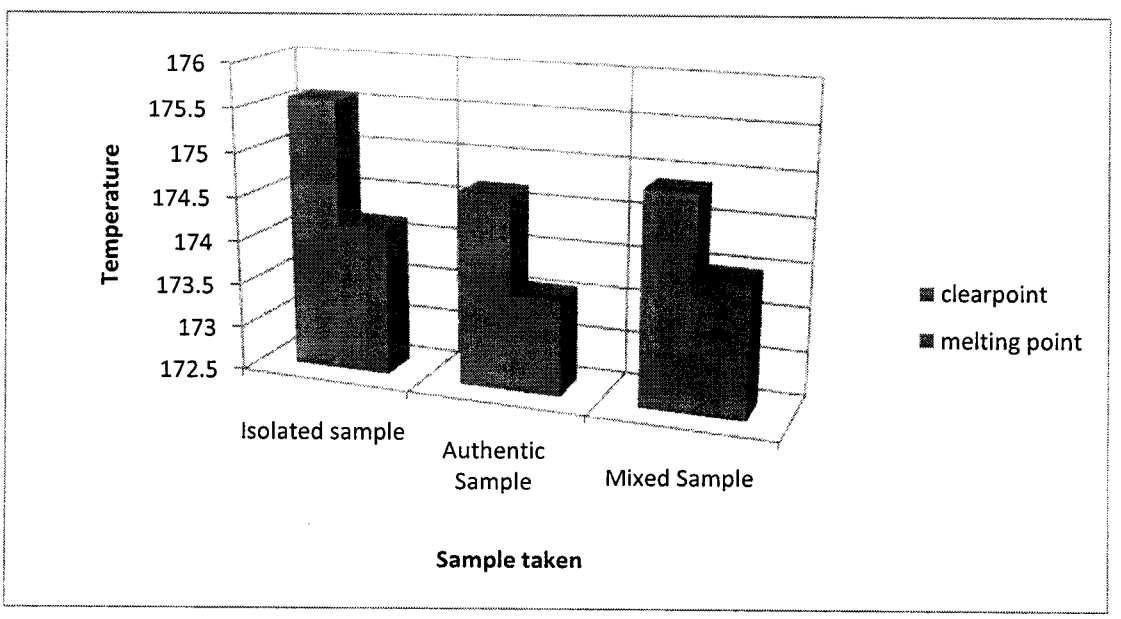


Fig. 4.2.1.1: Melting point & Clear point of Samples

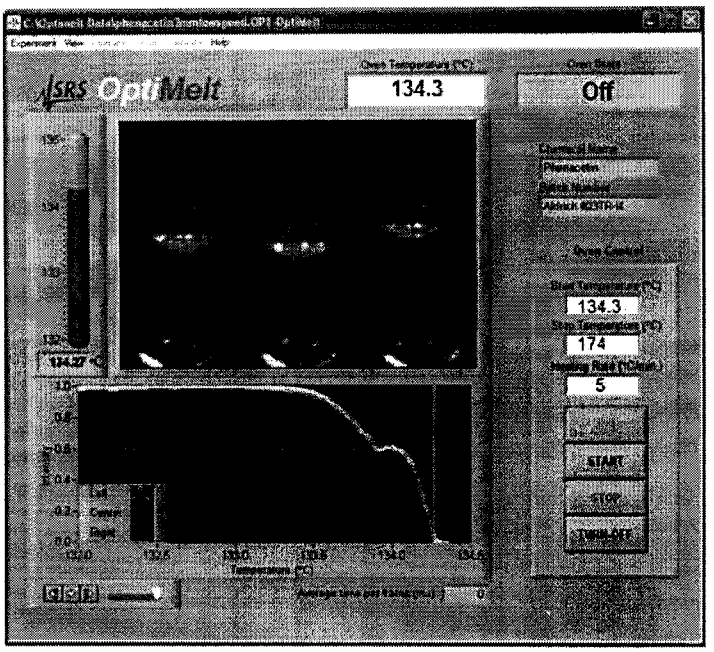


Fig. 4.2.1.2: Melting point of Catechin

4.2.2 Confirmatory test for catechin isolation

Thin Layer Chromatography was performed using the isolated sample and authentic sample and the results confirmed the isolated sample as catechin as both the samples (authentic & isolated) gave a single spot at R_f value of 0.27 (Fig. 4.2.1.1)

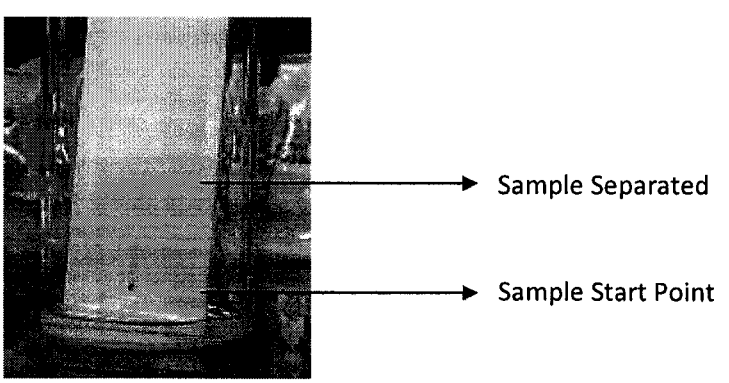


Fig. 4.2.2.1 Thin Layer Chromatographical analysis

4.3 Preparation of the cream

4.3.1 Preparation of Oil Phase

Table 4.3.1.1: Oil Phase preparation

Sample	Castor oil (ml)	Papaya Seed Oil (ml)	Emulsifier (ml)	Homogenization Temperature (°C)
1	100	5	3	61°C
2	100	10	3	63°C
3	100	15	3	67°C
4	100	20	3	67°C
5	100	25	3	71°C

Homogenization temperature was visualized and determined as shown in table 4.3.1.1.

The extent of homogenization was checked under microscopic examination using Nigrosine dye as shown in fig.

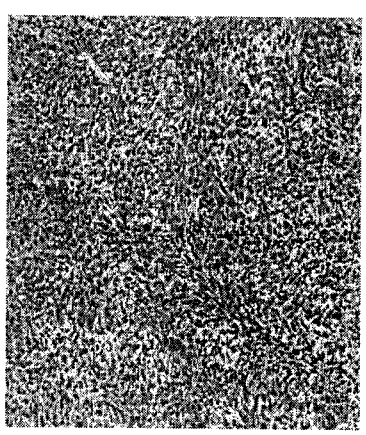


Fig. 4.3.1.1: Digital image of Extent of homogenization under a compound microscope

On microscopic examination, only the sample 3 gave globules of similar size. This was clearly visible in the digital image shown in fig 4.3.1.1.Hence owing to uniformity in globule size, sample 3 (100 ml of castor oil, 15 ml of papaya seed oil and 3 ml emulsifier) was selected for further processing.

4.3.2 Preparation of Lye/Water Phase

Table 4.3.2.1: Lye/Water Phase preparation

Sample	Lye (grams)	Distilled water (ml)	Homogenization Temperature (°C)
1	125	500	62°C
2	150	500	63.4°C
3	175	500	67°C
4	200	500	69.8°C
5	225	500	-
6	250	500	-
7	275	500	-

Based on table 4.3.2.1, sample 3 was taken for further processing and preparation of soap base as its homogenization temperature would be similar to the homogenization temperature of the sample taken for oil phase. This would result in proper blending.

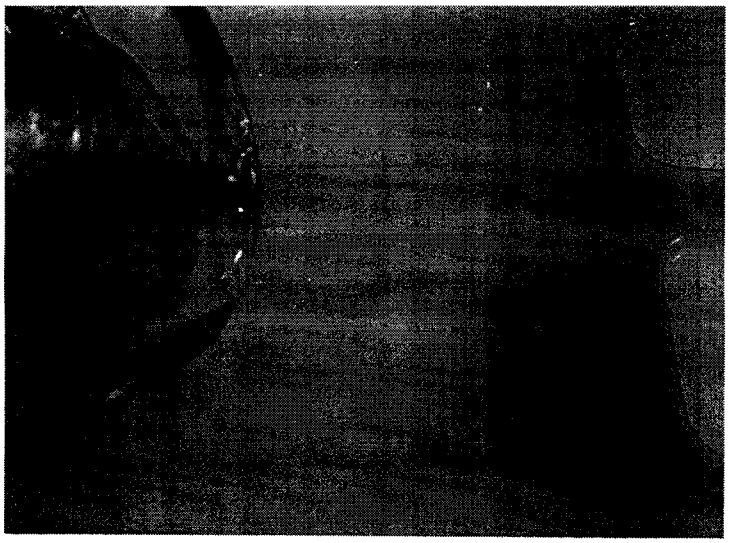


Fig. 4.3.2.1: Preparation of Lye phase

4.3.3 Preparation of cream base

Sample 3 from the tables, were taken for the preparation of cream base. The Lye/ Water Phase was added to the Oil Phase (W/O emulsion) as shown in fig. 4.3.2.1 and heated to 67°C for 1-5 hours and then kept at 45°C untouched for 48 hours (mould time).

Table 4.3.3.1: Homogeneity of emulsion

W/O emulsion	Heated at 67°C for (hours)	Mould time of 48 Hours Globule size on microscopic examination	Uniformity
Sample 1	1		Uniform
Sample 2	2		Uniform
Sample 3	3		Non Uniform
Sample 4	4		Non Uniform
Sample 5	5		Non Uniform

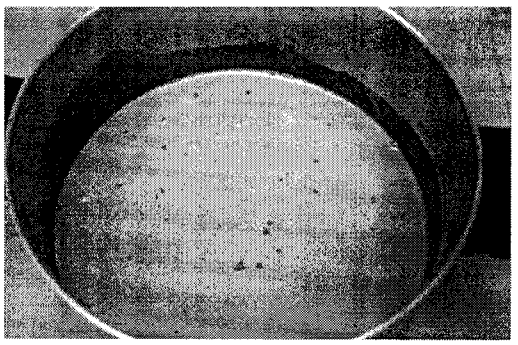
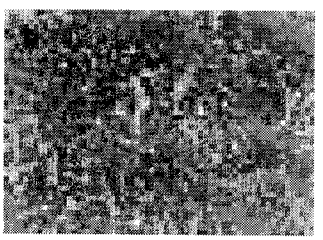
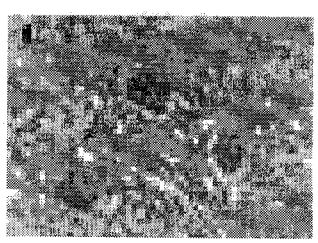


Fig. 4.3.3.1: Preparation of Cream Base

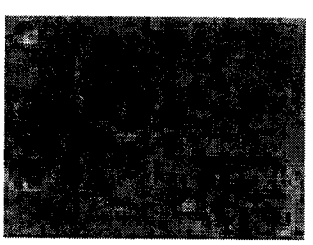
Sample 1



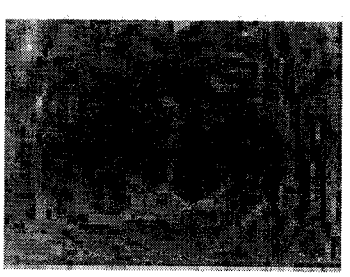
Sample 2



Sample 3



Sample 4



Sample 5

Fig. 4.3.3.2: Homogeneity of emulsion viewed under a compound microscope

The samples denote the fact that globules were of uniform size in samples 1 & 2 whereas the other samples denoted globules of varies shape and size.

4.4 Additives added

- Catechin
- Preservative
- Colorant
- Perfume

Table 4.4.1: Range of Additives to be added

Additive/100 ml	Catechin (grams)	Preservative (ml)	Colorant (ml)	Perfume (ml)
Range	1- 10	0.5- 1.0	0.5- 3	0.2- 1

Table 4.4.1 showed the range value of the additives to be added to the base cream in order to test further for analysis. Preservative and colorant were kept at a constant minimum level of 0.5 ml per 100 ml. Quantity of perfume to be added was crucial as it could damage the skin when used in higher quantities.

4.5 Analysis

Various samples were taken with varied proportions of catechin, preservative, colorant and perfume. The samples are mentioned in table 4.5.1 in terms of sample codes.

For varied values of catechin,

Table 4.5.1: Amount of catechin and perfume used in samples

S. No.	Catechin (grams)	Sample Code	Preservative (ml)	Colorant (ml)	Perfume (ml)
1	For 1 g,	S1	0.5	0.5	0.2
2		S2			0.4
3		S3			0.6
4		S4			0.8
5		S5			1.0
6	For 2 g,	S6	0.5	0.5	0.2
7		S7			0.4
8		S8			0.6
9		S9			0.8
10		S10			1.0
11	For 3 g,	S11	0.5	0.5	0.2
12		S12			0.4
13		S13			0.6
14		S14			0.8
15		S15			1.0
16	For 4 g,	S16	0.5	0.5	0.2
17		S17			0.4
18		S18			0.6
19		S19			0.8
20		S20			1.0
21	For 5 g,	S21	0.5	0.5	0.2
22		S22			0.4
23		S23			0.6
24		S24			0.8
25		S25			1.0

Table continued,

S. No.	Catechin (grams)	Sample Code	Preservative (ml)	Colorant (ml)	Perfume (ml)
26	6	S26	0.5	0.5	0.2
27		S27			0.4
28		S28			0.6
29		S29			0.8
30		S30			1.0
31	7	S31	0.5	0.5	0.2
32		S32			0.4
33		S33			0.6
34		S34			0.8
35		S35			1.0
36	8	S36	0.5	0.5	0.2
37		S37			0.4
38		S38			0.6
39		S39			0.8
40		S40			1.0
41	9	S41	0.5	0.5	0.2
42		S42			0.4
43		S43			0.6
44		S44			0.8
45		S45			1.0
46	10	S46	0.5	0.5	0.2
47		S47			0.4
48		S48			0.6
49		S49			0.8
50		S50			1.0

4.5.1 Determination of acid value, ester value, saponification value

Table 4.5.1.1: Determination of acid value, ester value, saponification value

S. No.	Sample Code	Acid Value KOH/g	Ester Value KOH/g	Saponification Number
1	S1	.098	.139	193
2	S2	.137	.194	196
3	S3	.137	.193	189
4	S4	.176	.247	167
5	S5	.135	.191	175
6	S6	.097	.1361	174
7	S7	.135	.190	169
8	S8	.135	.190	188
9	S9	.132	.186	189
10	S10	.133	.188	176
11	S11	.178	.251	178
12	S12	.143	.202	176
13	S13	.140	.196	163
14	S14	.067	.095	155
15	S15	.067	.095	142
16	S16	.134	.188	175
17	S17	.134	.189	170
18	S18	.176	.247	166
19	S19	.134	.189	159
20	S20	.038	.053	150
21	S21	.132	.187	181
22	S22	.127	.179	184
23	S23	.138	.194	177
24	S24	.136	.192	170
25	S25	.136	.192	166

26	S26	.127	.179	181
27	S27	.181	.256	173
28	S28	.178	.252	170
29	S29	.132	.1848	166
30	S30	.130	.182	168
31	S31	.135	.190	187
32	S32	.137	.192	175
33	S33	.135	.191	174
34	S34	.133	.188	170
35	S35	.133	.188	168
36	S36	.135	.190	185
37	S37	.135	.190	189
38	S38	.237	.334	193
39	S39	.134	.187	188
40	S40	.136	.192	199
41	S41	.133	.1875	169
42	S42	.137	.193	166
43	S43	.135	.191	169
44	S44	.136	.192	158
45	S45	.068	.099	151
46	S46	.068	.096	174
47	S47	.134	.188	170
48	S48	.135	.191	159
49	S49	.141	.199	162
50	S50	.133	.139	160

From the table, the acid value, ester value and saponification value were determined for the 50 samples of the cream. Few of them had high SAP number and hence not suitable for being used as cream. SAP number between 176- 187 (for castor oil) were the expected values. Deviations from this range were rechecked and were neglected after confirmation.

4.5.2 Determination of Unsaponifiable matter

S-sample, V- Unsaponifiable matter

Table 4.5.2.1: Determination of Unsaponifiable matter

S. No.	% Quantity of Unsaponifiable matter									
	S	V	S	V	S	V	S	V	S	V
1	S1	6.1	S11	1.85	S21	3.8	S31	4.3	S41	5.2
2	S2	6.3	S12	2.9	S22	4.4	S32	5.9	S42	5.9
3	S3	7.2	S13	5.7	S23	4.5	S33	6.0	S43	6.1
4	S4	7.8	S14	7.2	S24	6.1	S34	6.4	S44	6.6
5	S5	6.9	S15	6.3	S25	6.6	S35	7.2	S45	6.9
6	S6	9.0	S16	8.9	S26	4.5	S36	3.9	S46	5.7
7	S7	7.8	S17	9.0	S27	6.2	S37	5.7	S47	5.4
8	S8	6.9	S18	9.4	S28	6.75	S38	5.4	S48	5.9
9	S9	6.0	S19	9.7	S29	7.1	S39	5.9	S49	6.8
10	S10	4.20	S20	9.9	S30	7.7	S40	6.2	S50	7.2

The following table 4.5.2.1 denoted the quantity of unsaponifiable matter. Samples S10, S11, S12, S21, S22, S23, S26, S31 and S36 showed lower quantity of unsaponifiable matter. Hence, they were apt for being used for a cream as more unsaponifiable matter meant high non uniformity of emulsion globules.

4.5.3 Determination of acids and bases

Table 4.5.3.1: Determination of acids and bases, free and combined with weak bases and acids

S- Sample, V- % Free acid, or acid combined with a weak base/ % Free base, or base combined with a weak acid

S. No.	% Free acid, or acid combined with a weak base/ % Free base, or base combined with a weak acid									
	S	V	S	V	S	V	S	V	S	V
1	S1	19.4	S11	11.2	S21	8.9	S31	27.6	S41	34.6
2	S2	19.6	S12	14.8	S22	5.2	S32	28.1	S42	33.9
3	S3	22.1	S13	18.1	S23	15.1	S33	28.3	S43	33.6
4	S4	24.3	S14	17.9	S24	17.9	S34	28.7	S44	33.9
5	S5	24.0	S15	15.6	S25	19.1	S35	29.1	S45	34.2
6	S6	21.1	S16	22.0	S26	10.6	S36	31.0	S46	31.4
7	S7	20.8	S17	25.4	S27	15.1	S37	28.9	S47	34.5
8	S8	21.0	S18	27.6	S28	20.8	S38	33.0	S48	35.2
9	S9	23.4	S19	29.2	S29	19.4	S39	33.6	S49	37.2
10	S10	22.9	S20	29.0	S30	21.0	S40	34.1	S50	38.0

Table 4.5.3.1 denoted the extent of % free acids and bases present in the samples. It was measured by titration with acid and a base in order to determine the stability of the cream base. Low percentage of acids and bases were determined from many samples indicating the fact that the W/O emulsion had neutralized.

These samples S11, S12, S21, S22 and S26 with good SAP value, low amount of unsaponifiable matter and high neutralization were determined and taken for further analysis.

4.5.4 Determination of neutral fatty matter & total fatty acids

Table 4.5.4.1: Determination of neutral fatty matter & total fatty acids

S. No.	Sample	% Neutral fatty matter	% Total fatty acid
1	S11	84.0	62.0
2	S12	88.2	63.2
3	S21	92.6	67.1
4	S22	94.8	66.0
5	S26	83.9	58.2

Table 4.5.4.1 denoted amount of neutral fatty matter present in the selected samples. Of these, samples S12, S21 and S22 contained high percentage of neutral fatty matter showing high emulsion stability.

4.5.5 Determination of pH

Table 4.5.5.1: Determination of pH

S. No.	Sample	pH
1	S11	5.87
2	S12	5.43
3	S21	6.7
4	S22	6.54
5	S26	5.2

Skin care cream should possess pH between 6-8 for high efficiency as a stabilized cream. In table 4.5.5.1, Samples S21 and S22 showed pH within this range. pH range of 6-8 is

non irritant to skin and hence amongst the 50 samples examined so far, S21 &S22 showed positives signs of a stable cream for skin care.

4.5.6 Determination of Anti microbial activity

Table 4.5.6.1 : Microorganism count on application of cream S21

S. No.	Applied and kept untouched for (Hours)	Micobial count	
		Control	Test sample
1.	2	$2.8 * 10^8$	$9.8 * 10^6$
2.	3	$2.9 * 10^8$	$8.1 * 10^6$
3.	4	$3.3 * 10^8$	$2.1 * 10^6$
4.	5	$5.4 * 10^8$	$7.0 * 10^5$
5.	6	$6.6 * 10^8$	$8.9 * 10^4$

Table 4.5.6.2 : Microorganism count on application of cream S22

S. No.	Applied and kept untouched for (Hours)	Micobial count	
		Control/mm	Test sample/mm
1.	2	$2.0 * 10^8$	$1.1 * 10^7$
2.	3	$2.6 * 10^8$	$8.8 * 10^6$
3.	4	$3.0 * 10^8$	$2.1 * 10^6$
4.	5	$4.5 * 10^8$	$9.7 * 10^5$
5.	6	$7.1 * 10^8$	$8.9 * 10^4$

Table 4.5.6.1 & Table 4.5.6.2 showed the effectiveness of the cream by considerably reducing the microorganism count there by proving the fact that it has a good anti microbial property.

5. Conclusion

5. Conclusion

A skin care cream made using natural sources is known for its various beneficiary effects. Here, the formulation on samples S21 & S22 gave the expected results. The results were in accordance with the previous literature reviewed. Their Acid value, Ester value, SAP number, Neutral fatty matter, total fatty acids content, pH, free acid & base value were determined to be under the required standards of usage for a skin cream. S21 & S22 also showed positive results for Anti Microbial Activity and hence, it would prove to be a effective skin care cream against microorganisms. This was possibly owing to the use of catechin as an anti microbial agent in the current formulations.

The cream base as well showed high neutralization suggesting the fact that the samples were at a pH of 6-8 which is quite suitable for all types of skin sensitivity. The cream showed positive results for being a good emulsion and high stability as an emulsion suggested the fact that it could be preserved over a period of time. The cream had good homogeneity as the globules were uniform in size and distribution suggesting that it had high percentage of neutral fatty matter and very less amount of unsaponifiable which was a good indication for usage as a skin cream

As the experimental part of this Skin cream formulation was a successful attempt, it could now be tested under BIS regulated tests and measured to check its reactions. If the reports suggest that the cream answered these tests within the specified ranges, then various forms of animal testing would be performed. Following this, the cream would be sent to FDA for approval and finally, on approval, the product would be ready for human usage.

6. APPENDIX

6. Appendix

Acid value- The number of mg of potassium hydroxide (KOH, molecular weight = 56.1) required to neutralize the free acids in 1 g of sample. The acid value gives information about the quality of a raw material or product. Rancidity and ageing of fatty materials is indicated by an increase in acid value.

Alcoholic hydrochloric acid solution, *c* (HCl)=0.5 mol/l. Dilute 50 ml concentrated hydrochloric acid to 1000 ml with ethanol and mix. Standardize immediately before use by titrating a 50 ml aliquot with accurately standardized aqueous sodium hydroxide solution, *c* (NaOH) = 1.0 mol/1, using phenolphthalein indicator.

Alcoholic potassium hydroxide solution, *c* (KOH) = 0.5 mol/1. Dissolve 14Og potassium hydroxide in 150 ml water with continuous stirring and cooling. Cool to room temperature, dilute to 250 ml and mix thoroughly. Dilute 50 ml of this solution to 1000 ml with ethanol and mix. Protect from exposure to the atmosphere. Allow any insoluble matter (potassium carbonate) to settle out before use.

Alcoholic sodium hydroxide solution, *c* (NaOH) = 0.1 mol/1. Pipette 50 ml accurately standardized sodium hydroxide solution, c (NaOH) = 1.0 mol/1, into a 500 ml volumetric flask, dilute to volume with ethanol and mix.

Emulsion- An emulsion is liquid preparation containing two immiscible liquids, one of which is dispersed as globules (dispersed phase = internal phase) in the other liquid (continuous phase = external phase).

Ester value- The number of mg of potassium hydroxide required to saponify, i.e. turn into soap, the fatty esters in 1 g of sample.

Hydrochloric acid solution, *c* (HCl) = 1 mol/1. Cautiously pour 100 ml concentrated acid (into 500 ml water, dilute to 1000 ml and mix.

Saponification value- The number of mg of potassium hydroxide required to saponify the fatty acids and the fatty esters in 1 g of sample. Samples which contain esters of low molecular weight fatty acids will have higher saponification values. Generally, saponification value is inversely proportional to the mean of the molecular weights of the fatty acids in the glycerides present in the sample. The saponification value is of most use for detecting the presence of adulterants such as waxes or paraffin which have negligible values.

Sodium hydroxide solution, *c* (NaOH) = 1 mol/1. Dissolve 40 g sodium hydroxide pellets in water, cool. Dilute to 1000 ml and mix.

7. References

7. References

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