

# Formulation and Development of De Pigment Serum Incorporating Fruits Extract

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## 1. INTRODUCTION

Skin Serum is a skincare product you can apply to your skin after cleansing but before moisturizing with the intent of delivering powerful ingredients directly into the skin. Serum is particularly suited to this task because it is made up of smaller molecules that can penetrate deeply into the skin and deliver a very high concentration of active ingredients. This makes them a great tool for targeting specific skincare concerns, like pigmentation, signs of aging.

### 1. Skin

Skin is the outermost and is the most superficial part of the body. It constitute about 15 to 20% of the total body mass. The skin is an ever changing organ that contains many specialized cells and structures. As we age, changes occur in the structure of the skin that affect its appearance.

#### 1.1 Structure of Skin

The structure of skin varies at different site of the body. To understand the structure one may segregate into three basis layers –

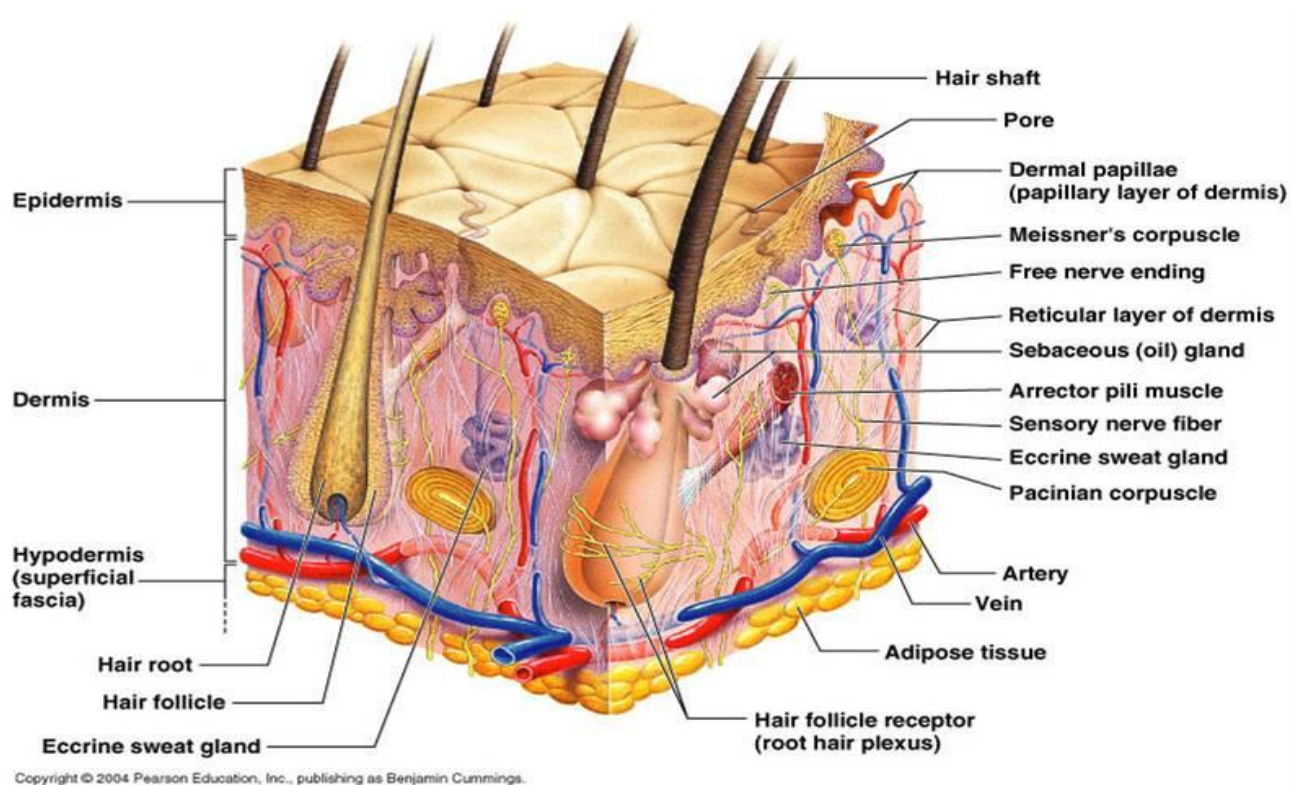


Fig.1 : Structure of Skin

## [A] Epidermis

The epidermis is the most superficial layer of the skin. It is derived from the ectoderm and composed of keratinising stratified squamous epithelium cells. It forms a protective barrier over the body's surface responsible for keeping water in the body and preventing pathogens from entering. It is thick at soles and palms. The epidermis also helps the skin to regulate body temperature.

Epidermis has four types of cells

- 1) keratinocytes (skin cells)
- 2) Melanocytes (pigment produce cells)
- 3) Langerhans cells (immune cells)
- 4) Merkel cells is a fourth less visible epidermal cells

### 1) keratinocytes

The epidermis consist primarily of keratinocytes, which comprises most of the epidermis which are characterized by numerous intercellular junctions. The keratinocytes become more mature or differentiated and accumulate keratin as they move upward.

The epidermis consist of four distinct layers –

#### a) Stratum Corneum

It is the uppermost layers and is composed of keratinised cells. The thin membrane consisting of dead nucleus, keratinized cell embedded in a lipid matrix.

#### b) Stratum Granulosum

The granular cells are so called because they acquire granular structures.

#### c) Stratum Spinosum

The stratum spinosum is also known as prickle cell layer and is found on top to the basal layer and together these two layers are termed the malpighian layer.

#### d) Stratum Germinativum

This layers contains the only cells (keratinocytes) within the epidermis that undergo cell division.

### 2) Melanocytes

These are found in the basal layer of the epidermis. Melanocytes manufacture the pigment melanin. Melanocytes appear as a small cells. They have thin cytoplasmic processes which extend between nearby

keratinocytes and serve to transfer melanosomes into adjacent keratinocytes and serve to transfer melanosomes into adjacent keratinocytes.

### 3) Langerhans Cells

Langerhans cells are antigen presenting cells which participate in the surveillance function of the immune system. These cells are smaller than keratinocytes.

### [B] Dermis

The dermis is the major component of human skin. It is composed of network of connective tissue. Tissue in dermis –

#### a. collagen

#### b. Elastin

### Layers of the dermis –

#### a) papilla layer

#### b) Reticular layer

a) **Papilla layer** – The upper papillary layer contains a thin arrangement of collagen fibers

b) **Reticular layer-** The lower reticular layer is thicker and made up of thick collagen fibers that are arranged parallel to the surface of the skin.

### [C] Hypodermis

The hypodermis lies below the dermis. The purpose is to attached the skin to underlying bone and muscles as well as supplying it with blood vessels and nerves. It consists of loose connective tissue and elastin. The main cell type are fibroblasts, macrophages adipocyte. Another name for hypodermis is subcutaneous tissue.

### Functions of Skin:

Skin performs the following functions:

**Protection:** an anatomical barrier from pathogens and damage between the internal and external environment.

**Sensation:** contains a variety of nerve endings that react to heat and cold, touch, pressure, vibration, and tissue injury.

**Heat regulation:** increase perfusion and heat loss, while constricted vessels greatly reduce cutaneous blood flow and conserve heat.

**Control of evaporation:** the skin provides a relatively dry and semi-impermeable barrier to fluid loss.

**Storage and synthesis:** acts as a storage center for lipids and water.

**Absorption:** oxygen nitrogen and carbon dioxide can diffuse into the epidermis in small amounts, some animals uses their skin for their sole respiration organ.

**Water resistance:** The skin acts as a water resistant barrier so essential nutrients aren't washed out of the body.

## 1.2 Skin Colour:

The color of the skin and hair is primarily due to the existence of pigment bodies known as melanin. Melanin is produced by the melanocyte cell present in stratum basal. There melanocyte present in basal layer is great abundance and the number does not differ greatly in people with more or less melanin pigment or colour in the skin, Melanin may be produced in either yellow, red or black colour and colour of the skin is determined by the amount and type of melanin produced. Melanin production is increased by exposure to sun and plays a role in protecting the skin from sunlight.

## 1.3 Pigmentation

### 1.3.1 Melanocytes:

In the basal layer of the epidermis there are approximately two billion specialized unicellular secretory glands known as Melanocytes. There are unique cell secret as amorphous pigment into surrounding epidermal cell which is largely responsible for the tineterial difference that exist in the skin, hair and eyes of man. The pigment secreted by there cell is not only important man's cosmetics appearance but also to his ability to survive without pigmentation, man would be obliged to live under total cover or resume in a sunless environment fortunately the normal skin is well with Melanocytes distributed over the entire body. The density of these cell varies being most dense on the exposed portion of the skin such as the for head and least on protected areas such as inner aspect of the thing.

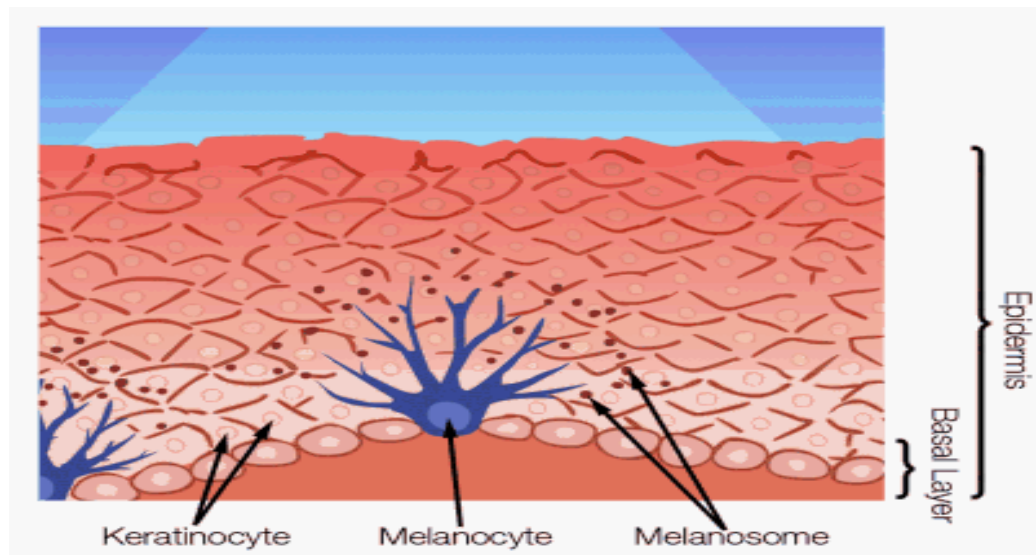


Fig. 2 : Melanocyte

Histologically and embryologically the melanocyte is uniquely distinct from epidermal cell. Melanocytes are derived from nervous tissue and morphologically appear as irregular branched dendritic shaped cell resembling ink blood Melanocytes occur. Normally in human skin scattered along the plan of the epidermal/dermal junction and in tube, hair pupal. In the latter area they are responsible for the colour of hair while in the former they are response for the he of the skin.

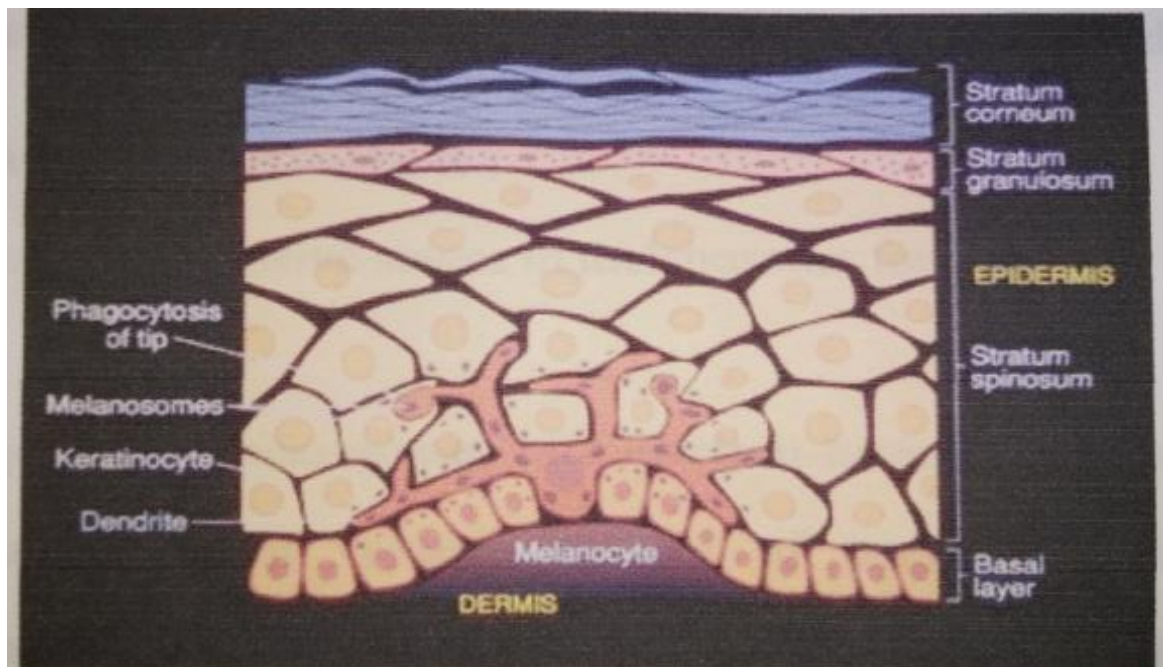


Fig. 3 : Position of Melanocytes of Skin

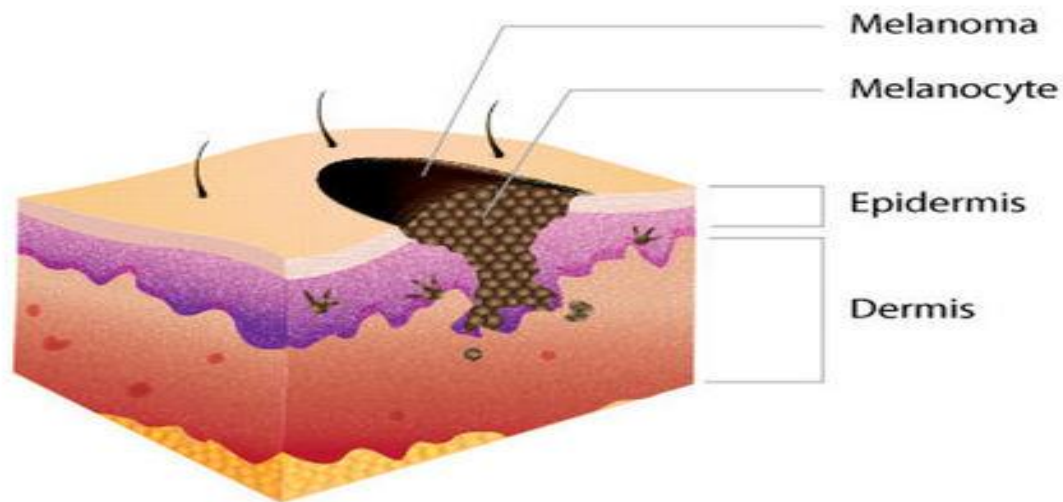


Fig. 4 : Melanocytes and Melanoma

### 1.3.2 melanosomes:

melanosomes rearrange themselves within the cell in responses to external such as UV rays or mutation in their transport system. melanosomes usually cluster together near the central of the cell but can rapidly redistribute themselves to the ends of dendrites (tooth like) processes protecting from the cell melanosomes involves long range transport from center along microtubule along the periphery short range capture and transport. Each melanocyte supplies melanin to approximately 30 nearby keratinocytes its dendrites.

### 1.3.3. Melanosome Formation:

There are 4 successive stages in melanosome formation.

#### Stage – I

In stage – I Premelanosomes are electron-lucent, membrane delimited spherical structures with variable amount of poorly organized internal membranes and no melanin. They are similar morphology to early multivesicular endosome.

#### Stage – II

In stage II the melanosome acquired an elliptical shape with intralayered fibers the ruin the length of the organel in an organized array, producing a striated appearance.

**Stage – III:**

In stage III there is regular periodic deposition of electron, opaque melanin on their fibers resulting in blackened and thickened striation and finally in stage – IV, the melanosome is completely melanised such that all the intralumental structure is occurred by the melanin. These striations likely function to detoxify eumelanin intermediates to sequester and concentrate melanins and in epidermal Melanocytes to prevent diffusion of melanin during transfer to keratinocytes. Integral membrane protein pommel 17 (also known as gp 100) is preferentially enriched in premelanosomes. Type- I and likely tyrosinase and Type-2 are enriched in later stage, pigmented melanosomes. Melanosomes have a low internal pH of 4-4.5. The non-higher melanised melanosomes are indulgent to the ratio of eumelanin to pheomelanin. More melanized large melanosomes show more. Clearly the presence of electronlucent bodies. The ability of melanosomes to store more melanin may not only be depended on the internal structure and composition of melanosomes but on the presence of 13 compared such glutathione that may react with melanin precursors and prevent them from binding to the melanosome matrix.

Melanosomes in keratinocytes of black skin are large individually dispersed throughout the cytosol and absorb more incoming light and refract less whereas those within keratinocytes of Caucasian skin are smaller and distributed in cluster hence less light is absorbed and more light is refracted. This disparity contributes different in skin pigmentation and photo protection.

**1.4 Skin of Pigment Secretion:**

The melanocyte is a secretory cell. The process of pigment secretion by melanocyte may be summarized as follows. In the cellular cytoplasm an aggregation of enzymatic process develops such that an intracellular granule called melanosome is formed. It is the melanosome that migrates towards the terminal end of dendritic branch where it becomes a pigment granule composed of almost 100% pigment totally devoid of enzymatic activity. It is these terminal portions of the dendritic containing the pigment granules that are assimilated by either the cortical cells of hair fiber of the epidermis, thereby imparting colour to these structures.

In human and types of pigment are formed by the melanosome one is the well known brown black pigment and other is the yellow, red pigment and pheomelanin.

**1.5 Tyrosinase, The Enzyme Behind The Dark Skin:**

Tyrosinase is multifunction glycosylated copper containing oxidase with molecular weight of approximately 80 to 70 kDa. In mammals, it is exclusively found in melanin cells. It is therefore, a good specific marker for these cells. Tyrosinase is synthesized in melanosomes ribosomes found on the rough endoplasmic reticulum. After synthesis, tyrosinase is glycosylated within golgi and on delivered to melanosomes via coated vesicles.



Tyrosinase is rate limiting the essential enzyme in the biosynthesis of the skin pigment melanin. As such it catalysis the different reaction in the biosynthesis pathway of melanin. The rate limiting steps in melanogenesis are the oxidation of tyrosine and DOPA. The quantity of melanin synthesized is thus proportional to the amount of tyrosinase activity present in the cell.

### **1.5.1 Tyrosinase –Related Protein (TYRP-1):**

TYRP related protein is the most abundant glycoprotein expressed specifically by Melanocytes. It is involved in maintenance of melanosome ultrastructure and affect melanocyte proliferation and melanocyte cell death. Result from our laboratory in the past have demonstrated that human Type-I exhibits tyrosine hydroxylase activity under low substrate. (L-tyrosine) conc. But no DOPA oxidase activity during umelanogenesis thereby establishing that Type-I function as DHICA oxidase in murine system. It is actively involved in the biosynthesis of black isolable 24 eumelanin in association with tyrosines and Tyrp-2. Tyrp-1 responsible for black hair coloration in mice. Murine Tyrp-1 has also been attributed with various other catalytic function including DCT, DHI oxidase DHICA oxidase. Mutation of the brown locus not only accelerated the degradation of mutant Tyrp-I but also wild typr tyrosinase expressed in there melanocyte. Tyrosinase activity is more stable in presence of both Tyrp-I and Tyrp-2 mutation of Tyrp-1 also ocultaneous albinism. (OCA) Type-B, which is associate with moderate hypo-pigmentation of skin, hair and eyes.

### **1.5.2. Tyrosinase Related Protein (TYRP-2):**

TYRP-2 also known as DOPA chrome tautomerase (DCT) is encoded of DCT\TYRP locus. It is a tyrp-1 membrane, protein that isomerase dopachrome to DHICA. Mutation of DCT in mice is responsible for stay phenotype characteristic by the production of DHICA-poor eumelanin Tyrp-2 is a protease sensitive enzyme. Activity for incubation for 15min at 65<sup>0</sup>C, broad pH optimum between 5.7-8.2. If activity is neither affected by metal, chelator, non blocked phenyl thiourea in point inhibitor of tyrosinase.

## **1.6 Chemistry of Melanin:**

The melanin are quinonoid polymer of somewhat uncertain structure. There are 2 main subdivision, pheomelanin yellowish and reddish brown pigment containing sulphur and eumelanin black or brown insoluble pigment derived from polymerization of tyrosinase oxidation. Two types of oxidation involved, namely oxygen addition to monophenols and dehydrogenation of diphenols.

### **1.6.1 Melanin:**

Melanin synthesis take place in distinct cytoplasmic organelles known as melanosomes located in melanocyte which inhabits the stratum basale of the epidermis. melanosomes originate from the endosomal compartment. The mature melanin filled melanosomes travel along the dendrites and are transferred to the neighboring keratinocytes. In protect from DNA from solar ultraviolet (UV) radiation. Thus, keratinocytes bearing the internalized melanosomes divide and move to the upper layer of the epidermis, causing the skin to look visibly pigmented. This association between one melanocyte and approximately 36 keratinocytes is defined as epidermal melanin unit (EMU) Epidermal pigmentation is a mosaic of the individual contribution of millions of

EMU overlapping each other resulting in a visually homogeneous skin color therefore mammalian pigmentation is a result of the synthesis of photo-protective melanocyte combined with efficient transfer to and processing of melanosomes by keratinocytes. As the keratinocytes differentiate and migrate to the upper layer of the epidermis, the melanosomes are degraded to form finer particles that may increase its effectiveness in shielding the epidermis from penetration and damage by U.V. light.(8)

### **1.6.2. Melanin Biosynthesis :**

Melanin biosynthesis (melanogenesis) is influenced by genetics. Environmental factor, diet and medication. The production of melanin by specialized cell melanocyte (in the basal layer of the epidermis in light skinned people). Occur through the action of the enzyme limiting step in melanogenesis, in the converts L-tyrosinase to melanin, through the action of tyrosinase copper and oxy act as catalyst, other enzyme also control melanin production, particularly in the presence of sulphur. Thus include the following.

Dopachrome oxidoreductase which control melanogenesis in absence of tyrosinase. It helps to convert dopachrome into 5,6 dihydroxyindole. tyrosinase which helps to maintain the balance in the biosynthesis of eumelanin and pheomelanin.

The currently accepted scheme for melanin biosynthesis in the skin pigmentation is attributed to the level of melanin produced and no. Melanocytes present.

Although light skinned and dark people same no. of Melanocytes present the rate of melanin production is greater in darker skin is resistant to enzymatic degradation. Increased production of melanin on one side of the skin and dramatically reduced decomposition of melanin on the other side result in darker skin tones in light skinned people.

Melanin granules synthesized in Melanocytes are then transferred from the cytoplasm of the Melanocytes to the basal cytoplasm of the keratinocytes. They thus form a protective covering in the inner layer of the epidermis absorbing U.V. rays and inhibiting their proportion.

Various type of inflammatory mediation such as leukotrienes and prostaglandins, cytokines and growth factor may influence melanin synthesis by affecting the proliferation and functioning of Melanocytes. This explains why inflammatory disease often induce hypopigmentation or hyper pigmentation. The enzyme protein kinase that phosphorylates protein may also influence the growth and differentiation of Melanocytes cytokines such as endothelins (also known as vasoconstrictive peptides) are also reported to accelerate melanogenesis.

Thus tyrosinase inhibitors, agent that increase keratinocytes turnover agent that inhibits the hormone of intermediates in melanin biosynthesis antioxidants that chelate metal ion (which catalyze tyrosinase's activity) cytokine regulation and genetic manipulation would also be beneficial in controlling melanin synthesis.

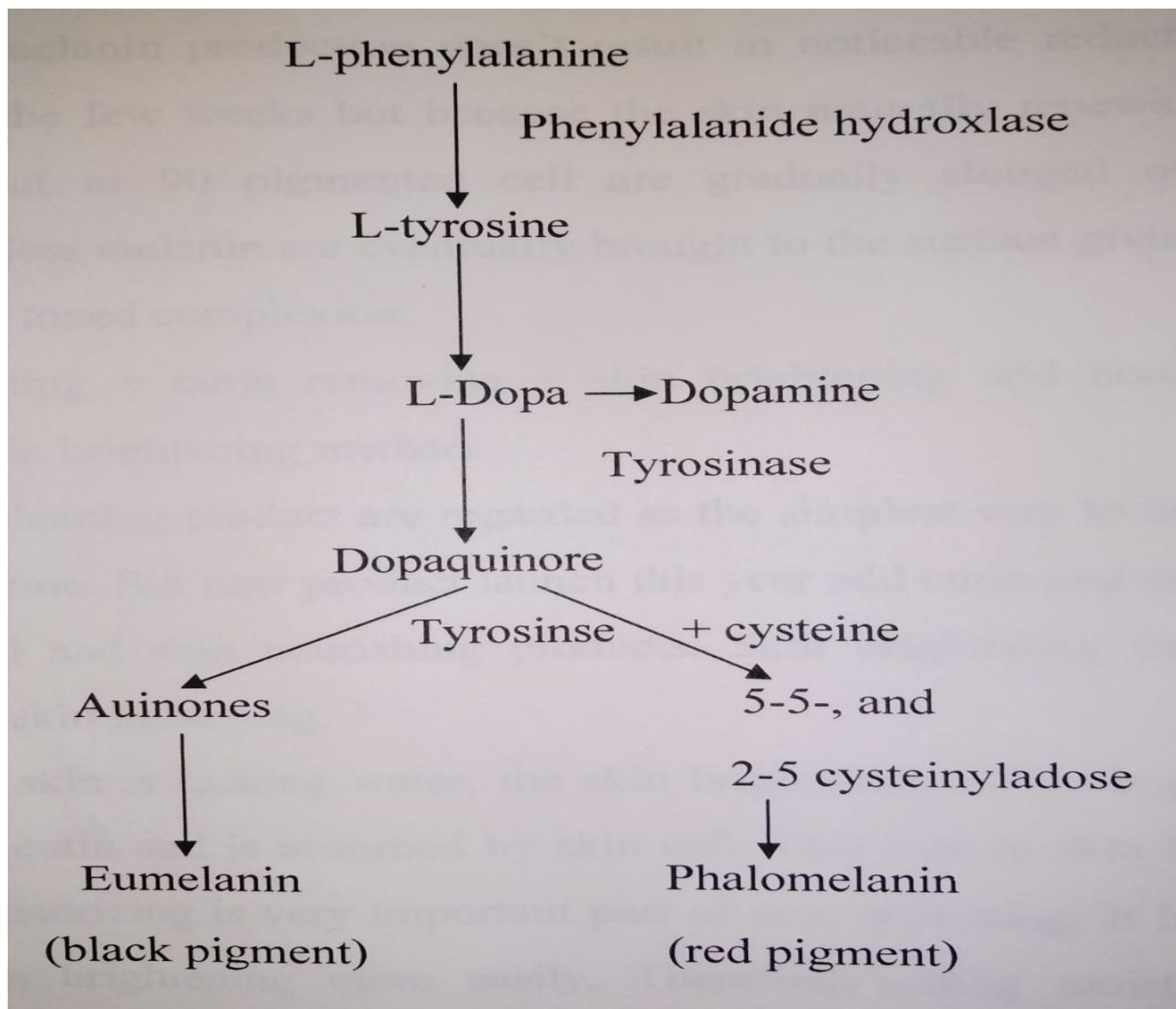


Fig. 5 : Formation of Melanin

**1.7 Skin Lightening:**

Skin whitening is the elimination of the melanin that acts as a self defence mechanism for the human skin against the exposure to ultraviolet to mean for more it entails inhibiting the further synthesis of melanin.

Skin lightening product has experienced a global boom and their rapid growth is showing no signs of slowing. Solar lentigines, commonly called age or liver spots and found on hand and face or other area frequently exposed to the sun are common form of hyper pigmentation. Melasma or chloasma spots are similar in appearance to age spots but are larger areas of darkened skin that appear most often as result of human changes, pregnancy.

Inhibiting melanin production doesn't result in noticeable reduction in pigmentation for the few weeks but because the skin naturally renews, itself every 28 days but or 90 pigmented cell are gradually sloughed off and keratinocyte with less melanin are eventually brought to the surface giving skin lighter, movement toned complexion.

Deep cleaning + cutin removing + skin brightening and nourishing product= Fast skin brightening method.

The deep cleaning products are regarded as the simplest way to brighten the skin in short time. But new product launch this year add cutin and removes function, material and skin nourishing products. Skin brightening and skin moisturizing real skin lightening.

When the skin is tacking water, the skin brightening active ingredient cannot penetrate cutin and is absorbed by skin cell. This lead to skin looking dull the skin moisturizing is very important part of skin whitening. It helps to achieve the skin brightening more easily. Therefore adding moisturizing ingredients in skin brightening product will improve skin whitening efficiency.

### **1.7.1 Mechanism of Skin lightening :**

- 1) Promotes skin tones by lightening the over production of melanin.
- 2) Block the melanin production process.
- 3) Inhibits the activity of dopachrome tautmerase.
- 4) Inhibits melanin producing inflammatory responses of skin.
- 5) Skin lightening elimination of melanin that act as self defense mechanism for human skin against the exposure to U.V. rays.
- 6) Skin lightening process making skin healthier improving the immunities if skin cell themselves.
- 7) Tyrosinase inhibitor by inactivating tyrosinase by chelating with its vital copperion.

### **1.7.2 Types of skin whitening product :**

- 1) Those containing sunscreen.
- 2) Those containing light reflection ingredients.
- 3) These containing ingredient that produces chemical change on skin.

### **1.7.3 Various skin lightener's s available in the market:**

Kojic acid, Vitamin-c, Arbutin, Melnostat, Licorice, Mulbery extract, Wild buchal extract, Dong Quai extract, Wild yam extract, Alpha hydroxy acid, Emblica extract, Retinol, etc.

#### **1.7.4 Beneficial effect of skin lightening agent :**

Natural method for light overall skin color and fading age, spot and Freckles hyper pigmentation and skin discolourisation. Even out discolourisation without causing skin hyper pigmentation.

Provides deep moisturizing which makes skin to look lighter smoother and more beautiful. Suitable for people of all nationalities and skin colour. Safe in use and given fast result even in 4-8 weeks depending on individual.

#### **1.7.5 Adverse effect of skin lightening agent :**

They have high potential and cause irritant contact dermatitis may be harmful by inhalation ingestion or it absorbed through skin irritant.

### **1.8 Moisturization:**

#### **1.8.1 Dry Skin:**

Dry skin is characterized by sensation of tightness with skin feeling rough and scaly and visible line developing. It is the dehydration of stratum corneum and is a condition especially seen among the elder people. As it worst it may look cracked.

#### **1.8.2 Causes Dry Skin:**

Skin dehydration is generally causes by following factor :

Friction, Weather, water, organic solvent, soap, surfactant.

Disturbed skin functions such as lack of protection from bacterial invasion.

No protection against penetration of foreign substance.

No maintenance of body temperature.

No regulation of the water content of the body.

#### **1.8.3 Moisture in Stratum Corneum :**

The stratum corneum have flexibility and protective function which are tightly linked with its moisture level depend basically on three factors.

The rate at which the water in the dermis reaches the stratum corneum.

The rate at which water is eliminated by evaporation.

The stratum corneum ability to retain H<sub>2</sub>O. This is tightly linked with the role if surface lipids film natural moisturizing factor and polar lipids and glycol lipids phospholipids, free amino acid which makes the well known lamellae in the intracellular space.

#### **1.8.4 Moisturizing Phenomenon :**

The functioning of skin and its mechanism are upset by changes in the environment and aging. There are 2 basic reasons for dry skin.

First is due to prolonged exposure to low humidity and air movement which modifies the normal hydration gradient of stratum corneum.

The II<sup>nd</sup> is due to physical and chemical changes in the skin due to process such as aging. For the purpose of moisturizing the stain oil the nature humectants components exhibits complementary function in the respect continuous and prolonged immersion in soap for detergent solution may contribute to dryness of stratum corneum.

The etiology skin surface lipids the horny layer the dissolution of the hygroscopic water soluble components in the corneum.

### **1.8.5 Concept of Moisture Balance :**

Organs, it is stated their ideas concerning the moisture balance and said the following. We have demonstrated that the horny layer NMF and lipid amount decrease skin ages resulting in reduction in this moisture retention capacity leading to hardening of the horny layer. But we can make up for reduction in moisture NMF and lipid dervening through again by supplying equivalent substance (water humectants and oil) in cosmetic.

### **1.8.6 Role of Moisturizer:**

Moisturizer are the material used for the prevention or relief of dryness as well for the protection of the skin. The approach to restoring water to dry skin has taken three different routes.

- Occlusion
- Humectancy
- Restoration

Deficient material which may be often combined. Occlusion consist in reducing the rate of transepidermal water loss through old or damaged skin or in protecting of a severally drying environment. It has been demonstrated that the occlusion of skin result in an immediate decrease in rate of water loss through epidermis. This has the desired effect of causing the stratum corneum to become more hydrated making it softer and more supply. However eventual effect of the extra hydration is to increase the diffusion coefficient of H<sub>2</sub>O across the epidermis.

A second approach to the moisturizing problem is the use of humectants to attract water from atmosphere, so supplementing the skin water content.

The third and perhaps the most valuable approach to moisturizing of skin is to determine the precise mechanism or the material miniaturization process to access what has gone wrong with it. In the case of dry skin and to reduce any material in which such research has shown damaged skin to the deficient.

### **1.8.7 Moisturizer helps to decrease appearance of skin wrinkling:**

What exactly does ‘decreasing’ the appearance wrinkles mean from standpoint? It means moisturizing the skin. The easiest ways to improve wrinkles are those of skin dehydration. Moisturizer can smooth down desquamating coenocytes and fill in the gap between the remaining coreocytes to create the impression of tactile smoothness moisturizers can create a optimal environment for heating and minimizing the appearance of lines of dehydration by decrease transepidermal water loss.

### **1.9. Serum:**

Serum is concentrated product widely used in cosmetology. The term come itself from professional cosmetology.

#### **1.9.1 cosmetic serum:**

Cosmetic serum is highly concentrated based on water or oil as any other cream. A serum, or other concentrated product containing ten times more of biologically active substance than creams, Therefore quicker and more effectively coping with cosmetic problems.

#### **1.9.2 serum effects:**

When concentrate are used, the skin immediately gets the amount of active substance in such from which assimilate easier . The active substance in high concentrations act in same way as they moisture, rejuvenate, lift up, etc. The only difference is that in case concentrate are used correctly and noticeable result will be ricked quicker.

According to effect produced all serum are strictly divided in following category:

- Lifting up
- Revitalizing
- Moisturizing
- Nourishing
- Anti inflammatory
- Something
- Anti stress

Serum act locally upon different body parts : face ,naje, decollate eyelids. Exceptional cases should be taken of the skin around where special preservatives and bases are used, and the doses of active constituent accurately calculated. Serum can be used irrespective of age. When using concentrate you can always get not only quick cosmetics effects. But also psychological satisfaction after the treatment because the will be seen practically immediately.

Serum or essence make up for what is lacking in conventional skin care cosmetics. In other words, they are positioned value added cosmetics product. There are many transparent, semi-transparent, viscous liquid type in market. Because the serum are used in small amount and must fulfill a lot of requirement of body.

### 1.9.3 Different types of serums and their features :

Types	Technology	Features
Transparent Or semi - transparent Lotion type.	Solubilization, Micro emulation, Liposomes, Disc like capsule.	In general contains more humactant than lotion.the texture may be adjusted through the selection of humactant and water soluble polymer and varing there combination. This is most general form of serum preparation.
Emulsion type	O/W type W/O type W/O/W type	As the type contain large amount of emollient ,it is suitable for preparation containing large amount of U.V. absorber and oily ingredients.the w/o type is suitable for preparation requires water repellence.
Oil type		In which, the texture is adjusted by solid or semi solid oils,and animals fats or plants oils in different proportions. As texture of this type is not good as that of other preparation it is disappearing from market
Two agent mix together type	In addition to above, spray dry, freezedry , Microcapsule technology are used.	In order to prevent instability in pharmaceutical agent and preparation or to affect a visual Change two agents are mixed together to use.they are liquid-liquid or liquid-powder combination.
Others	Lotion with powder type.much alcohol type.	Serum for t- zone which secrets a lot of sebums including sebum absorbing powder increases lasting power of make up essence having a germisidal effect for acne preparation.

Table No. 1 : Different Types of Serums and their Features



## 2. LITERATURE SURVEY

Survey is the documentation of a compressive review of the published and unpublished work from secondary sources data in the area of specific interest to the researcher. The library is a rich storage base for secondary data and researcher used to sped several weeks and sometimes months going through book. Journals, newspapers, magazines, conference proceeding doctor dissertation, master these, government publication and financial reports to find information on their research topic with computerized databases, now readily available and accessibly the literature search is much speedier and easier and can be done without entering the portals of library building.

**H. Dureja, D. Kaushik, M. Gupta, V. Kumar, V. Kumar:** showed that “Cosmeceuticals: Anemerging concept.”<sup>16</sup>

**N. Puizina :** reported that there are two main process that induce ageing i.e. Intrinsic and Extrinsic.<sup>17</sup>

**Chrmahini S.H et al :** reported that herbal and natural cosmeceutical products are safe to use and do not have any side effects. With man relearning the benefits of natural products, cosmeceutical products are increasingly being used by leading herbal. Manufacturers right from body lotion to face packs,from skin cleansers to fairness cream. They concluded that these reviews gives an overview from cosmeceutical value of natural ingredients and novel technologies for pigmentation.<sup>18</sup>

**Jain Amit, Dubey Subodh, Gupta Alka. Tomar Vivek:** reported the potential of herbs as cosmeceuticals.<sup>19</sup>

**Zoe Diana, Draelos :** reported that the easiest way to reduce wrinkles are those of skin hydration, moisturizers can create an optimal environment for healing and appearance of lines.<sup>20</sup>

**Lidia Maria Ravelo :** Perz et al. stated that lycopene is one of the most important and abundant carotenoids found in peach it has been shown to play a very important role in human nutrition, mainly due to its high antioxidant activity.<sup>21</sup>

**Lu. Michelle et al :** stated that a topical cosmetic composition for improving the asthetic appearance of skin comprising blend of one or more extracts of botanical selected from group of consisting peach extract and others.<sup>23</sup>

**L. Stussi, F Henry,et al :** stated that, olive oil has traditionally been used for skin care, hair care, nail care and various dermatological conditions. Olive oil contains essential unsaturated fatty acid in abundance. Beside it contains sterols, polyphenols, ferulic acid, linolic acid and high levels of vitamin E.<sup>24</sup>

**Dom Guillaume :** stated that olive oil is an ingredient which has posses antioxidant and moisturizing property, and can be used in the preparation of many cosmetic product.<sup>25</sup>

**Zoubida Charrouf** : stated that olive oil has been widely used by Moroccan's and currently there are no known acute or chronic toxicity levels.<sup>26</sup>

**Gupta Shyam K** : The present invention is to boasting of the skin whitening. The skin whitening benefits are synergistically increased by combining at least one of tyrosinase inhibitor, tyrosinase competitors and melanin and other colour bodies reducing agent and other benefits is to u.v. absorber and inhibitor. United state of patent application

**Kenya Ishida et al.** : Melanogenesis inhibitor stable, safe and has sufficient fair skin effect and for curing skin depigmentation. International Application PCI/JP 2004.

**Shyama Gupta** : Discovered that certain hydroxyaryl or polyhydroxyaryl compound that contain alkyl chain gives unexpected skin whitening effect and addition metal ion and certain antioxidant additionally increases skin whitening effect. United state Patent Application, Publication date : Dec. 8, 2005.

**M.J. Olsson J M. Gillbro**: Had been given the review on the melanogenesis and mechanism of skin lightening agent-existing and new approach. International Journal of Cosmetic Science, 2011, P.N. 33,210-221.

**Hyunsu Bae, Shoukat Parvez, Moonkyu Kang, Hwan-Suck Chung, Changwoon Chu, Noo-Chang Hong, Minkyu Shin**: All these researchers had given review on survey and mechanism of skin Depigmenting and lightening agent and said that tyrosinase catalyses three different reaction in the biosynthesis to L-DOPA and the Oxidation of L-DOPA to dopaquinone furthermore, in human dopaquinone is converted by a series of complex reaction to melanin. Bull Korean Chem, Soc. 2005 Vol. 26, No. 7, 1135.

**En-Qin Xia, Gui-Fang Deng, ya-Jun Guo and Hua-Bin Li**, "Biological Activities", according to this paper" Anthocyanins, flavanols, and resveratrol are the most important grape polyphenols because they possess many biological activities, such as antioxidant, cardio protective, anticancer, anti-inflammation, antiaging and antimicrobial properties. International Journal Molecular Science ISSN 1422- 0067, . International Journal Molecular Science 2010-11, P.N. 622-646

**Raymond E. Boissy Jody P. Ebanks** : Had reported about mechanism regulation skin pigmentation. The Rise and Fall of complex coloration. International Journal Molecular Science 2009.

### 3. RESEARCH ENVISAGED

#### 3.1 Rational Work

The concept of beauty and cosmetics is as ancient as mankind and civilization. Women are obsessed with looking beautiful. So, they use various beauty products that have herbs to look charming and young. Indian herbs and its significance are popular worldwide. An herbal cosmetic have growing demand in the world market and is an invaluable gift of nature. Herbal formulations always have attracted considerable attention because of their good activity and comparatively lesser or nil side effects with synthetic drugs. Herbs and spices have been used in maintaining and enhancing human beauty. Indian women have long used herbs such as Sandalwood and Turmeric for skin care, Henna to color the hair, palms and soles; and natural oils to perfume their bodies. Not too long ago, elaborate herbal beauty treatments were carried out in the royal palaces of India to heighten sensual appeal and maintain general hygiene. The herbal cosmetics manufactured and used commonly for daily purpose include herbal face wash, herbal conditioner, herbal soap, herbal shampoo etc. The industry is now focusing on the growing segment with a vast scope of manifold expansion in coming years. Herbal cosmetics are defined as the beauty products which posses desirable physiological activity such as healing, smoothing appearance, enhancing and conditioning properties because of herbal ingredient. Here we reported the introduction, classification, common herbs used in cosmetics. Herbal Cosmetics, here in after referred as Products, are formulated, using various permissible cosmetic ingredients to form the base in which one or more herbal ingredients are used to provide defined cosmetic benefits only, shall be called as “Herbal Cosmetics”.

Beginning 1990's cosmetic manufacturer adapted a term 'cosmeceuticals' to describe the OTC skin care products that claims therapeutic benefit by addition of plant based active ingredient such as alpha- hydroxy acid, retinoic acid, ascorbic acid and coenzyme Q10.(4) These active ingredients serves many purposes viz. increase in skin elasticity, delay in skin aging by reducing the wrinkles, protection against UV radiation by antioxidant property and to check degradation of collagen respectively.

#### 3.2 Objective of Work

Today serum are so popular that they constitute major portion of skin care products. Pigmentation of skin is caused by various factors such as free radical which are generated in our body and due to extra dry skin etc.

So serum are widely used in cosmetics field. These products helps to prevent all dark spots. This product on application reduces sagging of skin, pigmentation due to ageing. Sunlight play an important role in ageing skin photo ageing in severve causes lead to Actinic Keratosis and skin cancer.

These products also help by preventing wrinkle formation and sagging skin and improves the appearance of the skin. There are many natural antioxidants agents which prevent or slow pigment formation and also have the beneficial effect for skin tonning, moistening, and whitening etc.

There are many antioxidants available in nature such as Amla, carrot seeds, Tomato, orange, berry, spinach etc. This antioxidants rejuvenate the skin with anti ageing effect on skin.

By considering these points main attention was given to formulate product

To give antioxidant effect which reduce cell damage and prevent pigmentation To provide moisturizing effect to skin for soft and supple feel.

Natural agents not only have function of antioxidant and moisturization but it also have whitening, nourishing effects. So taking in view of this antioxidants property in consideration the main objective of this study is to formulate and evaluate the de pigment serum with fruit extract which will give antioxidant, moisturizing activity by using fruit extract.

### 3.3 Plan of Work

Peach fruit extract was selected as de pigment agent and incorporated them in the cosmetic product i.e. serum. The detail plan of work is given below:

CHAPTER – 1	:	INTRODUCTION
CHAPTER – 2	:	LITERATURE SURVEY
CHAPTER – 3	:	RESEARCH ENVISAGED

a) selection of drugs which have antioxidant activity and objective of work.

CHAPTER – 4	:	DRUG PROFILE AND EXCIPIENT
Peach fruit extract		

#) Excipients

CHAPTER – 5	:	MATERIAL AND EQUIPMENTS
CHAPTER – 6	:	EXPERIMENTAL

a) Selection of base formulation of serum

b) Formulation and optimization of serum

c) Incorporation and selection at different concentration of extract into serum

CHAPTER – 7	:	EVALUATION
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a) In Vitro studies

b) In vivo studies

CHAPTER – 8	:	RESULT AND DISCUSSION
CHAPTER – 9	:	SUMMARY AND CONCLUSION

CHAPTER – 10	:	BIBILOGRAPHY
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#### 4. DRUG PROFILE AND EXCIPIENT



Fig.6 : Peach Fruit



Fig.7 : Peach Fruit Extract

#### 4.1 Drug Profile

Active :Peach Fruit Extract

Biological name :Prunus persica

Classification :

Kingdom -Plantae

Division -Magnoliophyta

Class -Magnoliopsida

Order -Rosales

Family -Rosaceae

Genus -Prunus

Species -P. persica

#### Biological Source

The peach (*Prunus persica*) is a deciduous tree native to the region of Northwest China between the Tarim Basin and the north slopes of the Kunlun Shan mountains, where it was first domesticated and cultivated.[3] It bears an edible juicy fruit called a peach or a nectarine.

The specific epithet *persica* refers to its widespread cultivation in Persia, whence it was transplanted to Europe. It belongs to the genus *Prunus* which includes the cherry, apricot, almond and plum, in the rose family. The peach is classified with the almond in the subgenus *Amygdalus*, distinguished from the other subgenera by the corrugated seed shell.

Peach and nectarines are the same species, even though they are regarded commercially as different fruits. In contrast to peaches, whose fruits present the characteristic fuzz on the skin, nectarines are characterized by the absence of fruit-skin trichomes (fuzz-less fruit); genetic studies suggest nectarines are produced due to a recessive allele, whereas peaches are produced from a dominant allele for fuzzy skin.

#### Chemical Constituent:

Peaches are rich source of Vitamin A, C, K, beta carotene, potassium and magnesium that saves your skin from harmful UV rays. Peaches store a broad range of nutrients that are vital for the healthy functioning of the body. Peaches are a rich provider of vitamin A, beta-carotene, and vitamin C (ascorbic acid). They are also a good source of vitamin E (alpha-tocopherol), vitamin K (phylloquinone), vitamin B1 (thiamine), vitamin B2 (riboflavin), vitamin B3 (niacin), vitamin B-6, folate, and pantothenic acid. Peaches also offer a rich treasure of minerals such as calcium, potassium, magnesium, iron, manganese, phosphorous, zinc, and copper. Peaches are low in calories, contain no saturated fat or cholesterol, and are a good source of dietary fiber.

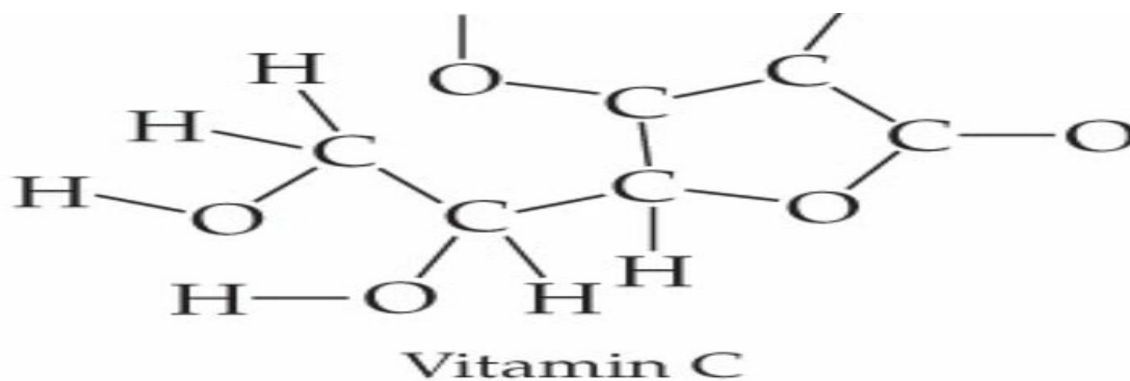
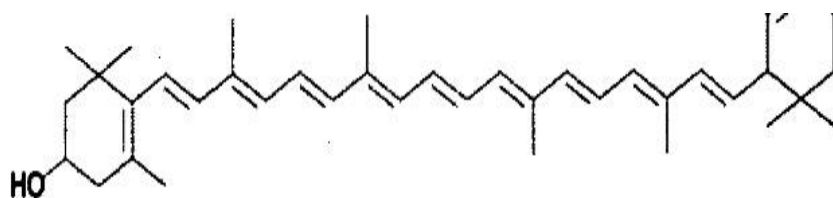
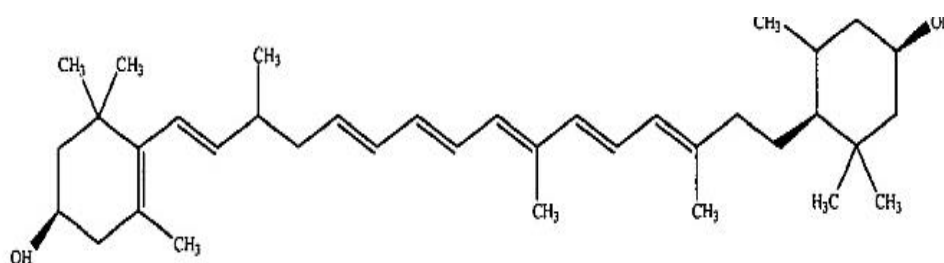


Fig. 8 : Vitamin C



Lutein

Fig. 9 Lutein



Zeaxanthin

Fig. 10 Zeaxanthin

**Description:**

Color - Light Yellowish to Orange Color.

Odor - Characteristic Odor.

Solubility - Soluble in Water.

PH - 4.48

Specific Gravity - 1.0276

**Uses:****Antioxidant activity :**

Vitamin C is a powerful antioxidant with radical scavenging activity very useful in the treatment of pigmentation. Vitamin C can neutralize free radicals generated by UVB radiation. Lutein and zeaxanthin also enhance the antioxidant properties of peach fruit.

**Softening and Transepidermal water loss regulatory activity:** Carbohydrates are active principles extensively used in cosmetics. Monosaccharides are hygroscopic, namely they absorb water thus contributing to keep a healthy moisture level in the horny layer.

**Vitamins and mineral replenishing activity. Revitalizing and Stimulant activity.**



Sr. no.	Test	Standard	Results
A.	Physico chemical test		
1	Description	Light yellowish or orange color liquid	Light yellowish or orange color liquid
2	Water solubility	Soluble	Complies
3	Propylene glycol solubility	Soluble	Complies
4	Alcohol solubility	-----	-----
5	Ph	3-6	4.48
6	Specific gravity	0.900 -2.0	1.0276
7	Dry residue	NMT - 5%	1.40%
8	Assay	Presence of sugar	Positive
B.	Microbiological test		
9	Total plate count	NMT -1000cfu/gm	Complies
10	Yeast/moulds	NMT -100cfu/gm	Complies
11	E.coli	Absent	Absent
12	Salmonella	Absent	Absent
13	Total coliforms	Absent	Absent

Table No. 2 : Certificate of Analysis

## 4.2 Excipients

### 1) Carbopol :

USP : Carbopol

**Chemical name and CAS registry number:** Carboxy methylene (54182 – 57 – 9) (2007 – 3) for carbopol 973 (76050 – 42 – 5 ) for carbopol 940

**Functional category :** Suspending agent, Emulsifying agent, binder, viscosity increasing agent.

**Application :** Carbopol are mainly used as in liquid or semi solid cosmetic formulations which includes cream, gels and ointments. Carbopol are also employed as a emulsifying agent in the preparation of oil in water emulsion

### 2) Disodium EDTA :

**Synonym :** Disodium dihydrogen ethylenediamine tetra acetate Disodium edentate, disodium salt, dehydrate

Chemical name :  $C_{10}H_{14}O_8N_2Na_2 \cdot 2H_2O$   
 CAS no. : 139 – 3  
 Molecular weight : 372.24  
 Functional category : Stabilizer, chelating agent.  
 Description : White crystals, it is odourless  
 Melting point :  $252^\circ C$   
 Solubility : 10gm in 100ml of water  
 Storage : Stored in well closed container.

### 3) Triethanol amine :

Synonym : TEA, Triethylamine, trihydroxytriethyl amine  
 Chemical name : 2,2,2-tris(2-hydroxyethyl)amine  
 CAS No. : (102 – 71 – 6 )  
 Empirical formula :  $C_6H_{15}NO_3$   
 Molecular weight : 149.19  
 Structural formula :  $N(CH_2CH_2OH)_3$   
 Functional category : Alkalizing agent, Emulsifying agent  
 Description : Clear colourless to pale yellow colour Viscous liquid having a slight ammoniacal Odour.

### 4) Glycerin :

Synonym : glycerin, glycerol, glycon G-100; 1,2,3 – propanetriol Trihydroxy propane glycerol  
 Chemical name : Propane-1,2,3-triol  
 Empirical formula :  $C_3H_8O_3$   
 Molecular weight : 92.09

Functional category	:	Humectant, emollient, solvent, plasticizer
Specific gravity	:	1.249
Description	:	Glycerin is clear, colourless, odourless, viscous, hygroscopic liquid.
Melting point	:	17.8°C
Boiling point	:	290°C
Solubility	:	It is slightly soluble in acetone, practically Insoluble in benzene, chloroform and oil, Miscible with ethanol.
Storage	:	Store in well closed container.

**5) Tween 20 :**

Chemical name	:	Polysorbate 20
Molecular weight	:	1310
Functional category	:	Solublizing agent
Description	:	Yellow oily liquid
Solublity	:	oil soluble

**6) Sodium benzoate :**

Synonym	:	benzoate of soda
Chemical name	:	Methyl 4-hydroxy benzoate
Emperical formula	:	C <sub>6</sub> H <sub>8</sub> O <sub>3</sub>
Molecular weight	:	152.15
Functional category	:	Antimicrobial preservative
Description	:	Colourless crystals or a white crystalline Powder. It is odourless and has slight Burning taste.
Melting point	:	125-128°C
Storage	:	Stored in well closed container.

**7) Water :**

Synonym	:	Aqua; hydrogen oxide
Chemical name	:	Water
CAS no.	:	7732-18-5
Empherical formula	:	H <sub>2</sub> O
Molecular formula	:	18.02
Description	:	Water is clear, colourless and tasteless Liquid.
Melting point	:	0°C
Boiling point	:	100°C
Solublity	:	Miscible with most polar solvent.
Storage	:	Water for specific purposes should be Stored in appropriate container.

**8) Olive oil :**

Synonyms	:	vegetable oil
Colour	:	yellowish green liquid oil
Odour	:	none
Melting point	:	–6.0 °C (21.2 °F)
Boiling point	:	300 °C (572 °F)
uses	:	use as a moisturizing agent
Storage	:	Stored in well closed contain

**9) vitamin E**

Synonyms	:	tocopherol
Color	:	clear liquid oil
Odour	:	none
Uses	:	use as a antioxidants

## 5. INGREDIENT AND EQUIPMENTS

### 5.1) List of materials required for serum :

Carbopol 940	:	S.D Fine chemicals Ltd., Mumbai
Triethanol amine	:	S.D Fine chemicals Ltd., Mumbai
Di sodium EDTA	:	S.D Fine chemicals Ltd., Mumbai
Tween 20	:	S.D Fine chemicals Ltd., Mumbai
Glycerin	:	S.D Fine chemicals Ltd., Mumbai
Sodium benzoate	:	S.D Fine chemicals Ltd., Mumbai
Water	:	MIDC, Amravati.
Olive oil	:	S.D Fine chemicals Ltd.,Mumbai
Vitamin E	:	Medical shop,Amravati.

### 5.2) List of Equipments

Precision balance	:	CA series contech
Mechanical stirrer	:	Shettal Scientific industry Pvt ltd.,Mumbai
pH meter	:	Digital Model 111E-E-1 Electronic india
Brook field Viscometer	:	S.M.S Scientific Industry Pvt.Ltd. Mumbai (DV-E-version1, E-34/03)

## 6. EXPERIMENTAL

### Formulation and Optimization of Base Formulation

#### 6.1 Formulation of serum base

Procedure –

Carbopol was dispersed in 80% of water along with EDTA and allowed to hydrate keeping overnight. After dispersing the TEA was added for desired consistency. Then one by one added remaining ingredients. At last add perfume in it.

##### 6.1.1 Formulation of Serum Base

Sr. No.	Ingredients	M1 For 100%	M2 For 100%	M3 For 100%
1	Water	82	80	75
2	Carbopol 940	0.3	0.3	0.3
3	Triethanol amine	0.1	0.1	0.1
4	Glycerin	4	7	8
5	Disodium EDTA	0.1	0.1	0.1
6	Tween 20	3	4	4
7	Olive oil	8	7	7
8	Vitamin E	1	1	1
9	Sodium benzoate	0.1	0.1	0.1
10	Perfume	1	1	1

Table No. 3 : Formulation of Serum Base

### 6.1.2 Optimization of Serum Base

Sr.no	Parameter	M1	M2	M3
1	Appearance	++	+++	++
2	Colour	+	+++	++
3	Consistency	++	+++	++
4	Spreadability	+	+++	+++
5	Feel	+	++	+

Table No. 4 : Optimization of Serum Base

From the above observation formula M2 was stable and it shows consistency, spreadability, and feel therefore it was selected and extract was added with different concentration and forward for in vitro study as in vivo study with human volunteers Here, + = good, ++ = Better, +++ = Best

### 6.2.3 Incorporation of peach fruit extract at different concentration in base formulation:

Sr No.	Ingredients	M1 For 100%	M2 For 100%	M3 For 100%
1	Water	78	77	76
2	Carbopol 940	0.3	0.3	0.3
3	Triethanolamine	0.1	0.1	0.1
4	Glycerin	7	7	7
5	Disodium EDTA	0.1	0.1	0.1
6	Tween 80	4	4	4
7	Olve oil	8	8	8
8	Vitamin E	1	1	1
9	Sodium benzoate	0.1	0.1	0.1
10	Perfume	1	1	1
11	Peach fruit extract	1	2	3

Table No. 5 : Incorporation of Peach Fruit Extract At Different Concentration in Base Formulation

## 7. EVALUATION

### 7.1 Evaluation of extract

#### 7.1.1 Preliminary phytochemical screening:

**Flavonoids:** To test solution add few drops of NaOH solution formation of dilute acid indicates presence of flavonoids.

**Glycosides:** A small amount of alcoholic extract of samples is dissolved in 1ml water and then aqueous sodium hydroxide is added. Formulation of yellow colour indicates the presence of glycosides.

**Alkaloids (Mayer's test):** 1.36gm of mercuric chloride is dissolved in 60ml and 5gm of potassium iodide is dissolved in 10ml of distilled water respectively. These two solvents are mixed and dilute to 100ml using distilled water. To 1ml of acidic aqueous solution of samples few drops of reagent is added. Formation of blue or green colour indicates the presence of alkaloids.

**Phenols (ferric chloride test):** To 1ml of alcoholic solution of sample. 2ml of distilled water followed by a few drops of 10% aqueous ferric chloride solution is added. Formation of blue or green colour indicates the presence of phenols.

**Tannins (lead acetate test):** In a test tube containing about 5ml of an aqueous extract a few drops of 1% solution of lead acetate was added. Formation of a yellow or red precipitate indicate the presence of tannin.

#### Lipids

In a test tube 5 drops of the sample was taken and a pinch of sodium hydrogen sulphate was added. Pungent odour emanates from the tube which indicates that glycerin is present which is produced by hydrolysis in fixed oil which shows the presence of lipids.

#### 7.1.2 Test for Antioxidant Activity of Extracts:

##### 1) Reducing Power method:

##### Principle –

This method is based on the principle of increase in the absorbance of the reaction mixture. Increase in the absorbance indicates increase in the antioxidant activity. In this, the antioxidant compound forms a coloured complex with potassium ferricyanide, trichloroacetic acid and ferric chloride, which is measured at 700nm. Increase in absorbance of reaction mixture indicates the reducing power of the samples.

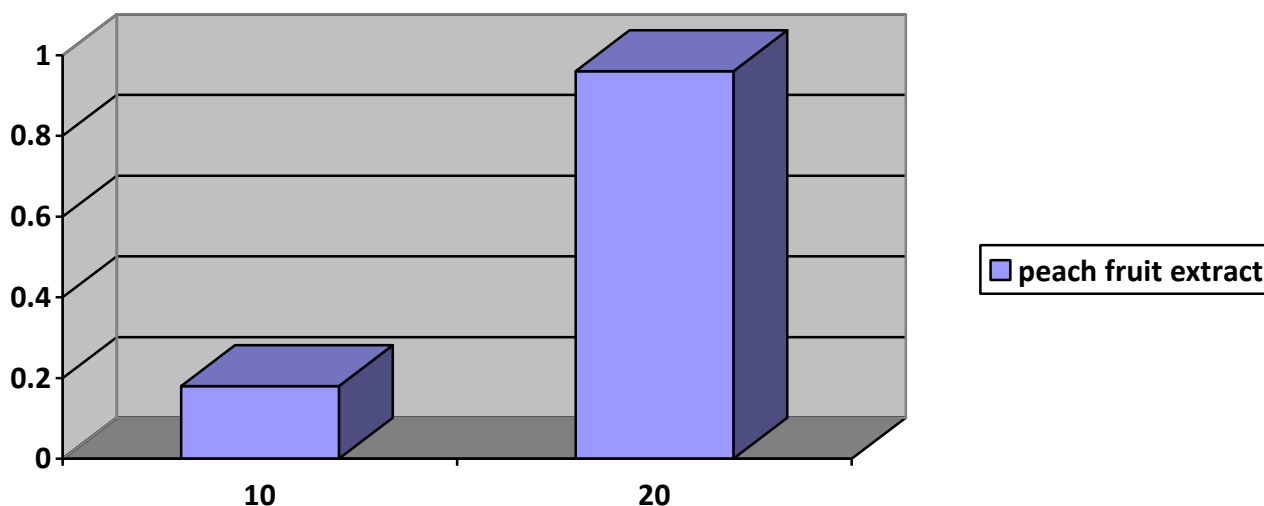
**Requirement -** UV Spectrophotometer, Incubator.



**Procedure** – The reducing power was assayed by taking different concentration of extract (1ml) from each other were mixed in different test tubes with 2.5 ml of phosphate buffer (pH-7) and 2.5 ml of 1% potassium ferric cyanide. The mixture was then incubated at 50°C for 20 minutes. Then 2.5 ml of trichloroacetic acid (10%) solution was added to the mixture, which was mixed for 15 minutes. Finally 1.25 ml of distilled water was mixed with 0.50 ml of FeCl<sub>3</sub> solution (0.1 w/v). The absorbance was measured at 700nm.

Sr. no.	Extract	Concentration (ug/ml) and absorbance	
		10	20
1	Peach fruit extract	0.18	0.96

Table No. 6: Determination of Antioxidant Activity of Extract



Graph No.1 : Determination of Antioxidant Activity of Extract

## 7.2 In Vitro Studies :

### [A] Determination of Ph

**Apparatus** :pH meter, preferably equipped with glass electrode.

**Principle** :The formulation of serum are meant for topical application. So their pH should be similar to that of skin. The skin has an acidic range and the pH of the skin serum should be in the range of 5 – 9 To ensure the required shelf life of skin serum, chemical inertness is essential i.e. it should neither be too acidic nor too alkaline. Based on above point it was through that the standard pH of skin should be in the range of 4 - 5.5

**Procedure :** Take 5gm of sample in a beaker and add 45 ml of distilled water in it. Mix it properly until the whole gel is dissolved in water, then note the pH of the sample mixture by using pH meter.

#### **[B] Determination of Viscosity :**

**Appartus :** Brook Field Viscometer.

**Principle :** The viscosity is the most important parameter in the evaluation of cosmetic product. Viscosity governs the many properties such as spreadability, pourability of the product from the container. As viscosity is affected by many factors such as change in temperature, change in manufacturing condition, quality of the raw material. Hence it is very important to measure the viscosity of product.

**Procedure :** The viscosity of serum was determined by using spindle no. 4 using brook field viscometer then all the operating conditions was set up. Then five readings were taken at different rpm and average of there will be the final reading. Viscosity was measured at 6 rpm in cps.

#### **[C] Determination of Spreadability Time :**

**Principle :** It is very important for any cosmetic product that after application the product must be easily spread over the skin. Spreadability is affected by many factors such as viscosity, temperature etc. The spreading time must be very less.

The apparatus consist of a wodden block, with a movable glass slide with one end tied to weighted pan rolled on pulley.

**Procedure :** 2 Gm of serum sample was placed on a surface. A slide was attached to a pan to which 20 gm weight was added. The time (seconds) required to seprate the upper slide from surface was taken as a measure of spreadability.

#### **[D] Microbial Examination of the Product :**

Cosmetics do not need to be sterile, but they must be adequately preserved. When consumers use cosmetics they repeatedly challenge the cosmetics with micro organism in salivaon dirty hands, in tap water. Microbial growth may occur in cosmetics and toiletry product like cream, lotion and gel and many more intended to be use as skin care preparation. Hence it is very important that the cosmetics product must be free from microbial contamination,so that it will ensure safety to product to the client. The cosmetic product must be safe and adequately preserved.

#### **Requirement :**

Media : Nutrient Agar

Apparatus : test tube, petri dish

**Procedure :** Sterilize the work area with disinfectant. Wash and dry thoroughly all the apparatus required. Prepare the dilution of the product take 1gm/ml of product and add to first test tube with pipette and shake it thoroughly then take 1ml from it in second test tube and prepare further dilution in same way.

#### **Total bacteria count :**

Weigh accurately required quantity of nutrient agar and add 50 ml of water in an autoclave conical flask. Autoclave it at 121°C for 15 min. When the temperature reduces to 45°C add 1ml of dilution of the product to autoclave petridish and add 20ml of nutrient agar medium and mix by rotating in the clock wise and anticlockwise direction Allow the plate to solidify. Incubate this plate for 48 hours at 37°C.

#### **Total Fungal Count :**

Pipette out in duplicate 1ml of pretreated sample aseptically into 5 sterile petridishes. Pour 15 to 20 ml of molten sabouraud's chloranphenicol agar (SCA) maintained at about 45°C mix the content of the plate by swirling. Allowing the plates to solidify, invert and incubated at 23+ 2 0 C for three days. Count the number of colonies in each plate.

#### **7.2.1 Stability study of serum**

The sample of serum was kept at 5°C, room temperature 40°C. The changes in physical appearance, colour, feel etc were studied.

SR.NO.	Parameters	M1	M2	M3
1	Appearance	Opaque	Opaque	Opaque
2	Colour	White	White	White
3	Spreadability	Good	Very good	Good

Table No.7 : Stability Studies of Serum

### 7.2.2 Accelerated Stability Studies :

#### [A] Cyclical Temperature Tests :

These tests are not carried out at fixed temperature and humidity. In this test, temperature was changed cyclically every day e.g. low-high-low-high to stimulate the changes in temperature daily.

Sr.no.	Parameter	M1	M2	M3
1	Freeze temperature	Stable	Stable	Stable
2	Room temperature	Unstable	Stable	Stable
3	High temperature	Unstable	Stable	Unstable

Table No.8 : Cyclic Temperature Test

### 7.3 Comparative study of formulated product and marketed product

The comparative studies were carried out to check and compare physical parameters of formulated product with marketed product.

The marketed product are selected for these purpose were :

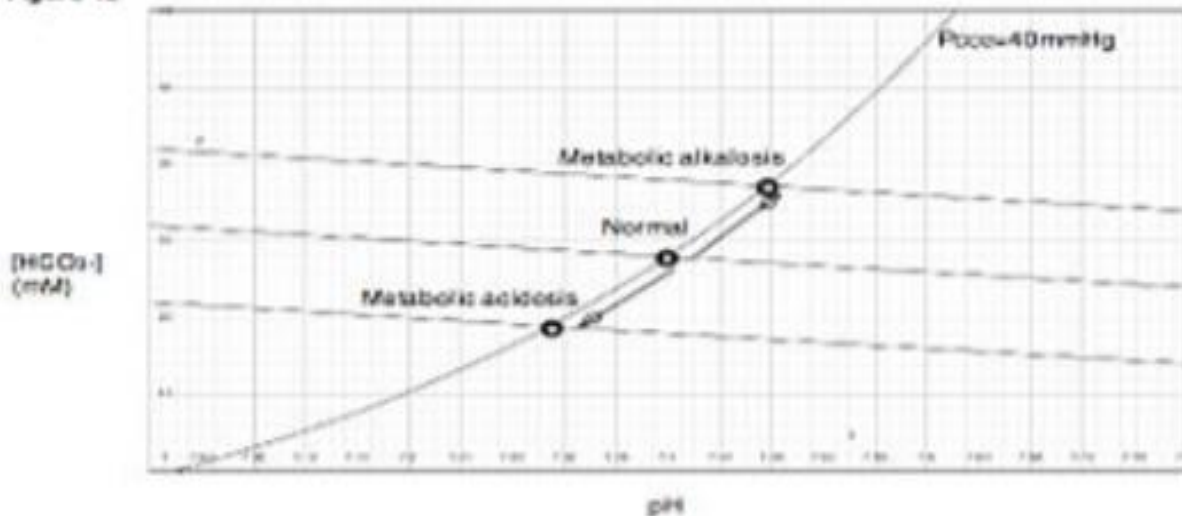
- 1) VLCC skin whitening serum,remove dark spots, lighten pigmentation.
- 2) Kaya, pigmentation reducing complex serum.
- 3) Lotus ,depigmentone serum.

These products were checked for their pH and Viscosity against formulated product.

Sr. No	Parameter	Formulated Product	Marketed Product
1.	pH	4.8	4.3

Table No. 9 : Comparative Between Formulated Ph and Marketed pH.

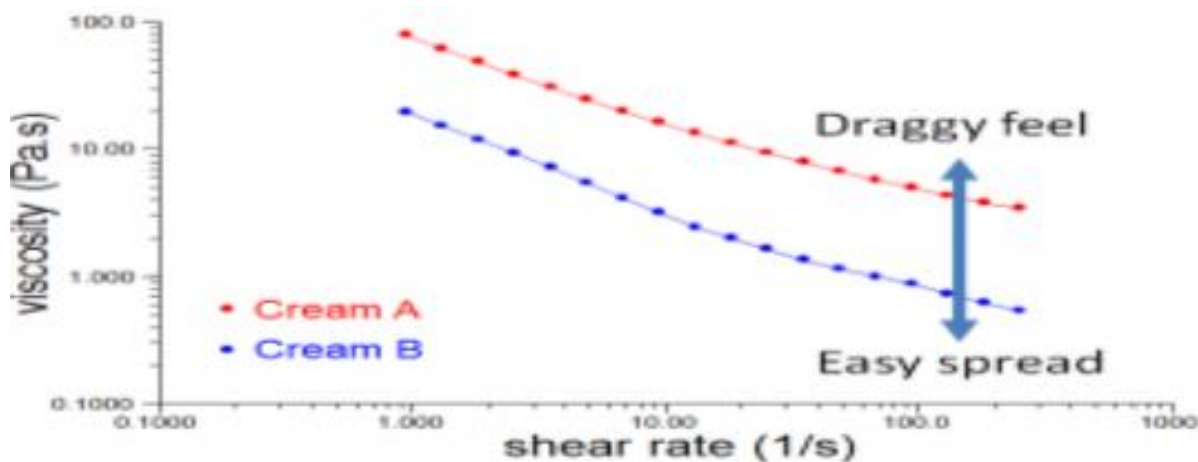
Figure 12



Graph No. 2 : Comparative between Formulated pH and Marketed pH.

Sr.no	Parameter	Formulated Product	Marketed Product
1.	Viscosity	13759	11870

Table No. 10 : Comparative between Formulated Viscosity and Marketed Viscosity.



Graph No. 3 : Comparative between Formulated Viscosity and Marketed Viscosity.

#### 7.4 In vivo studies:

##### [A] Patch test :

Patch test was performed on sensitive part of skin, e.g. bend of elbow, popliteal space of skin behind ears. The cosmetic was tested by applying to an area of 1 sq.cm of the skin. Central patches were also applied. The site of the patch was inspected after 24 hours. There was no reactions and then test was repeated once more on the same side. Since there was no reaction as the person was considered as not hyper sensitive and product pass the test.

##### Patch test for Serum:

Sr.no.	Parameter	M1	M2	M3
1	Immediately after removal of product	N.R.	N.R.	N.R
2	After 24 hrs	N.R.	N.R.	N.R
3	After 48 hrs	N.R.	N.R	N.R

Table No.11 : Patch Test of Serum

**N.R = No Reaction**

##### [B]Analysis of moisturizing property by using corneometer

Corneometer is device which is equipped with a moisture sensitive probe which is used to determine the accurate moisture content of stratum corneum. Hence it plays important role in determining the moisturizing activity of product on stratum corneum after its application on skin.

**Apparatus :** Corneometer equipped with a probe.

**Procedure :** First clean the hand with a soap and then dry it completely, then touch the probe on hand in order to note the initial reading of moisture of skin, then apply product on skin and wash it. Then again note the reading by touching the probe on the part of application of analysis of moisturizing property of serum.

##### [C] Determination of Melanin by using mexameter

**Apparatus:** Mexameter equipped with a probe

**Procedure :** First clean the hand with a soap and then dry it completely, then touch the probe on hand in order to note the initial reading of melanin contain in skin then apply product on skin then note the reading continuously 7 days by touching the probe on the part of application of analysis of melanin activity.

**[D] Photographic evaluation:**

Photographic evaluation is carried to see the effect of the product visually. To study whether the finished product were really effective formulated de pigment products have been subjectively studied. Four individual human volunteers of different age groups (30-50) were required to aassist in this research. The newly formulated de pigment products was requested to apply for 45 days. Photographs of their lower arm were taken before applying the products and then after 15 days, 30 days, 45 days of application of the antiageing products. The comparison can be easily made between two state side. Before applying the products and after applying the products. The difference between the skin before and after applying the formulated de pigment products were able to distinctly visualize easily.

**7.5 Test for antioxidant activity of serum:****1) Reducing Power method:**

**Principle** – This method is based on the principle of increase in the absorbance of the reaction mixture. Increase in the absorbance indicates increase in the antioxidant activity. In this, the antioxidant compound forms a coloured complex with potassium ferricyanide, trichloroacetic acid and ferric chloride, which is measured at 700nm. Increase in absorbance of reaction mixture indicates the reducing power of the samples.

**Requirement -**

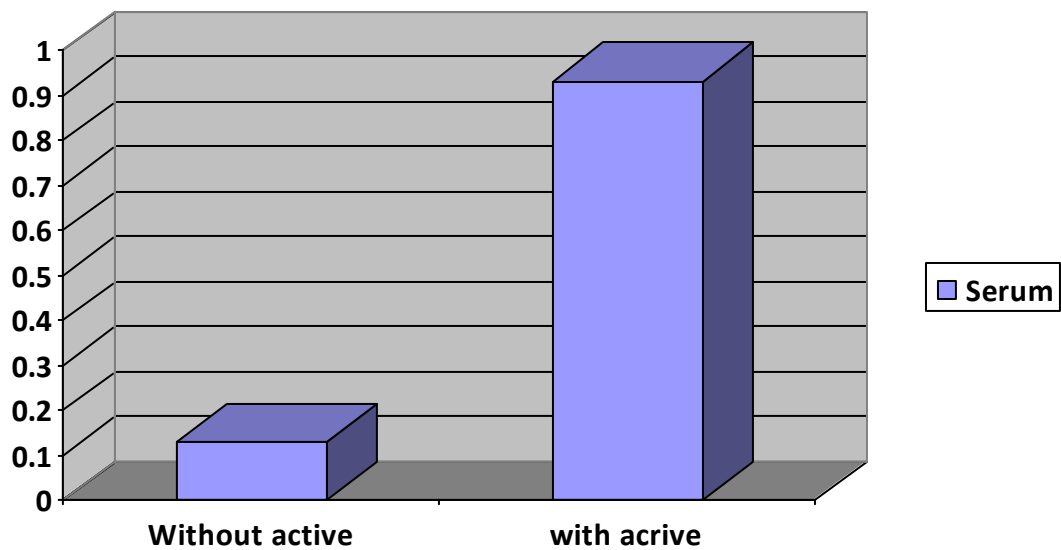
UV Spectrophotometer, Incubator.

**Procedure –**

The reducing power was assayed by taking different concentration of sample (1ml) from each other were mixed in different test tubes with 2.5 ml of phosphate buffer (pH-7) and 2.5 ml of 1% potassium ferric cyanide. The mixture was then incubated at 50°C for 20 minutes. Then 2.5 ml of trichloroacetic acid (10%) solution was added to the mixture, which was mixed for 15 minutes. Finally 1.25 ml of distilled water was mixed with 0.50 ml of FeCl<sub>3</sub> solution (0.1 w/v). The absorbance was measured at 700nm.

Sr. no.	Product	Absorbance	
		Without actives	With actives
1	Serum	0.13	0.93

Table No.12 : Antioxidant Activity of Serum



Graph No. 4. Antioxidant Activity of Serum



## 8. RESULTS

### Result and Discussion

#### 8.1 Evaluation of Extract:

##### 8.1.1 Preliminary Phytochemical Screening :

Sr. no.	Alkaloids	Flavonoids	Phenols	glycosides	Tannins	Lipids
2	Peach fruit extract	+	+	+	-	-

Here, + = Present , - = Absent

Table No. 13 : Preliminary Phytochemical Screening

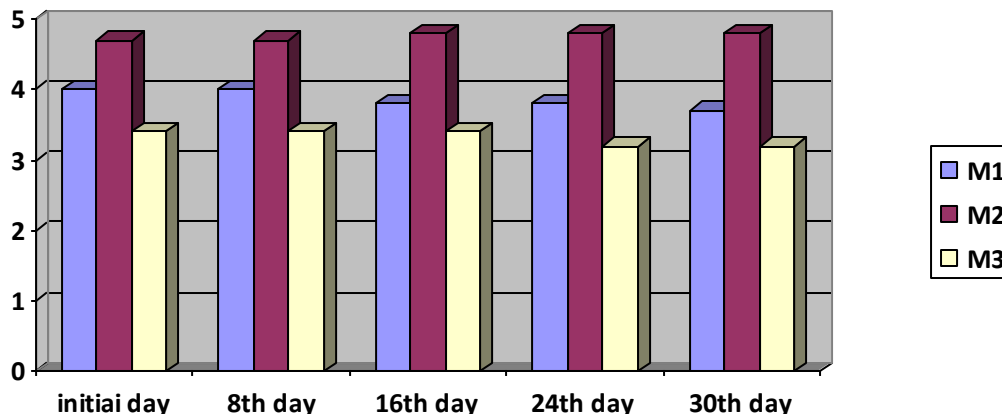
#### 8.2 in vitro studies :

##### [A] Determination of Ph

##### a) Determination of pH of serum incorporated with peach fruit extract:

Sr.no.	Time Interval	M1	M2	M3
1	Initial	4	4.7	3.4
2	8 <sup>th</sup>	4	4.7	3.4
3	16 <sup>th</sup>	3.8	4.8	3.4
4	24 <sup>th</sup>	3.8	4.8	3.2
5	30 <sup>th</sup>	3.7	4.8	3.2

Table No.14 : Determination of pH of Serum Incorporated with Peach Fruit Extract



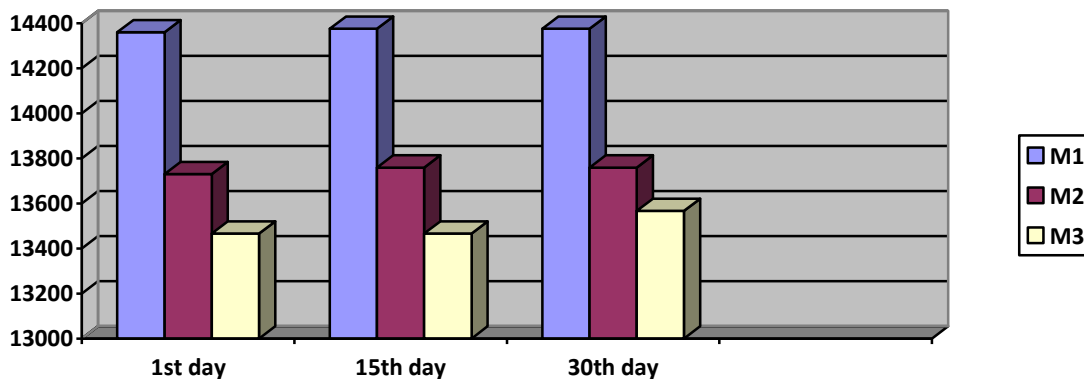
Graph No.5 : Graphical Representation of Ph of Serum with Peach Fruit Extract.

[B] Determination of Viscosity:

a) Determination of viscosity for serum incorporated with peach fruit extract :

Sr.no.	No. of days	M1	M2	M3
1	1 <sup>st</sup> day	14360	13730	13466
2	15 <sup>th</sup> day	14375	13759	13466
3	30 <sup>th</sup> day	14375	13759	13567

Table No. 15 : Determination of Viscosity for Serum Incorporated With Peach Fruit Extract

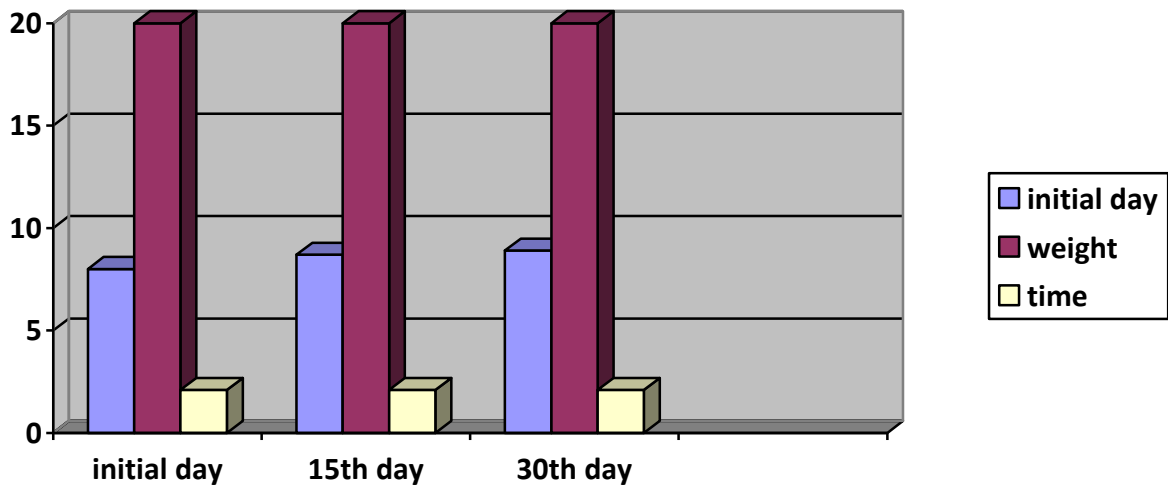


Graph No. 6 : Graphical Representation of Viscosity of Serum With Peach Fruit Extract:

**[C] Determination of Spreadability**

Sr.no.	Days of interval	Initial area	Weight	Time
1	Initial day	8cm	20gm	2.1 sec
2	15 <sup>th</sup> day	8.7cm	20gm	2.1 sec
3	30 <sup>th</sup> day	8.9cm	20gm	2.1 sec

Table No. 16 : Determination of Spreadability



Graph No.7 : Graphical Representation of Spread ability of Serum

**[D] Microbial Examination of Serum:**

Sr.no.	Test	Result	Specification	Unit
1.	Total microbial count	20 CFU/gm	NMT/100 CFU/gm	CFUgm
2.	Total fungal count	Nil	NMT/10 CFU/gm	CFUgm

Table No. 17 : Microbial Examination of Serum

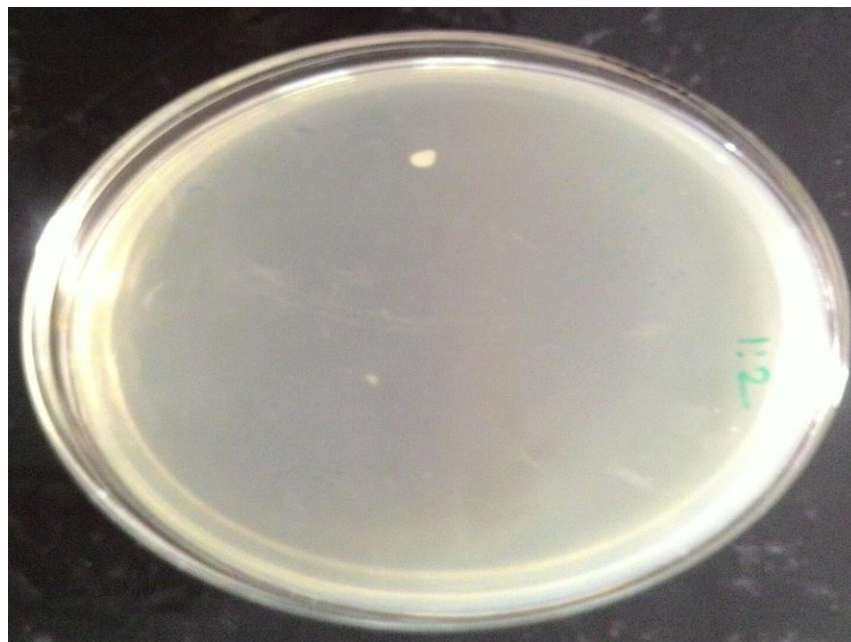


Fig. 11 : Total Microbial Count

**Result:**

The total microbial count of serum containing peach extract was found to be 20cfu/gm that is <100 cfu/gm. Therefore the serum passes the test.

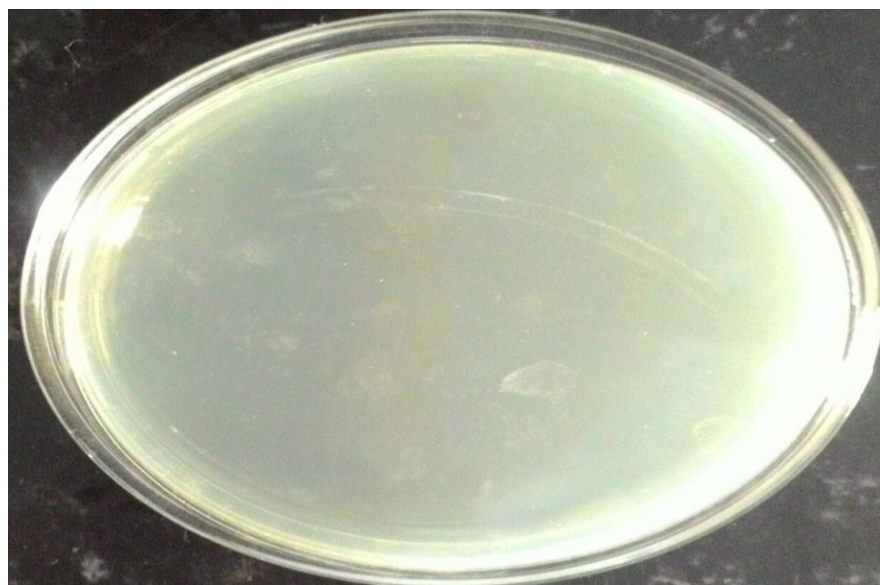


Fig. 12 : Total Fungal Count

**Result :**

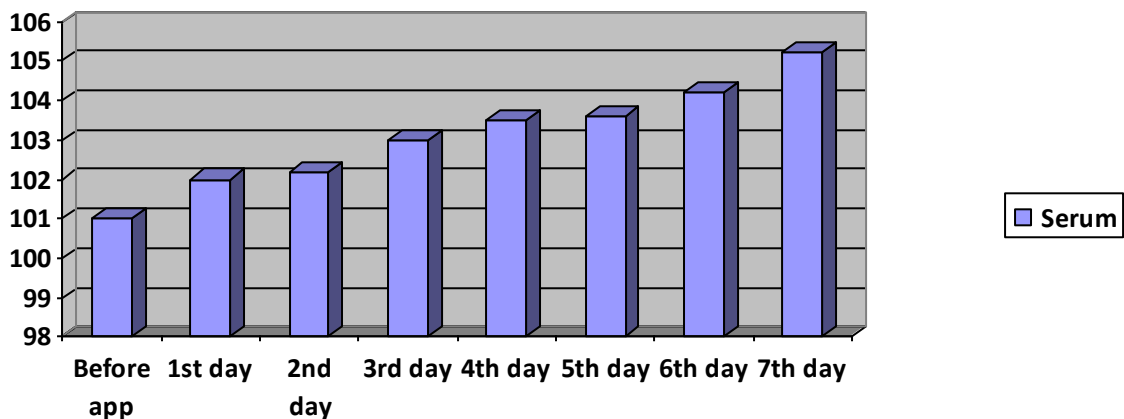
The total fungal count of serum containing peach fruit extract was to be nil. Therefore ,the serum passes the test.

**8.3 in vivo**

**8.3.1 Determination of Moisturizing Activity by Corneometer**

Product	Days	% of Moisture content
Serum	Before App	101
	1st day	102
	2nd day	102.2
	3rd day	103
	4th day	103.5
	5th day	103.6
	6th day	104.2
	7th day	105.2

Table No. 18 : Determination of Moisturizing Activity by Corneometer

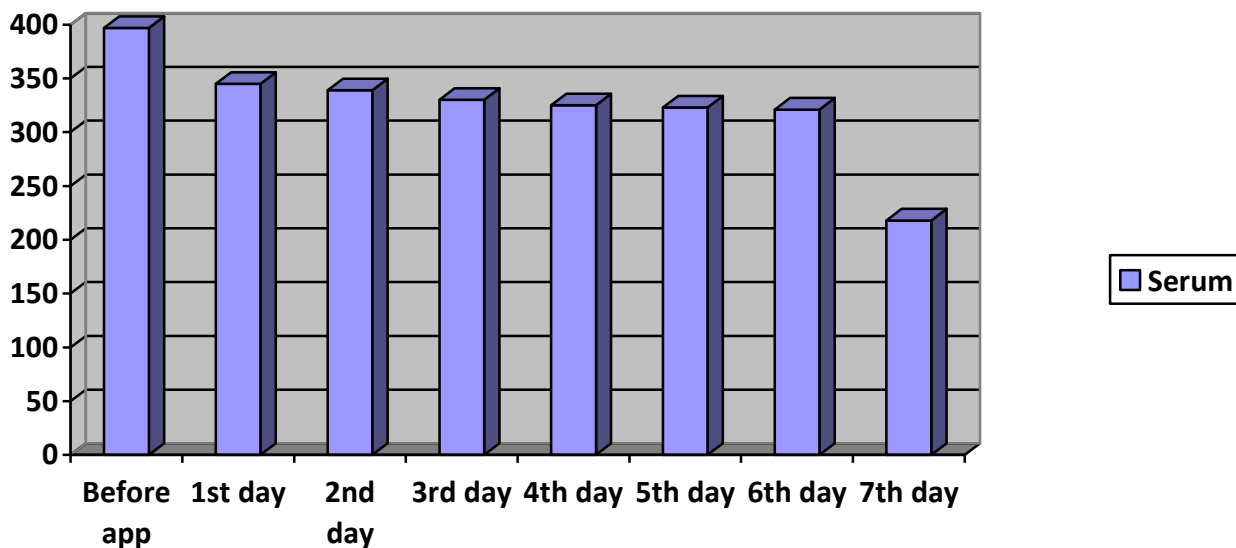


Graph No.8 : Graphical Representation of Determination of Moisturizing Activity of Serum

**8.3.2 Determination of Melanin by Mexameter**

Product	Days	% of melanin present
Serum	Before App	397
	1st day	345
	2nd day	339
	3rd day	330
	4th day	325
	5th day	323
	6th day	321
	7th day	218

Table No. 19 : Determination of Melanin by Mexameter



Graph No.9 : Graphical Representation of Determination of Melanin by Using Mexameter

**8.3.3 Photographic Evaluation**

**Before**



Fig.13 : The Skin Before Application

**After**



Fig. 14 : The Skin After Application

**Result-** The above photographs shows that the dark spots minimized within 15 days of application of the product.

## 9. SUMMARY

The present study was conducted with a view to formulate and evaluate on dark spots on face for formulation by using peach fruit extract. The serum was prepared with natural active agents. The concentration of active agent were kept in range of 0.5:2%, 1:3%, 2:4%, each were incorporated and three combination of each were prepared.

Antioxidant testing of extract was done by Reducing power method. The Antioxidant activity was determined by power reducing method which showed that high absorbance which indicates it has good antioxidant property. Moisturizing activity was determined by using corneometer. The moisture content of skin increases with the continuous use of product.

In the present work de-pigmentation formulation gave satisfactory good moisturizing, antioxidant property and this is achieved by the use of natural actives like peach fruit extract.

The serum was prepared by the conventional procedure and all the factors, parameters such as pH, viscosity, stability, microbial analysis were determined. It was also kept accelerated by stability testing for 30 days. The moisturizing property was determined by corneometer and conductivity method. Then the product i.e. serum was applied on human volunteers and progressive effective result were found. Thus photographs of before application and after application was taken out from photographs. Thus the formulation M2 of serum containing 2% peach fruit extract were found stable and gave most effective results.



## 10. CONCLUSION

At present because of availability of wide range of cosmetic products in market, consumers are giving special attention towards the selection of cosmetic product to develop a well standard formula, the new product viz. herbal de-pigment serum was formulated by incorporating active extract singly and also in combination for good effect.

Thus M2 of serum with peach fruit extract were found to be most effective and stable.

Thus, conclusion can be made that the serum containing peach fruit extract have been able to remove dark spots and other signs also moistens the skin without any side effect making skin soft smooth and supple.

## **11. FUTURE SCOPE**

The scope of present result is that the formulated product i.e. serum with different concentration of active showed an effective against dark spots property for longer time. serum also soothe and moisten the skin for long time. Development of de-pigment products can be done with different actives or herbal extract which will give more effective results. Formulation of serum can be done with novel technology.

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