2.1. Sun Protection Factor (SPF):

The Sun Protection Factor (SPF) of a sunscreen is a clinical measure of the effectiveness of a sunscreen; the higher the SPF, the more protection a sunscreen offers against UV-B (the ultraviolet radiation that causes sunburn). Introduced across several decades, the SPF method is based on a human end-point well-known in biology: the minimal erythema dose (MED), mainly due to the ultraviolet B (UVB) part of the sun spectrum radiations. MED is the minimal dose of UV B that causes skin burn/erythema. UV causes significant number of adverse effects in the exposed skin. UVB irradiation has been demonstrated to produce reactive oxygen species (ROS) in the cells and skin, which induces the synthesis of matrix metalloproteinases (MMPs), causing skin photoaging (Ho J N et al., 2005). UV irradiation increases ROS production via Protein kinase C delta (PKCdelta) signaling in primary murine fibroblasts (Bossi O et al., 2008). Ultraviolet-B (UVB) irradiation has been demonstrated to produce melanosomal damage in normal human melanocytes and increased intracellular melanin is essential for protection against UV radiations (Gidanian S et al., 2008, Kowalczuk C et al., 2001).

The SPF indicates the time a person can be exposed to sunlight before getting sunburn with a sunscreen applied relative to the time they can be exposed without sunscreen. For example, someone who would burn after 12 minutes in the sun would expect to burn after 2 hours (120 min) if protected by a sunscreen with SPF 10.

$$SPF = \frac{\text{Time of exposure before getting sunburn with a sunscreen}}{\text{Time of exposure before getting sunburn without a sunscreen}} = \frac{120}{12} = 10$$

The SPF can be measured clinically, by applying sunscreen to the skin of a volunteer and measuring how long it takes before sunburn occurs when exposed to an artificial sunlight source (Colipa, 2006). In the US, such an in vivo test is required by the FDA. Sun Protection Factor had so far been determined clinically in human volunteers, following the international Sun Protection Factor test method (Colipa, 2006).
In vitro determination of Sun Protection Factor was earlier done by spectroscopy for various sunscreens (Oliveira S L et al., 2008). Various comparisons were drawn for Clinical SPF and in vitro SPF determined by spectroscopy for various sunscreens. It was observed that the correlation was not convincing enough especially for those sunscreens where the SPF was very high (Santos E P et al., 1999 and Heinrich U et al., 2004).

2.2. Types of Sunscreens:

Ultraviolet Light Absorbers are molecules used in organic materials (polymers etc.) to absorb UV light to reduce the UV degradation (photo-oxidation) of a material. A number of different UV absorbers exist with different absorption properties. In sunscreens, ingredients that absorb UVA/UVB rays, such as avobenzone and octyl methoxycinnamate, are known as absorbers. They are contrasted with physical "blockers" of UV radiation such as titanium dioxide and zinc oxide. Sunscreen prevents the direct DNA damage that causes sunburn. Most of these products contain Sun Protection Factor (SPF) rating to show how well they block UVB rays. UV protection can be provided from within also. Many of the antioxidants like Ascorbic acid etc., are known to provide UV protection from within. In recent years, improved filtering substances have come into use in commercial sunscreen lotions that don't significantly degrade or lose their capacity to protect the skin as the exposure time increases (photostable substances) (Bissonnette R, 2010). Some of the sunscreen products containing photostable filters are Drometrizole trisiloxane, Bisoclitizole or Bemotrizinol and Ecamsule (Bissonnette R, 2010).

2.3. Protection from UV and importance of safe Sunscreens:

Medical organizations recommend that people protect themselves from UV radiation using sunscreen. However, some sunscreen chemicals produce potentially harmful substances if they are illuminated while in contact with living cells (Knowland J et al., 1993; Damiani E et al., 1999 and Xu C et al., 2001). The amount of sunscreen that penetrates through the stratum corneum may or may not be large enough to cause damage. The question whether UV filters act on or in the skin has so far not been fully answered. Despite the fact that an answer would be a key to improve formulations of sun protection
products, many publications carefully avoid addressing this question. In an experiment by Hanson et al., the amount of harmful reactive oxygen species (ROS) was measured in untreated and in sunscreen treated skin. In the first 20 minutes the film of sunscreen had a protective effect and the number of ROS species was smaller. After 60 minutes, however, the amount of absorbed sunscreen was so high, that the amount of ROS was higher in the sunscreen treated skin than in the untreated skin (Hanson K M, 2006). Sunscreen protects only against the direct DNA damage, but increases the indirect DNA damage (Knowland J et al., 1993; Damiani E et al., 1999 and Xu C et al., 2001). Some studies suggest that this may be the cause of the higher incidence of melanoma found in sunscreen users compared to non-users (Garland C et al., 1992; Autier P et al., 1995; Weinstock M A, 1999; Westerdahl J et al, 2000 and Vainio H and Bianchini F, 2000). Studies have demonstrated that UV-irradiated sunscreen components such as titanium dioxide (TiO$_2$) promote the photogeneration of reactive oxygen species (ROS) in cultured human skin fibroblasts (Shen B et al., 2006). Although TiO$_2$ is known to have high SPF as per the clinical SPF measurement in humans, it was observed to promote ROS in human skin fibroblasts. This indicates that although visible effects are not observed on skin during the clinical SPF testing, there is a probability that the cells do get affected which may be manifested eventually. Such intrinsic cell damages cannot be investigated in a clinical trial in humans. Moreover, the effect of the product may be visible after a long time. Hence, studies in cell culture can be fast and even the minute cell adversaries can be studied in a short period of time. However, clinical SPF testing has certain benefits like testing in wide variety of skin types from normal to highly sensitive skin. Only the significant compounds screened by in vitro methodology for UV protection, with no adverse effects in cells and with best efficacy can be finally studied clinically as a confirmative study. Hence, on ethical grounds the frequency of clinical testing can be reduced and reliable products with significant efficacy and no adverse effects can be put into the market. Synthetic sun screens have safety concerns and can cause phototoxicity. Synthetic sunscreen Titanium dioxide (TiO$_2$) resulted in TiO$_2$-photosensitized ROS generation in cultured human skin fibroblasts (Shen B et al., 2006). For an attempt to develop safe materials protecting solar UV induced skin damage plant extracts were evaluated for their inhibitory activities of free radical generation and arachidonic
acid/metabolites release from UVB-irradiated normal human keratinocytes (Ramos M F S et al., 2007 and Kim Y H et al., 2000). The safety of new ultraviolet filters is assessed by an initial in vitro screen including photostability, cytotoxicity, photocytotoxicity, genotoxicity, and photogenotoxicity tests (Nohynek G J and Schaefer H, 2005). Plant extracts having phenolic compounds provide protection from harmful UV irradiation (Cle C et al., 2008). A significant relationship between antioxidant capacity and total phenolic content was found, indicating that phenolic compounds are the major contributors to the antioxidant properties of these plants (Dudonne S et al., 2009). Antioxidants from plant extracts are screened for their efficacy in scavenging reactive oxygen species by in vitro methodologies (Bergman M et al., 2003).

Appreciable photoprotection can be obtained from the combination of topical vitamins C and E. Natural products may protect against skin cancer and photoaging (Lin J Y et al., 2003). Phenol-oxidizing peroxidases in plants concurrently contribute to UV protection as well as the control of leaf and plant architecture (Jansen M A et al., 2001). Phenolic sunscreens in soybean are highly responsive to the wavelengths that are most affected by variations in ozone levels, and that they play an important role in UV protection (Mazza C A et al., 2000). Dicaffeoylquinic acids present in plant extracts of Globe artichoke play a significant role in photoprotection (Moglia A et al., 2008). Carotenoids are efficient in photoprotection, scavenging singlet oxygen and peroxyl radicals. An increased consumption of carotenoids may contribute to life-long protection against UV-induced damage (Sies H and Stahl W, 2004). The principal UV-A absorbers in the cuticle of apple were identified as quercetin glycosides. On the shaded side of apple fruit, both UV-A and UV-B absorption by the peel is, to a large extent, governed by cuticular phenolics, whereas on the sunlit surface, the absorption of the peel in the UV-A range is determined mainly by vacuolar peel flavonoids (Solovchenko A and Merzlyak M, 2003).
2.4. Photoprotection and Biological strategies:

2.4.1. Topical Photoprotection:

There are different strategies for protection of skin against UV-dependent damage. Most simple ones are avoidance of sun exposure and wearing protective clothing as well as topical application of sunscreens, generally recommended during times of intense exposure, e.g., during holidays or stays at high altitude. Human skin is protected against UV radiation by melanins, endogenous pigments that scatter and absorb UV light (Ortonne J P, 2002). Upon sun exposure, pigmentation is enhanced by stimulated synthesis of melanin in the epidermal melanocytes (sun-tan). The risk for UV-related skin disorders is correlated with pigmentation; the darker the skin, the lower the risk. The use of sunscreens for topical protection is promoted as an integral part of skin cancer prevention programs (Moloney F J et al., 2002). In most of the sunscreens, UV-absorbing compounds and inorganic pigments like titanium dioxide or zinc oxide are combined. Thus, absorption, reflection, and light scattering are the chemical and physical principles of protection. The effectiveness of sunscreens to protect against UVB is denoted by the SPF, which is determined following standardized methods (Griffin M E and Bourget T D, 1997). SPF is calculated as the ratio of the MED measured on protected skin over the MED of unprotected skin. In the assay 2 mg of sunscreen are applied per cm$^2$ of skin (Ferguson J, 1997 and Griffin M E and Bourget T D, 1997).

2.4.2. After sun care:

“After sun care” actives are the ones which have significant benefits for UV damaged skin. These are especially antioxidant and anti-inflammatory actives that have the potential to reduce UV induced adversaries like generation of free radicals and inflammatory markers which damage the skin. All sunscreens or UV blockers need not necessarily be “After sun care” products and all “After sun care” products need not necessarily be sunscreens or UV blockers. Even though some of the actives do not show significant UV protection potential, they can be potential “After sun care” actives.
Although “After sun care” actives need not necessarily act as sun screens or UV blockers, they can reduce the effects of UV exposure. “After sun care” actives act by

- Protecting target molecules by acting as scavengers; e.g., antioxidants
- Repairing UV-induced damage by induction of repair systems
- Suppressing cellular responses; e.g., anti-inflammatory agents

2.4.3. Endogenous Photoprotection:

Systemic photoprotection through endogenous supply of components provides an important contribution to the defense against UV effects. Dietary micronutrients include efficient antioxidants capable of directly scavenging lipophilic and hydrophilic prooxidants or serving as constituents of antioxidant enzymes. Carotenoids, tocopherols, flavonoids and other polyphenols as well as vitamin C contribute to antioxidant defense and may also contribute to endogenous photoprotection. The levels of antioxidant vitamins and micronutrients in skin vary with respect to skin area and skin layer. High levels of carotenoids are found in skin of the forehead, palm of the hand, and in dorsal skin; lower levels are found in skin of the arm and the back of the hand. Vitamins E and C are lower in the dermis than in the epidermis. The stratum corneum contains amounts of tocopherol similar to that in the epidermis with increasing levels at inner layers (Thielle J J et al., 1998). Polyunsaturated fatty acids and retinoids play a role during inflammatory reactions and cellular signaling and might thus serve in systemic photoprotection. In vitro data provide evidence that dietary micronutrients like β-carotene interact with UVA in the cell and prevent the induction of photoaging-associated DNA mutations (Eiker J et al., 2003).

2.5. Conventional SPF testing and its limitations:

In vivo method is time-consuming, impossible to run all year round and expensive – not to mention the ethical problems of testing on humans. Moreover, it is an imperfect measure of skin damage because invisible damage such as free radical generation, generation of inflammatory markers, DNA damage, skin ageing and triggering of these
mechanisms which does not cause visible reddening or pain is not detected by the clinical SPF method. For example, as already mentioned above, someone who would show erythema after 12 minutes in the sun would show erythema after 2 hours (120 min) if protected by a sunscreen with SPF 10. But the invisible skin damage like free radical generation etc. would have occurred after 1 hour itself. So the actual SPF would probably be 5. As mentioned earlier, although TiO$_2$ was rated to have a high SPF, its effect on ROS generation on UV exposure could not be detected by the clinical SPF method. Hence, a methodology for UV protection with indications on intrinsic damage to the skin cells would be a prerequisite for conducting a confirmatory and elaborate clinical trial in humans.

2.6. In vitro testing for SPF:

There is a constant effort for developing in vitro models for determining SPF due to the lack of consensus for an in vivo method. For economical, practical and ethical reasons, a reliable in vitro measurement of the SPF which seemed particularly useful as a supplement to the in vivo SPF was so far based on the physical and optical determination of the reduction of the energy in the UV range, through a film of product which has previously been spread on an adequate substrate. It can be measured in vitro with the help of a specially designed spectrometer. In this case, the transmittance of the sunscreen is measured over all wavelengths in the UV-B range (290–350 nm) (Oliveira et al., 2008). According to the comparative in vitro and in vivo SPF determination reported where three sunscreen lotions containing 2, 4.5 or 7.5% Octyl methoxycinnamate (OMC) were studied, the results indicate that there was a good correlation between the in vitro and in vivo determinations for the sunscreen lotions examined (Santos et al., 1999). However, in case of sunscreen products which gave high SPF values of 25 and above, the correlations with in vitro spectrophotometric methods was not satisfactory (Heinrich et al., 2004). The main reason for this could be the absence of a living system in the spectrophotometric in vitro methodology. The most appropriate in vitro method so far adopted three-dimensional models like the dermal equivalent composed of a porous collagen-glycosaminoglycans-chitosan matrix populated by normal human fibroblasts and the skin
equivalent comprising of fully differentiated epidermis to study the deleterious effects of UV and photoprotection trials of sunscreen formulations (Augustin C et al., 1997). Human skin reconstructed *in vitro* is used as a model to study the keratinocyte, the fibroblast and their interactions, photodamage and repair processes (Bernerd F, 2005). Phototoxic effect of compounds is determined according to the Organization for Economic Co-operation and Development (OECD) guidelines *in vitro* in cell cultures (OECD, 2002). *In vitro* cell viability is determined by various techniques Sulforhodamine B staining, Neutral red uptake, and MTT staining (Vichai V et al., 2006, Repetto G et al., 2008 and Edmondson J M et al., 1988). The present study for the development of *in vitro* SPF determination in cell lines is followed in the same principles of the OECD guidelines.

2.7. Effect of UV on skin melanogenesis:

Ultraviolet B radiation (UVB) elicits an increase in melanin production in mammalian skin. The mechanisms regulating this process are not understood, although it is well documented that there is an increase in the number of melanin-producing melanocytes. The melanotropins like MSH are a family of peptides that increase the melanin content of melanocytes through an interaction with high affinity receptors. The effects of UVB on melanogenesis may be mediated through an increase in MSH receptor activity on melanocytes. UVB-irradiated cultures displayed 2-10-fold increases in MSH binding capacity over that of unirradiated control cultures (optimum doses 10-20 mJ/cm²). UVB and MSH potentiate one another in promoting cutaneous melanogenesis in both mice and guinea pigs. In the areas of guinea pig skin that received both UVB and MSH, there was a fivefold increase in active melanocytes/mm² over the sum of active melanocytes/mm² in areas receiving either MSH or UVB separately. UVB light causes an increase in MSH receptor activity on cutaneous melanocytes, thus increasing cellular responsiveness to MSH. Implicit in this mechanism is a transduction of radiant energy into chemical energy during the process of UVB-induced melanogenesis (Bologna J et al., 1989).

UV also causes free radical generation and these free radicals further regulate skin pigmentation (Bogdanov G et al., 1978). Variations in human pigmentation among different racial groups are due to differences in the production and deposition of melanin
in the skin. Although melanin synthesis is known to be controlled by the rate-limiting enzyme tyrosinase, the role of this enzyme as the principal determinant of skin pigmentation is unclear. Results from studies with human melanocyte cultures derived from different racial skin types reveal an excellent correlation between the melanin content of melanocyte cultures and the in situ activity of tyrosinase. Melanocytes derived from black skin have up to 10 times more tyrosinase activity and produce up to 10 times more melanin than melanocytes derived from white skin. However, the higher level of tyrosinase activity in melanocytes derived from black skin is not due to a greater abundance of tyrosinase. Results from immunotitration experiments and Western immunoblots reveal that the number of tyrosinase molecules present in white-skin melanocytes may equal the number found in highly pigmented black skin types. Moreover, approximately equivalent levels of tyrosinase mRNA are present in white and black skin cell strains. In contrast, melanocytes derived from red-haired neonates with low tyrosinase activity contain low numbers of tyrosinase molecules and low levels of tyrosinase mRNA. These results show that tyrosinase activity and melanin production in most light-skinned people is controlled primarily by a post-translational regulation of pre-existing enzyme and not by regulating tyrosinase gene activity. In contrast, melanocytes from red-haired (type I) people have low levels of tyrosinase protein and mRNA, suggesting that transcriptional activity of the tyrosinase gene is suppressed (Ken I et al., 1993). The expression of tyrosinase in melanocytes relates to skin pigmentation or depigmentation. Tyrosinase, a type I membrane glycoprotein, is synthesized and glycosylated in the endoplasmic reticulum (ER) and Golgi. The enzyme is subsequently transported to melanosomes where it participates in melanogenesis. Previous studies showed that the disruption of early ER N-glycan processing by deoxynojirimycin (DNJ), an inhibitor of α-glucosidase, suppresses tyrosinase enzymatic activity and melanogenesis (Hyunjung C et al., 2007). Absence of functional tyrosinase results in amelanotic melanoma (Karime A et al., 2001). Tyrosinase is a type I membrane glycoprotein essential for melanin synthesis. Mutations in tyrosinase and impairment of glycosylation result in albinism (Ujvari A et al., 2001). Inhibition of phenylalanine hydroxylase also deactivates tyrosinase but improper functioning of phenylalanine hydroxylase results in phenylketonuria (Eisensmith R and Woo S, 1992).
In mammals, adrenocorticotropic hormone and α-melanocyte-stimulating hormone (α-MSH) regulate melanogenesis, and thus skin pigmentation, by activating the cyclic adenosine 3′,5′ monophosphate (cAMP) pathway through the melanocortin type 1 receptor (MC1-R). One limb of this pathway involves a Protein kinase A (PKA)-dependent stimulation in the expression of the microphthalmia-associated transcription factor (MITF). MITF, in turn, stimulates expression of tyrosinase, the rate-limiting enzyme in melanogenesis (Khaled M et al., 2002). Prolonged exposure to high levels of cAMP results in accumulation of melanin and terminal differentiation of human melanocytes. Here we present evidence that activation of a cAMP pathway correlates with multiple cellular changes in these cells: increased expression of the transcription factor microphthalmia and increased melanogenesis (Maher M et al., 1999). The most common origin of red hair and pale skin in humans is found in a tiny pouch-like receptor, called MC1-R, on the surface of melanocytes. When the hormone α-MSH drops into the pouch, it causes a surge in the melanocyte's production of the chemical cAMP. cAMP then stimulates melanocytes to turn on a large number of genes, causing a pigment called melanin to be produced. If cAMP levels are low, the melanocytes make red/blond melanin. If cAMP levels are high, they make brown/black melanin. Less cAMP means less red/blond pigment production, which results in fair skin (http://news.biomedicine.org/biology-news-3/New-insight-into-skin-tanning-process-suggests-novel-way-of-preventing-skin-cancer-5019-4/).

Inhibitors of melanogenesis are screened for their efficacy by in vitro methodologies (Mas-Chamberlin C et al., 2004). Many melanocyte or skin equivalent models have been used to evaluate the potential efficacy of melanogenic compounds to regulate pigmentation (Lei T C et al., 2002). Skin equivalents are prepared by seeding mixtures of cultured human keratinocytes and melanocytes in various ratios onto de-epidermized dermis. Histological examination revealed a structure that closely resembled human interfollicular epidermis. Melanocytes, identified by their dendritic appearance, positive dopa reaction and positive staining with a melanocyte-specific antibody (MEL5), are located in the basal layer. Melanin is seen both in melanocytes and in neighbouring keratinocytes (Todd C et al., 1993). Hyperpigmentation frequently accompanies chronic
or acute inflammation. A number of inflammatory mediators have been shown to stimulate melanin synthesis in human melanocytes (Masaki Y et al., 2000).

Although many types of drugs with whitening effects are well known, neither the definite effect nor the mechanism underlying the effect has been elucidated (Nagaoki W et al., 1991).

### 2.8. Actives for UV protection and skin lightening:

#### 2.8.1. Synthetic sunscreens:

In an *in vitro* study, SPF was determined by applying 15 mg of product on the Polymethylmethacrylate (PMMA) plate. The following ranking was drawn up in ascending order of efficacy: 3-Benzylidene camphor (1.66) < oxybenzone (3.01) < octylsalicylate (3.12) < PABA (3.36) < polysilicone 15 (3.64) < methylene bis-benzotriazolyl tetramethylbutylphenol (3.68) < PEG25 PABA (3.81) < benzophenone-4 (3.85) < 4-methylbenzylidene camphor (4.22) < homosalate (4.33) < octyltriazone (7.80) < phenylbenzimidazole sulfonic acid (8.31) < octyldimethyl PABA (8.71) < octocrylene (10.41) < octyl methoxycinnamate (10.42) (Couteau C et al., 2007). Octyl methoxycinnamate (Fig. 2.1) was the most frequently used UV filter, present, in 49% of the sunscreen products (Rastogi S C, 2002). Therefore, **Octyl methoxycinnamate (OMC)** is considered as a significant sunscreen active and is a suitable active that can be used as a reference standard for screening methodologies for UV protection and SPF analysis.

![Figure 2.1: Structure of Octyl methoxycinnamate](image)
2.8.2. *Kaempferia galanga*, the natural source of Ethyl paramethoxycinnamate (EPMC):

*Kaempferia galanga* (Fig. 2.2) is a natural source of **Ethyl paramethoxycinnamate (EPMC)** (Fig. 2.4) which is structurally similar to OMC. *Kaempferia galanga*, commonly known as kencur, aromatic ginger, sand ginger, cutcherry or resurrection lily, is a monocotyledonous plant in the ginger family. It is found primarily in open areas in southern China, Taiwan, Cambodia and India, but is also widely cultivated throughout Southeast Asia. The rhizomes of aromatic ginger have been reported to include cineol, borneol, 3-carene, camphene, kaempferol, kaempferide, cinnamaldehyde, **p-methoxycinnamic acid (PMC)**, ethyl cinnamate and **ethyl p-methoxycinnamate (EPMC)** (Taufikkurohmah T, 2005). EPMC and **Para methoxycinnamic acid** (Fig. 2.3) are structurally similar to OMC and are therefore potential actives for UV protection efficacy. Supercritical carbon dioxide extraction of EPMC from *Kaempferia galanga* rhizome and its apoptotic induction in human HepG2 cells is reported. By using supercritical carbon dioxide extraction, the yield of EPMC identified by gas chromatography mass spectrometry (GC-MS) was as high as 2.5% with respect to the raw materials (Liu B et al., 2010). Apart from being a potential active for UV protection efficacy, unlike many other sunscreen actives, EPMC is know for its anti cancer activity. It was found that EPMC could inhibit the proliferation of the human hepatocellular liver carcinoma HepG2 cell line in a dose-dependent manner and induce the significant increase of the subG0 cell population. After treatment with EPMC, phosphatidylsereine of HepG2 cells could significantly translocate to the surface of the membrane. The increase of an early apoptotic population was observed by both annexin-fluorescein isothiocyanate (FITC) and propidium iodide staining. It was concluded that EPMC not only induced cells to enter into apoptosis, but also affected the progress of the cell cycle (Liu B et al., 2010).
2.8.3. Stilbene compounds from plants:

Stilbenes are C6 (aromatic)-C2-C6 (aromatic) compounds that are biogenetically produced through the mixed shikimate–acetate pathway. They have been found in nature as monomers and oligomers. Stilbenes have been vastly studied for their therapeutic applications like anti diabetic, anti cancer etc. Based on the literature evidences, stilbene compounds can be promising actives for cosmetic applications on account of their significant antioxidant and anti inflammatory potential. Therefore a study on the mechanism of action of stilbenes for cosmetic benefits such as UV protection and skin
lighten ing is of significance. Based on their mechanisms of action for UV protection and skin lightening in comparison to other potential actives, an appropriate ranking in the order of their efficacy can enable the claim substantiation for target specific cosmetic applications. Stilbenes are naturally present in various plant sources.

*Polygonum cuspidatum* (Fig. 2.5) also known as Japanese knotweed, is a rich source of stilbene compound Resveratrol (Fig. 2.6) replacing grape byproducts. Resveratrol is present in the roots of *Polygonum cuspidatum*. Many large supplement sources of resveratrol now use Japanese knotweed and use its scientific name in the supplement labels. The plant is useful because of its year-round growth and robustness in different climates. Resveratrol at 10µM increased cell proliferation and also achieved the most effective photoprotection (Caddeo C *et al*., 2008). Resveratrol imparts chemopreventive effects against UV-B exposure-mediated damages in SKH-1 hairless mouse skin via inhibiting Survivin and the associated events (Aziz M H *et al*., 2005). Resveratrol may be useful for the prevention of UVB-mediated cutaneous damages including skin cancer (Reagan-Shaw S *et al*., 2004). NF-kappaB pathway plays a critical role in the chemopreventive effects of resveratrol against the adverse effects of UV radiation including photocarcinogenesis (Adhami V M *et al*., 2005). Therefore, it is conceivable to design resveratrol-containing emollient or patch, as well as sunscreen and skin-care products for prevention of skin cancer and other conditions, which are believed to be caused by UV radiation (Aziz M H *et al*., 2005). Stilbenes are also known for significant skin lightening applications. (4-Methoxy-benzylidene)-(3-methoxy-phenyl)-amine, a nitrogen analog of stilbene, inhibited the tyrosinase activity, which converts dopa to dopachrome in the biosynthetic process of melanin, and showed a UV-blocking effect at UV-B band. The compound also exhibited SOD-like activity, which is involved in the protection against auto-oxidation and inhibited melanin production in melan-a cell line. Therefore (4-methoxy-benzylidene)-(3-methoxy-phenyl)-amine might be used as a skin whitening agent (Choi S Y *et al*., 2002). Piceid (5,4′-dihydroxystilbene-3-O-β-D-glucopyranoside), one of the stilbenes found in *Polygonum cuspidatum* inhibits tyrosinase activity and melanin production in a concentration-dependent manner (Jeong E T *et al*., 2010).
The effects of piceid on hyperpigmentation and inhibition of tyrosinase activity were better than those of arbutin, which is well known to inhibit melanin formation in melanocytes. In addition, piceid suppressed the mRNA and protein expression of the aforementioned enzymes and transcriptional factor in a concentration-dependent manner. Therefore, piceid represents a safe and new candidate for a skin-lightening agent (Jeong E T et al., 2010).

Gnetol a naturally occurring stilbene particularly found in the genus Gnetum, had a strong inhibitory effect on murine tyrosinase activity. The rich source of Gnetol, Gnetum is a genus of about 30-35 species of gymnosperms, the sole genus in the family Gnetaceae and order Gnetales. They are tropical evergreen trees, shrubs and lianas. Gnetum gnemon (Fig. 2.7), one such species of Gnetum is an easily available source of Gnetol (Fig. 2.8) in South east Asia. The leaves are glossy green and the fruit-like
strobilus consist of little but skin and a large nut-like seed 2–4 cm long inside. Gnetol (IC₅₀ 4.5μM) was stronger than kojic acid (IC₅₀ 139μM) as a standard inhibitor for murine tyrosinase activity. Moreover, gnetol significantly suppressed melanin biosynthesis in murine B16 melanoma cells (Ohguchi K et al., 2003) but was not studied in detail for its role in all the other mechanisms influencing pigmentation and also its efficacy for UV protection.
Stilbenes like **Pterostilbene** and **3-hydroxypterostilbene** are present in the heartwood extract of *Pterocarpus marsupium* (Fig. 2.9). *Pterocarpus marsupium* or the Indian Kino Tree is a medium to large, deciduous tree that can grow up to 30 metres tall. It is native to India, Nepal, and Sri Lanka, where it occurs in parts of the Western Ghats in the Karnataka-Kerala region. It is also known by the names Malabar Kino, *Benga*, *Bijiayasal* (in western Nepal), *Piasal* (Oriya), *Venkai*, and many others. **Pterostilbene** (Fig. 2.10) is a chemically related to resveratrol and is also found in blueberries and grapes. It belongs to the group of phytoalexins, agents produced by plants to fight infections. Based on animal studies it is thought to exhibit anti-cancer and anti-hypercholesterolemia properties. Apart from anti cancer properties, it exhibited significant antioxidant activity and anti inflammatory activity by inhibition of Matrix metalloproteinase 9 (MMP9) (Chakraborty A *et al*., 2010). Pterostilbene protected erythrocyte membranes against lipid peroxidation with an IC$_{50}$ value of 44.5 +/- 7.8 µM. Pterostilbene and Resveratrol protected the erythocytes against hemolysis and glutathione depletion. At lower concentrations, resveratrol with pterostilbene inhibited synergistically the oxidative injury of membrane lipids. At higher concentrations, an additive effect was observed. These protective effects may partially explain the health benefit of these bioactive microcomponents when together in the diet (Mikstacka R *et al*., 2010). Hydroxy derivatives of pterostilbene are also reported to have antioxidant potential (Amorati R *et al*., 2004). However, the cosmetic applications of Pterostilbene or its derivatives like 3-hydroxypterostilbene (Fig. 2.11) have not been explored for cosmetic applications such as UV protection and skin lightening.
Figure 2.9: *Pterocarpus marsupium* tree

Figure 2.10: Structure of *Pterostilbene*

Figure 2.11: Structure of 3’-hydroxypterostilbene
Similarly, Oxyresveratrol (Fig. 2.12) is a major stilbene compound present in the heartwood of *Artocarpus lakoocha* (Fig. 2.14). In 2009, research of this plant detected oxyresveratrol after ethanolic extraction (Maneechai et al., 2009), which exhibited strong tyrosinase-inhibitory activity, and its potential use as a skin-whitening agent (Likhitwitayawuid et al., 2006; Tengamnuay et al., 2006). *Artocarpus lakoocha* Roxb., Moraceae, is a valuable tropical tree species native to India and used for fruit, furniture, timber, and feed. The tree bark containing 8.5% tannin is chewed like betel nut and is also used to treat skin ailments. It yields a durable fiber good for cordage. The wood and roots yield a lavish color dye. *Artocarpus lakoocha* is a tropical tree widely distributed in the regions of South and Southeast Asia, including Nepal, India, Sri Lanka, Myanmar, southern China, Vietnam, Thailand, Malaysia and Indonesia. In Thailand, *A. lakoocha* is called ‘Ma-Haad’, and the dried aqueous extract prepared from the heartwood of this plant is known as ‘Puag-Haad’, which has been traditionally used as an anti-helmintic (Charoenlarp et al., 1989; Maneechai et al., 2009). In addition flavonoids found in species of *Artocarpus* possess strong antioxidation (Toshio et al., 2003), anti-inflammation (Wei et al., 2005), and antiplatelet aggregation (Lin et al., 1996).

Oxyresveratrol is known to have tyrosinase inhibitory effect with an IC$_{50}$ value of 1.2 μM on mushroom tyrosinase activity, which is 32-fold stronger inhibition than kojic acid, a depigmenting agent used as the cosmetic material with skin-whitening effect and the medical agent for hyperpigmentation disorders. Hydroxystilbene compounds of resveratrol, 3,5-dihydroxy-4’-methoxyxystilbene, and rhapontigenin also showed more than 50% inhibition at 100 μM on mushroom tyrosinase activity, but other methylated or glycosylated hydroxystilbenes of 3,4-dimethoxy-5-hydroxystilbene, trimethylresveratrol, piceid, and rhaponticin did not inhibit significantly. None of the hydroxystilbene compounds except oxyresveratrol exhibited more than 50% inhibition at 100 μM on L-tyrosine oxidation by murine tyrosinase activity; oxyresveratrol showed an IC$_{50}$ value of 52.7 μM on the enzyme activity. The kinetics and mechanism for inhibition of mushroom tyrosinase exhibited the reversibility of oxyresveratrol as a noncompetitive inhibitor with L-tyrosine as the substrate (Kim Y M et al., 2002).
However, the cosmetic applications of Oxyresveratrol and its derivatives like **Dihydro-oxyresveratrol** (Fig. 2.13) have not been explored for cosmetic applications such as UV protection. Based on their mechanisms of action for UV protection and skin lightening in comparison to other potential actives, an appropriate ranking in the order of their efficacy can enable the claim substantiation for target specific cosmetic applications.

![Figure 2.12: Structure of Oxyresveratrol](image)

![Figure 2.13: Structure of Dihydro-oxyresveratrol](image)

The seeds of *Artocarpus lakoocha* contain artocarps, the isolectins which exhibit high haemagglutination activity (Wongkham, 1995). **Artocarps** are also present in the heartwood extract of *Artocarpus lakoocha*. Artocarpin, a tetrameric lectin of molecular mass 65 kDa, is also extracted from the seeds of jackfruit (Jeyaprakash A A *et al.*, 2004). Artocarpin also has a promising role for skin lightening (Arung E T *et al.*, 2008). However, the cosmetic applications of Artocarps have not been explored for cosmetic applications such as UV protection. Based on the mechanisms of action for UV protection and skin lightening in comparison to other potential actives, an appropriate ranking in the order of their efficacy can enable the claim substantiation for target specific cosmetic applications.
2.8.4. Glabridin from the root (Licorice) of *Glycyrrhiza glabra*:

Glabridin is another significant skin whitener but not much explored for its UV protection efficacy. Glabridin is isolated from the root (Licorice/liquorice) of *Glycyrrhiza glabra* (Fig. 2.15). The liquorice plant is a legume that is native to southern Europe and parts of Asia. It is an herbaceous perennial and the additional sweetness in liquorice comes from glycyrrhizin, a compound sweeter than sugar.
Glabridin (Fig. 2.16) and its derivatives are known for tyrosinase inhibition (Jirawattanapong W et al., 2009). Glabridin is a unique compound possessing more than one function; not only the inhibition of melanogenesis but also the inhibition of inflammation in the skins (Yokota T et al., 1998). However, the cosmetic applications of Glabridin have not been explored for UV protection. Based on the mechanisms of action for UV protection and skin lightening in comparison to other potential actives, an appropriate ranking in the order of their efficacy can enable the claim substantiation for target specific cosmetic applications.
2.8.5. *Curcuma longa* (Turmeric) traditionally used in India for skin care:

*Curcuma longa* is a rhizomatous herbaceous perennial plant of the ginger family, Zingiberaceae which is native to tropical South Asia. Turmeric from *Curcuma longa* (Fig. 2.17) root is yellow in color and is being traditionally used in India for beautification and lightening of the skin. Active “Tetrahydrocurcumin” (THC) from turmeric extract, is white in color and is well known for its antioxidant (Portes E *et al.*, 2007) and skin lightening benefits. The role of curcuminoids, yellow phenolic compounds derived from turmeric roots as topical antioxidants has been traditionally known in Asia and recently validated in laboratory experiments. Curcuminoids are reported to protect normal human keratinocytes from hypoxanthine/xanthine oxidase injury in *in vitro* studies, and they can protect the skin against a broad range of physical, chemical and biological factors injuring the skin. Curcuminoids prevent free radical formation and scavenge free radicals in biological systems. This combined action was previously described as a Bioprotectant mechanism that protects the integrity of the living cell e.g. skin cell. The antioxidant effects of curcuminoids combined with their known inhibitory effects on cyclooxygenase 2 (Cox-2) render them useful as ingredients in anti-aging formulations and in topical formulations designed to maintain general skin health and integrity. Curcuminoids have also been found to inhibit the activity of tyrosinase, thereby preventing melanin formation with resultant lightening of the skin tone. Amongst the curcuminoids, curcumin was the strongest antioxidant, demethoxycurcumin the second strongest and bisdemethoxycurcumin the least effective. Curcuminoids nevertheless showed activity against oxidation. Curcuminoids act as a superoxide radical scavenger as well as singlet oxygen quencher and gives the antioxidant its effectiveness. Of the naturally accruing curcuminoids, one of the main metabolites of curcumin (Fig. 2.18), tetrahydrocurcumin (Fig. 2.19) is the most potent antioxidant (Jayaprakasha G K *et al.*, 2006).
Tetrahydrocurcumin is a color-free compound derived from curcumin, the yellow, parent compound in the process of hydrogenation. The process of hydrogenation of curcuminoids can also occur naturally in the gastrointestinal tract. The tetrahydrocurcuminoids with similar biological properties to curcuminoids combined
with the lack of yellow color, render them useful in achromatic food and cosmetic applications that currently employ conventional synthetic antioxidants. Like the curcuminoids, THC also showed significant antioxidant action in a number of \textit{in vitro} and preclinical studies. THC is valued as the ultimate metabolite of the curcuminoids \textit{in vivo}. Several independent studies validated the significant antioxidant effects of the tetrahydrocurcuminoids and protection of skin against free radicals and UVB rays (Majeed M and Badmaev V, 2003). However the mechanism of action of THC in UV protection and influence on various mechanisms for skin lightening has not been explored in detail.

\textbf{2.8.6. Piperlongumine from \textit{Piper longum} root:}

\textit{Piper longum} (Long pepper), is a flowering vine in the family \textit{Piperaceae}, cultivated for its fruit, which is usually dried and used as a spice and seasoning. Long pepper has a similar, but hotter, taste to its close relative \textit{Piper nigrum} - from which black, green and white pepper are obtained. \textbf{Piperlongumine}, an alkaloid from the root of \textit{Piper longum} (Fig. 2.20), showed an inhibitory effect on alpha-MSH-induced tyrosinase synthesis, documented by Western immunoblot analysis. However, piperlongumine (Fig. 2.21) did not show an inhibitory effect on tyrosinase activity or a direct depigmenting effect of melanin (Min K R \textit{et al}., 2004). Piperlongumine was not earlier studied for its role in various skin lightening mechanisms and its comparative efficacy with respect to other significant skin lightening actives.
2.8.7. Antioxidant and anti inflammatory plant actives unexplored for UV protection and skin lightening:

Inflammation and free radical damage that are aggravated by UV are some of the main factors that result in UV induced skin damage and skin darkening. Free radicals generated in the body due to stress conditions like UV exposure, pollution, unhealthy food habits and ageing, primarily damage the skin. They trigger the inflammatory markers that eventually cause skin damage. As a result, excess melanin is produced in a defense mechanism, resulting in pigmentation. Topically-applied antioxidants do have merit for all skin types to keep skin healthy and help prevent sun damage and improve cell function. Antioxidants have been conclusively shown to exert a positive effect on reducing skin irritation and inflammation, and that is a crucial step in creating or maintaining healthy, vibrant and wrinkle free skin. Hence, Antioxidant and anti inflammatory actives play a significant role in healthy skin (Rasik A M and Shukla A, 2000 and Kalka K et al., 2000). Although all antioxidant and anti inflammatory actives do not necessarily inhibit melanin synthesis directly, they do have a positive synergistic
effect for skin lightening. For example, Glutathione is a significant antioxidant and not a
direct inhibitor of melanin synthesis. However, when taken internally as a nutricosmetic,
it helps in skin lightening. Therefore, antioxidant potential and anti inflammatory
potential plays a significant role in UV protection and skin lightening. Many plant actives
with significant antioxidant and anti inflammatory potential have not yet been explored
for cosmetic potential exclusively for UV protection and skin lightening applications.
Some such plant actives are described below:

2.8.7.1. Hydroxychavicol from *Piper betle* leaves:

The Betel (*Piper betle*) is the leaf of a vine belonging to the Piperaceae family, which
includes pepper and Kava. It is valued both as a mild stimulant and for its medicinal
properties. The betel plant (Fig. 2.22) is an evergreen and perennial creeper, with glossy
heart-shaped leaves and white catkin. The Betel plant originated from South and South
East Asia (India, Bangladesh and Sri Lanka).

**Figure 2.22: Piper betle plant**

[Image of Piper betle plant]

**Figure 2.23: Structure of Hydroxychavicol**

[Chemical structure of Hydroxychavicol]

**Hydroxychavicol** (Fig. 2.23) from the leaves of *Piper betle* is a potent antitoxidant and an
anti inflammatory active (Sharma S *et al.*, 2009). It significantly inhibits cyclooxygenase
and reactive oxygen species (Chang M C et al., 2007). Hydroxychavicol has not been studied earlier for its UV protection and skin lightening applications.

2.8.7.2. *Citrullus colocynthis* seed extract:

*Citrus colocynthis* (Fig. 2.24), commonly known as the colocynth, bitter apple, bitter cucumber, egusi, or vine of Sodom is a viny plant native to the Mediterranean Basin and Asia, especially Turkey, Nubia, and Trieste. Its seed, which is edible but bitter, nutty-flavored, and rich in fat and protein, is eaten whole or used as an oilseed.

![Citrullus colocynthis plant and its active medicinal principle Colocynthin](image-url)
The oil content of the seeds is 17-19% (w/w), consisting of 67-73% linoleic acid, 10-16% oleic acid, 5-8% stearic acid, and 9-12% palmitic acid. The extract of *Citrullus colocynthis* is also well reported for antioxidant and anti-inflammatory potential. The methanolic fruit extract of *C. colocynthis* was found to have high antioxidant and free radical scavenging ability at a concentration of 2.5 mg/ml (Kumar *et al.*, 2008). It has significant anti-inflammatory and analgesic properties and is used as folk medicine (Marzouk *et al.*, 2010). Colocynthin (Fig. 2.24), a bitter crystalline glucoside is the active medicinal principle of *Citrullus colocynthis* fruits. *Citrullus colocynthis* has not been studied earlier for its UV protection and skin lightening applications.

2.8.7.3. *Nigella sativa* seed extract:

*Nigella sativa* (Fig. 2.25) is an annual flowering plant, native to southwest Asia. The fruit is a large and inflated capsule composed of 3–7 united follicles, each containing numerous seeds (Black cumin seeds). The seed is used as a spice. Black cumin seeds (Fig. 2.25) are traditionally used for a variety of conditions and treatments related to immune system support, as analgesic, anti-inflammatory, antiallergic, antioxidants, anticancer and for general well-being. Earlier it was reported that Thymoquinone (Fig. 2.26) and Thymohydroquinone (Fig. 2.27) from black cumin seeds had significant anti-cancer, antioxidant and anti-inflammatory properties (Ivankovic *et al.*, 2006; Al-Majed A A *et al.*, 2006 and Marsik P *et al.*, 2005). *Nigella sativa* has not been studied earlier for its UV protection and skin lightening applications.
Figure 2.25: Nigella sativa plant, flowers and seeds

Figure 2.26: Structure of Thymoquinone

Figure 2.27: Structure of Thymohydroquinone
2.8.7.4. *Eugenia jambolana* fruit extract:

*Eugenia jambolana* (Fig. 2.28) is an evergreen tropical tree in the flowering plant family Myrtaceae, native to Bangladesh, India, Nepal, Pakistan and Indonesia. The fruits called as *Jamun fruits* (Fig. 2.28) are rich source of Vitamin A and C (Luximon-Ramma *et al.*, 2010). The fruit extract showed significant antioxidant potential (Santos *et al.*, 2010). *Eugenia jambolana* fruit extract has not been studied earlier for its UV protection and skin lightening applications.

![Figure 2.28: Eugenia jambolana tree, leaves and fruits](image)

However, all antioxidants or anti inflammatory actives may not necessarily have applications for UV protection or skin lightening. *Ascorbic acid or Vitamin C* (Fig. 2.29), a known antioxidant and skin lightener and a chief active of most of the plant sources can be a significant active for a comparative study with respect to other plant actives on the mode of action for UV protection and skin lightening. The effects of Vitamin C are greater than those of multivitamin on mushroom tyrosinase inhibition and antioxidation (Choi *et al.*, 2010). Ascorbic acid is reported for skin lightening potential; however its role in UV protection and its effect on various modes of skin lightening mechanisms has not been studied in detail.
Ascorbic acid along with other known antioxidants like **Glutathione** (Fig. 2.30) and **Tocopherol or Vitamin E** (Fig. 2.31) are of significant importance for a comparative study with respect to other antioxidant plant actives, for cosmetic applications. Glutathione is the major endogenous antioxidant produced by the cells, participating directly in the neutralization of free radicals and reactive oxygen compounds, as well as maintaining exogenous antioxidants such as vitamins C and E in their reduced (active) forms (Scholz R W et al., 1989 and Hughes R E, 1964). Vitamin E is widely used as an inexpensive antioxidant in cosmetics and foods. Vitamin E is a family of α-, β-, γ-, and δ- (respectively: alpha, beta, gamma, and delta) tocopherols. Vitamin E is a fat-soluble antioxidant that stops the production of reactive oxygen species formed when fat undergoes oxidation (Herrara E and Barbas C, 2001). Of these, α-tocopherol has been most studied as it has the highest bioavailability (Brigelius-Flohe R and Traber M G, 1999). It has been claimed that α-tocopherol is the most important lipid-soluble antioxidant, and that it protects cell membranes from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reaction (Herrara E and Barbas C, 2001). This would remove the free radical intermediates and prevent the oxidation reaction from continuing.

*Figure 2.29: Structure of Ascorbic acid*
2.8.8. *Emblica officinalis* (Amla) fruit extract:

Ascorbic acid was also earlier thought to be the active in *Emblica officinalis* (Amla) extract responsible for the high antioxidant potential of Amla. *Emblica officinalis* Gaertn. (Euphorbiaceae), commonly known in India as Amla (Sanskrit name Amalaki) is used in Ayurveda for its cosmetic applications due to its antioxidant properties. It is also used as astringent, cardiac tonic, diuretic, laxative, liver tonic, anti inflammatory, hair tonic and digestive medicine. For several decades, the fruits of Amla (Fig. 2.32) had been claimed to be a rich source of Ascorbic acid and further its high antioxidant potential was earlier attributed to the presence of ascorbic acid (Scartezzini P et al., 2006). It was later reported that low molecular hydrolysable tannins emblicanins A and B contribute to the antioxidant potential of Amla (Pozharitskava O N et al., 2007). However, recent studies have confirmed that only trace amounts of Ascorbic acid are found in Amla extract and the earlier reported antioxidant hydrolysable tannins, emblicanins A and B, correspond to
1-O-galloyl- β-D-glucose (β-glucogallin) (Fig. 2.33) and mucic acid 1,4-lactone 5-O-gallate respectively (Majeed M et al., 2009). The trace amount of free Ascorbic acid in Amla extract suggests that the antioxidant effects exhibited by Amla fruits are due to gallic acid esters (Majeed M et al., 2009). Although fruits are reputed to contain high amounts of ascorbic acid, 445 mg/100g (Tarwadi K and Agte V, 2007), the specific contents are disputed and the overall antioxidant strength of amla may derive instead from its high density of tannins (Dharmananda S, 2003).

The fruit also contains other polyphenols: flavonoids, kaempferol, ellagic acid and gallic acid (Dharmananda S, 2003 and Habib-ur-Rehman et al., 2007). Although Amla extract is known to reduce UV induced erythema, the component in Amla fruits that has significant role in photoprotection was not studied earlier. Many chemical actives with significant photoprotection efficacy are also known to have side effects such as skin cancer.
Hence, natural actives are preferred over chemical actives for photoprotection applications. Hence, Amla extract composition and its mechanism of skin lightening and photoprotection efficacy for cosmetic applications is of significance.

2.8.9. **Natural ceramides having skin conditioning efficacy but unexplored for UV protection and skin lightening potential:**

**Natural ceramides** from plant extracts were another group of actives selected for the present study. Ceramides play an important role in skin conditioning. The long hydrophobic chains in ceramides are essential for maintaining the skin barrier function (Janusova B et al., 2010). Ceramides are the major lipid constituent of lamellar sheets present in the intercellular spaces of the stratum corneum. These lamellar sheets are thought to provide the barrier property of the epidermis. It is generally accepted that the intercellular lipid domain is composed of approximately equimolar concentrations of free fatty acids, cholesterol, and ceramides. Ceramides are a structurally heterogeneous and complex group of sphingolipids containing derivatives of sphingosine bases in amide linkage with a variety of fatty acids. Differences in chain length, type and extent of hydroxylation, saturation etc. are responsible for the heterogeneity of the epidermal sphingolipids. It is well known that ceramides play an essential role in structuring and maintaining the water permeability barrier function of the skin. In conjunction with the other stratum corneum lipids, they form ordered structures. An essential factor is the physical state of the lipid chains in the nonpolar regions of the bilayers. The stratum corneum intercellular lipid lamellae, the aliphatic chains in the ceramides and the fatty acids are mostly straight long-chain saturated compounds with a high melting point and a small polar head group. This means that at physiological temperatures, the lipid chains are mostly in a solid crystalline or gel state, which exhibits low lateral diffusional properties and is less permeable than the state of liquid crystalline membranes, which are present at higher temperatures. The link between skin disorders and changes in barrier lipid composition, especially in ceramides, is difficult to prove because of the many variables involved. However, most skin disorders that have a diminished barrier function present a decrease in total ceramide content with some differences in the ceramide pattern.
Formulations containing lipids identical to those in skin and, in particular, some ceramide supplementation could improve disturbed skin conditions. Incomplete lipid mixtures yield abnormal lamellar body contents, and disorder intercellular lamellae, whereas complete lipid mixtures result in normal lamellar bodies and intercellular bilayers. The utilization of physiological lipids according to these parameters has potential as new forms of topical therapy for dermatoses. An alternative strategy to improving barrier function by topical application of the various mature lipid species is to enhance the natural lipid-synthetic capability of the epidermis through the topical delivery of lipid precursors (Coderch L et al., 2003). However ceramides although known for skin conditioning are not studied for their cosmetic applications like UV protection and skin lightening. If ceramides from plant sources contain these benefits along with their skin conditioning properties, they could be complete cosmetic ingredients. Apple is a pomaceous fruit of the species *Malus domestica* (Fig. 2.34) in the rose family (Rosaceae), and is a perennial. It is one of the most widely cultivated tree fruits, and the most widely known of the many members of genus *Malus* that are used by humans. Oats (*Avena sativa*) (Fig. 2.35) is also another source of ceramides that has not been studied for UV protection and skin lightening application.

*Figure 2.34: Malus domestica (Apple)  Figure 2.35: Avena sativa (Oat) florets*
The common oat is a species of cereal grain grown for its seed, which is known by the same name. While oats are suitable for human consumption as oatmeal and rolled oats, one of the most common uses is as livestock feed because of its high nutritious value. The soluble fibre in whole oats comprises a class of polysaccharides known as beta-D-glucans. Beta-D-glucans, usually referred to as beta-glucans, comprise a class of indigestible polysaccharides widely found in nature in sources such as grains, barley, yeast, bacteria, algae and mushrooms. In oats, barley and other cereal grains, they are located primarily in the endosperm cell wall. Oat beta-glucan is a soluble fiber. It is a viscous polysaccharide made up of units of the monosaccharide D-glucose. Other than oat ceramides and betaglucans, which can possibly have high unexplored cosmetic potential, oats are also the source of Avenanthramides (Fig. 2.36), a type of oat phytoalexins that exist predominantly in the groats of oat seeds (Emmons C L and Peterson D M, 1999). Among a group of at least 25 avenanthramides (Collins F W 1989) that differ in the substituents on the cinnamic acid and anthranilic acid rings, three are predominant in oat grain: Bc (also called avenanthramide C or Av-C), Bf (also called avenanthramide B or Av-B) and Bp (also called avenanthramide A or Av-A) (Fig. 2.36). In vitro experiments indicate that they have significant antioxidant activities, with Bc > Bf > Bp (Peterson D M et al., 2002). In human and animal tests, this antioxidant capacity provides health benefits ranging from reduced rate of LDL oxidation (Chen C Y et al., 2004) to protecting against cancers and heart disease (Bailey G S and Williams D E, 1993). The results of human in vivo skin prick tests combined with an in vitro model, investigating the inhibitory activity of avenanthramides on the histamine net release from rat peritoneal mast cells stimulated by substance P showed a clear indication that avenanthramides found in oat extract play a major role in the reduction of itching and redness in skin. Because of the fundamental role of histamine in itch sensation, oat extracts with a standardized content of avenanthramides are useful materials to reduce histamine related itch sensation and redness in skin (Vollhardt J et al., 2000). Due to their high antioxidant and anti-inflammatory potential, Avenanthramides can have significant potential for UV protection and skin lightening applications that has so far not been explored.
2.8.10. Forskolin from *Coleus forskohlii* roots:

Forskolin is a labdane diterpene that is produced by the Indian Coleus plant (*Coleus forskohlii*). *Coleus forskohlii* (Fig. 2.37) is a tropical perennial plant related to the typical coleus species. It is interesting from a scientific and medicinal standpoint because it produces forskolin. Forskolin is a labdane diterpene that is produced by the Indian Coleus plant. Forskolin and its derivatives like Deacetylforskolin and Isoforskolin are present in the roots of *Coleus forskohlii* plant (Fig. 2.38). Forskolin (Fig. 2.39) is commonly used to raise levels of cyclic AMP (cAMP) in the study and research of cell physiology. Forskolin resensitizes cell receptors by activating the enzyme adenylyl cyclase and increasing the intracellular levels of cAMP. cAMP is an important signal carrier necessary for the proper biological response of cells to hormones and other extracellular signals. It is required for cell communication in the hypothalamus/pituitary gland axis and for the feedback control of hormones. It acts by activating protein kinase A.
Figure 2.37: *Coleus forskohlii* plants

Forskolin helps in tanning of skin via cAMP pathway and is also known to protect skin from UV induced damage. Melanin pigments provide efficient protection against ultraviolet B (UVB) radiation but DNA repair also plays a key role in eliminating UV-induced damage and preventing the development of skin cancers. In this study, we demonstrate that forskolin (FSK), an agent that increases intracellular levels of cAMP, protects keratinocytes from UVB-induced apoptosis independently from the amount of melanin in the skin.
Forskolin enhances the removal of the two major types of UVB-induced DNA damage, cyclobutane pyrimidine dimers and 6,4-photoproducts, by facilitating DNA repair. These findings suggest new preventive approaches with topical formulations of FSK or other bioactive agents that could be applied to the skin before sun exposure to increase its ability to repair DNA damage (Passeron et al., 2009). It is reported that topical application of forskolin to the skin of fair-skinned MC1R-defective mice with epidermal melanocytes resulted in accumulation of eumelanin in the epidermis and was highly protective against UV-mediated cutaneous injury. Forskolin-induced eumelanin production persisted through 3 months of daily applications, and forskolin-induced eumelanin remained protective against UV damage as assessed by minimal erythematous dose (MED). No obvious toxic changes were noted in the skin or overall health of
animals exposed to prolonged forskolin therapy. Body weights were maintained throughout the course of topical forskolin application. Topical application of forskolin was associated with an increase in the number of melanocytes in the epidermis and thickening of the epidermis due, at least in part, to an accumulation of nucleated keratinocytes. Together, these data suggest that short-term topical regular application of forskolin promotes eumelanin induction and over time, topical forskolin treatment is associated with persistent melanization, epidermal cell accumulation, and skin thickening. (Spry M L et al., 2009).

Forskolin as well as its derivatives are known for adenylate cyclase stimulating activity which further increases the levels of cAMP (Tatee T et al., 1996). Hence, Forskolin and its derivatives have similar properties and probably a similar UV protection profile for cosmetic applications. However, the comparative analysis of Forskolin and its derivatives Deacetylforskolin and Isoforskolin for protection from UV has not been earlier reported.

2.8.11. Popular Antioxidant and Anti inflammatory plant extracts – Actives of significance as nutricosmetics:

Antioxidants play a significant role in skin lightening and UV protection as UV induced free radicals are the major factors for causing skin damage and excessive melanogenesis. Topically-applied antioxidants have merit for all skin types to keep skin healthy and help prevent sun damage and improve cell function. Antioxidants do not just have skin care potential through topical applications but also through oral applications as they are well known for their Nutricosmetic benefits. The term nutricosmetics refers to nutritional supplements which can support the function and the structure of the skin. Many micronutrients have this effect. Vitamin C, for example, has a well established antioxidant effect that reduces the impact of free radicals in the skin. It also has a vital function in the production of collagen in the dermis. Other micronutrients e.g. some omega-3 fatty acids, carotenes and flavonoids protect the skin from the damaging effects of Ultraviolet (UV) light exposure, which may lead to accelerated skin ageing and wrinkle formation. A wide variety of polyphenols or phytochemicals, most of which are
dietary supplements, have been reported to possess substantial skin photoprotective effects. This review article summarizes the photoprotective effects of some selected polyphenols, such as green tea polyphenols, grape seed proanthocyanidins, resveratrol, silymarin and genistein, on UV-induced skin inflammation, oxidative stress and DNA damage, etc., with a focus on mechanisms underlying the photoprotective effects of these polyphenols. The laboratory studies conducted in animal models suggest that these polyphenols have the ability to protect the skin from the adverse effects of UV radiation, including the risk of skin cancers. It is suggested that polyphenols may favorably supplement sunscreens protection, and may be useful for skin diseases associated with solar UV radiation-induced inflammation, oxidative stress and DNA damage (Nichols J A and Katiyar S K, 2010). The polyphenolic compounds from green tea when tested against chemical carcinogenesis and photocarcinogenesis in murine skin, it was observed that green tea polyphenols provide protection against chemical carcinogenesis as well as photocarcinogenesis in mouse skin. Analysis of published studies demonstrates that green tea polyphenols have anti-inflammatory and anticarcinogenic properties. These effects correlate with the antioxidant properties of green tea polyphenols. The outcome of the several experimental studies suggests that green tea possess anti-inflammatory and anticarcinogenic potential, which can be exploited against a variety of skin disorders. Supplementation of skin care products with green tea may have a profound impact on various skin disorders in the years to come (Katiyar S K et al., 2000). Many plant extract so far have been studied for their nutricosmetic benefits i.e., for their role in cosmetic applications when taken orally. For example, Acerola fruit has a remarkably high vitamin C content, as well as antioxidants carotenoids and bioflavonoids (Lunceford N and Gugliucci A, 2005). In two studies comparing 14 tropical fruits from Brazil, acerola came out tops in antioxidant activity, ascorbic acid and total phenols (Heck C I and De Mejia E G, 2007). More functionally, a 2008 study found acerola extract significantly lightened the UVB-irradiated skin pigmentation of brown guinea pigs (Lanzetti M et al., 2008). Some of the plant extracts rich in antioxidant and anti-inflammatory actives with potential benefits as nutricosmetics are as follows,
2.8.11.1. *Punica granatum* (Pomegranate) extract:

*Punica granatum* (Pomegranate) is a fruit-bearing deciduous shrub or small tree growing between five and eight meters tall. In the Indian subcontinent's ancient Ayurveda system of medicine, the pomegranate fruit and its rind has extensively been used as a source of traditional remedies for thousands of years (Jindal K K and Sharma R C, 2004). Pomegranate juice provides about 16% of an adult's daily vitamin C requirement per 100 ml serving, and is a good source of vitamin B₅ (pantothenic acid), potassium and polyphenols, such as tannins and flavonoids (Nutritiondata.com, Schubert S Y et al., 1999). Punica granatum fruits (Fig. 2.40) are listed as high-fiber in some charts of nutritional value. That fiber, however, is entirely contained in the edible seeds which also supply unsaturated oils. People who choose to discard the seeds forfeit nutritional benefits conveyed by the seed fiber, oils and micronutrients. The most abundant polyphenols in pomegranate juice are the hydrolyzable tannins called ellagitannins formed when ellagic acid binds with a carbohydrate. Punicalagins are tannins with free-radical scavenging properties in laboratory experiments (Kulkarni A P et al., 2007) and with potential human effects (Heber DH, 2008). Punicalagins are absorbed into the human body and may have dietary value as antioxidants, but conclusive proof of efficacy in humans has not yet been shown (Seeram N P et al., 2006 and Mertens-Talcott S U et al., 2006). During intestinal metabolism by bacteria, ellagitannins and punicalagins are converted to urolithins which have unknown biological activity in vivo (Bialonska D et al., 2009 and Larrosa M et al., 2009). Other phytochemicals include polyphenolic catechins, gallocatechins, and anthocyanins, such as prodelphinidins, delphinidin, cyanidin, and pelargonidin (Plumb G W et al., 2002). The ORAC (antioxidant capacity) of pomegranate juice was measured as 2,860 units per 100 grams (Development of Accurate and Representative Food Composition Data). Many food and dietary supplement makers use pomegranate phenolic extracts as ingredients in their products instead of the juice. One of these extracts is ellagic acid (Fig. 2.41), which may become bioavailable only after parent molecule punicalagins are metabolized. However, ingested ellagic acid from pomegranate juice does not accumulate in the blood in significant quantities and is rapidly excreted (Seeram N P et al., 2004). Pomegranate rind extract
containing 90% ellagic acid showed significant skin whitening effect and also inhibited UV B induced skin pigmentation (Yoshimura M et al., 2005). **Pomegranate fruit extract** and **Pomegranate rind extract** are significant for study in developing products and techniques for UV protection and skin lightening.

*Figure 2.40: Punica granatum (Pomegranate) fruits and seeds*

*Figure 2.41: Structure of Ellagic acid*
2.8.11.2. *Terminalia belerica* fruit extract:

*Terminalia belerica* plants and other species of Terminalia are also reported for antioxidant properties (Sabu M C and Kuttan R, 2009) and hence are significant for study in developing products and techniques for UV protection and skin lightening. *Terminalia* is a genus of large trees of the flowering plant family Combretaceae, comprising around 100 species distributed in tropical regions of the world. This genus gets it name from Latin *terminus*, referring to the fact that the leaves appear at the very tips of the shoots. Trees of this genus are known especially as a source of secondary metabolites, e.g. cyclic triterpenes and their derivatives, flavonoids, tannins, and other aromatics. Some of these substances have antifungal, antibacterial, anti-cancer and hepatoprotective indications. It is reported that the methanolic extracts of the fruits of *Terminalia chebula*, *Terminalia belerica* (Fig. 2.42) and *Emblica officinalis* might be useful as potent sources of natural antioxidants. The ability of the extracts of the fruits in exhibiting their antioxative properties follow the order *T. chebula* > *E. officinalis* > *T. belerica*. The same order is followed in their flavonoid content, whereas in case of phenolic content it becomes *E. officinalis* > *T. belerica* > *T. chebula*. In the studies of free radical scavenging, where the activities of the plant extracts were inversely proportional to their IC$_{50}$ values, *T. chebula* and *E. officinalis* were found to be taking leading role with the orders of *T. chebula* > *E. officinalis* > *T. belerica* for superoxide and nitric oxide, and *E. officinalis* > *T. belerica* > *T. chebula* for DPPH and peroxynitrite radicals. Miscellaneous results were observed in the scavenging of other radicals by the plant extracts, viz., *T. chebula* > *T. belerica* > *E. officinalis* for hydroxyl, *T. belerica* > *T. chebula* > *E. officinalis* for singlet oxygen and *T. belerica* > *E. officinalis* > *T. chebula* for hypochlorous acid. In a whole, the studied fruit extracts showed significant efficacy in their antioxidant and radical scavenging abilities, compared to the standards (Hazra B et al., 2010).
2.8.11.3. Grape seed extract:

Another rich source of antioxidants are polyphenols. Polyphenols are also known as phenols or phenolics. As they are found occurring in nature, they are considered biomolecules. As many of polyphenols are found in plants, they are also described as phytochemicals. A grape (Vitis vinifera) is a non-climacteric fruit that grows on the perennial and deciduous woody vines of the genus Vitis. Grapes (Fig. 2.43) can be eaten raw or they can be used for making jam, juice, jelly, vinegar, wine, grape seed extracts, raisins, and grape seed oil. Polyphenols from Grape seeds (Fig. 2.44) are well reported for their antioxidant potential (Chao C L et al., 2010 and Hanausek M et al., 2010). Polyphenols from plant sources are therefore significant for study in developing products and techniques for UV protection and skin lightening. Grape polyphenols are also known to protect neurons against oxidative stress (Fujishita K et al., 2009).
2.8.11.4. Lotus seed extract:

*Nelumbo nucifera* (Fig. 2.45), also known as Indian Lotus, Sacred Lotus or simply Lotus, is a plant in the Nelumbonaceae family. This plant is an aquatic perennial and has distinctive circular seed pods. The dried seed heads (Fig. 2.45) resemble the spouts of watering cans. Under favorable circumstances its seeds may remain viable for many years. Lotus seed extract is known for its antioxidant potential. Total phenolic content in the seed extract was reported to be 7.61 +/- 0.04% (w/w). The seeds contain alkaloids, saponins, phenolics and carbohydrates. The Lotus seed extract exhibited strong free radical scavenging activity as evidenced by the low IC$_{50}$ values in both DPPH (1,1-diphenyl-2-picryl hydrazyl) (6.12 +/- 0.41 microg/ml) and nitric oxide (84.86 +/- 3.56 microg/ml) methods. Administration of HANN to Wistar rats at 100 and 200 mg/kg body weight for 4 days prior to carbon tetrachloride CCl$_4$ treatment caused a significant dose dependent increase (p < 0.05 to p < 0.001) in the level of superoxide dismutase (SOD) and catalase and a significant decrease (p < 0.05 to p < 0.001) in the level of thiobarbituric acid reactive substances (TBARS), when compared to CCl$_4$ treated control in both liver and kidney (Rai S *et al*., 2006). Procyanidins in Lotus seed extract also confer significant antioxidant potential to the extract (Xu J *et al*., 2010).
Lotus seed extract can be of significance for developing products and techniques for UV protection and skin lightening.

*Figure 2.45: Lotus flower and Lotus seeds*
2.8.11.5. Green tea extract:

_Camellia sinensis_ (Green tea) is the species of plant whose leaves and leaf buds are used to produce tea. It is of the genus Camellia, a genus of flowering plants in the family Theaceae. _Camellia sinensis_ (Fig. 2.46) is native to mainland China, South and Southeast Asia, but it is today cultivated across the world in tropical and subtropical regions. It is an evergreen shrub or small tree that is usually trimmed to below two metres (six feet) when cultivated for its leaves. It has a strong taproot. The flowers are yellow-white, 2.5–4 cm in diameter, with 7 to 8 petals. The seeds of _Camellia sinensis_ can be pressed to yield tea oil, a sweetish seasoning and cooking oil. The leaves are 4–15 cm long and 2–5 cm broad. Fresh leaves contain about 4% caffeine. The young, light green leaves are preferably harvested for tea production; they have short white hairs on the underside. Older leaves are deeper green. Different leaf ages produce differing tea qualities, since their chemical compositions are different. Usually, the tip (bud) and the first two to three leaves are harvested for processing. This hand picking is repeated every one to two weeks. Green tea extract is known for its anti-inflammatory and antioxidant properties (Galleano M et al., 2010 and Lambert J D et al., 2010). Green tea extract rich in polyphenols possesses high antioxidative and anti-inflammatory capacity, thus being protective in various models of acute inflammation (Relja B et al., 2011). Polyphenols from green tea are therefore significant for study in developing products and techniques for UV protection and skin lightening.
2.8.11.6. Cocoa polyphenols:

*Theobroma cacao* (Fig. 2.47) also *Cacao tree* and *Cocoa tree*, is a small (4–8 m or 15–26 ft tall) evergreen tree in the family Sterculiaceae (alternatively Malvaceae), native to the deep tropical region of the Americas. Its seeds are used to make cocoa powder and chocolate. The fruit, called a cacao pod, is ovoid, 15–30 cm (6–12 in) long and 8–10 cm
(3–4 in) wide, ripening yellow to orange, and weighs about 500 g (approximately 1.2 lb) when ripe. The pod contains 20 to 60 seeds, usually called "beans", embedded in a white pulp. The seeds are the main ingredient of chocolate, while the pulp is used in some countries to prepare a refreshing juice. Each seed contains a significant amount of fat (40–50%) as cocoa butter. Their most noted active constituent is theobromine, a compound similar to caffeine. Cocoa polyphenols from Cocoa beans (Fig. 2.48) are used in the present study for synergistic skin lightening potential and nutricosmetic potential on account of their high antioxidant and anti-inflammatory potential. Cocoa polyphenols modulate inflammatory mediators in patients at high risk of cardiovascular disease. These anti-inflammatory effects may contribute to the overall benefits of cocoa consumption against atherosclerosis (Monagas M et al., 2009). Cocoa is a rich source of dietary polyphenols. In vitro as well as cell culture data indicate that cocoa polyphenols may exhibit antioxidant and anti-inflammatory, as well as anti-atherogenic activity. Several molecular targets (e.g., nuclear factor kappa B, endothelial nitric oxide synthase, angiotensin converting enzyme) have been recently identified which may partly explain potential beneficial cardiovascular effects of cocoa polyphenols (Rimbach G et al., 2009). Polyphenols from Cocoa also are significant for study in developing products and techniques for UV protection and skin lightening.

2.8.11.7. Coffee bean extract:

Coffea arabica (Coffee) is a species of Coffea originally indigenous to the mountains of Yemen in the Arabian Peninsula, hence its name, and also from the southwestern highlands of Ethiopia and southeastern Sudan. It is also known as the "coffee shrub of Arabia", "mountain coffee" or "arabica coffee". Coffea arabica (Fig. 2.49) is believed to be the first species of coffee to be cultivated, being grown in southwest Arabia for well over 1,000 years. Wild plants grow to between 9 and 12 m tall, and have an open branching system; the leaves are opposite, simple elliptic-ovate to oblong, 6–12 cm long and 4–8 cm broad, glossy dark green. The flowers are white, 10–15 mm in diameter and grow in axillary clusters. The fruit is a drupe (though commonly called a "berry") 10–15 mm in diameter, maturing bright red to purple and typically contains two seeds (the
Coffee is a brewed drink prepared from roasted seeds, called coffee beans, of the coffee plant. They are seeds of coffee cherries that grow on trees in over 70 countries, cultivated primarily in Latin America, Southeast Asia, and Africa. Green unroasted coffee is one of the most traded agricultural commodities in the world. Due to its caffeine content, coffee often has a stimulating effect on humans. Today, coffee is the third most popular drink in the world, behind water and tea. Chlorogenic acid from the extract of Coffee beans (Fig. 2.50) is known to have high antioxidant potential (Richelle M et al., 2001). Chlorogenic acid (Fig. 2.51) is a hydroxycinnamic acid, a member of a family of naturally occurring organic compounds. It is an important biosynthetic intermediate and one of the phenols found in coffee. These are esters of polyphenolic caffeic acid and cyclitol (-)-quinic acid (Clifford M N et al., 2003). Coffee bean extract and its chief active, Chlorogenic acid is of significance for developing products and techniques for UV protection and skin lightening.

Figure 2.49: Coffea arabica plant
2.8.11.8. Rosmarinic acid:

Rosmarinic acid is also a well known antioxidant that can be of significance for cosmetic potential. Rosemary (Rosmarinus officinalis) (Fig. 2.52) is a woody, perennial herb with fragrant evergreen needle-like leaves. It is native to the Mediterranean region. It is a member of the mint family Lamiaceae, which also includes many other herbs. The
leaves are evergreen, 2–4 cm (0.8–1.6 in) long and 2–5 mm broad, green above, and white below with dense short woolly hair. Flowering, very common in a mature and healthy specimen, blooms in summer in the north; but can be ever blooming in warm-winter climates and is variable in color, being white, pink, purple, or blue. **Rosmarinic acid** (Fig. 2.53), is a natural polyphenol antioxidant carboxylic acid found in many *Lamiaceae* herbs used commonly as culinary herbs such as lemon balm, rosemary, oregano, sage, thyme and peppermint (Clifford M N, 1999). Chemically, rosmarinic acid is an ester of caffeic acid with 3,4-dihydroxyphenyl lactic acid. Because of the antioxidant activity of *Lamiaceous* herbs in laboratory test models they have been suggested to have beneficial effects in humans (Triantaphyllou K *et al.*, 2001). Rosmarinic acid has a number of interesting biological activities, e.g. antiviral, antibacterial, antiinflammatory and antioxidant. The presence of rosmarinic acid in medicinal plants, herbs and spices has beneficial and health promoting effects. In plants, rosmarinic acid is supposed to act as a preformed constitutively accumulated defense compound (Petersen M and Simmonds M S J, 2003). Rosmarinic acid from the leaf extract of *Rosmarinus officinalis* has significant antioxidant potential (Kelsey N A *et al.*, 2010). Rosmarinic acid also showed hyaluronidase inhibitory potential (Murata T *et al.*, 2011).

*Figure 2.52: Rosmarinus officinalis plant*
2.8.11.9. Tulsi extract:

Rosmarinic acid along with other important antioxidants is also present in the leaves of Tulsi, making Tulsi extract a significant cosmetic active for skin care studies. Ocimum tenuiflorum (Tulsi, Holy Basil) also called Ocimum sanctum (Fig. 2.54) is an aromatic plant in the family Lamiaceae which is native throughout the tropics and widespread as a cultivated plant. It is an erect, much branched shrub 30–60 cm tall with hairy stems and simple opposite green leaves that are strongly scented. Leaves have petioles, and are ovate, up to 5 cm long, usually slightly toothed. Flowers are purplish in elongate racemes in close whorls. There are two main morphotypes cultivated in India—green-leaved (Sri or Lakshmi tulsi) and purple-leaved (Krishna tulsi). Tulsi is cultivated for religious and medicinal purposes, and for its essential oil. It is widely known across South Asia as a medicinal plant and herbal tea, commonly used in Ayurveda, and has an important role within the Vaishnavite tradition of Hinduism, in which devotees perform worship involving Tulsi plants or leaves. Ocimum sanctum (Tulsi) leaves are used traditionally for various ailments on account of the high antioxidant and anti-inflammatory potential (Singh S et al., 2007). Some of the main chemical constituents of Tulsi are: Oleanolic acid, Ursolic acid, Rosmarinic acid, Eugenol, Carvacrol, Linalool, and β-caryophyllene (Merrily K and Winston D, 2007). Recent studies suggest that Tulsi may be a COX-2 inhibitor, like many modern painkillers, due to its high concentration of eugenol (1-
hydroxy-2-methoxy-4-allylbenzene) (Singh S, 1999 and Prakash P and Gupta N, 2005). Another study showed that Tulsi's beneficial effect on blood glucose levels is due to its antioxidant properties (Sethi J et al., 2004).

![Figure 2.54: Ocimum sanctum plant and leaves](image)

2.8.11.10. Mulberry extract:

Similarly the antioxidant actives from Mulberry are of significance for cosmetic studies. *Morinda citrifolia* (Mulberry), commonly known as great morinda or Indian mulberry, is a tree in the coffee family, Rubiaceae. *Morinda citrifolia* (Fig. 2.55) is native from Southeast Asia to Australia and is now distributed throughout the tropics. The plant bears flowers and fruits all year round. The fruit is a multiple fruit that has a pungent odor when ripening, and is hence also known as cheese fruit. It is oval in shape and reaches 4–7 centimeters (1.6–2.8 in) size. At first green, the fruit turns yellow then almost white as it ripens. It contains many seeds. Mulberry extract has significant antioxidant potential (Chang L W et al., 2010). Several polyphenols belonging to the coumarin, flavonoid and phenolic acid groups, and two iridoids were identified in Morinda juice and the juice demonstrated a mean range free radical scavenging capacity.
Furthermore, it also reduced carrageenan-induced paw oedema, directly inhibited cyclooxygenase COX-1 and COX-2 activities and inhibited the production of nitric oxide (NO) and prostaglandins E(2) (PGE(2)) in activated J774 cells, in a dose dependent manner (Dussossoy E et al., 2011).

Figure 2.55: Morinda citrifolia plant and fruits

2.8.11.11. Aloe vera extract:

*Aloe vera* (Fig. 2.56) widely distributed in Africa, India, and other arid areas is a stemless or very short-stemmed succulent plant growing to 60–100 cm (24–39 in) tall, spreading by offsets. The leaves are thick and fleshy, green to grey-green, with some varieties showing white flecks on the upper and lower stem surfaces (Fig. 2.57). The margin of the leaf is serrated and has small white teeth. The flowers are produced in summer on a spike up to 90 cm (35 in) tall, each flower pendulous, with a yellow tubular corolla 2–3 cm
(0.8–1.2 in) long. *Aloe vera* is claimed as a cosmetic and alternative medicine for skin soothing, moisturising, and healing properties. *Aloe barbadensis* (Miller), *Aloe vera*, has a long history of use as a topical and oral therapeutic. The plant is the source of two products, gel and latex, which are obtained from its fleshy leaves. *Aloe vera* products contain multiple constituents with potential biological activity (Boudreau M D and Beland F A, 2006). *Aloe vera* extract is also known to have significant antioxidant properties (El-Shemy H A *et al*., 2010).

![Figure 2.56: Aloe vera plant](image1)  
![Figure 2.57: Aloe vera succulent leaves](image2)

### 2.8.11.12. Coriander seed oil:

*Coriander* (*Coriandrum sativum*) (Fig. 2.58) is an annual herb in the family Apiaceae. Coriander is native to southern Europe and North Africa to southwestern Asia. It is a soft, hairless plant growing to 50 centimetres tall. The leaves are variable in shape, broadly lobed at the base of the plant, and slender and feathery higher on the flowering stems. The flowers are borne in small umbels, white or very pale pink, asymmetrical, with the petals pointing away from the center of the umbel longer (5–6 mm) than those pointing towards it (only 1–3 mm long). The fruit is a globular dry schizocarp 3–5 mm diameter.
All parts of the plant are edible, but the fresh leaves and the dried seeds are the parts most commonly used in cooking. The dry fruits known as coriander seeds (Fig. 2.59) are also called dhania in India. Types with smaller fruit are produced in temperate regions and usually have a volatile oil content of around 0.4-1.8%, and are therefore highly valued as a raw material for the preparation of essential oil. The essential oil content of the dried seeds varied from 0.1% to 0.36%. Thirty-four different compounds were identified in the essential oil of all accessions. Linalool (40.9-79.9%), neryl acetate (2.3-14.2%), gamma-terpinene (0.1-13.6%) and alpha-pinene (1.2-7.1%) were identified as main components in the oil of the coriander accessions (Nejad Ebrahim S et al., 2010). Tocopherols, phospholipids, phytosterols, and phenols are the most important natural antioxidants in crude oils. Black cumin (Nigella sativa), coriander (Coriandrum sativum), and niger (Guizotia abyssinica) crude seed oils extracted with n-hexane, and its various fractions like neutral lipids, glycolipids, and phospholipids when investigated for their radical scavenging activity towards 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical by spectrophotometric method, it was found that Coriander seed oil and its fractions exhibited the strongest radical scavenging activity compared to black cumin and niger seed oils (Ramadan M F et al., 2003).
2.8.11.13. *Boswellia serrata* extract:

*Boswellia serrata* (Fig. 2.61) of the family Burseraceae is known as the Indian frankincense or *Salai*. It is found in Rajasthan and Madhya Pradesh in India. **Boswellic acids** are a series of pentacyclic triterpene molecules which are produced by plants in the genus *Boswellia*. Like many other terpenes, boswellic acids appear in the resin (Fig. 2.62) of the plant which exudes them; it is estimated that they make up 30% of the resin of *Boswellia serrata*. Boswellic acids have significant anti-inflammatory potential. The boswellic acids are organic acids, consisting of a pentacyclic triterpene, a carboxyl group and at least one other functional group. Alpha-boswellic acid and beta-boswellic acid, $C_{30}H_{48}O_{3}$ both have an additional hydroxyl group; they differ only in their triterpene structure. Acetyl-alpha-boswellic acid and acetyl-beta-boswellic acid, $C_{32}H_{50}O_{4}$, replace the hydroxyl group with an acetyl group. Analysis of boswellic acids shows that there are four major β-boswellic acids involved in the inhibition of 5-lipoxygenase and related anti-inflammatory events. These are: β-boswellic Acid, Acetyl- β-Boswellic Acid, 11-keto- β-Boswellic Acid, Acetyl-11-keto- β-Boswellic, listed in the order of increasing anti-inflammatory properties. One of the boswellic acids, Acetyl-11-keto-beta-boswellic acid (AKBBA) (Fig. 2.60), exhibits superior anti-inflammatory properites (Liang Y H *et al.*, 2010). AKBBA showed significant efficacy for topical anti-inflammatory and anti-arthritic applications (Goel A *et al.*, 2010). Based on the anti-inflammatory activity of Boswellic acids, they can be of significance for inhibiting skin pigmentation induced by inflammation.

*Figure 2.60: Structure of Acetyl-11-keto-beta-boswellic acid*
2.8.11.14. Garcinia cambogia and Garcinia mangostana extract:

*Garcinia* is a plant genus of the family Clusiaceae native to Asia, Australia, tropical and southern Africa. Many species of *Garcinia* have fruit with edible arils, but most are eaten locally; some species' fruits are highly esteemed in one region, but unknown just a few hundred kilometres away. The best-known species is the purple mangosteen (*G. mangostana*), which is cultivated throughout Southeast Asia and other tropical countries. Garcinol from *Garcinia cambogia* fruit (Fig. 2.63) extract and Mangostin from *Garcinia mangostana* fruit (Fig. 2.64) extract are known for antioxidant and anti-inflammatory properties (Padhye S, 2009; Koeberle A *et al.*, 2009; Shih Y W *et al.*, 2010 and Pothitirat W *et al.*, 2010). Based on the anti-inflammatory activity of garcinol (Fig. 2.65) and mangostin (Fig. 2.66), they can be of significance for inhibiting skin pigmentation induced by inflammation.
Figure 2.63: Garcinia cambogia fruit  
Figure 2.64: Garcinia mangostana fruit

Figure 2.65: Structure of Garcinol

Figure 2.66: Structure of Mangostin
2.8.11.15. Saffron:

**Saffron** is a spice derived from the flower of the saffron crocus (*Crocus sativus*), a species of crocus in the Iridaceae. A *C. sativus* flower (Fig. 2.68) bears three stigmas, each the distal end of a carpel. Together with their styles—stalks connecting stigmas to their host plant—stigmas are dried and used in cooking as a seasoning and colouring agent. Saffron contains more than 150 volatile and aroma-yielding compounds. It also has many nonvolatile active components, many of which are carotenoids, including zeaxanthin, lycopene, and various α- and β-carotenes. Saffron (Fig. 2.69) is the best example for a nutricosmetic as it has high antioxidant potential and tyrosinase inhibitory potential (Li C Y *et al*., 2004 and Karimi E *et al*., 2010). Saffron is traditionally used in India for beautification. In the present study Saffron has been used to study its usage as nutricosmetic with reference to its antioxidant potential.

![Saffron flowers](image1)

![Structure of Saffron](image2)
2.8.12. Coenzyme Q\textsubscript{10}: 

Other than plant sources, naturally occurring antioxidants like Coenzyme Q\textsubscript{10} (CoQ\textsubscript{10}), a significant antioxidant present in the mitochondria of eukaryotic cells can play a significant role for skin care. Presence of CoQ\textsubscript{10} is relatively much lower in plants. CoQ\textsubscript{10} (Fig. 2.67) also known as ubiquinone, ubidecarenone, coenzyme Q, and abbreviated at times to CoQ\textsubscript{10}, CoQ, Q10, or Q, is a 1,4-benzoquinone, where Q refers to the quinone chemical group, and 10 refers to the number of isoprenyl chemical subunits in its tail. This oil-soluble, vitamin-like substance is present in most eukaryotic cells, primarily in the mitochondria. It is a component of the electron transport chain and participates in aerobic cellular respiration, generating energy in the form of ATP. Ninety-five percent of the human body’s energy is generated this way (Ernster L and Dallner G, 1995; Dutton P L et al., 2000). Therefore, those organs with the highest energy requirements—such as the heart, liver and kidney —have the highest CoQ\textsubscript{10} concentrations (Okamoto T et al., 1989; Aberg F et al., 1992 and Shindo Y et al., 1994). Coenzyme Q10 acts as an important antioxidant in the body. The topical and peroral administration of network antioxidants, such as vitamin E and C, coenzyme Q10, alpha-lipoic acid and glutathione, enhance antiaging effect (Puizina-Ivic N et al., 2010).

Figure 2.69: Structure of Coenzyme Q10
2.8.13. Phytoestrogens:

Most of the aged women especially after menopause lose the glow in their skin due to the lowered estrogen levels. **Phytoestrogens** are xenoestrogens which means foreign substances functioning as the primary female sex hormone not generated within the endocrine system but consumed by eating phytoestrogenic plant products. Also called "dietary estrogens", are a diverse group of naturally occurring nonsteroidal plant compounds that, because of their structural similarity with estradiol (17-β-estradiol), have the ability to cause estrogenic or/and antiestrogenic effects. Phytoestrogens therefore have efficacy in increasing bone strength and brightness of the skin like estrogens. Phytoestrogens in combination with significant skin lightening actives can synergistically enhance the effect by bringing out a fair glow on the skin. **Soybean** or **Soya bean** (*Glycine max*) (Fig. 2.70) is a species of legume native to East Asia which is a rich source of phytoestrogens. The plant is classed as an oilseed rather than a pulse. The pods (Fig. 2.70), stems, and leaves are covered with fine brown or gray hairs. The leaves are trifoliolate, having 3 to 4 leaflets per leaf. The fruit is a hairy pod that grows in clusters of 3–5. Soybeans contain isoflavones, genistein and daidzein (Fig. 2.71), which are a type of phytoestrogen, that are considered to be useful in the prevention of cancer. Soy's content of isoflavones is as much as 3 mg/g dry weight. Isoflavones are polyphenol compounds, produced primarily by beans and other legumes, including peanuts and chickpeas. Isoflavones are closely related to the antioxidant flavonoids found in other plants, vegetables and flowers. Isoflavones such as genistein and daidzein are found in only some plant families, because most plants do not have an enzyme, chalcone isomerase which converts a flavone precursor into an isoflavone. Soybeans and processed soy foods are among the richest foods in total phytoestrogens (wet basis per 100g), which are present primarily in the form of the isoflavones daidzein and genistein (Thompson L U *et al.*, 2006). Combinations with Soya isoflavones were also studied for synergistic benefits in skin lightening as Isoflavones, which are phytoestrogens, have a structural and functional similarity to human estrogen and have been consumed by humans world-wide throughout history. Of all plant estrogens, soy isoflavones have been studied most. Because of the extensive consumption of soy-foods in Asia and Japan, there is reasonably
knowledge on long-term safety aspects, as well as on possible interactions between soy isoflavone consumption, disease prevention and skin conditioning (Brouns F, 2002; Schmid D and Zulli F, 2002).

Figure 2.70: Soya plant and beans

Figure 2.71: Structure of Soya isoflavones
2.8.14. Cell proliferation and Collagen enhancers:

Some actives do not directly help either in UV protection or skin lightening but in combination with other actives may have significant synergistic enhancement in the UV protection or skin lightening activity. These actives act as enhancers on account of their properties like cell rejuvenation and collagen enhancement. Actives with no significant antioxidant, anti inflammatory, UV protection or skin lightening potential but yet with a significant cell proliferation or collagen enhancement potential can have a significant effect in skin lightening. Therefore, the combination of cell proliferation enhancers or collagen enhancers along with skin lightening actives is of significance for cosmetic research. They help in skin rejuvenation and when taken in combination with skin lighteners, help in rejuvenation of the top layer of skin cells, with an effect that the darker skin is continuously replenished by fresh lightened skin cells. Therefore the process of skin lightening is quickened. For example, Retinoids influence pigmentation by speeding up turnover in the skin, gradually eliminating anything sitting on the top layers. This sloughing process automatically begins to slow down in our mid twenties. Retinoids reverse that effect by producing a faster rate of cell turnover as well as eliminating abnormal melanin in the top layer of skin. Retinoids are therefore useful in treating melasma and acne scars by reducing the amount of excess melanin and distributing it more evenly.

2.8.14.1. Liquid endosperm of Coconut, a nutritional active:

Some of the actives help in cell proliferation and rejuvenation by providing nutrients for the skin. One such active showing significant benefit for cell rejuvenation is the liquid endosperm of Coconut (*Cocos nucifera*) also called tender coconut water. *Cocos nucifera* (Fig. 2.72) belongs to the family Arecaceae and is widely grown in South Asia. Coconut liquid endosperm or tender water of the coconut (Fig. 2.73) contains concentrated amounts of growth hormones like Cytokinins which are involved in the cell growth and differentiation and in other physiological processes. When cytokinins are present, the cells can replicate to form a perfect copy of its DNA. If they are not present, imperfect cells are formed resulting in future chronic illnesses.
Coconut water is rich in important minerals like Calcium, Pottasium, Magnesium and Sodium which also promote cell growth. Due to its high nutritional value, minerals and growth hormone concentration, it is used by nature to nurture the growing embryo of the coconut. Hence, Coconut water extract rich in proteins (1.2%), Carbohydrates (85%), Sodium (0.5%), Pottasium (4%), Magnesium (0.3%), Cytokinins (0.003%), Indole-3-acetic acid (0.001%), Shikimic/quinic acids (25-30 Meq/mg) and RNA-P (25-30µg/g). The RNA-phosphorus (RNA-P) content of coconut water was found to be consistently high. The role of RNA in amino acid transport and respiratory metabolism of living cells is well known (Pandalai K M, 1958).
Coconut water is also used as an intravenous hydration fluid in some developing countries where medical saline is unavailable (Campbell-Falck D et al., 2000).

2.8.14.2. *Centella asiatica* extract:

*Centella asiatica* (Gotu Kola) (Fig. 2.74) is a small herbaceous annual plant of the family Mackinlayaceae or subfamily Mackinlayoideae of family Apiaceae, and is native to India, Sri Lanka and other parts of Asia. The stems are slender, creeping stolons, green to reddish green in color, interconnecting one plant to another. It has long-stalked, green, reniform leaves with rounded apices which have smooth texture with palmately netted veins. *Centella asiatica* leaf extract rich in triterpenes, Asiaticosides (Fig. 2.75) and
madecassoside has significant wound-healing activity through several mechanisms including antioxidative activity, collagen synthesis and angiogenesis (Liu M et al., 2008). Centella asiatica extract also exhibited significant anti allergic and anti inflammatory properties (George M et al., 2009).

Upon treatment with *Centella asiatica*, maturation of the scar is stimulated by the production of type I collagen. The treatment also results in a marked decrease in inflammatory reaction and myofibroblast production (Widgerow A D et al., 2000). *Centella asiatica* extract due to its collagen enhancement properties can in combination with other actives have significant synergistic enhancement in the UV protection or skin lightening activity.
2.8.15. Peptides for skin care:

Peptides are taking a significant importance in the field of cosmetics. Pentapeptides help in maintaining healthy skin and prevention of skin ageing. Pentapeptide KTTKS, a subfragment of type I collagen propeptide, has been demonstrated to promote the extracellular release of collagen in fibroblasts. KTTKS (Lysine-Threonine-Threonine-Lysine-Serine) promotes the expression of type I collagen and maintains its mRNA stability in a process associated with the upregulation of Transforming growth factor, TGF-beta, which is a gene associated with upregulation of collagen (Tsai W C et al., 2007). The palmitoyl pentapeptide, palmitoyl-lysine-threonine-threonine-lysine-serine (pal-KTTKS) is a synthetic material that was designed as a topical agent to stimulate collagen production and thus provide a skin anti-wrinkle benefit. Pal-KTTKS was well tolerated by the skin and provided significant improvement vs. placebo control for reduction in wrinkles/fine lines by both quantitative technical and expert grader image analysis. In self-assessments, subjects also reported significant fine line/wrinkle improvements and noted directional effects for other facial improvement parameters (Robinson L R et al., 2009). L-ascorbic acid (Vitamin C) and its peptides are both useful compounds for collagen biosynthesis in cosmeceuticals. The instability of these compounds, however, limits their applications in cosmetics. It is reported that a novel compound, Stabilized Ascorbyl Pentapeptide (SAP), which physically is much more stable than L-ascorbic acid in water, inhibits tyrosinase and melanin synthesis comparable to that of L-ascorbic acid. Importantly, the SAP compound displays no cytotoxicity at a high concentration (5 mM). The ability of SAP to promote collagen biosynthesis is greater than that of L-ascorbic acid or the KTTKS peptide alone (Choi H I et al., 2009). Therefore a conjugate of a skin lightener Ascorbic acid with a collagen enhancer KTTKS peptide resulted in potential candidate that helped in skin lightening as well as inhibiting skin ageing. Based on these findings in the present study, peptides conjugated to potential actives were selected for the present study to emphasize on the integration of various skin lightening mechanisms for a synergistic potential.

Peptide sequences conjugated to triterpenes like Oleanolic acid (Fig. 2.78) can have significant potential for skin care since as Oleanolic acid (Fig. 2.77) is known to
have significant antioxidant and anti inflammatory activity (Allouche Y et al., 2010). Oleanolic acid is a naturally occurring triterpenoid, widely distributed in food and medicinal plants. *Salvia officinalis* (Garden sage, Common sage) (Fig. 2.76) is one such medicinal plant which is a small perennial evergreen shrub, with woody stems, grayish leaves, and blue to purplish flowers. It is a member of the family Lamiaceae and is native to the Mediterranean region, though it has naturalized in many places throughout the world. It has a long history of medicinal and culinary use, and in modern times as an ornamental garden plant. The plant grows to approximately 2 ft tall, with lavender flowers most common, though they can also be white, pink, or purple. The leaves are oblong, grey-green on the upper side and nearly white underneath with many short soft hairs. The strongest active constituents of sage are within its essential oil, which contains cineole, borneol, and thujone. Sage leaf contains tannic acid, oleanolic acid, ursolic acid, cornsone, cornsolic acid, fumaric acid, chlorogenic acid, caffeic acid, niacin, nicotinamide, flavones, flavonoid glycosides, and estrogenic substances and all the diterpenoids and triterpenes have significant antioxidant potential (Mathe I et al., 2007).
Similarly a peptide conjugates with synthetic actives like Thiodipropionic acid, a white crystalline solid with significant antioxidant benefits (Diamante C et al., 2010) can also have significant potential for skin care.

2.8.16. Probiotics for skin care:

Apart from just plant actives, certain probiotics can have significant potential for skin care applications. It has been clinically-proven that Probiotics have a positive effect on UV-exposed skin. Studies have shown that skin cell regeneration after UV exposure is accelerated by using Probiotics as a complement to sun protection cream (www.research.nestle.com). The Tyndallized Bacteria Complex was demonstrated to have the properties of stimulating synthesis of endogenous ceramides, deep hydration, nutrition and protection, immunostimulation, soothing irritation and antimicrobial efficacy (www.verattiva.com). Probiotic bacteria *Bacillus coagulans* (Fig. 2.79), a lactic acid producing bacteria is one such probiotic bacteria that can be of significance for skin care.

![Figure 2.78: Bacillus coagulans](image)
2.8.17. Tetrahydropiperine (THP), an enhancer of bio-availability of actives:

Apart from chemical conjugation of actives, physical combination of various actives with the integration of various mechanisms of action can have a significant synergistic effect on the skin lightening potential. The actives in combination with **Tetrahydropiperine (THP)** (Fig. 2.81), an enhancer of bioavailability (Matkari Y et al, 2006) for the adequate availability of actives to the target sites for enhanced efficacy is of significance for cosmetic applications. Tetrahydropiperine, a natural aryl pentanamide is a derivative of piperine, an alkaloid found in black and long pepper (*Piper longum*) (Madhusudhan P and Vandana K L, 2001). The fruit of the pepper (Fig. 2.80) consists of many minuscule fruits, each about the size of a poppy seed, embedded in the surface of a flower spike that closely resembles a hazel tree catkin. The alkaloid piperine in the fruits contributes to their pungency. THP from *Piper longum* in combination with skin lightening or sunscreen actives can make the actives more bio available at the target site of skin damage thereby enhancing the skin repair process. Such studies were not earlier done for skin care applications.

*Figure 2.79: Piper longum plant and dried fruits*

*Figure 2.80: Structure of Tetrahydropiperine*
2.8.18. Reference standards for skin lightening:

Significant skin lightening actives other than Ascorbic acid are Kojic acid (Fig. 2.82) and Arbutin which are used in research as reference standards for skin lightening. Kojic acid is a chelation agent produced by several species of fungi, especially *Aspergillus oryzae*, which has the Japanese common name *koji* (Yabuta T, 1924). Kojic acid is a by-product in the fermentation process of malting rice, for use in the manufacturing of sake, the Japanese rice wine. It is a mild inhibitor of the formation of pigment in plant and animal tissues (Kahn V, 1995). It is used on cut fruits to prevent oxidative browning, in seafood to preserve pink and red colors, and in cosmetics to lighten skin. It is also used in skin diseases like melasma (Bandyopadhyay D, 2009).

![Figure 2.81: Structure of Kojic acid](image1)

Arbutin (Fig. 2.83) also known as Bearberry extract is both ether and a glycoside; a glycosylated hydroquinone extracted from bearberry plant in the genus *Arctostaphylos*. It inhibits tyrosinase and thus prevents the formation of melanin. Arbutin inhibits melanin production in B16 cells induced with alpha-MSH and decreases tyrosinase activity in a cell-free system (Lim J Y *et al.*, 2009).

![Figure 2.82: Structure of Arbutin](image2)
Arbutin is therefore used as a skin-lightening agent. Arbutin is found in wheat, and is concentrated in pear skins. Bearberry Extract is used in skin lightening treatments designed for long term and regular use. It is more expensive than traditional skin lightening ingredients like hydroquinone which was reported to have safety concerns and is now banned in many countries.

An important industrial chemical, hydroquinone (HQ) is also a ubiquitous chemical readily available in cosmetic and nonprescription forms for skin lightening. It is considered one of the most effective inhibitors of melanogenesis *in vitro* and *in vivo*. HQ causes reversible inhibition of cellular metabolism by affecting both DNA and RNA synthesis. The cytotoxic effects of HQ are not limited to melanocytes, but the dose required to inhibit cellular metabolism is much higher for nonmelanotic cells than for melanocytes. Thus, HQ can be considered a potent melanocyte cytotoxic agent with relatively high melanocyte-specific cytotoxicity Azelaic acid also has an antiproliferative and cytotoxic effect on melanocytes. The latter effect occurs because of a rather potent inhibition of thioredoxin reductase, an enzyme involved in mitochondrial oxidoreductase activation and DNA synthesis. (http://emedicine.medscape.com/article/1068091-overview). Hence HQ and Azelaic acid were not selected as an appropriate reference standard.