CHAPTER 1 INTRODUCTION
PREAMBLE
The present chapter introduces the theme of the research. A detailed background about the current status of the cosmetic as well as nutraceutical products is introduced in the chapter. The chapter also highlights the use of plants in traditional therapeutic systems and tremendous potential of their actives to exert a pharmacological effect. The use of synthetic chemical components has been reported to exert adverse effects on the physiology either due to their continual and irrational use or even their innately associated undesirable effects. Thus raised concerns about the safe use of therapeutics as well as cosmetics have created a need for continual research in these areas. The present chapter thereby provides an elaborate idea about the root problem encountered and the purpose of the present research work undertaken. It thus provides an effective solution for the problem. For ease of understanding the chapter is subdivided into subtopics relevant to the concern areas.

Chapter 1.1 GENERAL INTRODUCTION
1.1.1 BRIEF INSIGHT ABOUT USE OF COSMETICS:
Don't you want to look beautiful? Attractive and possess an impressive personality? The answer for all such questions being asked either to the men or women is always a big YES. Then here lies a question about how you groom yourself, what do you wear, how do you communicate etc. The key point in corporate world is grooming one's facial looks to be impressive along with the efficient communication; to work on the first part is much simpler than the second hence the use of cosmetics comes into the picture.

   Globalization has made world's population more urbanized. Personal appearance is becoming more and more individualized, and people desire living and staying young longer. There is no age limit to dream beautiful look, as the unwavering truth of human being is that the younger people do not want to age and the older people continuously desires to appear younger. For this reason more and more people seek to enhance their looks by using means like cosmetics. For centuries, civilizations have used forms of cosmetics products to look attractive and elegant. Although written recorded history dates back only six millennia, the history of skin decoration and care is much longer, perhaps
as long as mankind itself. The growing health and beauty consciousness has thus created excellent opportunities for growth in the global beauty industry thereby challenging associated professionals such as formulation scientists, dermatologists, manufacturers of cosmetic products and also dieticians. Nanotechnology in cosmetics has thus aroused. The nano size of the cosmetic constituents can be spread uniformly on the skin surface and provide a enhanced protection like in case of sunscreen formulation. Also due to the smaller size the particles could permeate much more deeply than the micron size used conventionally. Also they were found to be retained in the skin layers for longer time thus the effects were provided for more time period. The overall elegance of the product also improved.

Technology today is viewed and recognized as a double ended sword. So is the use of nanotechnology in cosmetics. Although beneficial and been explored tremendously in the last decade, it has been reported that the dermal exposure of nanomeric particles of drug to be retained on surface, produces more deleterious effects to skin internal structures by penetration into deeper layers to maximum levels and also absorption to a greater extent in general systemic circulation. Hence the need for choosing the nano formulation approach should be ruled out initially. The toxicity of such products on topical application to the internal organs should also be evaluated.

Cosmetic formulations are being used right from the morning as one gets up like toothpaste or followed by soap, face wash etc. till the night like night massage cream. Hence no consumers can say that do not used a single cosmetic. Be it a routine hygiene or a product providing attractive looks the list is never ending. Cosmetic Industry is thus blooming day by day due to constant consumer demand. The skin is the superficial organ of the body on which the cosmetics are used. The skin over a period of time shows wrinkling a primary sign of aging. This could be natural or could be premature. In order to look younger and glowing, the skin should be maintained healthy with routine hygienic procedures. However the detrimental factors like unprotected sun exposure, genetics, ever growing stress and the exposure to environmental toxins can show wrinkling effect leading to premature ageing. The first factor responsible for the premature skin aging can be easily controlled while the rest are ambiguous. An overview of the skin anatomical
structure could thus give a detail understanding of the underlying cause of aging phenomenon.

1.1.1.1 Structure of human skin:
The human skin is the superficial and easily visible organ. The total surface is about 2 square meters weighing approximately 4.5-5 kg. The thickness of the skin layer varies depending on the mechanical wear and tear encountered at each part of the body. The anatomical structure reveals the two important layers of the skin. The transverse sectional view of skin as sketch is given in Chapter no. 5.1, Figure no. 5.1.1.1.1.1. The outermost layer exposed to the environment is the epidermis and comprises the epithelial tissue. While the second layer is dense and comprises a connective tissue matrix. This layer is called as dermis. Further a fatty tissue layer is present beneath the dermis, called as hypodermis. Cosmetic dermatology lays interest specifically on the epidermis as it is the permeability barrier that prevents loss of water and electrolytes (Pillai et al., 2010; Tortora et al., 2009).

1.1.1.1.a Epidermis:
The outermost epidermal layer mainly comprises stratified squamous epithelium which contains keratin protein. It is characterized by four different types of cell depending on its functions as keratinocytes, melanocytes, Langerhans cells, and Merkel cells. About 90% of epidermal cells are keratinocytes, responsible for the production of water repellent keratin protein synthesis. They provide a lipid barrier to the entry of all chemicals. The next types of cells are melanocytes, responsible for the synthesis of melanin. The pigment provides a specific color to the skin and also functions as ultraviolet radiation absorber. Thus the cells synthesized melanin protects the DNA structures from the oxidative stress induced by ultraviolet light exposure. Further the Langerhans cells are responsible for preventing the invasion of the antigenic components through the skin. Thus it serves as a defensive role. Merkel cells are present to a lower extent; however they functions as a sensor for touch.
The epidermal layer is present as four layers throughout the body. They are arranged as *Stratum basale, Stratum spinosum, Stratum granulosum*, and a thin *Stratum corneum*. However in regions which are subjected to more mechanical work and high friction, the thickness of the epidermal layer is higher and also reveals the presence of additional layers of cells. They are arranged as *Stratum basale, Stratum spinosum, Stratum granulosum, Stratum lucidum*, and a thick *Stratum corneum* (Tortora *et al.*, 2009).

1.1.1.1.b Dermis:

The dermis lies between the epidermis and the subcutaneous fat. The whole mass of the dermis may constitute 15-20% of total body mass. Made up of collagen and elastin fibres, its functions include supporting the epidermis and the annexes i.e. hairs and nails, and regulating temperature (Tortora *et al.*, 2009).

1.1.1.1.c Hypodermis (Subcutaneous):

The innermost layer hypodermis beneath the dermal matrix is not considered as important in cosmetic application as it just provides cushioning effect and acts as a shock absorber to the body. It mainly comprises of adipose tissue (Tortora *et al.*, 2009).

1.1.1.2 Physiological effects of UV radiation:

1.1.1.2.a Useful effects:

Vitamin D, essential for healthy bones, is synthesized in the skin following irradiation by sunlight. Over-production of Vitamin D is harmful but is prevented by the natural Ultraviolet radiation (UV) attenuating mechanisms of the skin such as pigmentation and keratinization. Tanning, or the increase of pigmentation in the skin, is the most common biological reaction following exposure to UV radiation. The melanin formed in this process acts to absorb the incident UV radiation, thus protecting the skin. It mainly prevents the generations of UV induced free radicals and further limits the potential
oxidative stress and UV induced damage to the cells. However the concentration of melanin produced in the skin may not be adequate to prevent the oxidative damage to the skin. Keratinization, like pigmentation, is a natural defensive response of the skin to UV radiation. The keratin protein synthesis is induced on exposure to UV radiations which is responsible to scatter the light, hence keratin then serves as UV protectant.

1.1.1.2.b Harmful effects:

Ultraviolet radiations (UVR) or solar radiations are electromagnetic radiations with wavelength in the range of 200 nm - 400 nm. These radiations are classified according to the wavelength as UV-A (320-400 nm), UV-B (290-320 nm) and UV-C (100-290 nm). Prolong and unprotected ultraviolet radiation could exhibit marked short term as well as long term effects on the skin. UV-C is absorbed by the Earth's ozone layer. UV-B and UV-A however is able to penetrate the ozone layer and affect the radiation exposed skin (Sonawane et al., 2010). The skin protects itself from the UV-A and UV-B radiation by absorption of the radiation by chromophores. Depending on the wavelength absorbed, UV radiation interacts with various skin cells at different depths. Energy from the UV-B rays is mostly absorbed by the epidermis and affects the keratinocytes, the UV-A rays affect the epidermal keratinocytes as well as the dermal fibroblasts. UV-B radiation may affect the immunologic functions of the skin along with its fatal damage to the DNA, thus inducing uncontrolled growth of cells exhibiting cancerous overgrowth in 65% cases (Mishra et al., 2011). These radiations are both erythemogenic (capable of producing redness) and pigmentogenic. In contrast UV-A capable of penetrating up to the deep skin layers can damage the DNA and tissue via the production of reactive oxygen species (ROS). These radiations are pigmentogenic but produce little erythema. Erythema, or sunburn, is often the most noticeable reaction to UV radiation, particularly in fair-skinned people. It develops 2-3 hours after initial exposure and reaches a maximum 10-24 hours later. The mechanism is thought to involve the photochemical generation of unstable radical species which combine to form irritants such as peroxides, which subsequently give rise to erythema.

Ultraviolet radiations thus penetrating the skin if not protected can accelerated the cell damage by inducing free radical generation, leading to increased production of
inflammatory mediators followed by increased inflammation. Further the damage to the dermal elastic matrix can cause the skin to lose the flexible nature and produce wrinkles. If the exposure prolongs then the melanin synthesis is also increased leading to hyperpigmentation. The mechanism thus involved in premature ageing on prolonged exposure to UV radiation is breakdown of the collagen and elastin fibres of the skin by enzymes collagenase and elastase and deterioration of the texture of skin. Further degenerative changes have also been reported in the skin cells, fibrous tissues, blood vessels, most serious cases skin cancers could erupt (Maithri et al., 2012). The dreadful effects of UV radiations on skin could be explained in nutshell in Chapter no. 5.1, Figure no. 5.1.1.1.2.1. To avoid all such detrimental consequences it is therefore recommended protecting the skin against ultraviolet radiation exposure. Use of safety clothing, hats or total avoidance of radiation exposure may not be a feasible option in routine practice and corporate job hence use of sunscreen could be considered as most feasible.

1.1.2 INTRODUCTION TO SUNSCREENS:
Rising consumer awareness about global warming and changing intensities of radiations reaching the Earth’s surface have raised concerns among them. Recent statistics reveal that Sunscreens or sun care products have captured upto 10% of global skincare market. These topical formulations containing sunscreen agents prevent or protect the skin from direct contact of sunrays thereby minimizing the harmful effects of the solar radiations on the skin on application. Such sunscreen agents are categorized as UV light absorbers and UV light reflectors. UV light absorber comprises organic compounds like benzophenone derivatives, cinnamate derivatives etc. able to absorb more than 95% of the radiations due to its specific chemical structure. The energy absorbed by the organic agents is converted to non-damaging energy and dissipated primarily as heat. On the other hand UV reflector comprises inorganic compounds like titanium dioxide, zinc oxide which are able to scatter radiation based on particle size. The photons thus scattered by them are reflected back out of the skin. Increasing the particle size of the inorganics increases the reflecting potential; however the overall product appearance turns opaque on application to skin.
hence they do not favor consumer acceptance. Thus the widely used sunscreens products include UV absorbers as potential sunscreen agents.

Currently an exhaustive list exists about the different UV absorbers which can be used in the formulation; however non-toxicity and dermatological acceptability are important factors to be considered in their selection. A sunscreen product protects different individuals to the different extent. This depends on the individual skin type as described by Fitzpatrick scale tabulated in Chapter no. 5.2, Table no. 5.2.1.1.2.1 This scale was developed by Thomas Fitzpatrick, a Harvard Dermatologist to classify the response of different skin types to ultraviolet radiations. Plough Corporation further developed the sun protection factor (SPF) system to define the relative effectiveness of sunscreen agents to protect the skin. However later US FDA recommended SPF as a means of numerically identifying the efficacy of various sunscreen products and to provide consumers a guide to the products suitable for their individual skin types.

The SPF value determined \textit{in vivo} is given as,

\[
\text{SPF value} = \frac{\text{MED (PS)}}{\text{MED (US)}}
\]

Where,

\begin{align*}
\text{MED (PS)} & = \text{Minimum erythemal dose for protected skin.} \\
\text{MED (US)} & = \text{Minimum erythemal dose on skin without any product.}
\end{align*}

Larger the Sun protection factor value, greater is the sun protection offered. A detail description of SPF suggested for each skin type is also mentioned in Chapter 5.2, Table no. 5.2.1.1.2.1. \textit{In vivo} evaluation is critical, costly and has raised ethical issues hence \textit{in vitro} SPF determination is being accepted worldwide. Every skin is unique, as the extent of the melanin produced in the skin varies depending on the environment in which the individual dwells in. As reported in various literatures and also in the earlier topics melanin protects the skin against harmful effects of the ultraviolet radiation exposure. So each skin might reveal a different response on exposure to radiations. Not all the consumers are aware about the selection criteria of the sunscreen product and which will
be suitable for their particular skin type. Hence the need for a particular sunscreen formulation with an appropriate SPF value should be ruled out using the opinion of the dermatologist.

1.1.2.1 Does use of sunscreens solve the problem?

As mentioned in the earlier topics use of sunscreens is the most feasible option available to protect the skin exposure to harmful ultraviolet radiations however the safety of such products must be evaluated before the use. The sunscreens are available easily in any departmental stores, medical retail shops, malls etc. The choices available are also many. The selection of the best and safe can be recommended only by the healthcare person. Hence the opinion of the dermatologist to use a particular product must be considered.

Today's out of the total sunscreens available in the market some of them contain mainly constituents which are capable of undergoing degradation on exposure to ultraviolet radiations. Further such constituents along with the degraded products are also able to penetrate the skin layers and enter the general circulation. Cosmetics are merely applied for topical skin effects however if they are penetrated into the deep layers, should be restricted in the skin layers thereby preventing systemic absorption. The reality is completely different. They are been found to move still deeper inside providing greater concentrations near the blood vessels and thus being diffused inside. Hence the conventionally used sunscreens have been relooked nowadays with respect to photostability, major concern considering the overexposure to UVR as cause of global warming. Photoinstability leads to phototoxic reactions, wherein the photolabile molecule on absorption of energy from ultraviolet light and dissipates in the form of heat to the cellular structures. Further phototoxic response occurs by production of photo adducts with biological molecules or through formation of reactive oxygen species (ROS), such as singlet oxygen ($^1$O$_2$) and superoxide (O$_2^-$) thereby selectively modifying oxidative reaction mechanisms (Onoue et al., 2006).

In fact, different adverse reactions like photosensitivity reactions either phototoxic or photoallergic in nature, generation of reactive oxygen species and simulating
estrogenic effects have been revealed on continual use of photounstable formulations comprising sunscreen actives (Gordon et al., 2005, Bambal et al., 2011). Absorption of UV-A by the chromophores acts indirectly by transferring energy to generate ROS. UV-B rays have a more direct effect on the absorbing chromophores. About 50% of the total photodamage results from the formation of free radicals. Apart from the ultraviolet radiation induced skin reactions the generation of endogenous mitogens, most importantly reactive oxygen species (ROS) and other byproducts like free radicals of physiological cellular metabolism reactions are also required to be overcome by the skin.

### 1.1.2.2 Photodamage:

The skin is very much susceptible to damage caused by photon or ultraviolet light exposure as it is the superficial organ. The human skin on chronic exposure to ultraviolet radiations can lead to induction of oxidative stress thereby generating the reactive oxygen species. These free radicals further cascade towards the abnormal and uncontrolled cell growth leading to skin cancer in individuals (Goihman M. 1996). The less serious consequences include wrinkling, scaling, dryness and hyper/hypo-pigmentation. The photochemical reaction is based on the basic law of photochemical absorption, established by Grotthus and Draper in 1818 that a photochemical reaction is possible only on absorption of the electromagnetic radiation. Further the theory states that due to the absorption of the ultraviolet radiations the molecules produce an excited state chemical or metabolite, and further follows either of the pathways leading to photosensitization. In the first pathway free radicals are generated while in the second pathway singlet oxygen is produced, which further oxidizes the biomolecules, damage their critical cellular components and initiate erythrogenic mediator’s release.

### 1.1.2.3 Reactive Oxygen Species:

Species able to exist as an independent moiety and comprising unpaired electrons are commonly termed as free radicals. Reactive oxygen species consisting of superoxide anion (O$_2^-$), peroxide, hydroxyl radical (OH$^-$), hydroxyl ion, and singlet oxygen (^1O$_2$), an excited state of molecular oxygen are the unstable moieties commonly encountered during the oxidative stress. They exhibit a high energy state and hence donate electrons to
the neighbouring molecules to become stable. They can be sequestered by antioxidants to reduce the oxidative damage to the cellular skin structures.

1.1.2.4. Physiological damage to the cells from free radicals:
Exposure to UV-B radiations can produce DNA damage. UV-A induced ROS generation and further damage caused to the DNA is mediated by formation of a modified guanine nucleotide (8-hydroxyguanine), lysis of single-strandes as well as oxidation of pyrimidine bases (Cadet J. *et al.*, 2011; Kielbassa *et al.*, 1997). Ultraviolet radiation generated ROS also target the lipid cellular phospholipid membranes and proteins causing generation of lipid peroxides protein by products as carbonyl derivatives. Eventually all three effects can be detrimental to apoptosis.

1.1.2.5 Cutaneous damage from free radicals:
1.1.2.5.1. Photoaging:
Harman D, stated that ageing related symptoms were observed due to the accumulation of free radicals. Exposure to ultraviolet radiations generate ROS further cascade the release of proinflammatory cytokines leading to erythema and growth factors (Fisher *et al.*, 2002; Wlaschek *et al.*, 1995). Important factors include the activation protein-1 (AP-1), nuclear factor-B (NF-kB) thereby up-regulating the key matrix metalloproteinases (MMP) such as MMP-1, MMP-3, MMP-8, as well as MMP-9. Proteases further degrade the collagen and elastin fibers of the extracellular matrix (Sardy *et al.*, 2009). Also, Ultraviolet radiation induced ROS showed decline in the transforming growth factor-β-expression, which further reduces production of collagen while enhancing production of elastin (Kawaguchi *et al.*, 1997; Bernstein E. 2002; Uitto J. 2008).

1.1.2.5.2. Suppression of immune response:
UV-A and UV-B are capable of initiating the suppression of the immune response in the skin. Although the mechanism of UV-A immunosuppression is yet to be known completely, a ROS dependent mechanism is put forth. UV-A induced ROS produces lipid peroxidation, disrupts the redox potential, and also initiate AP-1 and NF-kB transcription, further eventually activating downstream cytokines (Interleukin-4 and Interleukin-10),
responsible therein for systemic immunosuppression (Ulrich S. 2005; Shreedhar et al., 1998).

1.1.2.5.3. Photo carcinogenesis:
Ultraviolet induced ROS interferes with normal cell signaling as it affects the expression of signal transduction genes (Valko et al., 2007). Halliday G. also examined DNA from human actinic keratoses and squamous cell carcinomas revealing significant ROS mutations. A large number of mutations were ROS induced on the p53 gene, thereby suggesting that ROS can be a driving precursor or as a mutagen to malignancy (Stolze et al., 1999). Thus the data provides an experimental based evidence that the ROS which are formed on exposure to ultraviolet radiations exert a damaging effect on the skin cells thereby leading to cancer.

1.1.2.6 Combating the oxidative stress by use of Antioxidants:
Antioxidants are described as molecule able to delay or inhibit the lipid oxidation and other molecules thereby inhibiting the initiation or propagation of oxidative chain reactions and also able to prevent or repair the oxidative cell damage (Tachakittirungrod et al., 2007). It acts through various mechanisms such as, reactive oxygen species (ROS) scavenger, reducing power, chelation of metal ions, as an antioxidative enzyme, and also inhibition of oxidative enzymes (Shahidi 1997). Recent literature revealed a sizable research on antioxidant activity of the herbal extracts. The phytochemicals serving as antioxidants obtained from plants, particularly flavonoids and other polyphenols, have been reported to exhibit antioxidant activity by inhibiting the propagation of free radical reactions (Tachakittirungrod et al., 2007). Also, the use of synthetic antioxidants has raised significant concern due to their reported toxicity (Valentao et al., 2002). Thus to protect the cells and organ of the body against reactive oxygen species, humans have researched a highly sophisticated and complex antioxidant protection system. It includes a variety of components, both endogenous and exogenous in origin, functioning interactively and synergistically to neutralize free radicals.
1.1.2.6.1 Antioxidants obtained \textit{in vivo}:  
These include enzymes catalyzing the removal of oxidants. The endogenously obtained superoxide dismutase, superoxide reductase, catalase, and glutathione peroxidase are majorly produced antioxidants.

1.1.2.6.2 Antioxidants obtained through external source:  
These antioxidants such as vitamins, trace elements, and phytoantioxidants are not synthesized by our body hence provided externally. Vitamin E (tocopherol) helps in inhibition of the peroxidation of membrane lipids. It also works in conjunction with other antioxidants such as vitamin C and selenium. Vitamin C is a water-soluble and exhibits a strong antioxidant activity by scavenging free radicals. Selenium like trace elements are important cofactors for the activity of antioxidant enzymes (Vile \textit{et al}., 1994 and Dernbach \textit{et al}., 2004). The skin is well equipped with an elaborate system of antioxidants and enzymes thereby protecting from the oxidative stress generated by sunlight exposure. However, the normal ageing process and environmental stress can deplete the epidermis of the protective antioxidants. Hence, it is beneficial to replenish the skin"s reservoir by external application of antioxidants containing formulations. The stability of the molecule needs to be taken care of. Thus, antioxidants need to be formulated in a way that maximizes their delivery to the skin.

1.1.2.6.3 Externally supplied antioxidants:  
1.1.2.6.3.a Ascorbic acid:  
Vitamin C (L-ascorbic acid) is found to significantly enhance the synthesis of collagen and also provides photoprotection. Continual photoprotection provided by vitamin C rendered the normal skin structure in photodamage skin, also the synthesis of collagen and inhibition of MMP-1 was revealed to reduce the wrinkling of the skin, and the tyrosinase inhibition and anti-inflammatory activity resulted in depigmenting solar lentigines (Dayan N. 2008).
1.1.2.6.3.b Vitamin E:
Vitamin E (α-tocopherol) provides photoprotection (Fryer M. 1993). Its topical application has been shown to reduce short term as well as long term photodamage. Topically applied, only the natural forms of vitamin E, alpha tocopherol and tocotrienol was found to effectively reduce the skin roughness, the length and depth of wrinkles (Mayer et al., 1993). Topical application also revealed to increase the hydration of the stratum corneum (Dayan N. 2008). It contains good amounts of linoleic acid (43-53%), Tocopherols and phenolics, accounting to 59% of the antioxidant effects.

Apart from these the vitamins carotenoids, proanthocyanidin, caffeic acid, ferulic acid etc plants constituents have also been reported to exhibit antioxidant as well as photoprotective effects. Carotenoids are found to prevent the lipid peroxidation induced by the exposure of the skin to ultraviolet radiations. They are also effective against sunburn. Cyanidin, pelargonidin, nasunin, delphinidin etc. constituents obtained from the fruits belonging to the class anthocyanin exhibit inhibitory activity on the generation of free radicals during ultraviolet radiation exposure to skin. Grape seed Proanthocyanidins are reported to provide effective antioxidant mediated mechanisms against tumor growth. Not only are the isolated compounds effective but the whole plant extracts are also effective. *Tomato extract, Prunus amygdalus* extract, *Pongamia pinnata* extract, *silybum marianum* extract were also reported to be effective as antioxidants. Other known examples of plant extracts with antioxidant activity includes; *Terminalia chebula*, Marjoram, Wormwood, Liquorice, Accacia, Lemon, Thyme, Shatavari, Marigold etc.

1.1.2.7 Plant isolates and extracts for photoprotective activity:
Aware of the fact that UV exposure generates free radicals leading to oxidation of tissues, lipids and proteins and that plant do not move i.e they are immobile, it was obvious that plants would certainly develop defence mechanisms for their survival. Hence the objective was to search for phytoantioxidants. Ayurvedic literature recognizes a substantial number of plants such as *Boerhavia diffusa, Bacopa moneira, Carum carvi, Eulopia campestris, Ocimum sanctum, Zingiber officinale* with skin anti-inflammatory and antiaging activity but there is lack of scientific data on their photoprotective activity.
Tea polyphenols (*Camellia sinensis*), curcumin (*Curcuma longa*), silymarin, apigenin, resveratrol have shown efficacy against UV radiation induced inflammation and skin cancer i.e. photocarcinogenesis. Tea polyphenols mainly constitute Epigallocatechin-3-gallate, (-) epigallocatechin gallic acid, catechins etc. while silymarin constitutes polyphenolic and flavonoids. The flavonoids exist as a mixture of different flavanolignans like silybin, silidianin, silychristin and isosylibin. Further caffeic and ferrulic acids which are cinnamic acid derivatives of natural origin obtained from fruits, grains and vegetables. Further genistein isoflavone and quercetin are also reported to exhibit photoprotective ability. Carnosic acid from rosemary and sage are probable photoprotectants and hence need to be tested for topical photoprotective activity (Svobodova *et al.*, 2003).

Topical as well as oral ingestion of *Polypodium leucotomes*, *Capparis spinosa*, *Culcitium reflexum*, bark of *Pinus pinaster* comprising polyphenols and phenolic compounds, *Ginkgo biloba*, grape seed extract (GSE), root extract obtained from *Krameria tiandra*, *Sanguisorba officinalis* constituting tannins which prevent the dermal elastic nature, *Aloe barbadensis* extract and red oranges are all evaluated for their photoprotective ability.

The above data thus encouraged the search for phytoantioxidants with either a polyphenol (Flavonoid) or with terpene background. Multiple isoprene units lead to the formation of terpenes. Eg. Vitamin E is a 20 isoprene compound and should be able scavenge the free radicals generated upon exposure of human skin to UV radiation.

Thus it may be understood that the plants have a great potential to be used as therapeutics and also in cosmetics. There are many plants that are yet to be explored for their utilities. A detail literature reports were reviewed and a simple as well as fundamental approach was revaled to prevent the skin ailments like erythema, sunburn, oxidative damage to the DNA structures leading to skin cancer like harmful effects, produced on exposure of the skin to ultraviolet radiations either in the UV-A or UV-B range and mediated manly through oxidative stress. Thus in the present research an attempt shall be made to screen the natural, cheap, safe herbal extracts for effective sunscreens activity mediated through its antioxidant mechanism. An elaborate and extensive research methodology thus planned may rule out all the possible shortcomings.
encountered in development of a effective formulation. The product is required on the skin surface to exert a ultraviolet radiation protective effect hence development of a topical formulation could be the best approach.

1.1.3 BRIEF INSIGHT INTO SCENARIO OF ANTI-ACNE AGENTS:

Apart from ultraviolet radiation induced skin ailments one of the major problems affecting the consumers at large is Acne. Acne basically develops due to clogging of the pores eventually leading to infestations of micro-organisms. The pictorial representation of the acne condition is given in Chapter no. 5.1, Figure no. 5.1.1.3.1. Acne condition is described as presence of inflammatory lesions and invasion of bacteria Propionibacterium acnes, Staphylococcus epidermidis in the follicular canal along with alteration of the overall sebum turnover. P. acnes bacteria are capable of secreting various proteins, including digestive enzymes. These enzymes further digest the sebum and also involved in the other nutrients acquisition. They are also capable of destabilizing the cell layers forming the follicle walls. The inflammation is mainly triggered by the damage to the cells, formed byproducts during its metabolism and debris produced by bacteria during the rapid growth of Propionibacterium acnes in the follicles. Researchers reported that healthy pores are specifically colonized only with P. acnes hoewever the unhealthy ones include the non-pore resident Staphylococcus epidermidis universally. Current treatment of acne majorly includes drugs like retinoid, isotretinoin and antibiotics; the widely used marketed antiacne formulations are listed in Chapter no. 5.2, Table no. 5.2.1.1.3.1. Although retinoids and isotretinoin have proven medical benefits they also have major disadvantages associated with the single drug therapy. Thus most largely prescribed ones include oral and topical antibiotics, however the invading bacteria are found to develop resistance with continuel use (Bahmani et al., 2008) following repeated or subclinical doses as shown in Chapter no. 5.2, Table no. 5.2.1.1.3.2. This growing incidence of bacterial resistance to antibiotics represents a major medical, socio-economic challenge for health care researchers. Thus these bacteria"s are termed as “Superbugs”, disease-causing microorganisms resistant to multiple antibiotics. “This serious threat is now not a prediction for the future, because it”s happening right now in every region of the world and also has the potential to affect
anyone, of any age, in any country”, states a 2014 World Health Organization report. More than as a skin ailment the growing concern about acne is the tremendous psychological impact on the image conscious professionals as shown in Chapter 5.1, Figure no. 5.1.1.3.2. The acned face does not provide an hygienic look at the same time the itching and irritation of the acned, pimpled face causes discomfort to the patient. Moreover psychological based case studies reported that the confidence of the person with acned face is very low and hence no one desires even single acne on their face. Although stress, hormonal changes as well as environmental factors also can lead to acne formation, the major cause is the infection by the invading acne causing organisms. Also the growing incidence and reports about the resistance to the microorganisms towards the earlier and effective antibiotics have raised a concern in healthcare team. Thus there is a continual need for newer anti-acne molecules providing an improved therapeutic action and greater consumer satisfaction.

Several Indian medicinal plants have being evaluated for antimicrobial activity against etiologic agents of acne vulgaris. Arjuna extracts, cucumber gel, manjishta and vetiver extracts are proven effective herbal anti-acne drugs. Ethanolic extracts of the roots of *Hemidesmus indicus*, stems of *Coscinium fenestratum*, roots of *Tephrosia purpurea*, roots of *Euphorbia hirta*, bark of *Symplocos racemosa*, seeds of *Curcubito pepo* and also fruits of *Eclipta alba* have being reported to exhibit strong inhibitory effects against *Propionibacterium acnes* and *Staphylococcus epidermidis* (Kumar *et al*., 2007).

Thus it may be understood that the plants have a great potential to be used as actives in acne treatment. There are many plants that are yet to be explored for their utilities. Thus in the present research an attempt shall be made to screen the natural, cheap, safe herbal extracts for effective antiacne activity.

### 1.1.4 NEED FOR A NUTRACEUTICAL PRODUCT:

Nutraceuticals, term was coined by Stephen DeFelice using two different words “nutrition” and “pharmaceutical”, in 1989, and is defined as a food or food product that provides health and medical benefits, including the prevention and treatment of disease.
Globally this market is blooming and it supplies products which are incorporated in every consumer’s diet in one or the other form. The important reason been that consumers in this fast and paceful life are unable to find time to eat nutritionally and adequate balanced diet, they dwell on fast foods and hence encounter various lifestyle diseases like diabetes, cardiac hypertension, obesity etc. In fact, as per the euromonitor scale, in developing nations, malnutrition related mortality factor is nearly 40 percent. Thus the there is a tremendous need for nutraceutical products, to balance the nutritional intake of the individuals. Dietary phytochemicals are widely used as pharmaceuticals beneficial for human health and other commercial products. Epidemiologic evidence has suggested that certain plant polyphenols, flavonoids, phytoestrogens, terpenoids, phytosterols, fibres, promote good health and help prevent the occurrence of chronic and fatal diseases, like cardiovascular diseases, cancer, diabetes etc. The phytoconstituents have been reported to act through various mechanisms like antioxidant, promoting blood flow, maintaining the cholesterol level etc. However the exact mechanism of action for each of the nutritional database needs further research.

Traditional Indian fruits like pomegranate, grapes, chickoo as well as exotic superfruits known for their antioxidant properties and concentrated vitamin content including mangosteen, goji, acai and cupuacu packed with high impact health benefits, tropical and ethnic influences, sustainability and niche appeal have interested the consumers at large. Consumers are aware of the link between eating healthily and staying fit, many struggle to eat the recommended amounts of fruits, vegetables and dietary supplements needed to achieve this. Nutraceutical products readily available in the market thus fill this gap and provide healthy solutions. Nutraceuticals are thus a growing segment of the global consumer market.

Also the major reason for the global demand for such nutraceutical products is that the consumers desires a nutrient rich product readily made available to them. Every individual if asked for something they don’t get, the answer given by the middle class as well as the rich class of the population is the time. Cooking a nutritious and adequately balanced food is never achieved in a span of 10-20 minutes, which is a normal minimum time required to cook the food. However if the supplements are made readily available to
them the time is saved and the healthy diet is also made available. Considering the varying demands of the consumers, the healthcare system such products are thus formulated. Consider an example of the fruit juice readily made available in the tetrapack containers in the market. To obtain the necessary nutrients from the fruits the fruits need to be washed, cut and grinded in a mixer to make the fruit juice available. However, the ready-made tetrapack juices can be served in a very less time period. Not only the time is saved but also the ready available juices could serve a multiple nutrient from multiple fruits in a single glass. Thus, the nutritional value of such product could be increased by using multifunctional components.

1.14.1 Formulation Challenges of Nutraceutical Products:

Until recently, the manufacturing and quality control of these products which include dietary supplements, botanicals, fish and animal-based supplements has been largely unregulated. The challenges in formulation of nutraceuticals get tougher when one considers the complexity of multiple active ingredients, natural matrix effects and isolation of representative marker compounds. Nutraceuticals products providing health and medicinal benefits, which include the prevention and treatment of diseases in addition to the basic nutritional value found in foodstuff are been introduced to a large extent in the market. Nutraceuticals have evolved particularly due to the interest of the present generation as they generally look out for some quick recipes. However, due to inadequate nutrition provided in the some quick recipes, the fortification of the food as a concept at the same time easy to cook is evolved.

Formulating solid-dose nutraceutical products, such as tablets, capsules and powders, can cause technical challenges. These challenges need to be explored. So far the regulations for nutraceutical product manufactureing were less stringent, however now the regulations are changing with the inclusion of FSSAI i.e. the Food Safety and Standards Authority of India. However, the undesirable odor, taste of the product and stability of the nutrient itself during formulation as well as during absorption process are a concern. Moreover, the development of a stable nutraceutical product is a difficult challenge faced
by the manufacturers. The incorporation of the fortifying nutraceutical supplements in product thus needs to be approached specially with due considerations relative to the aspects of the product like appearance, its dosage level, its storage, its bioavailability, its efficacy and finally its cost. The most common multivitamin formulas comprises upto 40-50 active ingredients and two to eight excipients including coating ingredients. The presence of high active ingredients in nutraceutical formulations thus affect the overall particle size, flow, compressibility, moisture sensitivity, ingredient interaction, content uniformity and quality control (QC) testing of the product thus posing a challenge in assigning the quality standard to the product. Nutraceutical formulations are likely to have more actives present in higher weight. Many new ingredients enter the market, but information is sparse regarding their physical, chemical and mechanical properties. Based on this lack of information, formulation of nutraceutical products often depends on the firsthand experience of the formulator. Multivitamin ingredients often react with each other, and one or more displacement reactions may take place. However, it is difficult to investigate which ones are reacting due to many minerals and active ingredients in the same formula. The nutraceutical industry uses a wide variety of botanical and botanical-derived ingredients, so variation in ingredient characteristics is inevitable. Ingredient materials, such as herbal extracts and dried herb powders, vary based on the region where the crop has grown, season grown in and other factors. Even if a formulator has run a product 10 times in the past, it can still have issues with tablet integrity or color variations. Some ingredients are suitable neither for tablets nor for capsule formulations. These ingredients are not suitable for compression because they may stick to dies and punches. Nor are they suitable for capsule production because they will not flow unless excessive lubricants are added, which may create secondary problems such as capsule brittleness.

This may explain the research objectives of formulating a stable nutraceutical product containing the fruit pulp.
CHAPTER 1.2- A REVIEW ON Manilkara zapota AND Cymbopogon citratus

Plants as a source of medicinal actives have been reported in literature since ancient times. The phytoconstituents further obtained from these plants may act individually or synergistically to help provide total health care. In the present literature Manilkara zapota (L.) P. Royen and Cymbopogon citratus commonly called as lemongrass plant are reviewed so as to develop effective pharmaceutical products using them.

1.2.1 Manilkara zapota:

1.2.1.1 SYNONYM:

Manilkara achras F., Manilkara zapotilla G., Achras sapota L., Achras zapota L., Sapota achras M.

1.2.1.2 SOURCE:

Manilkara zapota L. Royen (commonly called as sapota, chikoo plant) belonging to the Sapotaceae family is a tropical fruit plant. It is cultivated throughout India (most extensive in coastal India such as Maharashtra, Gujarat, Andhra Pradesh, Madras and Bengal States), though it is native to Mexico and Central America. Manilkara zapota (L.) P. Royen is extensively found to be planted in Virar-Vapi belt, the western region of Maharashtra-Gujarat border, north of Mumbai as a source of its fruits.

1.2.1.3 TAXONOMICAL CLASSIFICATION:

Kingdom : Plantae

Order : Ericales

Family : Sapotaceae

Genus : Manilkara

Species : Manilkara zapota
1.2.1.4 VERNACULAR NAMES:

*Baramasi* (Bengal and Bihar, India); sapodilla plum (India); *sapota*, tree potato, *chikoo* (India); *chiku* (Malaya, India).

1.2.1.5 DESCRIPTION:

The *Manilkara zapota* is a slow-growing evergreen, glabrous tree, 8-15 m in height. Its possess ornamental leaves, they are evergreen, glossy and alternate as well as spirally clustered at the tips of the forked twigs; they are elliptic and pointed at both ends as well as firm about 7.5-11.25 cm long and 2.5-4 cm wide. Its flowers are small, bell-like, with 3 brown-hairy outer sepals and 3 inner sepals enclosing the pale-green corolla and 6 stamens. They are borne on slender stalks at the leaf bases. The fruit may be nearly round, oblate, oval, ellipsoidal, or conical; varies from 5-10 cm in width.

Several cultivars distinguished merely by shape prevail in India. Kalipatti, Calcutta Special, Pilipatti, Bhiripatti, Jumakhia, Dholadiwani, Fingar, Gavarayya, Guthi, Kali, Vanjet etc. are some of the cultivars.

1.2.1.6 SEASON AND YIELD:

The main season in India is December to March. A productive tree bears around 1000 fruits in its tenth year of plantation. Around 30-35th year the tree yields 2500-3000 fruits annually.

1.2.1.7 PHYTOCONSTITUENTS:

**Leaves:** Unsaturated fatty acids 32.32 %, oleic acid (13.95%), linoleic acid (10.18 %) and linoleic acid (5.96 %). Also flavonoids and phenolic compounds like lupeol acetate, oleanolic acid, apigenin-7-O-α-L-rhamnoside, myricetin-3-O-α-L-rhamnoside and caffeic
acid are present (Shazly et al., 2012). Saponins, alkaloids and terpenoids are also present (Islam, Md. Ekrakul et al., 2013).

**Bark:** Flavonoids, polyphenolics, saponins, alkaloids, glycosides and terpenoids are present (Islam, Md. Ekrakul et al., 2013).

The chemical structures of some of the phytoconstituents are given below.

![Chemical structures](image)

**Unripe fruits:** Tannins, lupeol-3-acetate, 4-caffeoylquinic acid (cryptochlorogenic acid) and triterpenoid acyl derivatives like β-amyrin-3-(3"-dimethyl)butyrate.

**Ripe fruits:** Moisture ranged from 69.0 - 75.7%; ascorbic acid from 8.9 - 41.4 milligrams per 100 grams; total acid, range from 0.09 to 0.15 percent; pH, 5.0 - 5.3; total soluble solids, 17.4° - 23.7° Brix; carbohydrates, glucose ranged from 5.84 - 9.23%, fructose 4.47 - 7.13%, sucrose 1.48 - 8.75%, total sugars 11.14 - 20.43%, starch 2.98 - 6.40% and tannin content 3.16 - 6.45%. Ten polyphenolic antioxidants are also reported (Aaradhya et al., 2007; Kennelly et al., 2011).

**Chicle:** Sticky latex constitutes 15 percent rubber and 38 percent resin.

### 1.2.1.8 PHARMACOLOGICAL ACTIVITIES:

Antioxidant, anti-inflammatory, antidiarrheal, antibacterial, anti-diabetic, hypocholesteremic, analgesic, anti-tumor, antinociceptive etc. activities have been reported in the literature exhibited by *Manilkara zapota* plant.
1.2.1.9 THERAPEUTIC USES:

The plant has been reported to exhibit wide varieties of therapeutic effects. The seeds are aperients, diuretic tonic and febrifuge. Bark decoction is used as tonic in diarrhea (Mohiddin et al., 1992) and also efficacious against peludism. The leaves are effective against cough, cold as well as diarrhea as reported by Mohiddin et al. 1992. Further the antimicrobial and antioxidant activities were reported from the leaves as reported by Nair and Chanda 2008; Kaneria et al., 2009. Analysis of the chemical composition juice obtained from sapota was reported to be one of the rich source of various constituents like sugars, proteins, ascorbic acid, phenolics, carotenoids and minerals like iron, copper, zinc, calcium and potassium. The sapota juice can thus be used as a nutritive product, comprising a rich source of polyphenols. Fruits were also identified and found to be a rich source of antioxidants and used extensively to overcome oxidative stress. Traditional Indian medicine reports that the young sapota fruits decoction can be effective to stop diarrhea. Further the young fruits and flowers infusion could be drunk to relieve complaints of pulmonary diseases. The crushed sapota seeds are reported to exhibit diuretic action and also revealed to expel stones in the bladder and kidney. A paste of the seeds is reported to be effective on application on stings and bites from venomous animals. The sticky latex, Chicle is also reported to be used in tooth cavities as crude filling (Aaradhya et al., 2007).

Thus based on elaborate literature reporting the multifunctional properties exhibited by Manilkara zapota plant, it is thought worthwhile to develop a nutritional and nutraceutical product with multi-therapeutic potential which will benefit the society at large.

Also the Manilkara zapota plants have been planted tremendously in the Institute garden since 20 years for its fruits. The plant at a glance is represented in the Chapter 5.1, Figure no.5.1.1.2.1.9.1. It therefore provides extensive availability of the leaves, bark and fruit material for conducting the research work. Thus the present research aims to use the opportunity of the Institute serenity and screen Manilkara zapota (L.) leaves and bark extract for their cosmetic utility as well as fruits to develop Nutraceutical product.
1.2 *Cymbopogon citratus*:

1.2.2.1 SYNONYM:

*Cymbopogon flexuosus*, *Cymbopogon nardus* and *Cymbopogon winterianus*.

1.2.2.2 SOURCE:

*Cymbopogon citratus* commonly called as lemongrass oil is native to warm temperate and tropical regions like India and South Asia.

1.2.2.3 TAXONOMICAL CLASSIFICATION:

Kingdom : Plantae  
Order : Poales  
Family : Poaceae  
Subfamily : Panicoideae  
Tribe : Andropogoneae  
Subtribe : Andropogoninae  
Genus : Cymbopogon  
Species : *Cymbopogon citratus*

1.2.2.4 VERNACULAR NAMES:

Common names include lemon grass, lemongrass, barbed wire grass, citronella grass, *cha de Dartiga longue*, *gavati chaha* (Marathi), etc. East Indian lemon grass (*Cymbopogon
flexuosus) is called as Cochin grass or Malabar grass while West Indian lemon grass (Cymbopogon citratus) is known as serai in Malaysia and Brunei.

1.2.2.5 DESCRIPTION:

Lemongrass is perennial herb growing upto 2 m tall with a magenta color base stems. The photograph of the herb is given in Chapter 5.1, Figure no. 5.1.1.2.2.5.1.

1.2.2.6 PHYTOCONSTITUENTS:

Lemongrass oil chemical composition varies as per the species. The widely reported phytoconstitutents includes monterpenes like citral, myrcene, citronellal, geranyl acetate, nerol, geranial, geraniol, neral, limonene,linalool, cis-ocimene, piperitone,α-terpeneol, thujane, isointermedol, borneol, α-pinene, β-pinene, β-sitosterol, hexacosanol, triacontanol, methylheptenone, methyleugenol andcymbopogonol (orientin, isoorientin, isoscoparin, swertiajaponin, chlorogenic acid, caffeic acid). East Indian lemongrass oil contains citral (65-85%) as major component while Cameroonian lemongrass oil contains geranial (33%) as major content (Md. Faiyazuddin et al., 2013). The chemical structures of some of the phytoconstituents are given below (Ganjewala, D. 2009).

![Chemical structures of some phytoconstituents](image-url)
1.2.2.7 PHARMACOLOGICAL ACTIVITIES:

Lemongrass oil has been reported to exhibit antioxidant, anti-inflammatory, antimicrobial, antifungal, diuretic, remedy against cold, relief from headache, migraine, muscle strains, depression, ease bowel movements in constipation, nerve tonic, antiseptic, insect repellant, pesticide, culinary use, anti-cancer etc. therapeutic properties.
1.2.2.8 THERAPEUTIC USES:

Lemongrass oil is extensively used in Ayurvedic medicines against nasal congestion. Flavor ant and perfumery use. Lemongrass herb possess aroma hence is been used for its odor also as coolant that is used to increase perspiration, relieve fever and also help treat minor, feverish illnesses. It is know for its invigorating and antiseptic properties. Lemongrass oil exhibits good anti-depressant activity, it is also reported to tone and fortify the nervous system and can be used in bath for soothing muscular nerves and pain. It is also used in skin care products as antibacterial, astringent, antiseptic, antiacne and as soothing agent. It is refreshing, cleansing and stimulating tonic on the body and added to shampoos thereby aids in adding a shine to the hair.
CHAPTER 1.3 – PROBLEM ON HAND

Herbals as a source of effective medicines are acceptable to the patients at large as they consider and are revealed through extensive literature that plants contain natural substances thereby promoting health and alleviating varying illness. For years so long plants have played a significant role to maintain the human health and improve the quality of human life. They have also have served humans by providing valuable components to be used as medicines, seasonings, health drink cum beverages, cosmetics as well as dyes. They are also reported to exhibit minimum or negligible side effects and are been considered to be safe for consumption. An extensive literature survey revealed that developing a herbal based cosmeceutical product could not only cater consumer needs but also provide a better efficacy and safety over the traditionally and age old conventional cosmetics. Further a nutraceutical product could enhance the dietary nutrition by supplementing the essential nutrients which are generally not received through routine diet.

1.3.1 DEVELOPMENT OF A COSMECEUTICAL PRODUCT:

Compared to the global statistics India is characterized by very basic sales in beauty and personal care products since past. However the current situation is changing; Indian cosmetic market scenario is improving drastically and along with rising consumer demands cosmeceutical sector is flourishing in terms of manufacturing and export. Infact many of the industries supply the key cosmetic ingredients to the leading groups globally. Also India been a hub of medicinal plants have also provided extensive research scope for newer potential actives. Thus it is apt to review the utility of the herbal extracts available in India for newer applications in cosmetics.

Alcoholic and acetone extracts of the leaves of *Manilkara zapota* (L.) R. are reported to reveal the presence of phytoconstituents like flavonoids and phenolic compounds (Shazly *et al*., 2012). Methanolic extract obtained from the leaves of the plant revealed the presence of flavonoids which exhibited significant antioxidant activity (Rao *et al*., 2014). As mentioned earlier sunscreen formulations mostly contains UV light absorbers. Flavonoids, one such constituent, also natural substance extracted from plants like green tea Polyphenols, *Aloe barbadensis* extract etc. have been considered as
potential sunscreen resources (Shenoy et al., 2010). Ethanolic extract of *M. zapota* (L.) leaves is reported to exhibit anti-oxidant effect owing to the presence of flavonoids and polyphenolic constituents (Chanda SV, *et al.*, 2010, Islam *et al.*, 2012). Reactive oxygen species are formed in the skin structures due to exposure to Ultraviolet radiations. Hence the use of antioxidant sunscreens could be the most beneficial option. Inspite of so many reports about *M. zapota* extracts researched and proved to provide a high antioxidant activity by virtue of the presence of phenols and flavonoids, its use as a sunscreen has not been studied. Hence the present research aims to explore antioxidant activity of *M. zapota* extracts to develop a topical sunscreen formulation.

The extracts obtained from the *Manilkara zapota* (L.) leaves and bark is reported as possessing antimicrobial activity against bacteria and fungi due to presence of terpenoids, flavonoids and glycosides (Karim *et al.*, 2011) however its activity against acne causing organism is yet to be explored hence the present research aims to screen the extracts for its anti-acne activity. Essential oils or volatile also called as ethereal oils are referred as aromatic and oily liquids derived from plant material and have being reported for medicinal and cosmetic applications. These oils composed upto 20–60 low molecular weight components mainly terpenes, terpenoids and aromatic, aliphatic constituents (Bakkali *et al.*, 2008). *Cymbopogon citratus* commonly called as lemongrass belongs to the family Poaceae or Gramineae. Although many species of the herbs do exist, the availability of the herb in the Institute surrounding so as to procure them easily also needed to be considered. Oil extracted from its leaves is been reported to possess strong antibacterial potential exhibiting low minimum inhibitory concentration (MIC) value of 0.125 % v/v against acne causing organisms (Luangnarumitchai *et al.*, 2007). Lemongrass oil also possess the ability to promote penetration of the chemical components through the skin layer. Hence when a topical formulation comprising lemongrass oil is used it can be advantageous in enhancing the permeation of poorly penetrating molecule across the the dead stratum corneum into the lower dermal matrix. Also lemongrass oil can offer protection from ultraviolet radiation induced skin cancers. Taking advantage of this fact, lemongrass oil could be included in the present formulation to enhance the therapeutic potential of the *M. zapota* extract. *Manilkara zapota* (L.) leaves and bark extract, which otherwise when used as a single extract might be required
in high concentrations to exhibit the therapeutic effect; thus the addition of the lemongrass oil to it may also reduce the overall dose.

1.3.2 DEVELOPMENT OF A NUTRACEUTICAL PRODUCT:
The Indian nutraceuticals sector is growing at a high pace among other sectors of Indian Food and Pharmaceuticals market and is also assumed and predicted to sustain its high growth in the future years. The burgeoning affluent middle class consumers in the country is becoming health conscious tremendously. Also the awareness about the positive impact of consuming nutraceuticals and dietary supplements is the generation next step among the young Indians. It is a preordained fact that affluence been one of the important causes of lifestyle diseases prevailing, and which nutraceuticals and dietary supplements should definitely address. However, India represents merely 2% of the global nutraceutical market and is way behind in terms of per capita spend of nutraceuticals with just USD 2.5 compared to the global average of USD 21. The nutraceutical market is poised to double in the next five years and could rise five-fold by 2020, as per a recent statistics. It is therefore fitting that the current research focuses on development of a nutraceutical product.

Literature review reports sapota fruit, commonly called as chikoo fruit to be consumed mainly due to its sweet taste, and nutritive purpose. Analysis of the chemical composition of *Manilkara zapota* (L.) fruit, sapota juice reveals that it is one of the rich sources of sugars, proteins, ascorbic acid, phenolics, carotenoids and minerals like iron, copper, zinc, calcium and potassium (Aradhya *et al*., 2007). Fruits are also identified as rich sources of antioxidants and used to overcome tremendous oxidative stress (Prasanna *et al*., 2012). Catechin, epicatechin, leucocyanidin, leucodelphinidin, leucopelarginidin, chlorogenic acid, and gallic acid like polyphenols are present in fruits (Aaradhya *et al*., 2012). Thus due to its high contents of the nutrient, sapota fruits could be consumed thereby combating the deficiency of the nutrients. However stability of the fruit pulp if stored at ambient or refrigeration temperature is a challenge hence the present research work aims to formulate a stable nutraceutical product using *M. zapota* fruit pulp.
Both the Cosmeceuticals as well as Nutraceuticals field are a part of Pharmaceuticals. The regulatory requirements for the individual fields differ extremely. In order to be easily monitored the classification is provided as the cosmeceutical and nutraceuticals depending on their application and purpose in the maintenance of the health of the human body. Overall the authority and rights to control the functioning of the fields are held with the Food Drug Association which is the main regulatory body of Pharmaceuticals. Thus in the present study both the formulations thought to be formulated are termed as pharmaceutical dosage forms. However a recommended dose is required to exert the desired therapeutic effect. Hence the thorough screening for utility and appropriate Pharmaceutical dosage forms needs to be formulated using *Manilkara zapota* extracts. Thus an attempt was made to formulate the pharmaceutical dosage forms from *Manilkara zapota* plant extracts based on a thorough review on their constituents, ethnopharmacological use and contemporary literature reports which has been detailed in the next chapter.
CHAPTER -1. 4 OBJECTIVES OF THE RESEARCH

1. With an objective to overcome the unwanted reactions associated with the use of chemical components, plant extracts could be a natural, cheap, safe, and effective sunscreen agent if found to absorb the harmful ultraviolet radiations. Thus the present research work aims:

   i. To screen the sunscreen activity of *M. zapota* (L.) leaves and bark extracts.

   ii. To screen the most promising extract of the above extracts in combination with lemongrass oil for any changes in the spectra.

   iii. To determine photo stability of the most promising *M. zapota* (L.) extract in combination with lemongrass oil.

2. To screen *M. zapota* (L.) leaves and bark extracts against the acne causing organisms like *Propionibacterium acnes* and *Staphylococcus epidermidis* in combination with lemongrass oil.

3. To formulate and characterize a topical o/w cream comprising the most promising *M. zapota* (L.) extract and lemongrass oil.

4. To determine the sun protection factor offered by the topical cream.

5. To evaluate the topical formulation for dermal safety.

6. In the current fast moving and stressful journey of life, time is very precious and every individual compromises his basic need of balanced and healthy food with some ready and quick recipes which may not provide the daily essential nutrition thus deteriorating own’s body functions. The basic intension of the research work is to use the nature’s gift of *M. zapota* (L.) fruit sapota and formulate a product which could provide a natural supplement of dietary requirements and maintain healthy lifestyle. Thus the present research aims:

   i. To formulate and characterize a spray dried/freeze dried Nutraceutical powder of *M. zapota* (L.) fruit, sapota juice/pulp.

   ii. To evaluate the formulation for nutritional value.
CHAPTER -1. 5 SCOPE OF THE RESEARCH

Herbal formulations have been proven as a potential, safe and efficacious alternatives to the Allopathic medicines. The side effects, allergies, overdose all such disadvantages of the modern Allopathic medicines are raising the concern in the patient group and hence their acceptance unless in emergency condition is declining. Patients nowadays also use other allied medicine systems like Homeopathy, Ayurvedic etc. systems as an alternative medicine. This means that the Herbals have a tremendous market not only in India but Globally.

The extracts obtained from *Manilkara zapota* (L.) P. Royen are proven to exhibit antioxidant and antibacterial action. Both the key therapeutic activities are essential for the exhibiting effective sunscreen and anti-acne activity respectively. There is a high demand for extracts exhibiting multifunctional activity. Hence the present research will definitely be beneficial in providing information about newer important applications of the *Manilkara zapota* (L.) P. Royen extracts in Cosmetic skin care area. Also exploring the indigenous plants as potential sun protective and anti-acne agents is a need of the hour. It will not only benefit the consumers but also Indian herbal based products shall gain a global market. It can also help generate Intellectual property thereby broadening the spectrum of sunscreens and anti-acne agents currently available.

The Nutraceutical formulation of the *Manilkara zapota* (L.) P. Royen fruits will help to stabilize the product from deterioration and will provide multiple essential nutrients in a single source. The research in this area is beneficial not only to the consumers but also the nutraceutical market in general.
CHAPTER -1. Six organizations were the research work is carried out.

Profile of the Institute:

The major research work was carried out at the St. John Institute of Pharmacy and Research, Paghar. The institute is a catholic minority Institute run by Aldel Educations Trust. The trust is a Christian Religious Minority Trust established in the year 2007. St. John Institute of Pharmacy and Research conducts a four year full time degree course of Bachelor of Pharmacy (B. Pharm.) since 2008 and has also commenced the two year full time Diploma in Pharmacy (D. Pharm.) since 2013. The Institute is recognized by Pharmacy Council of India (PCI), approved by All India Council for Technical Education (AICTE), and Directorate of Technical Education (DTE) as well as affiliated to the University of Mumbai. The Institute is equipped with various sophisticated equipments and possesses all the required facilities for conducting the research work undertaken under the titled, “Formulation, characterization and evaluation of pharmaceutical dosage forms comprising Manilkara zapota extract”.

The present research work was conducted at the Pharmaceutics Laboratory of the Institute.

Product Formulated:
In the present research work the Pharmaceutical dosage forms formulated using Manilkara zapota extracts are as follows;

1. Topical O/W cream evaluated for its sunscreen and anti acne activity using Manilkara zapota hydroalcoholic leaves extract for Cosmetic purpose.
2. A nutraceutical freeze dried powder comprising Manilkara zapota fruit pulp.

The other Organizations were the work was carried out partly:
The equipments which were not available inhouse and required in the study were accessed from the other Institutes. Also thw facility like Animal house was not available in house hence performed in the other Institutes. With prior concern from their respective Organisation heads.
1. FT-IR (Fourier Transformed Infra Red) spectra of the crude hydroalcoholic extracts of the *Manilkara zapota* leaf and bark were performed at Shri Vile Parle Kelavani Mandal’s Narsee Monjee Institute of Management Studies, (SVKM’s NMIMS) School of Pharmacy and Technology Management, Mumbai.

2. The SPF Analysis was performed using UV-2000S Ultraviolet Transmittance Analyzer at Quality Assurance Unit of The Kelkar Education Trust’s, Scientific Research Centre, Cosmeceutical Division, Mulund, Mumbai, by Ms. Rutuja Patil, HOD, Quality Assurance and Ms. Yamini Patil, Junior Scientist.

3. Animal Studies were conducted at Bombay College of Pharmacy, Kalina, Santacruz, Mumbai.

4. Spray drying was done using Spray mate (Spray dryer), JSIL Instruments, at Dr. L.H. Hiranandani College of Pharmacy, Ulhasnagar, Mumbai.

5. Freeze drying of the sample were performed using Virtis Bench top Lyophilizer (SP Industries, USA) Model: 2K XL, at Bombay College of Pharmacy, Mumbai.

6. Elemental analysis of the freeze dried powder of the fruit pulp juice was using Inductively Coupled Plasma Atomic emission Spectroscopy, at IIT Bombay.

**Processes used:**

The processes used in formulating the products are:

1. Topical O/W cream evaluated for its sunscreen and anti acne activity using *Manilkara zapota* hydroalcoholic leaves extract for Cosmetic purpose.
   **Process:** Extraction of the extract, phytochemical screening, evaluation of sunscreen and antiacne activity, Formulatio of the cream by fusion method, Characterization and Safety evaluation.

2. A nutraceutical freeze dried powder comprising *Manilkara zapota* fruit pulp.
   **Process:** Preparation of the fruit pulp, standardization of the pulp, spary drying and freeze drying, characterization of the product, evaluation of nutritive value and safety evaluation of the product.