CHAPTER 6.1- SUMMARY AND CONCLUSIONS

6.1.1 SUMMARY:

The present research work is a systematically structured project which has not only exploited the known facts about the plant *Manilkara zapota* (L.) P. Royen for its therapeutic utility but also explored the topical application of the extracts obtained therein. A summary of the individual chapters is provided herewith which shall give a better understanding of the present research undertaken.

CHAPTER 1: INTRODUCTION

Introduction chapter is broadly categorized as,

1.1 General Introduction,
1.2 A review on plant drugs *Manilkara zapota* (L.) P. Royen and *Cymbopogon citratus*,
1.3 Problem on hand
1.4 Research Objectives
1.5 Scope of research.
1.6 Organization where the work is carried out

1.1 General Introduction:

This topic mainly introduces about the use of cosmetics by mankind. People have become very much conscious about their looks and hence they use cosmetics to a large extent, as a result Cosmetic Industry is flourishing tremendously. The outermost or the superficial organ of the body is the skin and cosmetics are applied specifically on the skin. The premature skin ageing and allergies to the skin makes the face dull. An overview of the skin structure in brief is thus discussed so that the basics in formulations are clear. Further the exposure of the skin largely to the ultraviolet radiations exerts harmful effects in the skin structure. Hence use of sunscreen preparations are must. A detail description about the sunscreens, sun protection factor, photodamage to the sunscreen agents and reactive oxygen species is discussed. The topic specifically emphasizes the cellular damage that occurs due to oxidative stress and ways to overcome the damage. The use of antioxidants is thus elaborated.
Extensive information about the exogenous antioxidants that could be supplemented to the skin to prevent the cell damage is thus provided. These exogenous antioxidants are mainly obtained from herbal source hence various herbs, fruits, extracts and specific phytoconstituents exhibiting antioxidants are discussed. Thus the topic highlights the aim of selecting an antioxidant herb that could be screened for sunscreen activity. The underlying objective mainly was to explore the fact that oxidative damage to skin can lead to premature skin ageing hence the sunscreen formulation which protect the skin against ultraviolet radiation should be an antioxidant.

The second very important area discussed in the topic is about the facts of Acne, the condition which aggravates due to the infiltration by the micro-organisms like *Staphylococcus epidermidis* and *Propionibacterium acnes*. The growing incidence of resistance towards the antibiotic drugs used to treat acne has led to a continuous need to discover new molecules. The discussion also emphasizes the examples of various plant extracts evaluated against the acne causing organisms.

Further the newer area wherein the pharmaceuticals ventured into and are prospering is the nutraceuticals. The present introduction thus provides an elaborate note on the deficiency of healthy eating habits and use of the nutraceutical formulations to combat the deficiencies. Today’s market, food shopping mall and also street shops are very much flooded with such kind of products and their daily sales are increasing continuously. Plant fruits have very good amount of nutrients like antioxidants, flavonoids, vitamins, dietary fibres, sugars, carbohydrates, proteins etc. which can supplement a regular diet with the essential nutrients. Hence the topic emphasizes the need for formulating such nutraceutical product as well as to enhance the stability of the product made available from the fruits.

### 1.2 A review on plant drugs *Manilkara zapota* (L.) Royen and *Cymbopogon citratus:*

The plant drugs selected for the research work were *Manilkara zapota* (L.) Royen and *Cymbopogon citratus*. Both the plant sources have been reported to possess multi-functional therapeutic uses. Their detail information is thus provided in the topic. The
major highlights of the topic are the extensive availability of these herbal materials and the varying phytoconstituents present in them.

1.3 Problem on Hand:
The topic specifically highlights the need for development of the topical skin care product using *Manilkara zapota* (L.) P. Royen and *Cymbopogon citratus*. The rationale behind the selection of the plant material is highlighted. The need for nutraceutical product using *Manilkara zapota* (L.) P. Royen fruits is also discussed in detail.

1.4 Research Objectives:
The topic specifically highlights the key areas in which the research work shall progress. The objectives thus stated in the chapter provide a direct scheme of research.

1.5 Scope and Significance of Research:
The topic mainly stresses the need for selecting the above plants, *Manilkara zapota* (L.) P. Royen and *Cymbopogon citratus* in the present research. Also the significance of the indigenous plants to formulate the pharmaceutical dosage forms which shall broaden the spectrum of therapeutics available today. The cosmeceutical and nutraceutical product will thus gain a global market. That will not only increase the contribution of India in such areas of products but the Indian economy will improve. Thus the society at large will benefit.

1.6 Organization where the work is carried out:
The topic provides gist information about the place where the research work was undertaken. The details regarding the Institute are provided in the topic. Also the basic products formulated are mentioned. Further some experiments which were not able to be conducted at the Institute were performed in other organizations. Those details are also provided.
CHAPTER 2: REVIEW OF LITERATURE

The chapter provides extensive information on the literature reviewed in the key area like sunscreen, antioxidant sunscreen, acne and nutraceuticals. Around 125 reviews are provided in the topic, which includes original research articles, reviews on the specific topics, methodology and also important noting about *Manilkara zapota* (L.) P. Royen and *Cymbopogon citratus* mainly, though allied herbs were also mentioned in the key areas. The reviews were obtained by referring various research papers, patents as well as from books.

CHAPTER 3: METHODOLOGY

The detailed plan of the research work undertaken is mentioned in the topic 3.1. Further this chapter mainly provides elaborate experimental procedures used to screen and evaluate the extracts obtained from *Manilkara zapota* (L.) P. Royen. All the above details are given under 3.2.

3.2 Experimental:

The experimental work initiated with a thorough literature review about the pant *Manilkara zapota* (L.) Royen. The plant was then procured and sent for authentication to Dr. Harshad Pandit. Further the crude powder was standardized for the proximate values like determination of extractive values and ash values as per the procedures laid down by Indian Pharmacopoeia. A preliminary research was then conducted to check if the plant extracts possess sunscreen activity by measuring the spectrums of the extracts obtained from different solvents after Soxhlet extraction. Based on the spectra thus obtained the solvent for extraction was decided. The method of extraction was selected further. Then the hydroalcoholic extracts of *M. zapota* leaves and bark were screened for phytochemical investigations like extraction yield and chemical constituents present. The chemical test were performed for determination of presence of triterpenoids, saponins, steroids, phenols, flavones, flavanones-coumarins, anthocyanins, carbohydrates, alkaloids, quinones, tannins, proteins amino acids and glycosides. Further thin layer chromatography technique was used to identify and ascertain the phytoconstituents like flavonoids, steroidal
glycosides, alkaloids, coumarins, phenolics and terpene glycosides using appropriate solvent systems.

The *M. zapota* leaves and bark extracts after phytochemical screening were characterized for physical properties like appearance, color, odor, consistency and solubility. FT-IR i.e the Fourier transformed Infra Red spectrum of the extracts were then recorded using Potassium bromide pellet method to understand the functional groups present in them. Further a quantitative phytochemical analysis was performed to determine the total phenol and flavonoid content. The content of the total phenol was estimated using Folin-Coicalteu’s reagent method employing Gallic acid as a standard while the content of total flavonoid was estimated using Aluminum chloride colorimetric method employing Quercetin as a standard. Although the antioxidant activity of the extracts had been reported in ethanolic, methanolic, aqueous and acetone extracts, the hydroalcoholic solvent, water : ethanol in the ratio 3:7 was also screened for antioxidant activity determined by various assays. DPPH scavenging capacity of the extracts was determined using UV visible spectrophotometer employing ascorbic acid as a standard. Hydroxyl radical scavenging activity was performed using deoxyribose degradation method using UV visible spectrophotometer employing a known reference standard as ascorbic acid. Also Superoxide anion free radical scaventing activity of the extracts were determined using reduction of nitro blue tetrazolium assay method by UV visible spectrophotometer employing gallic acid as a standard.

The lemongrass oil was initially obtained by steam distillation of *Cymbopogon citratus* leaves using Clevenger’s apparatus. The plant was then sent for authentication to Dr. Harshad Pandit. The preliminary studies were conducted using the extracted oil. Further the lemongrass oil was procured from Dr. K. S. Laddha, Yucca Enterprises, Mumbai. It was subjected to characterization studies to determine the weight per millilitres, refractive index and optical activity as part of standardization process as per the procedures laid down by Indian Pharmacopoeia.

The first major objective of the research was thus undertaken and the *M. zapota* leaves and bark extracts were screened for the *in vitro* sunscreen activity. The spectrum of the extracts were recorded and compared to that of the spectrums of
standard sunscreens para-amino benzoic acid and benzophenone; marketed formulations SUNKARE 50® Gel and EKRAN 30® Lotion using UV Visible spectrophotometer. The leaves extract showed very good absorption spectra hence the potentiation of its activity was evaluated by the addition of lemongrass oil. Further the photostability of the extract was determined using various *in vitro* phototoxicity tests. Candida yeast test was performed using the test extract and comparison of photoxicity using standard phototoxic drug psoralen containing marketed formulation. Spectra measurements of the test extract initially and after irradiation to an Ultraviolet radiation were also performed and compared to that of the spectrums of standard sunscreens para-amino benzoic acid and benzophenone; marketed formulations SUNKARE 50® Gel and EKRAN 30® Lotion using UV Visible spectrophotometer. Further Photosensitized peroxidation of the linoleic acid was performed in presence of the test extract, the marketed formulation FACEGUARD® cream and the extent of peroxides formed were determined using Thiobarbituric acid assay by UV Visible spectrophotometer.

The second major objective of the research was then undertaken i.e to determine the antiacne activity of the *M. zapota* leaves and bark extracts. Cup plate method was used and the antiacne activity of the hydroalcoholic *M. zapota* leaves and bark extracts were determined against *Staphylococcus epidermidis* and *Propionibacterium acnes*. The bacteria seeded petriplates of *Staphylococcus epidermidis* were incubated in Nutrient agar media in presence of the test extracts in aerobic conditions at 37°C for 24 hours. While that of the bacteria seeded petriplates of *Propionibacterium acnes* were incubated in brain Heart Infusion agar media in presence of the test extracts in anaerobic conditions at 37°C for 48 hours. Later the zones of inhibitions were measured. The test procedures were repeated using lemongrass oil in combination with the extracts. For comparison the standard antibiotics Clindamycin and Erythromycin and marketed formulation FACECLIN® cream was also used. Further the minimum inhibitory concentrations were for both the extracts were also determined using agar dilution method.

The third objective was then undertaken, to formulate and characterize the topical o/w cream comprising the most promising extract in combination with lemongrass
oil. The fusion method was used to formulate the cream using triethanolamine as an \textit{in situ} emulsifier. Further the cream was characterized for appearance, texture, skin staining, odor, pH, homogeneity, spreadability, \textit{In vitro} diffusion study, rheology and stability analysis. The protocols of the characterization studies are mentioned in detail in the Chapter 4, Methodology. Later the fourth objective of determination of Sun Protection Factor of the cream was undertaken. The sample was sent for analysis using UV-2000S Ultraviolet Transmittance Analyzer at KET"s Scientific Research Centre, Cosmeceutical division, Mumbai. In order to meet the specified standards the cosmeceutical cream formulation has to be validated using Bureau of Indian Standards. Hence the prepared formulation was subjected to validation test like thermal stability, determination of pH, total fatty substance content and determination of residue and limit tests for microbial load, lead and Arsenic.

The fifth objective of determining the dermal safety of the prepared formulation was undertaken. The cutaneous toxicity of the cream was evaluated using Acute Dermal Irritation study as per OECD guideline 404. The test formulation was applied on the shaved skin on the posterior side of the Female Sprague Dawley rats. The irritation potential of the cream was determined in comparison with the placebo cream using the Draize"s score. Further the Acute Dermal Toxicity of the cream was carried out using OECD guideline 434. The animals used were Female Sprague Dawley rats. The test formulations were used at the dose of 1000 mg/KG and 2000 mg/KG of the hydroalcoholic \textit{M. zapota} leaves extract in combination with 0.5 \% v/v Lemongrass oil. The dermal irritation reactions were observed upto 14 days and scored in comparison of the individual cream with the placebo creams using the Draize"s score. During the study the changes in body weights, behavioral changes were noted and at the terminal stage the animals were sacrificed and major organs were excised to determine their organ to body weights. The statistical analysis of the \textit{in vivo} observations were done using student"s t test.

The sixth objective of the research, to formulate the stable nutraceutical powder using \textit{M. zapota} fruit pulp and evaluating its nutritional value was then undertaken. The ripe fruits were procured from the local garden of St. John Institute of Pharmacy and Research, St. John Technical Campus, Palghar, Maharashtra, India. The ripe
fruits were processed further to obtain the fruit pulp and then standardized using the test procedures like determination of pH, total solid content and ascorbic acid content. Further the nutraceutical powder was formulated using a spray drying technique and freeze drying technique, the best product of these two techniques was selected based on flow characteristics using Carr’s index, Hausner’s ratio and Angle of repose. The product was also evaluated for taste by human volunteers and moisture content by subjecting to heat in an oven. Further the nutraceutical potential or the nutritional value was determined using Elemental analysis, antioxidant value and Ascorbic acid content. The Elemental analysis was performed at Indian Institute of Technology, Bombay (IIT Bombay), Powai using Inductively Coupled Plasma (ICP) - Atomic Emission Spectroscopy. The antioxidant value of the freeze dried powder was evaluated by determining the DPPH scavenging activity using UV visible spectrophotometer and compared to the standard ascorbic acid. Also the determination of reducing power of the freeze dried powder in comparison the ascorbic acid standard helped to evaluate the antioxidant activity. Further the actual ascorbic acid content which could be exhibiting the antioxidant activity was also determined by titrating the test sample with the 0.05 M Iodine solution. The values of all the above in vitro tests were expressed as ± standard deviation.

Finally the freeze dried powder was evaluated as per the guidelines stated under OECD guideline no. 429 for Acute Oral Toxicity test in Sprague Dawley rats at a dose of 5000 mg per kilogram of the body weight of animals. Purified water was used as vehicle control. The animals were observed for fourteen days for any behavioral changes, weight change etc. further the animals were sacrificed and major organs were excised to determine their organ to body weights. The statistical analysis of the in vivo observations were done using student’s t test. Further the stability of the nutraceutical product was determined for a period of three months at room temperature. The moisture content, pH and ascorbic acid content was determined as per the procedures followed earlier.
CHAPTER 4: RESULTS AND DISCUSSION

An extensive review of the literature about the plant *Manilkara zapota* (L.) P. Royen belonging to the family *Sapotaceae* provided an in depth information about its various therapeutic utility and hence it was selected in the present research work.

The plant material was procured from the local garden of St. John Institute of Pharmacy and Research, (St. John Technical Campus) District Palghar, Maharashtra India. Further the plant material like leaves, bark and fruits were authenticated and confirmed that the plant material belongs to *Manilkara zapota* (L.) P. Royen synonym *Achras sapota* L. commonly known as chicle, Sapodilla, Chiku of the family *Sapotaceae*, by Dr. Harshad Pandit, Head of the Department of Botany, Guru Nanak Khalsa College of Arts, Science and Commerce, Mumbai. Further the specimen of the sample is deposited in the herbarium of their Department. The coarsely grinded powder of the leaves and bark were then standardized by determining various proximate values like extractive values and ash values. The results are tabulated in the Chapter no. 5. Based on the preliminary research conducted it was observed that the hydroalcoholic extracts of leaves and bark of *Manilkara zapota* (L.) P. Royen possesses sunscreen activity hence the solvent hydroalcohol was selected as extracting solvent. Further the comparison of the spectrums obtained by soxhlet extraction and maceration extraction techniques of the hydroalcoholic leaves extract helped to decide that maceration technique could be employed in the research as both the spectra were identical. The hydroalcoholic leaves and bark extracts thus obtained by extraction using maceration process yield upto 26.5 %w/w and 51.36 % w/w respectively. The phytochemical investigations performed to screen the presence of various phytoconstituents using simple chemical test reveal the presence of phenols, flavonoids-flavones, glycosides, coumarins, alkaloids, tannins, terpenoids etc. the phytoconstituents were also confirmed using Thin Layer Chromatography. The hydroalcoholic leaves and bark extracts characterized for physicochemical properties revealed a solid mass of dark green and brown color respectively. The other results are tabulated in the Chapter no. 5. Further the FT-IR spectra thus obtained confirmed the presence of phenolic like phytoconstituents having polar functional groups and aromatic ring structures. The extracts on quantitative phytochemical screening revealed the total phenol content of 330.77 ± 0.71 mg/g and 333.08 ± 0.96 mg/g of the hydroalcoholic
leaves and bark extract respectively, expressed as Gallic acid equivalent per gram of the dry extracts. The test extracts thus revealed very good amount of phenols. The values obtained using the standard curve of Gallic acid was very close in both the extracts. Also the total flavonoid content computed to be 183.89 ± 2.3 mg/g and 47.39 ± 1.2 mg/g of the hydroalcoholic leaves and bark extract respectively, expressed as Quercetin equivalent per gram of the dry extracts. The test extracts thus revealed very good amount of flavonoids. Comparatively leaves extract constitute more flavonoids content than the bark extract. The results are tabulated in the Chapter no. 5.

The antioxidant activity of the extracts by DPPH scavenging potential revealed that the hydroalcoholic leaves extract exhibited a lower IC$_{50}$ compared to bark extract although ascorbic acid exhibited comparatively lower IC$_{50}$ at low concentration. Further the hydroalcoholic leaves extract exhibited high hydroxyl radical scavenging activity compared to bark extract however Gallic acid activity was at very low concentration. Superoxide anion free radical scavenging activity of leaves and bark extract was similar, however Gallic acid exhibited strong scavenging ability comparatively.

*Cymbopogon citratus* (DC). Stapf. Synonym *Andropogon citratus* DC family Poaceae commonly known as Lemon grass, Gauti chai, Hirva cha. Leaves were obtained from St. John Technical Campus, District Palghar, Maharashtra, India. Leaves of the plant were separated, coarsely grinded and used for the extraction of volatile oil using Clevenger”s apparatus and used for preliminary studies. The plant was authenticated and confirmed that the plant material belongs to *Cymbopogon citratus* (DC). Stapf. Synonym *Andropogon citrates* DC family Poaceae commonly known as Lemon grass, Gauti chai, Hirva cha by Dr. Harshad M. Pandit, as earlier and the specimen no. #100314 is deposited in the Herbarium of the Department of Botany. Further studies were performed using Lemongrass oil sample procured from Dr. K. S. Laddha, Yucca Enterprises, Antop Hill, Mumbai. Characterization studies revealed the authenticity of the sample procurred.

Ultraviolet spectrum obtained for screening the sunscreen activity using hydroalcoholic *M. zapota* leaves extract gave a $\lambda_{\text{max}}$ at two peaks 274 nm and 264 nm wavelength further the absorbance was fairly good from 290-380 nm which is specifically UV-B and UV-A region. The spectra obtained from the leaves extract covered a wide wavelength with good absorbance at the same concentration than the bark
Lemon grass oil also showed a $\lambda_{max}$ at two peaks 272 nm and 281 nm extending further with a good UV radiation absorption from 290-380 nm. Further combination of the leaves extract and lemongrass oil samples led to a bathochromic shift in $\lambda_{max}$ to 324 nm and the spectrum showed very good UV absorption in the range 290-380 nm. Para amino benzoic acid and benzophenone used as a standard sunscreen agent showed a $\lambda_{max}$ at 265 nm and 257 nm respectively; also the curve degrades very rapidly up to 310 nm thereby showing negligible absorbance over 320 nm. The marketed formulation SUNKARE 50® Gel and EKRAN 30® Lotion exhibited a $\lambda_{max}$ at 308.5 nm and 309 nm respectively. Hence it was decided that the leaves extract could possess a potential sunscreen activity, thus the further photostability was determined using leaves extract in combination with lemongrass oil.

The yeast test using *Candida albicans* as revealed that *M. zapota* leaves extract on irradiation did not produce any phototoxic compounds hence the growth of *C. albicans* was not affected. However the known phototoxic drug psoralen containing Melanocyl® cream yielded phototoxic compounds on irradiation which affected the growth. The results thus indicate the photostability of the *M. zapota* leaves extract. UV Spectra measurement revealed that on irradiation the test sample i.e. *Manilkara zapota* leaves extract did not show a significant change in the absorption spectra particularly in the wavelength range 260-350 nm however the PABA sample was affected to a major extent in the wavelength range 260-300 nm, indicating its photo instability. However the benzophenone spectra remained unaffected indicating high photostability. Marketed formulations also revealed no significant change in the spectra. Lemongrass oil alone was not stable on irradiation however combination of leaves extract and lemongrass oil led to photostabilization. Photo labile drugs are known to cause lipid peroxidation in membrane lipids due to the degraded metabolites, leading to formation of ROS in lipids. ROS is also one of the major causative factors for increased MDA formation. Thus measurement of extent of such hydroperoxide formation with plain extract on exposure to UV radiation will provided a great input in determining the degree of photostability of the extracts. Linoleic acid lipid peroxidation test revealed that the extract on irradiation produced less MDA, a peroxidation product comparable to standards Quercetin and Gallic acid which are the effective flavonoids and polyphenols used as photo protective and antioxidant,
indicative of its photostability. The readings were also close to the marketed product Faceguard® containing photostable synthetic sunscreens, Uvinul A Plus (Diethylamino Hydroxy benzoyl Hexyl Benzoate), Tinosorb S (Bemotrinizol) and Octinoxate. The results thus obtained from the *in vitro* phototoxicity studies conducted revealed the potential photostable sunscreen activity of the hydroalcoholic *M. zapota* leaves extract. The phytoconstituents responsible for such activity were mainly the flavonoids, coumarins and polyphenolic compounds which are able to absorb the ultraviolet radiations as they have been confirmed in the earlier literature. Hence in the present study the similar constituents from the leaves extract were concluded to exhibit the sunscreen activity.

Cup plate method revealed that the hydroalcoholic extract of the *M. zapota* leaves showed good whereas the bark showed a moderate growth inhibition against *S. epidermidis* exhibiting a highest zone of inhibition at 1\% w/v and 2 \% w/v respectively. Although the zone of inhibition at 4 \% w/v were found to be comparable to that of 2 \%w/v but such high concentration of the extract possessed a solubility problem. The zone of inhibition obtained using Clindamycin and Erythromycin were comparatively very high at the same concentrations. Further addition of 0.5 \% v/v lemongrass oil in appropriate ratio (1:1) to the individual hydroalcoholic *M. zapota* leaves and bark extracts separately increased the diameter of the growth inhibition compared to just lemongrass oil. The results thus obtained on combining the above key components were attributed to the synergistic activity of the hydroalcoholic leaves and bark extract individually along with lemongrass oil. However the activity of the *M. zapota* leaves against *Propionibacterium acne* was very good compared to bark extract as it was very close to that of the antibiotics Clindamycin and Erythromycin. Further addition of lemongrass oil to leaves extract led to potentiation of the activity. Hence the combination of hydroalcoholic extract of *M. zapota* leaves and lemongrass oil was decided to proceed further for formulation development. The agar dilution method using hydroalcoholic leaves and bark extract of *M. zapota* individually revealed a respective MIC value of 0.05 \% w/v and 0.2 \% w/v against *S. epidermidis* also against *P. acnes* the MIC value was 0.1 \% w/v and 0.2 \% w/v which was comparatively very high to that of the standard antibiotics currently used to treat acne conditions. The leaves extract thus exhibited
higher growth inhibitions against both organisms and on addition of lemongrass oil the zone of inhibitions was greater than that of zone of inhibitions showed by the standard antibiotic Erythromycin. Also the results obtained using marketed formulation FACECLIN® Cream was comparable to that of the leaves extract. Thus it was decided worthwhile to proceed further towards formulation development using only the leaves extract and lemongrass oil rather than the bark extract. The presence of flavonoids, coumarins, polyphenols, terpenoids and saponin glycosides in the hydroalcoholic leaves and bark extract of *M. zapota* could be attributed for such activity.

An O/W cream was formulated by fusion method. The concentration of the individual components of the cream was decided based on the consistency and the stability of the cream product. Thus a 1%w/w *M. zapota* leaves extract containing O/W cream using triethanolamine stearate as an emulsifier and glycerin as humectants along with the preservatives parabens was prepared. Characterization of the cream revealed very good light greenish more specifically “Arylide yellow” colored lemongrass oil perfumed non staining formulation with appropriate pH around 6.8, visual evaluation of the cream revealed a homogenous dispersion of extract, absence of agglomeration and complete dispersion of other excipients. For a good spreadability, the cream should behave like a good lubricant exhibiting maximum slip and minimum drag. Lesser the time taken by two plates to cover a fixed distance, lesser the friction between the glass surfaces and the cream, more spreadable is the cream. The glass plate containing 0.5 gm of cream was able to slide in less than 10 seconds a distance of 5 cm, thus indicating a very good spreadability index. *In vitro* Diffusion studies revealed that the formulation containing the leaves extract and lemongrass oil could diffuse to a maximum value of 50-60% of the initial concentration at the end of 10 hours indicating the ability to provide maximum protection to the superficial skin. Rheological characteristics of the cream determined using Brookefield Viscometer showed an increase in viscosity at less than a linear rate with increasing shear rate. Thus the cream exhibits a non-Newtonian pseudoplastic rheology. The cream was found to be stable at room temperature (25°C) as well as at 40°C and revealed no signs of instability like, separation, drastic content difference and also stable against microbial growth. The SPF Analysis was performed at KET’s Scientific Research Centre, Mulund, Mumbai. The cream formulation thus
prepared gave a fairly good SPF value, also the UV-A/UV-B ratio was found to be 0.662 which means a good protection factor in UV-A as well as UV-B region with a Boots star rating 3 and the critical wavelength determined was 385.11. Validation of the formulation for quality standard as per the Bureau of Indian Standard guidelines also revealed that the product exhibited a good thermal stability and hence passes the test when exposed to 45 ± 1°C for 48 hours. The product did not show any oil separation or phase separation. The pH of the formulation was found to be 6.8 which within the limits 4-9 hence pass the test. The total fatty substance is above the minimum limit 5, hence passes the test. The total residue was estimated which exceeds the minimum value 10 hence the product passes the test. The product passes the microbial limit since as per the limit not more than 1000 cfu/gm of the microbial load detected. The product passes the limit test for lead since the opalescence produced by test solution was very much less than the standard lead solution of 20 ppm. The product passes the limit test for arsenic since the stain produced by the test sample was not intense than that of the standard stain of 2 ppm.

The formulation was then evaluated for Toxicological studies by Acute Dermal Irritation test. The formulation was applied (0.4g) as a non-occlusive dressing on dorsal shaved area (approx. 1 cm²). Unscheduled deaths were not observed as the study progressed. No test formulation related clinical signs and significant changes in the body weight of rats were not observed as they were weighed and the weights noted at regular intervals during the entire study period. The histopathological examination of skin samples of formulation site did not reveal any lesions of pathological significance and was found to retain normal physiology as that of the control site. The test formulation (1 % w/w *M. zapota* leaves extract cream) thus was concluded to show no major toxicological dermal reactions as seen from histopathological examination of skin. The histopathological examination of skin samples were performed by Dr. Sanjay Pawar, M.V.Sc. Pathology Registration no. 7238. This also proved that 0.5 % w/v concentration of lemongrass oil was also safe to use and non-irritant.

Further Acute Dermal Toxicity test was conducted. The formulation (0.3 g) of each dose was applied as a non-occlusive dressing on dorsal shaved area (approx. 1 cm²). There were no unscheduled deaths in any groups during the study period. No test formulation related clinical signs also significant changes in body weight of animals were
not observed in any groups during the time course of the study period. There were no
abnormal findings on gross necropsy in both groups on day 15 after the treatment. On
statistical analysis was performed using student’s t test at a 95% confidence limit and the
ratios was found to be non-significant with respect to each other. The histopathological
examination of skin samples of formulation site did not reveal any lesions of pathological
significance and was found to retain normal physiology as that of the control site.
However some infiltration was seen hence it was concluded that the test formulation at
dose I shows mild dermal reactions while test formulation at dose II shows moderate
dermal reactions as inferred from this test. The irritation reactions were considered due to
the presence of constituents like tannin which are known to be skin irritants. Lemongrass
oil comprises citral which is reported to be a primary skin irritant at a concentration
above 5 % v/v hence the concentration of the oil in the cream formulation was very low,
just 0.5 % v/v.

*M. zapota* fruits obtained from St. John technical campus and authenticated. The
prepared pulp of the ripe fruits was standardized and found to possess pH 3.9 on dilution
with water during its measurement. Solid content was determined to be 15%w/w and
ascorbic acid content of fresh pulp was 0.176 % w/w. The spray drying technique did not
yield a solid powder form due to the presence of sticky mucilaginous mass. Hence freeze
drying of the sample was done. The taste of the product was sweet. The powder was
characterized for flow properties by determining the Carr’s Index, Hausner’s ratio and
Angle of repose. The moisture content was also determined. The powder did not possess
very free flowing property due to the presence of mucilaginous mass in the pulp.
However the flow properties were improved by addition of 0.5 %w/w microcrystalline
cellulose. The moisture content was found to be less than 2%w/w. Elemental analysis of
the freeze dried fruit pulp analyzed using Inductively Coupled Plasma Atomic emission
Spectroscopy, at IIT Bombay revealed very good potassium, calcium, iron etc. elemental
composition. Ascorbic acid content however was reduced to 26.68 ± 4.5 mg/100 gms of
freeze dried sample.

Antioxidant potential of the *M. zapota* fruit juice was determined using DPPH
radical scavenging assay and total reducing power assay. Fruit juice exhibited a fairly
good DPPH scavenging activity with an IC_{50} value as 65.54 ± 0.004 µg/ml and total
reducing power to be good when compared to standard ascorbic acid. Test substance was appropriately diluted with water and administered at a dose of 5000 mg/kg as a single oral dose. None of the rats exhibited abnormalities in gait, fur, eyes, mucous membranes or abnormal behavior. Hence it was concluded that the test product did not exhibit any gross toxicological signs in acute oral toxicity study. Further the stability of the nutraceutical product determined over a period of three months. The results are tabulated in the Chapter no. 5.

Based on the extensive research conducted and the results obtained it was thus considered that the cosmeceutical formulation so prepared using hydroalcoholic leaves extract of *Manilkara zapota* at 1% w/w concentration in combination with 0.5 % v/v of Lemongrass oil (1:1) is a potential remedy against acne causing organisms as well as providing photostable sunscreen effect. The formulation is effective as sunscreen and antiacne. The formulation not only possesses desirable consistency, texture but is also stable at room temperature. Also the dermal toxicity and irritancy evaluated in animals revealed no harmful and sensitive reactions at the test dose. The nutraceutical formulation so prepared exhibited good nutritional value in terms of elemental and antioxidant ascorbic acid content. The freeze drying of the pulp led to stabilization of the fruit pulp as revealed from stability studies. The acute oral toxicity performed at a test dose of 5000 mg/Kg also showed no abnormal reactions thus concluding it to be safe for oral consumption.

**CHAPTER 5: DATA ANALYSIS**

The chapter mainly provides the Figures and Tables from the present research work.

**6.2 CONCLUSIONS:**

Herbal remedy is one of the ancient methods used to alleviate the human ailments. Once again through the present research work it is revealed that the herbal medicines not only possess the ability but a great potential as effective therapeutics.
The authenticated plant materials *Manilkara zapota* (L). P. Royen leaves, bark and fruits were used in the present research work. The hydroalcoholic leaves and bark extracts thus obtained using maceration technique on phytochemical evaluation concluded to yield majorly the phytoconstituents like flavonoids, phenols, tannins, alkaloids, coumarins, steroidal glycosides etc. The extracts also possessed very good antioxidant abilities as confirmed using various assay methods.

The sunscreen potential screening of the hydroalcoholic leaves and bark extracts using appropriate methodology helped to conclude that the leaves extract possessed very good sunscreen activity compared to the bark extract. The addition of 0.5 % v/v lemongrass oil further potentiated the activity. *In vitro* phototoxicity studies conducted also concluded the photostability of the hydroalcoholic leaves extract in presence of lemongrass oil.

Further the antiacne activity of the hydroalcoholic leaves and bark extracts using the cup plate method and determination of minimum inhibition concentration concluded its effectiveness against the acne causing organisms. Although the hydroalcoholic leaves and bark extracts both possess antiacne activity as evaluated against *S. epidermidis* and *P. acnes*; the leaves extract was continued further with the formulation development due to its results showing greater zone of inhibitions against the microorganisms at similar doses.

The results of the above experimental studies were taken into consideration and the final formulation was prepared as an O/W cream using hydroalcoholic leaves extract of *Manilkara zapota* at 1%w/w concentration in combination with 0.5 %v/v of Lemongrass oil (1:1) and triethanolamine stearate as an *in situ* emulsifier. Based on the extensive research conducted and the results obtained, it can be concluded that the cosmeceutical formulation so prepared using hydroalcoholic leaves extract of *Manilkara zapota* at 1%w/w concentration in combination with 0.5 %v/v of Lemongrass oil (1:1) is efficacious and a potential remedy against acne causing organisms as well as providing photostable sunscreen effect. The formulation not only possesses desirable consistency, texture but also good spreadability. The *in vitro* diffusion studies of the cream helped to conclude the maximum permeation of approximate 60% and thus its retention in the skin layers. The stability of the product thus observed at three different temperature conditions
indicated a desirable stability at the respective storage conditions evaluated. However, storage at 25°C should be suitable, as from the results obtained it was noted that the change in the contents could be possibly due to evaporation of the lemon grass oil. This evaporation rate could be more if stored at 40°C hence recommended storage could be at room temperature. Further the sunscreen effect of the prepared formulation was quantified employing the sun protection factor determination, which was estimated to be 2.7. This value was concluded to be good enough as it is obtained using a combination of single herb and lemongrass oil at a very low concentration compared to marketed sunscreen formulations using multiple extracts at very high concentrations thereby claiming a greater sun protection value. The Dermal Irritancy of the prepared cream product evaluated in animals revealed no harmful and sensitive reactions at the test dose of the hydroalcoholic leaves extract 1%w/w and lemongrass oil 0.5%v/v in the ratio 1:1. Further the Acute Dermal Toxicity studies of the prepared cream conducted in animals also evidenced the safety of the product even at a dose of 1000 mg as well as 2000 mg of the hydroalcoholic leaves extract per kilogram of the body weight of the animal and lemongrass oil 0.5%v/v in the ratio 1:1. Hence the present research work conducted to formulate a topical product comprising Manilkara zapota extract is not only effective but also safe. Also the cream formulation can be concluded to provide dual action on skin one as a photostable sun protective and other as effective antiacne. Further as the formulated cream product possess sunscreen effect, it can also be concluded to be capable of preventing the harmful effects of the ultraviolet radiations such as pre-mature skin ageing on the healthy human skin. Thus the skin can look younger, soft and retain the skin flexibility for a longer time, the features which are usually lost due to pre mature ageing. Also as the formulated cream being oil in water emulsion (O/W) in nature the occlusive effects of the skin shall be very limited hence its application will never give an oily look as well promote excessive sweating. Thus the formulation applied skin will provide a fresh and healthy attractive face.

The nutraceutical formulation so prepared by freeze drying the pulp obtained from the ripe fruits of Manilkara zapota exhibited good nutritional value in terms of elemental and antioxidant ascorbic acid content as evidenced from the comparison with various marketed nutraceutical products. The presence of whole fibre in the powder is an
added benefit compared to the plain juices of other ready juices available in the market. The Acute Oral Toxicity of the freeze dried powder performed at a test dose of 5000 mg per kilogram of the body weight of the animal also showed no abnormal reactions thus concluding it to be safe for oral consumption. The stability of the fruit pulp was thus enhanced which could retain the nutritional value of the product. Also the product was prepared without any preservative and color.

The topical cream formulation of which the photostable sunscreen activity mediated by antioxidant mechanism was established is not only efficacious but also may provide protection against Ultraviolet radiation induced premature skin ageing thus concluding its against antiageing property. Both the above products are indigenous, natural; also can be concluded to be effective and very important safe for use as evidenced from its experimental results. Formulation of the topical cream comprising *Manilkara zapota* (L). P. Royen leaves extract as cosmeceutical and freeze dried powder comprising *Manilkara zapota* (L). P. Royen fruits as nutraceutical product required very few processing steps hence the large scale manufacturing of such products can be considered to be feasible. Also the products were formulated using very few ingredients and the extract obtained from crude drug was also easy to obtain. The products possess multifunctional activity with only two active components as extract and lemongrass oil in topical cream and pulp of a single fruit in nutraceutical powder. The overall cost of the products can be considered to be very less compared to any novel drug delivery systems hence they can also be called as cost effective and thus has a great commercial potential. The society at large will definitely benefit by these products.

Thus the present research work can conclude that it has provided extensive information and evidenced the benefits of the plant materials obtained from *Manilkara zapota* (L). P. Royen leaves, bark, fruits and their formulations.
CHAPTER 6.2-RECOMMENDATIONS

Based on the extensive literature survey about the plant *Manilkara zapota* (L.) P. Royen which belongs to the family *Sapotaceae* and the research work undertaken under the title “Formulation, characterization and evaluation of pharmaceutical dosage forms comprising *Manilkara zapota* extracts” the following recommendations could be given;

1. The prepared topical cream formulation comprising 1% w/w hydroalcoholic leaves extract of *Manilkara zapota* and 0.5% v/v lemongrass oil in the ratio 1:1 possesses a potential sunscreen activity exhibiting a good SPF value, Boots star rating and UV-A/UV-B ratio.

2. The hydroalcoholic leaves extract of *Manilkara zapota* used in the product is an effective antioxidant can be used as sun protective which is also photostable.

3. Thus the product can be recommended to prevent ultraviolet radiation induced premature skin ageing by providing effective antioxidant sunscreen agent to the superficial layers of the skin.

4. The topical cream formulation comprising 1% w/w hydroalcoholic leaves extract of *Manilkara zapota* and 0.5% v/v lemongrass oil in the ratio 1:1 is effective against acne causing organisms *Staphylococcus epidermidis* and *Propionibacterium acnes*. the addition of lemongrass oil has led to potentiation of its activity.

5. The topical cream formulation is stable on storage at room temperature, possess requisite quality as per Bureau of Indian Standards and most importantly safe to use on skin.

6. The Nutraceutical product containing the freeze dried *M. zapota* fruit pulp possesses very good nutritive value and is safe for consumption.

Thus both the pharmaceutical dosage forms formulated comprising *Manilkara zapota* extracts can be recommended as multifunctional, natural, efficacious, safe and also cost effective products which will benefit the end users.
CHAPTER 6.3 FUTURE SCOPE

During the course of the present research work, formulation of stable and efficacious products comprising Manilkara zapota (L.) P. Royen extracts were executed. However a research work is never limited. There is always a tremendous scope for future research. So here are some of the key areas which could be addressed from the present research work in the further research.

6.3.1 Cosmeceutical Product:
To potentiate the sunscreen and antiacne activity of the hydroalcoholic leaves extract 0.5 \% v/v lemongrass oil was incorporated in the topical cream.

The limitations of the present research work could be overcome by future research approaches as follows:

1. The volatility or the evaporation rate of the lemongrass oil can be reduced by microencapsulating the oil in a coating material, as this technique has an important application of converting the liquid core material into solid form by coating.

Microencapsulation as the name suggest is the method of encapsulating the core material which exist in micron size. The techniques used for microencapsulation could be co-acervation phase separation, air suspension, spray drying etc. However the method suitable for encapsulating the liquid volatile oil and which can be feasible in large scale manufacturing at the same time be cost effective must be selected. The process requires high shear imparting system so that the size of the core materials must be reduced to micron size prior encapsulation.

Also the important thing to be considered is that the overall cost of the product will definitely increase. Formulating a microencapsulated lemongrass oil and dispersed in a cream base may also provide altered release rates in the formulation so such release studies need to be performed more extensively. The parameters to coat the lemongrass oil should be carefully studies in order to provide efficacious product.
2. The *in vivo* repeated patch photostability test could be performed to determine the photosensitivity of the product. As this test provides multiple application to the animals followed by irradiation to ultraviolet light which can clearly indicate the allergic potential of the product due to multiple exposure to ultraviolet radiations.

### 6.3.2 Nutraceutical Product:

The limitations of the present research work in nutraceutical product could be overcome by future research approaches as follows:

1. The nutraceutical product can be worked on further for enhancing the flow properties.
   The flow properties further could be enhanced by providing a smooth film coat using polymers or pelletization. However the overall cost of the final product must be ruled out.
CHAPTER 6.4- LIMITATIONS OF RESEARCH WORK

6.4.1 Cosmeceutical Product:

The pharmaceutical products thus formulated comprising *Manilkara zapota* extracts do have some limitations as were realized on thorough analyses of the results obtained. To potentiate the sunscreen and antiacne activity of the hydroalcoholic leaves extract 0.5% v/v lemongrass oil was incorporated in the topical cream. This volatile oil was found to evaporate during the storage period hence the limitations are given below.

The limitations are as follows:

1. The topical formulation, cream during storage for stability studies, it was noted that the lemongrass oil been a volatile oil, the content was reduced due to evaporation; this might affect the therapeutic effectiveness as sunscreen and antiacne could decrease over a period of time if the rate of evaporation is not controlled.

6.4.2 Nutraceutical Product:

The nutraceutical product formulated by freeze drying possess a flow property which might not be adequate enough.

The limitations are as follows:

1. The nutraceutical powder possesses less free flowing ability which might affect the uniformity in the content weight during packaging.