2.1 REVIEW OF LITERATURE

Literature survey was done for the selection of the photoprotective herbs as well as the novel systems which could be prepared for the effective administration of the selected herbal extracts to be used as photoprotectives. We have categorised various papers in different sections on the basis of vesicular systems (liposomes, ethosomes, transfersomes and niosomes), herbs as photoprotectives and various evaluation parameters used for the assessment of the formulations used as photoprotectives like Sun Protection Factor determination, bioengineering techniques and biological techniques. The literature has been arranged in chronological order.

Vesicular systems related literature

1. Alvi et al, 2011 performed the comparative study of transfersomes, liposomes, and niosomes for topical delivery of 5-fluorouracil to skin cancer cells: preparation, characterization, in-vitro release, and cytotoxicity analysis. Skin permeation and retention showed better permeability and retention than the nano vesicular dosage form.

2. Manosroi et al, 2011 investigated transdermal absorption enhancement of gel containing elastic niosomes loaded with gallic acid in the semipurified fraction isolated from Terminalia chebula Retz. (Combretaceae) galls. This study has demonstrated the potential of niosomes, especially elastic niosomes, for the enhancement of chemical stability and rat skin transdermal absorption of gallic acid in the semipurified fraction from T. chebula galls, which will be beneficial for topical antiaging application.

3. Agrawal and Kaur, 2010 studied the inhibitory effect of encapsulated curcumin on ultraviolet-induced photoaging in mice. Encapsulation of curcumin into elastic vesicles makes this agent amenable to topical dosing and circumvents the problem of poor topical availability that limits the
utility of free curcumin. The photoaged mice model showed promising results for curcumin loaded elastic vesicles.

4. Gupta et al, 2010 prepared liposomes, niosomes and phytovesicles of pure curcumin and incorporated into carbopol gel to make feasible for topical application on skin. They found that these vesicles provided enhanced antiaging, antioxidant and anti-wrinkle effect.

5. Rathore et al, 2010 prepared miconazole incorporated different novel carriers such as liposomes, ethosomes and compared their in vitro skin permeation studies with plane ointment using skin model. In vitro skin permeation study results showed that the steady state fluxes of drug was higher in case of ethosomal suspension incorporated ointment as compared to liposomal ointment. Hence, concluded that ethosomes shows better skin permeation as compared to liposomes.

6. Takahashi et al, 2009 reported that liposomes encapsulating Aloe vera leaf gel extract significantly enhance proliferation and collagen synthesis in human skin cell lines. Also proved that the bioavailability and skin care properties of Aloe Vera leaf gel extract will be significantly enhanced by liposome encapsulation, and the present liposomal Aloe Vera leaf gel extract should have a great potential as an effective skin care formulation.

7. Patel et al, 2009 reported in their studies that transfersomes formed from PC: Span 80 in the ratio 85:15 (in m mol) is a promising approach to improve the permeability of Curcumin in period of time. They also concluded that transfersome entrapped curcumin gel gives better permeation as compare to plain drug gel.

8. Bhaskaran and Lakshmi et al, 2009 prepared niosomes containing salbutamol sulphate using Span 60 as the surfactant, by employing different techniques namely, thin film hydration, hand shaking, ether injection, lipid layer hydration and trans membrane pH gradient method. They reported
that niosomes with thin film hydration and trans membrane pH gradient method were with high entrapment efficiency.

9. **Caddeo, 2008** investigated the possibility of improving the efficacy of resveratrol, a polyphenol with strong antioxidant and free-radical scavenging properties, on cell proliferation and photoprotection by **liposomal** incorporation. Liposomes prevented the cytotoxicity of resveratrol at high concentrations, even at 100 μM, avoiding its immediate and massive intracellular distribution, and increased the ability of resveratrol to stimulate the proliferation of the cells and their ability to survive under stress conditions caused by UV-B light.

10. **Sinico et al, 2008** reported the influence of **liposomal** incorporation on both the stability and the in vitro (trans) dermal delivery of verbascoside was evaluated. Results showed that liposomes promoted drug accumulation into the stratum corneum but they did not give rise to any significant transdermal verbascoside delivery. Finally, results obtained from a 1, 1-diphenyl-2-pierylhydrazyl (DPPH) radical assay demonstrated that liposomes did not interfere with the radical scavenging activity of verbascoside.

11. **Jigar et al, 2007** explored extensively for topical application of **niosomes** to enhance skin penetration as well as to improve skin retention of drugs. They demonstrated prolongation of drug release, an increase in amount of drug retention into skin and improved permeation across the skin after encapsulation of Erythromycin into niosomal topical gel.

12. **Dubey et al, 2007** studied that **ethosomes** are possible vehicle for transdermal delivery of methotrexate, an anti-psoriatic, anti-neoplastic agent. The study confirmed that ethosomes are a very promising carrier for the transdermal delivery of methotrexate. The enhanced accumulation of drug via ethosomal carrier within the skin might help to optimize targeting of this drug to the epidermal and dermal sites, thus creating new
opportunities for well-controlled and modern topical application of methotrexate in the treatment of psoriasis.

13. **Golmohammadzadeh, et al, 2007** had performed the determination of SPF and moisturizing effects of liposomal and conventional formulations of Octyl Methoxycinnamte (OMC) as a sunscreen. The results of study showed that Multilamellar liposomes prepared by fusion method is a good vehicle for OMC as a sunscreen since it provides proper SPF and increase the moisture content of the skin.

14. **El-Samaligy et al, 2006** prepared silymarin encapsulated hybrid liposomes which shows successful preparation with efficient encapsulation of silymarin. Incorporation of silymarin into liposomal dosage form administered buccally can improve its bioavailability. In this connection to improve the bioavailability of silymarin through its incorporation in a stable liposomal buccal dosage form, using commercially available soybean lecithin.

15. **Hung, 2006** incorporated Resveratrol, the main active polyphenol in red wine, into various combinations of emulsions and liposomes to examine its physicochemical characteristics and cardiovascular protection and concluded that encapsulation by the emulsion-liposome blends is a potent way to enhance the preventative and therapeutic benefits of resveratrol.

16. **Betz et al, 2005** had done *in vivo* comparison of various liposome formulations for cosmetic applications and reported that liposomes and liposome formulations have been implied for skin moisturization, due to the potential occlusive effect of the phospholipid film deposited on the skin surface. In order to increase the skin water content significantly, egg phospholipids are suggested to be used for the preparation of the topical formulation.

17. **Lee et al, 2005** indicated that cholesterol in liposomes greatly increases the incorporation efficiency of retinol and the stability of incorporated retinol.
18. **Li et al, 2005** prepared liposome-encapsulated *curcumin* and studied in vitro and in vivo effects on proliferation, apoptosis, signaling, and angiogenesis. The activity of liposomal curcumin was equal to or better than that of free curcumin at equimolar concentrations. In vivo, curcumin suppressed pancreatic carcinoma growth in murine xenograft models and inhibited tumor angiogenesis.

19. **Lee et al, 2003** studied the effect of edge activators on the formation and transfection efficiency of *ultradeformable liposomes* (UL) and reported that following topical application onto mice, DNA complexed with UL containing either sodium cholate or sodium deoxycholate showed substantial transdermal absorption. In contrast, DNA complexed with Tween 80-based UL did not show *in vivo* transdermal absorption.

20. **Touitou et al, 2001** investigated the efficiency of transcellular delivery into Swiss albino mice 3T3 fibroblasts of molecules with various physico-chemical characteristics from *ethosomes*, phospholipid vesicular carriers containing ethanol and proved that the system is a promising candidate for the delivery of biological and chemical compounds to both skin and cultured cells.

21. **Maghraby et al, 2000** said that incorporation of surfactants *sodium cholate* or *Span 80* into phosphatidylcholine liposomes was even better than incorporation of the penetration enhancer, oleic acid, for the skin delivery of the model lipophilic drug oestradiol. For optimum effects, the components should be in the form of vesicles. The overall results indicate that a penetration enhancing mechanism of liposome components is not the only or indeed the main factor operating. Liposome components in solution, however, have a definite additive effect with a possible synergism in some cases (surfactants or ethanol with PC).
Herbs related literature

22. Himesh et al, 2011 determined the qualitative and quantitative profile of curcumin in ethanolic extract of *Curcuma longa*. They reported total phenolic content as 11.24 mg GAE/g. Also performed simultaneous estimation of curcuminoids by HPLC method.

23. Thring et al, 2009 investigated the anti-ageing and anti-oxidant properties of 23 plant extracts, in which green tea and pomegranate extracts showed the presence of Anti-collagenase, anti-elastase and anti-oxidant activities.

24. Camouse et al, 2009, have experimentally determined that topical application of green tea and white tea extracts prevent simulated solar radiation-induced oxidative damages to DNA and Langerhans cells that may lead to immune suppression and carcinogenesis.

25. Cuili and Qin, 2008 studied on optimum extraction technology of Aloin from Aloe and adopted HPLC method for the estimation of Aloin. The result show the best reflux extraction technology is efficient, simple and rapid.

26. Ashawat et al, 2008 prepared and characterized herbal creams for the improvement of skin viscoelasticity and hydration. The cosmetic cream formulations were designed by using ethanolic extracts of *Glycyrriza glabra, Curcuma longa, Psorolea corlifolia, Cassia tora, Areca catechu, Punica granatum, Embelica officinale, Centella asiatica, Cinnamom zeylanicum* and fresh gel of *Aloe vera* in varied concentrations.

27. Wu, 2008 developed quercetin-loaded nanoparticles (QUEN) by a nanoprecipitation technique with Eudragit E (EE) and polyvinyl alcohol (PVA) as carriers, and to evaluate the antioxidant effects of quercetin (QU) and of its nanoparticles. The release of the drug from the QUEN was 74-fold higher compared with the pure drug. In addition, the antioxidant activity of the QUEN was more effective than pure QU on DPPH
scavenging, anti-superoxide formation, superoxide anion scavenging, and anti-lipid peroxidation.

28. Ashawat et al, 2007 prepared and characterized herbal cosmetic cream comprising extracts of *G. glabra*, roots of *C. longa*, *C. tora*, *A. catechu*, *P. granatum*, fruits of *E. officinale* leaves of *C. asiatica*, dried bark of *C. zeylanicum* and fresh gel of *A. vera* for the protection of skin against UV induced aging.

29. Plianbanchang et al, 2007 studied the efficacy and safety of curcuminoids loaded solid lipid nanoparticles facial cream as an antiaging agent. As curcuminoids are easily degraded by acid and alkali hydrolysis, oxidation and photodegradation; solid lipid nanoparticles (SLN) promotes its stability, prolongs the release.

30. Ashawat et al, 2007 confirmed the *in vitro* antioxidant activity of ethanolic extracts of *C. asiatica*, *P. granatum*, *G. glabra* and *A. catechu* and suggested that the combination of chemical with extract as antioxidant can be utilized in pharmaceutical and cosmetic formulation or chemical antioxidant can be replaced by herbal natural antioxidants.

31. Zhang et al, 2006 reported that green tea extract and (-)-epigallocatechin-3-gallate (EGCG) exhibit antiangiogenic activities in various experimental tumor models.

32. Ashawat et al, 2006 evaluated ultraviolet absorption ability of *Boerhavia diffusa* and expressed in terms of SPF values.

33. Kim et al, 2004 showed the protective effects of dietary soy isoflavones (genistein) against UV-induced skin aging in hairless mouse model. This is due to the inhibitory effects on UV induced MMP-1 expression and the subsequent collagen degradation.
34. **Wissing and Muller, 2003** confirmed the higher effectiveness of cream containing SLN on skin hydration and viscoelasticity as compared to conventional creams.

35. **Huang et al, 2007** reported that (-)-epigallocatechin-3-gallate (EGCG) is a potent agent against UVB- induced damage in HaCaT Keratinocytes.

36. **Siddiqui et al, 2010** reported that the origin of nano-encapsulation based chemoprevention approaches could be traced to Mukhtar and co-workers who also coined the term nanochemoprevention for the first time. This group utilized the multi-functionality of biodegradable polylactic acid (PLA–polyethylene glycol (PEG) nanoparticles to incorporate the ‘EGCG’ the chemopreventive agent from green tea.

37. **Lei et al, 2003** Pharmacokinetic study of ellagic acid in rat after oral administration of pomegranate leaf extract. Quantification of ellagic acid, the principal bioactive component of pomegranate leaf extract, in rat’s plasma following oral administration of pomegranate leaf extract was achieved by using a high-performance liquid chromatographic method. The pharmacokinetic profile indicates that ellagic acid has poor absorption and rapid elimination after oral administration pomegranate leaf extract, and part of it was absorbed from stomach.

38. **Egawa et al, 2002** studied the effect of exposure of human skin to a dry environment and they found a decrease in the stratum corneum water content and, related to this lack of water, a deterioration of the skin texture and the formation of fine wrinkles.

**Evaluation Parameters related literature**

39. **Huang et al, 2011** performed antioxidant activities and UV-protective properties of melanin from the berry of *Cinnamomum burmannii* (CBM) and *Osmanthus fragrans* (OFM) Results from the sun protection factor (SPF) *in vitro* determination of melanin-bearing gel formulations indicated that the SPF value of every formulation increased with amount of melanin,
which suggested the presence of additional compounds with sunscreen activity in the melanin extract; the synergistic effect of CBM on the SPF of gel formulations was greater than that of OFM. Both of them exhibited significant antioxidant activities.

40. **Kaur and Saraf, 2010** evaluated ultraviolet absorption ability of volatile and nonvolatile herbal oils used in sunscreens or cosmetics and express in terms of SPF values. It can be observed that the SPF values found for nonvolatile oils were found in between 2 to 8 and for volatile oils were in between 1 to 7. Among the fixed oils taken, SPF value of olive oil was found highest. Similarly among essential oils, SPF value of peppermint oil was found highest.

41. **Dutra et al, 2004** determined the SPF values of sunscreens emulsions containing chemical and physical sunscreens by UV spectrophotometry applying Mansur mathematical equation. They proposed UV spectrophotometric method which is simple, rapid, employs low cost reagents and can be used in the in vitro determination of SPF values in many cosmetic formulations. The proposed methodology may be useful as a rapid quality control method. It can be used during the production process, in the analysis of the final product, and can give important information before proceeding to the in vivo tests.

42. **Wissing and Muller, 2003** studied on the *in vivo* performance of a conventional o/w cream and solid lipid nanoparticles (SLN) incorporated cream. Influences on skin hydration and viscoelastic properties were investigated with validated devices (*Corneometer®* and *Cutometer®*). After an application period of 4 weeks, significant changes in skin hydration were detected for both formulations. The SLN-enriched cream was significantly more effective than the conventional cream (+24% for the cream and +31% for the SLN-cream).

43. **Sayre et al, 1979** did comparison of *in vivo* and *in vitro* testing of sun screening formulas. By all methods used, the combination of 7% octyl dimethyl para-aminobenzoic acid and 3% oxybenzone provided the most
protection from U.V. light. While estimates of the effectiveness of all products were much too high when calculated by the isopropanol solution method, the hairless mouse epidermis technique seems to be an accurate tool for predicting product efficacy in vivo.

44. Akhtar et al, 2011 evaluated various functional skin parameters using a topical cream of Calendula officinalis extract and reported that the topical non-invasive application of Calendula officinalis cream showed a positive rejuvenating effect on human skin. As it produced decrease in skin melanin content and skin erythema while increase in skin hydration and Skin sebum by creams (base and formulation).

45. Kaur and Saraf, 2011 studied skin parameters like melanin, erythema, skin hydration, and sebum score of six body sites namely volar forearm, cheek, chin, forehead, neck and post auricular skin of Asian (Indian) population with different skin colour and types to depict the formulation to be used for taking care. The measurements were taken using Mexameter (erythema and melanin), Corneometer (skin hydration) and Sebumeter (sebum score).

46. Akhtar et al, 2010 studied the effect of cream formulation of fenugreek seed extract on some mechanical parameters of human skin. Both the cream base and the cream containing fenugreek extract demonstrated significant (p < 0.05) improvement in all mechanical parameters related to skin elasticity, ageing, hydration and fatigue but the effect of the extract cream was more pronounced in some cases.

47. Kapoor and Saraf, 2009 performed the age dependent studies as various skin parameters using the noninvasive bioengineering suction device Cutometer, the age differences of various skin properties of human were evaluated. Study concludes that skin properties like elasticity, pliability, firmness, and extensibility varies individually and according to the age.

48. Kapoor and Saraf, 2009 had assessed the efficacy study of sunscreens containing various herbs for protecting skin from UVA and UVB sunrays.
Have compiled rapid, non-invasive technologies to investigate the sunscreens containing various herbs like Aloe vera, jojoba, cucumber, wheat germ, olive etc for their efficacy in protecting skin from UVA and UVB sunrays. Results of the study scientifically verified that herbs are having enough potential to protect skin to protect skin from harmful sunrays and it is worthwhile for consumers to use herbal sunscreens.

49. Belo et al, 2006 studied the moisturizing effect of cosmetic formulations containing Aloe vera extract in different concentrations assessed by skin bioengineering techniques. The results show that freeze-dried Aloe vera extract is a natural effective ingredient for improving skin hydration, possibly through a humectant mechanism. Consequently, it may be used in moisturizing cosmetic formulations and also as a complement in the treatment of dry skin.

2.2 RESEARCH ENVISAGED

After detailed literature survey we could find the prominent evidences of antioxidant, antiaging, nature of the herbs Camellia sinensis, Curcuma longa, Punica granatum and Aloe vera hence these herbs were selected for the study. We could find that some of the studies on conventional dosage forms like creams, lotions, gels but few works was seen which could actually predict the impact of their novel formulations on skin properties as well as their photoprotective nature.

Similarly we could see that the research had established the fact that vesicular systems like liposomes, ethosomes, niosomes and transfersomes enhance the penetration, so this fact was taken to develop novel systems of herbal extracts which could cause the penetration of the extracts photoprotective phytoconstituents deeper to the dermal layer so that the degradation caused due to the harmful ultraviolet radiations could be prevented as well as the morphological skin characters like skin hydration, viscoelasticity etc could also be improved. In the literature we find evidences for the antioxidant and photoprotective ability of extracts of Camellia sinensis, Punica granatum but most of the work is with oral use as antioxidant. Topical use with novel delivery systems for enhanced
penetration was the approach used by this study. *Aloe vera* has generally been used only for its moisturizing effect either alone or as liposomes but its use as photoprotective needs more study so we have tried to develop other systems also for *Aloe vera*.

Our aim will be to develop stable novel vesicles of the extracts of photoprotective herbs incorporate them into creams, completely evaluate the important physicochemical, psychometric parameters of the creams and then study their effect on human skin in the terms of skin hydration, viscoelasticity, sebum content, erythema and melanin content. We are also trying to study their effect biologically by checking the biochemical parameters and through histological studies.

Since most of the cosmetic work has been with the chemicals and conventional type of formulations, so our work will be a small step towards development of stable herbal photoprotective formulations. We are aiming to develop formulations which could be patentable and marketed. Our work will be a contribution to develop stable herbal cosmetic formulations using novel approaches. As Chhattisgarh is the land of medicinal herbs so there is availability of various photoprotective herbs which will be basis for selection of most appropriate and effective herbal extract showing photoprotective effect. We have tried to use conventional knowledge of cosmetics and inculcate newer scientific facts to develop more acceptable and stable herbal photoprotective formulation. The amalgamation of use of properties of phytocconstituents along with the characteristics of novel delivery systems are used as base for the formulation of photoprotective formulations with better and enhanced efficacy. The important objectives of the work are mentioned below:

- To develop herbal cosmetic formulations using novel approaches.

- Prepare stable formulations with herbal extracts.

- Develop important tool for combating the deleterious effects of ultraviolet radiations like photoaging and photocarcinogenesis.
• Develop cosmetic formulations which could produce photoprotective effects like improved skin hydration, viscoelasticity and reduced erythema, melanin and sebum level.

• To produce cosmetic formulations, this could have sustained effect.

• Our approach is to use various vesicular nano systems which could not only correct morphological defects but also go deeper to the cellular level of dermis and cause increased collagen and elastin synthesis so that harmful effects of ultraviolet radiations could be combated.

• To provide complete study from extraction of herbs to development of novel herbal cream formulations.

• To compare photoprotective ability of various herbal extract loaded novel vesicular systems.

2.3 PLAN OF WORK

The envisaged work was undertaken according to following plan:

1. Literature survey

2. Identification and selection of herbs

   The photoprotective herbs selected are as follows:-

   • Leaves of *Camellia sinensis* (Green tea)
   • Rhizomes of *Curcuma longa* (turmeric)
   • Gel of *Aloe vera* leaf
   • Seeds of *Punica granatum* (Pomegranate)

3. Extraction and phytochemical screening of extracts

   • Preliminary phytochemical analysis
   • Antioxidant activity determination of extracts
- Total Phenolic Content determination of extracts
- *In vitro* sun protection factor determination of extracts
- Standard Curve preparation of standards and estimation of main constituent from the extract
  - (-) Epicatechin: *Camellia sinensis* (Green tea),
  - Curcumin: *Curcuma longa* (turmeric),
  - Aloin: *Aloe vera*
  - Ellagic acid: *Punica granatum* (Pomegranate)

4. Preparation of various novel vesicular formulations
   - Liposomes
   - Transferosomes
   - Ethosomes
   - Niosomes

5. *In vitro* evaluations of vesicular formulations
   - Vesicular size and Poly dispersity index determination
   - Zeta potential determination
   - Vesicular shape and morphology by Transmission Electron Microscopy (TEM)
   - Entrapment Efficiency Determination

6. Optimization and Preparation of Cream Formulations
   - Preparation of base cream
   - Preparation of cream formulations with empty novel vesicles
   - Preparation of cream formulations with herbal extract loaded vesicular formulations
   - Stability Studies

7. *In vitro* evaluations of Cream Formulations
   - Physicochemical evaluations of creams
   - Psychometric evaluations of creams
   - *In vitro* sun protection factor (SPF) determination of creams
8. In vivo evaluations of cream formulations

- Efficacy studies by Bioengineering Methods
  - Skin viscoelasticity determination by Cutometer
  - Skin hydration measurement by Corneometer
  - Skin erythema determination by Mexameter
  - Skin melanin determination by Mexameter
  - Skin sebum determination by Sebumeter

- Biological Studies
  - Biochemical Analysis
  - Histological studies

9. Data Analysis

10. Compilation and Thesis Writing

11. Publication and Patenting

2.4 ACTIVITY CHART

A systematic month wise activity chart has been prepared depicting the duration of the activities performed as per plan of work during the tenure of research. The work was started with the review of literature on the basis of which we had selected four photoprotective herbs namely C. longa, C. sinensis, P. granatum and A. vera. Accordingly four vesicular systems were selected namely liposomes, ethosomes, transfersomes and niosomes and their herbal extract loaded novel vesicular systems were developed. Fig.2F-1 shows the Activity Chart showing various activities performed during the research tenure.
<table>
<thead>
<tr>
<th>S. No.</th>
<th>Particulars of Activity</th>
<th>Time in months</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Review of Literature and selection of herbs and systems,</td>
<td>2 months</td>
</tr>
<tr>
<td>2</td>
<td>Procurement of Ingredient,</td>
<td>3 months</td>
</tr>
<tr>
<td>3</td>
<td>Extract preparation of selected photoprotective herbs,</td>
<td>2 months</td>
</tr>
<tr>
<td>4</td>
<td>Photoprotective Extracts Analysis,</td>
<td>3 months</td>
</tr>
<tr>
<td>5</td>
<td>Optimization and Preparation of various novel vesicular formulations,</td>
<td>5 months</td>
</tr>
<tr>
<td>6</td>
<td>In vitro evaluations of novel vesicular formulations,</td>
<td>3 months</td>
</tr>
<tr>
<td>7</td>
<td>Optimization and Preparation of Cream formulations,</td>
<td>2 months</td>
</tr>
<tr>
<td>8</td>
<td>Physicochemical and Psychometric evaluations of creams,</td>
<td>4 months</td>
</tr>
<tr>
<td>9</td>
<td>Physical Stability of Creams,</td>
<td>6 months</td>
</tr>
<tr>
<td>10</td>
<td>In vitro SPF determination of creams,</td>
<td>4 months</td>
</tr>
<tr>
<td>11</td>
<td>Efficacy studies assessing skin properties</td>
<td>4 months</td>
</tr>
<tr>
<td>12</td>
<td>Photoprotective activity assessment</td>
<td>4 months</td>
</tr>
<tr>
<td>13</td>
<td>Statistical analysis of in vitro data and publication preparation and communication,</td>
<td>4 months</td>
</tr>
<tr>
<td>14</td>
<td>Complete data analysis and Compilation.</td>
<td>10 months</td>
</tr>
</tbody>
</table>

**Fig. 2F-1. Activity Chart showing various activities performed**