DEVELOPMENT AND EVALUATION OF TOPICAL ETHOSOMAL FORMULATION COMPOSED OF *ORTHOSIPHON STAMINEUS* EXTRACT FOR MELANOMA

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by

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This thesis is dedicated to

My wonderful angel, my mother

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Praise is to Allah, The Most Merciful, who gave me the determination and the strength to see this work through.

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LIST OF ABBREVIATIONS

°C	Celsius
µl	Microliter
3D	Three- dimensional
5-FU	5-fluorouracil
AJCC	American joint committee on cancer
ALD	Alkaline phosphatase
ALT	Alanine amino transferase
AMP	Antimicrobial peptide
ARASC	Animal Research and Service Centre
AST	Aspartate amino transferase
ATR/FTIR	Attenuated total reflection/Fourier-Transform Infrared Spectrometry
AU	Arbitrary unit
AUC	Area under curve
bFGF	Basic fibroblast growth factor
CBD	Canabidiol
CLSM	Confocal Laser Scanning Microscope
C_{max}	Maximum concentration
COX	Cyclooxygenases
DMSO	Dimethyl sulfoxide
DMSO	Dimethyl sulfoxide
DNA	deoxyribonucleic acid
DTIC	Dacarbazine
Ea	Activation energy

EC	Endothelial cells
EC	Endothelial cell
ECM	Endothelial cell medium
ECM	Extra-cellular matrix
ET	Ethosome
EUP	eupatorin
EX	ExtractFGF
FDA	Food and drug administration
FGF	Fibroblast growth factor
GGT	Gamma glutamyl transferase
H	Hours
HIF	Hypoxia-inducible factors
HIF	Hypoxia inducible factor
HIV	Human Immunodeficiency Virus
HPLC	High performance liquid chromatography
Hz	Hertz
ICH	International Conference on Harmonization
IFN	Interferon
IL-1	Interleukin-1
IL-2	Interleukin-2
IL-7	Interleukin-7
IPA	Isopropyl alcohol
K	Reaction rate constant
KDa	Kilo Dalton
LD_{50}	Lethal Dose, 50

LOD	Limit of detection
LOQ	Limit of quantification
Lyso-PC	Lysophosphatidylcholine
MIR	mid infrared
MMP	Matrix metalloproteinase
MTT	3-(4, 5-dimethylthiazol-2-yl)- 2,5 diphenyltetrazolim bromide
NCI	National Cancer Institute
NSCLC	Non-small cell lung cancer
OD	Optical density
OECD	Organization for Economic Cooperation and Development
OS	Orthosiphon stamineus
PBS	Phosphate-buffered saline
PC	Phosphatidylcholine choline
PDGF	Platelet fibroblast growth factor
PE	Plating efficiency
PG	Propylene glycol
PIGF	Placenta growth factor
RA	rosmarinic acid
RCC	Renal cell carcinoma
RH	Relative humidity
rpm	revolutions per minute
SC	Stratum corneum
Sin	sinesitin
TEM	Transmission electron microscopy
TGF-α	Transforming growth factor alpha

THP	Trihexyphenidyl hydrochloride
TKI	Tyrosine kinase inhibitor
TMF	3′-hydroxy-5,6,7,4 ′ -tetramethoxyflavone
UV	Ultra Violet
VEGF	Vascular endothelial growth factor
VEGFR-1,2	Vascular endothelial cell receptors -1,2
VPF	Vascular permeability factor
w/v	weight of solute in gram and volume of solution in mL
WHO	World Health Organization

PEMBANGUNAN DAN PENILAIAN FORMULASI ETOSOMAL TOPIKAL TERDIRI DARIPADA EKSTRAK *ORTHOSIPHON STAMINEUS* UNTUK MELANOMA

ABSTRAK

Melanoma adalah salah satu jenis kanser kulit yang paling agresif dengan kadar kematian yang tinggi jika tidak dirawat. Sebatian berasaskan herba boleh digunakan sebagai alternatif dalam terapi kanser seperti melanoma kerana sifat anti-oksidan dan anti-radang yang tinggi. Ciri-ciri ini boleh menghentikan angiogenesis, iaitu suatu proses pembentukan saluran darah baru yang penting untuk pertumbuhan tumor dan metastasis. Walau bagaimana pun bioketersediaan yang lemah kerana polaritas tinggi fitokimia mengehadkan keberkesanannya. *Orthosiphon stamineus* (O.S) adalah herba ubatan yang terkenal di Asia Tenggara dan ekstrak pelarut unik herba ini telah menunjukkan aktiviti antiangiogenik dan juga terbitan ini kini dalam percubaan klinikal untuk kanser payu dara dan kolon. Dalam kajian ini, ekstrak O.S yang diseragamkan kepada 6% asid rosmarinic diformulasikan ke dalam bentuk formulasi liposomal yang dikenali sebagai etasom. Kedua-dua ekstrak dan perumusan itu dinilai untuk keberkesanannya terhadap melanoma *in vitro* dan *in vivo* serta sifat-sifat farmakokinetik, toksikologi dan fiziko-kimia dinilai. Kaedah rehidrasi filem nipis digunakan untuk mempersiapkan formulasi gel liposomal fleksibel yang terdiri daripada ekstrak terpiawai daun O.S Kajian Kromatografi Cecair Prestasi Tinggi (HPLC) mendedahkan kecekapan pemerangakapan sebanyak 80% untuk asid rosmarinic (RA) dalam vesikel etosom. Saiz zarah purata vesikel teroptimum ialah 138.6 nm. Ekstrak O.S termuat gel etosomal pada kepekatan 1% menunjukkan aliran elasto-plastik yang betul dengan tekanan hasil tertentu serta kekerapan bebas G 'dan G

", yang boleh memudahkan ekstrak O.S.mencapai penelapan (permeasi) kulit yang berkesan dan maksimum. Saiz zarah purata vesikel teroptimum ialah 138.6 nm. Ekstrak O.S termuat gel etosomal pada kepekatan 1% menunjukkan aliran elasto-plastik yang betul dengan tekanan hasil tertentu serta kekerapan bebas G 'dan G ", yang boleh memudahkan ekstrak O.S mencapai penelapan (permeasi) kulit yang berkesan dan maksimum. Hayat/Tempoh simpan formulasi akhir gel adalah 2.5 bulan pada suhu 4ºC. Ekstrak O.S termuat vesikel ethosomal menunjukkan hampir 98% penembusan transdermal bertambah baik jika dibandingkan dengan gel hidro-ethanolic konvensional O.S ekstrak. Hasil kajian penelapan (permeasi) yang dipertingkatkan dan penyaringan FTIR kulit menunjukkan bahawa etanol membubarkan lapisan lipid membran kulit dan membuka jurang kecil dan penelapan drug/ubat dipertingkatkan. Hasil ini mendedahkan bahawa ekstrak O.S termuat vesikel ethosomal boleh dianggap sebagai ejen terapeutik kulit yang berkesan bagi rawatan penyakit kulit. Hasil kajian ketoksikan dermal akut dan berulang *in vivo* menunjukkan bahawa kompleks etosom O.S mempunyai profil keselamatan yang baik sebagai formulasi aplikasi topikal tanpa kesan toksik muncul dalam analisis histologi organ-organ penting. Kajian farmakokinetik dan pemendapatan kulit memperlihatkan peningkatan bioketersediaan sebatian aktif berbanding ekstrak mentah O.S Akhirnya, kajian model *in vivo* melanoma yang dijalankan ke atas tikus albino Swiss menyokong keputusan *in vitro* anti-kanser. Hasilnya memperlihatkan kesan antikanser formulasi etosomal-O.S topikal yang dipertingkat untuk mengendalikan tumor melanoma yang malignan dan mencegah metastasis. Keseluruhan penemuan penyelidikan terkini menunjukkan bahawa kadar penembusan yang dipertingkat formulasi gel O.S-ethosomal disebabkan oleh fleksibiliti dan kestabilan dalam vesikel menyebabkan kesan anti melanoma ekstrak secara *in vitro* dan *in vivo* yang lebih baik.

DEVELOPMENT AND EVALUATION OF TOPICAL ETHOSOMAL FORMULATION COMPOSED OF *ORTHOSIPHON STAMINEUS* EXTRACT FOR MELANOMA

ABSTRACT

Melanoma is one of the most aggressive types of skin cancer with high rate of fatality if left untreated. Herbal-based compounds can be useful as alternative in cancer therapy such as melanoma due to their high anti-oxidant and anti-inflammatory properties. These characteristic features can halt angiogenesis, a process of new blood vessels formation which is crucial for tumor growth and metastasis. However, poor bioavailability due to the high polarity of the phythochemicals limits their effectiveness. *Orthosiphon stamineus* (O.S) is a popular medicinal herb found in South East Asia and a unique solvent extract of this herb has been shown to have antiangiogenic activity. In this study, the extract of O.S standardized to 6% rosmarinic acid was formulated into a liposomal type of formulation known as ethosome. Both the extract and the formulation were evaluated for their effectiveness towards melanoma *in vitro* and *in vivo* and pharmacokinetics, toxicology and physico-chemical properties were evaluated. Thin-film rehydration method was employed to prepare the flexible liposomal gel formulation composed of standardized extract of O.S leaves. High Performance Liquid Chromatography (HPLC) studies revealed 80% entrapment efficiency for the rosmarinic acid (RA) in the ethosomal vesicle of the extract as marker compound. The average particle size of optimized vesicle was 138.6 nm. O.S extract-loaded ethosomal gel at concentration of 1% demonstrated proper elasto-plastic flow with specific yield stress as well as frequency independent G′ and G″, which could facilitate achieving effective and maximum skin permeation of O.S

extract. Shelf life of final gel formulation was 2.5 months at 4ºC. O.S extract loaded-ethosomal vesicles showed almost 98% improved transdermal penetration when compared to that of the conventional hydro-ethanolic gel of O.S extract. The enhanced permeation study results and FTIR screening of the skins suggests that the ethanol dissolved the lipid layers of skin membranes and opened up small gaps and enhanced drug permeation. These results revealed that O.S extract loaded-ethosomal vesicles can be considered as an effective dermal therapeutic agent for the treatment of skin ailments. *In vitro* anticancer and anti-angiogenic studies in melanoma (B16F10) and endothelial (EA.hy926) cell lines respectively, demonstrated significant improvement in ethosomal vesicle efficacy compared to O.S crude extract alone. *In vivo* acute and repeated dose dermal toxicity studies results showed that ethosomal complex of O.S has a good safety profile as a topical formulation with no toxic effect appeared in histological analysis of vital organs. Pharmacokinetic and *in vivo* skin deposition studies demonstrated enhanced bioavailability of active compounds compared to crude O.S extract. Finally, *in vivo* melanoma model study that was conducted in Swiss albino mice supported the *in vitro* anticancer results. The results exhibited enhanced anticancer effect of topical ethosomal-O.S formulation to control malignant melanoma tumor and prevent metastasis. Altogether the discoveries of present research shows that the enhanced penetration rate of O.S-ethosomal gel formulation due to its flexibility and stability in vesicle caused the improved anti-melanoma effect of the extract *in vitro* and *in vivo*.

CHAPTER 1

INTRODUCTION

1.1 Cancer

Cancer disease involves the abnormal growth of cells that have the ability to invade other organs. Normal human cells grow through the process of cell division. Cancer cells are able to ignore some critical cell signals that naturally inform cells to suppress programmed cell death. The result is abnormal cell proliferation leading to tumor growth.

1.2 Skin Cancer

Skin cancer generally occurs more frequently than other types of cancers. Generally it starts cells located in epidermis layer of skin which can metastasize to other organs (Ko *et al.*, 2010).

Skin cancer usually appears due to some specific mutations in cell DNA which can be stimulated by ultraviolet light (UV). It can be better controlled if diagnosed early, otherwise it can be developed into full blown tumors (D'Orazio *et al.*, 2013). Skin cancer is not an inherited disease but based on some research people with less pigmentation are at higher risk of getting skin cancer, so from this point of view genetic can play a role (Scherer *et al.*, 2010). Moreover, the risk of skin cancer increases in patients suffering from some unfrequented syndromes with genetic origin (Lomas *et al.*, 2012).

Skin tumor are categorized in 3 groups based on the shape and epidemiology and risk of lethality of cancer: basal cell carcinoma, squamous cell carcinoma and melanoma.

1.2.1 Basal Cell Carcinoma

Basal cell carcinoma is the least malignant and most common form of skin cancer, accounting for 80% of cases in the United States (Scotto *et al.*, 1983). With this type, stratum basal cells proliferate and invade the dermis and hypodermis. The cancer lesions usually occur on sun-exposed areas of the face and appear as shiny, dome-shaped nodules that eventually develop into ulcer in the center with a pearly edge (Feuerstein *et al.*, 2008). Basal cell carcinoma grows fairly slow and rarely metastasize without it being noticed (Randle, 1996). This type of skin cancer is fully curable by surgical excision in 99% of cases (Smeets *et al.*, 2004).

1.2.2 Squamous Cell Carcinoma

Squamous cell carcinoma is the second most common form of skin cancer and arises from keratinocytes in the stratum spinosum (D'Orazio *et al.*, 2013). The lesion usually appears as a scaly red papule, is small, round, and elevated. Most often, they arise on the head (ears, scalp, lip), and hands. Squamous cell carcinoma will grow rapidly and metastasize if not removed (Stein *et al.*, 2005). The chances of a complete cure are good if the lesion is caught early and removed surgically (or by radiation therapy).

1.2.3 Melanoma

Melanoma is the most rapidly-increasing type of cancer in the world, doubling approximately every 10-20 years in fair-skinned populations (Leiter *et al.*, 2014). Like many other types of cancers, occurrence raises with age. Young women are the fastest rising melanoma population (Argenziano *et al.*, 2010). Some other names of this cancer are malignant melanoma and cutaneous melanoma.

Most melanoma cells produce melanin; therefore, melanoma neoplasms are generally brownish or black in color. However, some types of melanoma can produce melanin but do not do so, so lesions may appear pink, brown, or perhaps white in color.

Melanoma commonly originates in three specific types of skin cell (Eisinger *et al.*, 1985), namely squamous cells, basal cells and melanocytes; although statistically there is a greater occurrence in melanocytes (Bonazzi *et al.*, 2012). Melanomas might occur anywhere on the skin; but they are most likely to begin on the trunk area (chest as well as back) in men, or on the legs in women. Melanoma is unusual among African Americans; however, when it happens, survival time is typically shorter than for Caucasians (Cormier *et al.*, 2006). Melanoma patients with weak immune systems such as those going through organ transplants or HIV patients are at higher risk of death (Vajdic *et al.*, 2009).

Melanoma (tumor of melanocytes) is the most risky type of skin malignancy since it is very metastatic and impervious to chemotherapy. Currently, it represents 2-3% of skin tumor cases, however the rate is expanding quickly (3-8% every year in the United States) (Saladi *et al.*, 2005). Melanoma normally appears suddenly and around 33% of cases originate from existing skin moles (Cummins *et al.*, 2006). It normally shows up as a spreading darker spot that spreads rapidly to nearby blood and lymph vessels (Egan, 2005).

The best chance to survive melanoma is early discovery and diagnosis. When the lesion become more than 4 mm thick, the possibility of survival is poor (Shields *et al.*, 2002). Standard treatment for melanoma incorporates surgical extraction along with immunotherapy (inoculating the body against the tumor cells) (Kanazawa *et al.*, 2000).

1.3 Angiogenesis

Angiogenesis is the growing of new blood vessels out of the available vasculature. This multi-step process happens in life in both normal and abnormal physical condition, starting before birth and until old age (Moore *et al.,* 2011). Capillary vessels are required in virtually all tissues in order to diffuse nutriments. Alterations in metabolic process cause relative modifications in angiogenesis and, consequently, relative modifications in the capillaries (Cassell *et al.*, 2002). Oxygen is a crucial factor in such regulation. Hemodynamic factors are vital for existence of blood vessel networks as well as structural adjustments of vessel wall membranes (Pries *et al.*, 2014).

Activation of angiogenesis is generally therapeutic in ischemic cardiovascular disease, peripheral arterial disorder, and also wound healing (Fadini *et al.*, 2010). Reducing or hindering angiogenesis could be considered as a treatment for tumors, ophthalmic diseases, rheumatoid arthritis, and even some other health conditions (Folkman, 1995). Capillary vessels develop and then regress in normal tissues because of physiological needs (Moulton, 2001). Any activity can induce angiogenesis in the heart as well as skeletal muscle (Chinsomboon *et al.*, 2009). Insufficient physical activity results in blood vessels regression (Nybo *et al.*, 2001).

Figure 1.1: Process of angiogenesis multistep (Bryan *et al.*, 2007)

Capillaries develop in adipocytes as a patient gains weight, and they regress upon weight loss (Kern *et al.,* 1995). Obviously, angiogenesis happens through lifespan (Giusti *et al.*, 2016).

1.3.1 Mechanism of Physiological Angiogenesis

As an embryo forms, new blood vessels are produced by a procedure known as vasculogenesis. Additional modification of vascular network continues throughout angiogenesis. That is a complicated multi-step procedure wherein new blood vessels are generated out of the available ones. Dysregulation in the process of angiogenesis leads to continuous blood vessel formation, which is a critical process in tumor growth ; thus, angiogenesis can be a suitable target for the treatment of tumors (Thijssen *et al.*, 2006). Hence, the purpose of using anti-angiogenic factors in cancer therapy aims to interrupt critical stages of angiogenesis (Carmeliet, 2000).

1.3.2 Tumor Angiogenesis

In tumor angiogenesis, cancer cells rely on new blood vessels to nourish them once the tumor exceeds $1mm^3$ in diameter. This occurs when a state of hypoxia exists in the central region of the tumor (Zeng *et al.*, 2015). The state of hypoxia inside the tumor stimulates the abnormal physiologic conditions affecting the balance between pro- and anti-angiogeneic mediators. If the balance shifts towards pro-angiogenic mediators, tumor growth increases. If the balance moves towards anti-angiogenic mediators, tumor growth slows down (Gacche *et al.*, 2014). Tumor angiogenesis involves various mediators, but vascular endothelial growth factor (VEGF) and its signaling function as the rate-limiting step of this process (Rosen, 2002). Growth and modification of new blood vessels not only serves to supply the tumor tissue with oxygen and nutrients, but they can also serve as a means for cancer cells to metastasize (Figure1.2) (R. K. Jain, 2005). However, the complexity of the interaction between tumor and vasculature is profound, and much research remains to be done in this area.

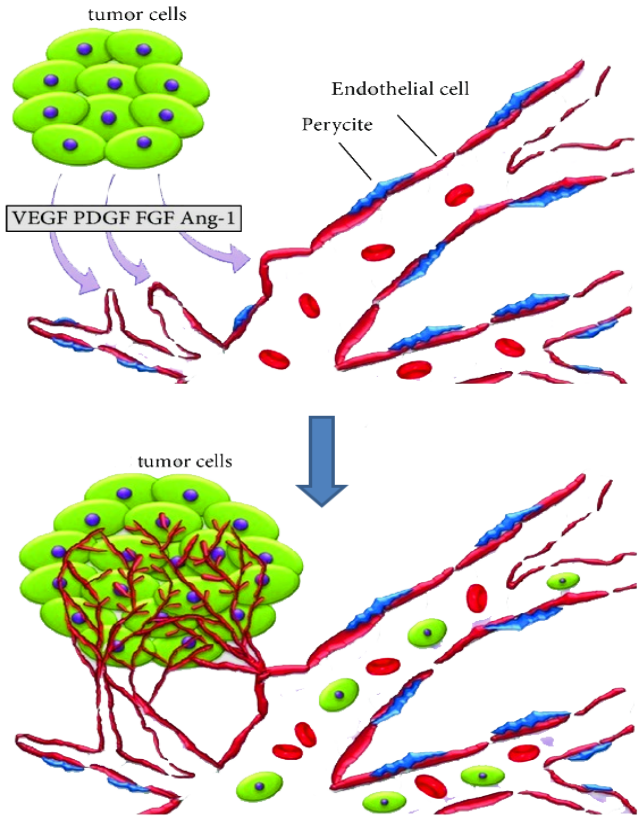

Figure1.2: Tumor-neovascularization process (Conti *et al.*, 2013)

Angiogenesis-dependence has been reported in many different types of cancer; these respond well to anti-angiogenic therapies. They include cancers of the colon, breast, lung, and bladder as well as renal cell carcinoma and non-small-cell lung cancer (NSCLC). Additionally, some of these cancers require VEGF for their survival. Anti-angiogenic therapeutic strategies block or hinder angiogenesis through two major mechanisms: blocking the receptor tyrosine kinases intracellularly or neutralizing angiogenic factors such as VEGF or its receptors (Ferrara, 2002).

1.3.3 Regulation of Tumor-Angiogenesis

The growth of a vascular supply is critical not just for development and differentiation of organs during embryogenesis; it is also important for wound healing

(Gerwins *et al.*, 2000). Angiogenesis is involved in the pathogenesis of a wide variety of diseases: proliferative retinopathies, psoriasis, tumors and rheumatoid arthritis (Pandya *et al.*, 2006). Hypoxia is a significant factor of homeostatic systems to regulate angiogenesis which connects vascular oxygen source to metabolic requirement (Dewhirst *et al.*, 2008). Molecular studies of tumor-angiogenic pathway, highlights the importance of hypoxia-inducible factor (HIF) as an important transcriptional controller of VEGF molecules. Various tumor-angiogenesis controllers have been identified, such as fibroblast growth factor-a (aFGF), bFGF, transforming growth factor-alpha (TGF-alpha), TGF-beta, angiogenin, and interleukin-8 (IL-8) (Miyake *et al.*, 2011; Weis *et al.*, 2011). Recently it was discovered that vascular endothelial growth factor (VEGF) is an endothelial-cell-specific mitogen. VEGF is highly effective with regard to vascular endothelial cells and, in contrast to bFGF, contributes to the role the molecule performs in the control of tumor angiogenesis. Various other members of the VEGF gene family have been identified, such as placenta growth factor (PIGF), VEGF-B, VEGF-C and VEGF-D. There is strong evidence demonstrating that VEGF acts as a critical factor in growth of tumor and metastasis (Saharinen *et al.*, 2011).

1.3.4 VEGFs and VEGF Receptors

VEGF, first known as vascular permeability factor (VPF), was discovered by Senger et al., as a part of tumor-secreted factors and inducing leakage of skin blood vessels (Weis *et al.*, 2005). Later in 1989, Ferrara *et al*. isolated VPF, which was then renamed as VEGF (Ribatti, 2007). VEGFs are a family of secreted dimeric glycoproteins that include VEGF-A (commonly referred to as VEGF), VEGF-B, VEGF-C, VEGF-D in mammals, and VEGF-E and VEGF-F found in other species such as viruses and snake venom, respectively (Ylä-Herttuala *et al.*, 2007). The VEGF

family also includes PlGF- 1 and 2 (Ferrara, 2010). The effects of these factors are mediated through binding to their receptors (VEGFR-1, VEGFR-2 and VEGFR-3 (Partanen *et al.*, 2000). VEGFR-1 and -2 interacts with neuropilin-1 while VEGFR-3 only associates with neuropilin-2 (Suarez *et al.*, 2006).

Diversity on the effects of VEGF receptors arises from receptor dimerization potentials (Robinson *et al.*, 2001). Dimerization between VEGFRs 1 and 2 as well as VEGFRs 2 and 3 have been shown to mediate some of the physiological effects of VEGF superfamily members (Holmes *et al.*, 2007). Furthermore, VEGFRs isoforms each lead to discrete effects compared to their counterparts in ECs (Bottos *et al.*, 2009): VEGFR-1 activation leads to a 'decoy effect' as a VEGF sequestrate or VEGF-trap (Al-Husein *et al.*, 2012); while VEGFR-2 binding to its ligand leads to proliferation, migration, survival, and angiogenesis (Cross *et al.*, 2003). Similar to VEGFR-2, VEGFR-3 mediates these cellular processes but primarily in lymphatic blood vessels (Cross *et al.*, 2003).

1.4 Treatment of Melanoma

Melanoma is a highly angiogenic dependent type of cancer. Treatment for melanoma is limited are in spite of improvements in immunotherapy as well as targeted therapy (Olszanski, 2014). For patients with surgically resected, dense (2 mm) initial melanoma with or even without localized lymph node metastases, the most common adjuvant treatment is interferon (IFN). However, the benefits of IFN are debatable, as tumors may recur and survival rates are not certain. For single-drug treatment in patients with stage IV metastasis, the American Joint Committee on Cancer (AJCC) recommends dacarbazine (Fischkoff *et al.*, 2005). However the treatment success rate for dacarbazine and its oral analogue, temozolomide (Middleton *et al.*, 2000), is only 15%, and even this is usually temporary. Amongst other treatment alternatives,

immunotherapy by using high-dose interleukin (IL)-2 obtains long-term, long-lasting, complete results in a small ratio of patients. However, the drug remains unproven in an official phase III, randomized proportional study on humans (Agarwala *et al.*, 2002). Bio chemotherapy thus has some positive effect, but has not been shown to significantly enhance survival rates due to its high toxicity, as opposed to chemotherapy alone (Ives *et al.*, 2007). Obviously, new treatment options are required.

In recent years, enhanced drug delivery technologies have been researched, to overcome the limitations of the traditional techniques. Drug delivery for cancer chemotherapy is a significant factor in the development of new techniques. Lipoproteins as drug delivery systems are a desirable field of investigation (Sharman *et al.*, 2004). Lipophilicity as well as molecular size is the most crucial criteria in enabling drug molecules to cross the normal membrane so they can be consumed efficiently after administration. For better bioavailability, herbal products need to have proper balance between hydrophilicity (to dissolve into the gastro-intestinal liquids) and lipophilicity (to pass lipidic bio-membranes). Numerous phytoconstituents including glycosilated polyphenolics, flavonoids, glycosides, tannins, in spite of remarkable water solubility and bioactivity *in vitro*, reveal significantly less *in vivo* activity because of their weak lipid solubility or inappropriate molecular size, causing inadequate absorption and poor bioavailability (Manach *et al.*, 1996). Standardized herbal extracts containing polar phytoconstituents such as flavonoids, terpenoids, tannins, xanthones utilize novel drug delivery systems, demonstrating greater absorption as they cross the biological membrane, which results in improved bioavailability (Cornwell *et al.*, 1993). Moreover more active compounds will reach their targeted destination in the body at an effective dosage, than is the case with traditional herbal extracts or phytomolecules (Borm *et al.*, 2004). Therefore, the

medicinal effect is better, more recognizable and longer-lasting (Vintiloiu *et al.*, 2008). So improving novel drug delivery systems may result in improved treatment of cancer patients using herbal active compounds and extracts.

Melanoma is the sixth most common cancer in the U.S., with approximately 44,200 latest patients declared in 1999 (Parkin *et al.*, 1999). This figure has increased hugely since then, with over 1.4 million cases in 2015 (Ferlay *et al.*, 2015). The subcutaneous type (B16) is commonly used for the investigation of treatment in several other tumor types (Marty *et al.*, 2006).

The most crucial part of cancer metastasis is the distribution of melanomas to the lymphatic vessels that surround the mass of the tumor tissue (Dadras *et al.*, 2003). At the same time, or shortly afterwards, melanoma cells may metastasize to invade the lungs, the liver, brain tissue, and other sites (Steeg, 2006). Exposure to ultraviolet radiation is known to cause genetic changes in skin, which modulate the cutaneous immune response and increase production of several growth factors (Matsumura *et al.*, 2004). This causes uncontrolled proliferation of melanocytes, which, in turn, dramatically increases the consumption of oxygen and nutrients, eventually leading to cell starvation and hypoxia (Haass *et al.*, 2005). To fulfill this increasing demand, additional vasculature needs to be developed. Thus, to increase the blood supply, the tissue begins to produce a spectrum of growth factors that trigger the process of angiogenesis (Claffey *et al.*, 1996). Studies using human melanoma xenograft models in nude mice indicated that melanoma tumor cells serve as a source of several growth factors, including but not limited to vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF). Both of these growth factors have a powerful stimulation effect on angiogenesis (Claffey *et al.*, 1996). Science has made considerable advances in recent years towards understanding the molecular

mechanisms of tumor growth. Modern technology makes it possible to detect and treat various human cancers in ways never imagined before, but our knowledge about the mechanisms behind cutaneous tumor angiogenesis and metastasis is still in its infancy (Mahabeleshwar *et al.*, 2007).

Angiogenesis in melanoma is stimulated by a variety of growth factors. Among these are VEGF, bFGF, acidic FGF, platelet-derived growth factor (PDGF), and transforming growth factors and (TGF- and) (Ferrara, 2002). Angiogenesis serves as a key point in melanoma tumor growth and metastasis (Mahabeleshwar *et al.*, 2007).

1.4.1 Chemotherapy

Chemotherapy is commonly used to treat advanced stage of melanoma and it is also often used in palliative treatment options for stage IV metastatic cancer (Mac Manus *et al.*, 2001); dacarbazine is the most frequently used principal chemotherapeutic drug for metastatic melanoma therapy (Middleton *et al.*, 2000). Both of conventional chemotherapies and radiotherapy are generally inefficient in metastatic melanoma patients (Engell-Noerregaard *et al.*, 2009).

1.4.2 Biochemotherapy

Biochemotherapy (e.g., cisplatin, vinblastine, and dacarbazine combined with IFN- IL-2) increases response rates but has not been shown to significantly improve survival compared to chemotherapy alone in randomized phase II and III trials (S. Bhatia *et al.*, 2009).

Despite decades of clinical research, patients with advanced melanoma continue to have a poor prognosis, and no agents have shown statistically significant improvement in overall survival in a phase III trial in patients with metastatic

melanoma (Atkins *et al.*, 2008). For high-risk, resected disease, adjuvant therapy with IFN- has been shown to consistently increase relapse-free survival, as well as overall survival in some studies (Cameron *et al.*, 2001). Serum levels of angiogenin, VEGF, bFGF, and IL-8 were significantly increased in melanoma patients compared to healthy controls (Ugurel *et al.*, 2001).

The effective management of malignant melanoma therapy has remained centered around the surgeon. The arrival of anti-angiogenic agents as the 'fourth' cancer treatment joining the ranks of surgery, chemotherapy and radiotherapy has been a source of renewed hope.

1.4.3 Medicines Discovered To Treat Melanoma

Metastatic melanoma is very impervious to chemotherapy, radiation treatment, hormonal treatment and current immunotherapeutic methodologies (Miller *et al.*, 2016). All known therapeutic methods revealed no noteworthy effect on survival with new medications. Angiogenesis is a legitimate focus in melanoma therapeutics. Hindrance of angiogenesis through the focusing of vascular endothelial development factor (VEGF) has long been utilized as a part of the treatment of tumors, although its antitumor action remains ineffective (A. Sharma *et al.*, 2005).

1.4.4 Anti-angiogenesis Therapy

Angiogenesis is characterized as the development of new blood vessels from a previous vasculature and is as prerequisite for tumor survival and movement past a couple of hundred microns in diameter (Fukumura *et al.*, 2008). Altogether for a cell to survive it must be guaranteed to have a steady supply of both oxygen and supplements and thus it cannot be more than a couple of hundred micrometers from

the closest vein (Lesart *et al.*, 2012). For a tumor to multiply the size, it must create satisfactory levels of pro-angiogenic development components to initiate neighborhood angiogenesis (Bergers *et al.*, 2003). It is this development which characterizes angiogenesis as one of the signs of malignancy (Carmeliet, 2003).

Malignant melanoma has been reported as an angiogenic tumor type, unmistakably showing new vessel arrangement as an essential advance in infection movement from atypical melanocytes, through outspread development to the forceful vertical development stage (Garcia-Barros *et al.*, 2003).

Vascular Endothelial Growth Factor (VEGF) exists in mammals as a few related groups of glycoproteins including VEGF-A, B, C, D, and E, which intervene through enactment of the class III tyrosine kinase receptors VEGFR-1, VEGFR-2, and VEGFR-3 (Hiratsuka *et al.*, 2002). VEGF-A, which is regularly alluded to as VEGF, is thought to be a standout amongst angiogenesis factors, and is overexpressed in all known cancerous tumors (Martin *et al.*, 2012). VEGF attaches to VEGFR-2 to initiate the angiogenic reaction, with actuation bringing about endothelial cell expansion and movement, alongside lumen development and expanded vessel dilatation and porousness (Lyden *et al.*, 2001).

There were great expectations in early research that angiogenic treatments would transform oncology practice, but in point of fact advances in the treatment of melanoma have been limited. Some few patients had no reaction, while others showed a slight response immediately follow treatment. There are many possible factors which may have influenced these results, and it is conceivable that every patient will respond in a unique way, requiring tailored interventions to bypass these complications (R. K. Jain *et al.*, 2009).

Dacarbazine is an alkylating agent affirmed by the United States Food and Drug Administration (FDA) for the treatment of melanoma. Dacarbazine is considered a standout amongst the best chemotherapy operators for metastatic melanoma (Pitts *et al.*, 2000).

Temozolomide is an alkylating operator identified with dacarbazine, which has superb oral bioavailability. It was recently approved through Phase III human trials (Herrlinger *et al.*, 2017).

IL-2 is a lymphokine that fortifies T-cell multiplication and capacity. It was affirmed by the FDA in 1998 for the treatment of metastatic melanoma. High measurement IL-2 appears to profit a few patients with metastatic melanoma, with finish and fractional reactions rates of 6% and 10%, individually (Chong, 2008).

Bevacizumab, Axitinib, Lenvatinib (E7080) and Etaracizumab (MEDI-522) are a portion of the medications in antiangiogenic treatment against melanoma. Nonetheless, more examinations are expected to assess the utilization of them in melanoma (Velho, 2012).

1.5 Potential Targets and Respective Anti-Angiogenic Agents

According to the mechanism of action, anti-angiogenic agents are classified into ″(1) endothelial growth factors inhibitors, (2) EC signal transduction inhibitors, (3) inhibitors of EC proliferation, (4) inhibitors of matrix MMPs, (5) inhibitors of EC survival, and (6) inhibitors of endothelial bone marrow precursor cells″ (Gasparini *et al.*, 2005). Depending on their targets, they are typically categorized as direct, indirect or mixed agents with anti-angiogenic effects. Further, the agents are defined as exclusive if they were deliberately designed to block angiogenesis as the sole mechanism of action; an example of such an agent is bevacizumab. Those that were

introduced as anticancer agents that were later found to target angiogenesis are known as inclusive agents (for example, bortezomib) (Chau *et al.*, 2008).

The NCI (National Cancer Institute) included a mechanistic classification of angiogenesis inhibitors (Efferth, 2005). They are classified into (1) agents that directly inhibit endothelial cells (integrin antagonists are included) (2) or those capable of interfering with signaling cascades and finally (3) agents that inhibit the ability of endothelial cells to breakdown extra-cellular matrix (ECM) (M. K. Gupta *et al.*, 2003). Some of the inhibitors modify the classification to include a ″miscellaneous″ group and put integrin inhibitors in a different category (Al-Husein *et al.*, 2012).

1.6 Anti-Angiogenic Agents In Cancer Therapy

Three major classes of agents which target VEGF have been developed: monoclonal antibodies, VEGF decoy receptor, and small molecule tyrosine kinase inhibitors (TKIs) (Ferrara *et al.*, 2003). These agents are currently in clinical practice or investigation as a monotherapy or in combination with cytotoxic chemotherapy or radiation (R. K. Jain *et al.*, 2006).

1.7 Mechanism of Anti-Angiogenic Therapy

Anti-angiogenic therapy can lead to reduced vessel permeability and blood perfusion, and vascular shrinkage which decreases the possibility of a tumor receiving oxygen and nutrients (Wedam *et al.*, 2006). Theoretically, anti-angiogenic therapy may return tumor blood vessels to their normal state, and improve the quality and delivery of cytotoxic therapeutic agents (R. K. Jain *et al.*, 2007). Hence, anti-angiogenic agents function by reducing the permeability of blood vessels in tumors,

and the dispersion of interstitial fluids. This leads to a decline in interstitial pressure, and, eventually, reduces tumor hypoxia (Maeda *et al.*, 2009).

If anti-angiogenic therapy is used along with cytotoxic agents, the effect of these agents increases, while tumor vessels are simultaneously normalized (Damge *et al.*, 1990).

A number of anti-angiogenic therapeutic agents are undergoing clinical trials. These agents can be classified into three categories: The first comprises the endothelial cell growth inhibitors, the preeminent of which is endostatin. This class of angiogenesis inhibitors induces apoptosis and inhibits endothelial cell growth (Decker, 1998). The second category includes drugs, which act as angiogenesis-signaling blockers, an example of which is Avastin®. They are inhibitors of the basic fibroblast growth factor (bFGF) and VEGF (Folkman, 2002). The third category is composed of the receptor blockers which inhibit ECM destruction, like the inhibitors of MMPs, which act by inhibiting the receptor activities of multiple growth factors (Guba *et al.*, 2002).

1.8 Traditional Therapeutic Plants for the Treatment of Cancer

Some kinds of plants obtain special effects in cancer treatment and control since long time ago. Previous reports of studying anticancer effect in such plants expressed numerous natural constituents that reveal anticancer effects. The significant toxic side effects of chemotherapeutic medicines frequently causes intolerable difficulties to patients that prevents them to continue treatment. Numerous therapeutic methods come up with cancer treatment using herbal products. Currently, there are four categories of anticancer products in market, which derived from herbal resources. These therapeutic constituents include vinca alkaloids, taxanes, epipodophyllotoxins as well as

camptothecin (Pan *et al.*, 2010). However, so many plants have potential to be used as anticancer or chemo-protective product because of containing specific active ingredients in different percentages. Several researches have concentrated on particular characteristics of herbs that can protect the patients from chemotherapy side effects. *Abrus precatorius* is one of the plants that its chemo-protective effects was studied in Yoshida sarcoma model of rats (M. Bhatia *et al.*, 2013; Gul *et al.*, 2013). Furthermore, in other study, the anti- sarcoma effects of *Albizzia lebbeck* in mice model was investigated (R. Jain *et al.*, 2010).

Anticancer plants contain numerous compounds with antioxidants, antiangiogenic and anti-inflammation effects. These therapeutic properties causes their cancer suppression effects. Transdermal ingenol mebutate, derived from *Euphorbia*, was approved in 2012 by the U.S. Food and Drug Administration (FDA) for treatment of pre-cancer lesions (Francis *et al.*, 2013). Even though, the possible toxicity effects and safety of most of these herbal products have not been evaluated in long term clinical trial studies, they are available without any proper regulation in some countries which needs to be controlled (Ekor, 2014).

Paclitaxel is an alkaloid extracted from *Taxus brevifolia* that has shown anticancer effect. It was investigated that paclitaxel blocks proliferation and suppress tumor growth. However, in further studies its toxic effects evaluated to be significant. In order to avoid its side effects another group designed and evaluated dermal paclitaxel-ethosomal formulation to treat skin cancer (Paolino *et al.*, 2012).

One of the active compounds in *Silybum marianum* is silymarin. Some of the pharmacological effect of this compound is anti-oxidant as well as anti-inflimmatory effects. Silymarin has reported as anticancer compound specifically in breast and

prostate cancer. In other studies dermal formulation of silymarin demonstrated anticancer results in different stages of skin cancer (F'guyer *et al.*, 2003).

Orthosiphon stamineus is one of therapeutic herbs that has demonstrated promising anticancer and antiangiogenic impacts in different angiogenic-dependent tumors like colon and breast cancer (Dolečková *et al.*, 2012; Sahib *et al.*, 2009).

1.8.1 *Orthosiphon stamineus*

O. stamineus or Misai Kucing (the local name for O.S) is a Malaysian therapeutic plant which grows well in numerous nations especially in Southeast Asia (L. Price *et al.*, 2008). Several studies have demonstrated the pharmacological possibilities of this plant, since *O. stamineus* is used traditionally for treating maladies including urinary, cardiovascular, and liver and digestive problems.

1.8.1(a) Traditional Uses of *O. stamineus*

Orthosiphon stamineus Benth. (Lambiaceae) is an imperative restorative herb. Different *in vitro* and *in vivo* models have been used to utilize the bioactive phytochemicals, including the flavonoids, terpenoids and basic oils, in this herb (Ramesh *et al.*, 2014). Early traditional practitioners utilized the leaves of *O. stamineus* (O.S) as a diuretic, and fused them into arrangements intended to assist the evacuation of kidney stones (Y.-S. Zhong *et al.*, 2012). They were also used for a remarkable range of other ailments, ranging from diabetes mellitus to tonsillitis, and even such things as epilepsy, syphilis, gout and psoriasis (Akowuah *et al.*, 2005). *O. stamineus* has pharmacological properties which can be helpful in various pathophysiological conditions. Many studies on the phytochemical and pharmacological effects of *O.stamineus* have been conducted (Abdullah *et al.*, 2009).

1.8.1(b) Pharmacological Properties of *O. Stamineus*

The pharmacological impacts of O.S are due to the presence of polyphenols, glycosides, lipophilic flavones, triterpenes, and diterpenes in its concentrates, showing various bioactive compounds, for example, rosmarinic corrosive (RA), sinensetin (SIN), eupatorin (EUP), betulinic acid, olenolic acid, 3′-hydroxy-5, 6, 7, 4′-tetramethoxyflavone (TMF) and a few caffeic acid subordinates (Ho *et al.*, 2010) .

A phytochemical analysis of O.S has shown that it includes monoterpenes, diterpenes, triterpenes, saponins, flavonoids, basic oils and a few long-chain natural acids, which still should be investigated and recognized (Sosa *et al.*, 2005). Pharmacological analysis of various O.S extracts show that the plant has a range of useful effects. These include cell reinforcement, antimicrobial and antitumor properties, nephroprotective benefits, and as an inhibitor of angiogenesis (Akowuah *et al.*, 2005).

Eupatorin displayed cell development restraint and apoptosis enlistment especially in tumor cells in spite of being a nonspecific inhibitor of a few protein kinases (A. Gupta *et al.*, 2014). It has been demonstrated that eutaporin is cytotoxic, and can inhibit the process of angiogenesis, possibly through restraint of VEGFRs (Dolečková *et al.*, 2012).

Certain concentrations of O.S suppressed the key angiogenic factor of VEGF both *in vitro* and *in vivo* (Foaud Saleih R Al-Suede *et al.*, 2014). This could be attributed chiefly to the polyphenolic substances, which are rich in cancer-prevention properties. These agents include: eutaporin, caffeic acid subsidiaries, sinensetin, 3'-hydroxy-5,6,7,4'- tetramethoxyflavone, polymethoxylated flavonoids and terpenes (Pang *et al.*, 2014). Flavonoids and phenolics have extremely encouraging

antiangiogenic properties (Lai *et al.*, 2004). Other non-flavonoid polyphenols, for example, rosmarinic acid and betulinic acid also add to the anti-angiogenic impact of polyphenol-rich plants (Ahamed *et al.*, 2012). In extensive preclinical models, the angiogenic procedure appears to have induced a significant delay in the development of tumors through the hindrance of VEGF; there are also clinical advantages to this approach (H. X. Chen *et al.*, 2009). Diterpenes confined from *O. stamineus* appear to exhibit antiproliferative effects against liver metastatic murine colon 26-L5 carcinoma cells (Awale *et al.*, 2001). Additionally diterpenes reduced the tumor-promoter-incited irritation of 12-O-tetradecanoylphorbol-13-acetic acid derivation in mice (Schmidt *et al.*, 1989). Moreover, it fundamentally repressed key steps of angiogenesis in endothelial cells, for example, movement and tube arrangement (Ullah *et al.*, 2008).

1.8.1(C) *Orthosiphon Stamineus* Is Rich in Anticancer Phytoconstituents

Rosmarinic acid (RA) is a dynamic caffeoyl ester found in *O. stamineus*. It inhibits several critical steps of angiogenesis including proliferation, migration and tube arrangement of endothelial cells. It also reduced retinal neovascularization in a mouse model of retinopathy (Y. Sharma *et al.*, 2017). RA restrained neutrophil elastase discharge and reduced thrombin movement (Porath, 2005). RA is considered a solid cancer prevention agent, mitigating antimicrobial effects, and the cell reinforcement action of rosmarinic acid is more grounded than that of vitamin E (Alayoubi *et al.*, 2015; K. S. Rao *et al.*, 1968). RA is the dynamic standard of numerous compounds in *Lamiaceae* family, with antioxidant properties (Gülçin *et al.*, 2007). Rosmarinic acid has shown a powerful anticancer, lipid peroxidative and apoptotic impact in DMBA-incited skin carcinogenesis (Hur *et al.*, 2004; Sharmila *et al.*, 2012). Comparative tests demonstrated the anticancer effect of *Perilla frutescens*, a plant in which RA is an

active ingredient, by means of two free systems: hindering the tumor's reaction to the drug, and reducing the activity of oxygen radicals (Banno *et al.,* 2004). Another investigation on human tumor cell line demonstrated that RA might have an effect against COX-2 initiation by AP-1-inciting operators in both disease and nonmalignant mammary epithelial cells (Scheckel *et al.,* 2008).

Methoxylated flavonoids (eupatorin and sinensetin) are in a critical class of bioactive compounds discovered richly in *O. stamineus* (Akowuah *et al.*, 2004). Eupatorin inhibits *in vitro* proliferaton of cancer cells (Androutsopoulos *et al.*, 2008). Research has demonstrated the capacity of eupatorin to nonspecifically repress numerous protein kinases (Sak *et al.,* 2015), which makes it a promising operator in anticancer research (Androutsopoulos *et al.,* 2008). Another comparable investigation announced that eupatorin strongly initiates apoptosis in different malignancy cell lines and smothers disease cell multiplication in organotypic 3D cell culture (Androutsopoulos *et al.,* 2008).

Tetramethoxyflavone has been shown to cause the activation of protein PKCε. Down-regulation of the instigated PKCε level by zapotin (a flavonoid) is associated with enhanced apoptosis (Toton *et al.*, 2012). Another study of tumor cell lines demonstrated that tetramethoxyflavone showed the cytotoxic property by enhancing the mitochondrial membrane potential in K562 and K562/adr in human blood growth cells (Khajapeer *et al.*, 2016). Comparable studies exhibited that tetramethoxyflavone has a high inhibitory impact on the multiplication of human nasopharygeal carcinoma (CNE) cells and initiates the apoptosis of CNE cells by decreasing the mitochondrial membrane potential and enhancing the outflow of Caspase3 and Caspase9 (Cao *et al.,* 2014). Another comparable study discovered that tetramethoxyflavone causes cell demise through an apoptotic pathway (Sergeev *et al.*, 2006).

Sinensetin prompts apoptosis and CYP1-interceded antiproliferation in human tumors cells (Ahamed *et al.*, 2012). An investigation revealed that sinensetin is expected as a novel and powerful second-age flavonoid chemo-sensitizer, since sinensetin has high therapeutic properties of being a non-transportable tumor inhibitor (Choi *et al.*, 2004).

1.8.2 Challenges in Topical Drug Delivery Systems

Skin is an organ consists of multiple layers with three different histological tissues. Epidermis is the outer layer of skin, which makes the skin tone and acts like a waterproof layer (Chisholm *et al.*, 2012). The second layer is the dermis, which consists of dense connective cells, follicles of hair, and sweat glands. Further subcutaneous tissue mass is called the hypodermis, consisting of fat and connective tissues (Khavkin *et al.*, 2011). Human skin is an effective, selective barrier to chemical permeation, although the skin as a route for delivery can offer many advantages, including avoidance of first-pass metabolism, lower fluctuations in plasma drug levels, targeting of the active ingredient for a local effect, and good patient compliance (Amnuaikit *et al.*, 2005).

Generally, the epidermis (especially the stratum corneum) controls most small, hydrophilic, and non-electrolytic diffusion of drugs through systemic blood circulation. So, to enhance the flux of the drug, the barrier interference is reduced. Several techniques have been utilized recently to conquer skin barrier characteristics (Alexander *et al.*, 2012).

A large portion of the bioactive constituents of phytomedicines are flavonoids (e.g., anthocyanidins from bilberry, catechins from green tea, silymarin). Numerous flavonoids are ineffectively ingested; the poor assimilation of flavonoid supplements

is likely because of two components. First is having various ring particles that make them too substantial to be in any way consumed by straightforward dispersion. Furthermore, flavonoid atoms commonly have poor miscibility with oils and different lipids, which restricted their capacity to pass through the skin (Montenegro *et al.*, 2007).

The main issue that needs to be considered in dermal and transdermal delivery of herbal products is their solubility whether in water or lipid. The phytoconstituents which are water soluble have less chance to permeate to stratum corneum (SC) becasuse it is composed of insoluble bundled keratins surrounded by a cell envelope, stabilized by cross-linked proteins and covalently bound lipids (Satyam *et al.*, 2015). For example polyphenol compounds due to their hydrophilicity are not thoroughly able to penetrate to SC, so it shows weaker efficacy compared to its oral administration (Saraf, 2010). Several approaches have been developed to weaken this skin barrier (Rothbard *et al.*, 2000), allowing for increasing the bioavailability of topical drugs and many cosmetic chemicals is the use of vesicular systems, such as liposomes (Elsayed *et al.*, 2006). Even though lipid soluble botanical active compounds can penetrate through SC but some particular enhancers ease this transfer by coating in some vesicles (Chanchal *et al.*, 2008).

Liposomal topical drugs even though improved the percentage of drug penetration through SC but this enhancement was not too much because morphological stability of such vesicles were rational(Liang *et al.*, 2015). In a comparison study between different types of topical vesicles results revealed that liposome can entrap less active compound which has potential to have leakage. It showed conventional liposomal vesicles can be considered as big size of vesicle that can consequently cause the lower bioavailability (Bragagni *et al.*, 2012).

Some research groups worked on designing similar vesicles to conventional liposomes but with elastic morphology properties with high penetration rate and no leakage to enhance the bioavailability of topical botanical drugs as much as possible (Ascenso *et al.*, 2015). So ethosome as flexible vesicle was introduced which could increase the stability of most of drugs with different molecular weight for dermal and transdermal application purpose (Jaiswal *et al.*, 2016).

1.8.3 Definition of Ethosome Complex

Ethosome was created by Touitou et al. in 1997 as extra novel lipid vesicles made out of ethanol, phospholipids, and water (Elsayed *et al.*, 2006). This kind of vesicle has the potential to enhance the skin conveyance of different medications (Godin *et al.*, 2004). Ethanol is a proficient permeation enhancer that acts by influencing the intercellular part of the SC.

Ethosomal transporters are flexible vesicles that are composed of hydroalcoholic or hydro/glycolic phospholipids, ethanol (moderately high concentration), and water (Elsayed *et al.*, 2006). The high concentration of ethanol increases porousness of the SC to provide an entrance for the medication upon topical application. This ethosomal system may be used to deliver different types of drugs, such as acyclovir, salbutamol, Insulin, cyclosporine, fluconazole, minodixil, and more (Mahale, 2011). Methods for preparation of the vesicles can utilize cold, heat, or rehydration methods (Pratima *et al.*, 2012). The size of ethosomal vesicles can be controlled, to some extent, using sonication (Satyam *et al.*, 2015).

1.9 Ethosome Technology

The primary problem of topical medications is poor penetration of compounds into the human skin (Moser *et al.*, 2001). .Ethosomes are phospholipid-based versatile