

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

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EXTRACT FOR MELANOMA**

by

MANSOUREH NAZARI VISHKAEI

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This thesis is dedicated to

My wonderful angel, my mother

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TABLE OF CONTENTS

ACKNOWLEDGEMENT	ii
TABLE OF CONTENTS	iii
LIST OF TABLES	ix
LIST OF FIGURES	xiii
LIST OF ABBREVIATIONS	xx
ABSTRAK.....	xxiv
ABSTRACT.....	xxvi

CHAPTER 1 - INTRODUCTION

1.1 Cancer.....	1
1.2 Skin Cancer	1
1.2.1 Basal Cell Carcinoma	2
1.2.2 Squamous Cell Carcinoma.....	2
1.2.3 Melanoma	2
1.3 Angiogenesis.....	4
1.3.1 Mechanism of Physiological Angiogenesis	5
1.3.2 Tumor Angiogenesis.....	5
1.3.3 Regulation of Tumor-Angiogenesis.....	6
1.3.4 VEGFs and VEGF Receptors	7
1.4 Treatment of Melanoma.....	8
1.4.1 Chemotherapy	11
1.4.2 Biochemotherapy	11
1.4.3 Medicines Discovered To Treat Melanoma.....	12
1.4.4 Anti-angiogenesis Therapy	12
1.5 Potential Targets and Respective Anti-Angiogenic Agents.....	14

1.6	Anti-Angiogenic Agents In Cancer Therapy	15
1.7	Mechanism of Anti-Angiogenic Therapy	15
1.8	Traditional Therapeutic Plants for the Treatment of Cancer	16
1.8.1	<i>Orthosiphon stamineus</i>	18
1.8.2	Challenges in Topical Drug Delivery Systems	22
1.8.3	Definition of Ethosome Complex	24
1.9	Ethosome Technology	24
1.9.1	Drug Absorption in Ethosome Technology	25
1.9.2	Different Categories of Ethosome	26
1.9.3	Therapeutic Potential of Ethosomes	27
1.9.4	Mechanism of Action of the Ethosomal Drug Delivery System	31
1.9.5	Bioavailability Enhancement in Ethosome	32
1.9.6	Stability of Ethosome	33
1.10	Hypothesis	35
1.11	Objectives of the Study	36

CHAPTER 2 - MATERIALS AND METHODS

2.1	Preparation of Ethosome Gel Formulation	41
2.2	Characterization	42
2.2.1	Entrapment Percentage	43
2.2.2	Particle Sizes and Zeta Potential	43
2.2.3	HPLC	43
2.2.4	Transmission Electron Microscopy (TEM)	44
2.2.5	Evaluation of Rheological Properties of Ethosome Gel	45
2.2.6	Fourier-Transform Infrared Spectrometry	46
2.2.7	Differential Scanning Calorimetry	46
2.2.8	Viscosity	46

2.2.9	<i>In Vitro</i> Skin Permeation Study	47
2.2.10	FT-IR Analysis of Mice Skin.....	47
2.2.11	Penetration Study	48
2.3	Stability Study.....	48
2.3.1	High Performance Liquid Chromatography	49
2.3.2	Analysis of Chemical Kinetic Parameters	49
2.4	<i>In Vitro</i> Anticancer Studies	51
2.4.1	Cell Lines and Media Cultures	51
2.4.2	Cell Culture and Daily Monitoring	51
2.4.3	Cell Viability Test: Using MTT to Analyze the Anticancer Potential of ET against That of EX in Skin Cancer and Normal Cell Lines	52
2.4.4	Colony formation assay in B16F10 cell line	54
2.4.5	Cell Migration Assay: B16F10 Cells Treated With ET and EX.....	55
2.4.6	Cell invasion assay of B16F10 cells	56
2.4.7	Hanging Drop Assay.....	57
2.5	<i>In Vitro</i> Antiangiogenic Properties of Endothelial Cells	57
2.5.1	Endothelial Cells Migration Assay	58
2.5.2	Endothelial cell tube formation assay	58
2.6	<i>Ex Vivo</i> Antiangiogenic Evaluation of ET and EX Using Rat Aortic Ring Assay.....	59
2.6.1	Experimental Animals	59
2.6.2	Rat Aortic Ring Assay Protocol.....	60
2.7	<i>In vivo</i> Pharmacokinetic Study	61
2.7.1	Plasma Sample Treatment	61
2.7.2	Development and Validation of HPLC Method	61
2.7.3	Calibration Curves (Linearity Ranges)	62
2.7.4	Recovery of Plasma Extraction.....	62
2.7.5	Limit of Detection (LOD) and Limit of Quantification (LOQ).....	63

2.7.6	Experimental Animals	63
2.7.7	Ethical Approval	63
2.7.8	Determination of Pharmacokinetic Parameters	64
2.7.9	<i>In vivo</i> Skin Deposition study	65
2.7.10	Statistical Analysis.....	65
2.8	<i>In Vivo</i> Toxicity Study	65
2.8.1	Acute Dermal Toxicity	65
2.8.2	Repeated Dose Dermal Toxicity.....	66
2.9	Anticancer Effect of Ethosomal Formulation of <i>O.Stamineus</i> Extract Compared to Crude Extract <i>in Vivo</i> Melanoma Tumor Animal Model	67
2.9.1	Animals.....	67
2.9.2	Development of Subcutaneous Melanoma Tumors in Albino Mice	67
2.9.3	Treatment and Tumor Size Measurement.....	68
2.9.4	Euthanasia and Tumor Collection.....	69
2.9.5	Biochemistry Indexes	69
2.10	Statistical Analysis	69

CHAPTER 3 - DEVELOPMENT AND CHARACTERIZATION AND ANTIANGIOGENIC AND ANTICANCER EFFECT OF ETHOSOMAL FORMULATION OF *O.STAMINEUS* EXTRACT AGAINST MELANOMA

3.1	Introduction.....	71
3.2	Materials and Methods	72
3.3	Results.....	73
3.3.1	Entrapment Analysis.....	73
3.3.2	Rheological study	74
3.3.3	Phytochemical study of ET compared to EX.....	80
3.3.4	Accelerated Stability Study of Ethosome gel formulation of O.S extract.....	89

3.3.5	<i>In Vitro</i> and <i>Ex Vivo</i> Anticancer and Antiangiogenic Studies.....	99
3.4	Discussion.....	118

CHAPTER 4 - PHARMACOKINETIC STUDY

4.1	Introduction.....	122
4.2	Materials and Methods.....	124
4.3	Results.....	124
4.3.1	Development and Validation of HPLC Method	124
4.3.2	Calibration Curves, Linearity Ranges.....	125
4.3.3	Plasma Recovery after Extraction.....	126
4.3.4	LODs and LOQs	127
4.3.5	Pharmacokinetic Study of ET and EX	127
4.3.6	<i>In Vivo</i> Skin Deposition Study.....	135
4.4	Discussion.....	138

CHAPTER 5 - TOXICOLOGY STUDIES

5.1	Introduction	141
5.2	Materials and Methods	142
5.3	Results.....	142
5.4	Discussion.....	148

CHAPTER 6 - *IN VIVO* ANTITUMOR STUDY OF ETHOSOMAL FORMULATION OF *O. STAMINEUS* EXTRACT COMPARED TO CRUDE EXTRACT IN MELANOMA CANCER MODEL 150

6.1	Introduction.....	150
6.2	Materials and Methods	151
6.3	Results.....	151
6.3.1	<i>In Vivo</i> Antitumor Model Study	151

6.3.2	Effect of Ethosomal Formulation of <i>O.Stamineus</i> Extract on Body Weight.....	157
6.3.3	Histopathological Examination.....	158
6.3.4	Biochemical Analysis of Treatment	160
6.4	Discussion.....	164

CHAPTER 7 - GENERAL DISCUSSION

7.1	<i>Orthosiphon Stamineus</i> Standardized Extract and Melanoma	167
7.2	Optimized Flexible O.S-Ethosomal Vesicle for Topical Delivery	170
7.3	Drug Permeation and Penetration Development of O.S-Ethosomal Formulation.....	173
7.4	Screening of Anticancer and Anti-Angiogenic Potentials of O.S Phytosome Formulation and Crude Extract	174
7.4.1	Effects on Cell Viability (MTT Assay)	174
7.4.2	Angiogenesis Assay: Rat Aortic Ring Assay	174
7.4.3	<i>In Vitro</i> Antitumorigenic Properties of O.S Ethosome in Melanoma Cells (B16F10).....	174
7.4.4	Enhanced Anti-Angiogenic Efficacy of O.S Ethosomal Formulation	176
7.5	Toxicity Profile of O.S-Ethosomal Formulation	179
7.6	<i>In Vitro</i> and <i>In Vivo</i> Anti Melanoma Studies	181
CHAPTER 8 GENERAL CONCLUSION		189

REFERENCES.....	191
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APPENDICES

LIST OF PUBLICATIONS

LIST OF TABLES

	Page
Table 2.1 List of chemicals.....	37
Table 2.2 List of equipment and apparatus.....	39
Table 2.3 Gradient elution program used in separation of <i>O. stamineus</i> marker compounds	44
Table 3.1 Entrapment percentages of different formulations of ethosome along with size and electrical charge of particles.	74
Table 3.2 Effect of acrypol concentration on yield stress obtained from flow curve test (n=3).....	76
Table 3.3 Entrapment percentage of active compounds in O.S ethosome.....	81
Table 3.4 Viscosity of O.S ethosome 1 and 2% gel in room temperature	82
Table 3.5 Permeated concentration of EX and ET gel after topical application in Franz diffusion cell using mice skin.	88
Table 3.6 Calibration equations of markers as reference for the evaluated HPLC method.....	91
Table 3.7 Remaining percentage data of marker compounds: rosmarinic acid (RA), 3'-hydroxy-5,6,7,4'-tetramethoxyflavone (TMF), sinensitin (SIN) and eupatorin (EUP) in ethosomal formulation of <i>Orthosiphon stamineus</i> extract (ET) stored for 6 months under different storage conditions	92
Table 3.8 Rate constant (K), activation energy (Ea) and pre-exponential factor (A) of the RA, TMF, SIN and EUP of ethosomal formulation of <i>Orthosiphon stamineus</i> ethanolic extract (ET) stored at different temperatures.....	96
Table 3.9 Shelf life (t ₉₀) of the markers in ethosomal formulation of <i>Orthosiphon stamineus</i> extract (ET) at different storage conditions	97
Table 3.10 Changes in size, potential, pH, viscosity, color and weight loss of ET gel in 6 month.	98

Table 3.11	Percentage of EA.hy926 and B16F10 cell lines viability after treatment with ET and EX refer to ethosomal formulation and crude extract of O.S, respectively with concentrations 50 and 100µg/ml. Statistical analysis was conducted by one-way ANOVA.	99
Table 3.12	Antiangiogenic effect of O.S ethosome in different concentrations on rat aortic ring assay following 5 days of treatment. Data shows percentage inhibition on the growth of new blood vessels.ET25, 50, 100, 150, 200 and 250 stands for ethosomal formulation of extract in concentrations 25 to 250 µg/ml respectively. EX25, 50, 100 and 200 stands for crude extract of O.S in concentrations 25 to 200 µg/ml.EE50and EE100 stands for empty ethosome in concentration 50 and 100 µg/ml respectively. Sur100 stands for suramin in concentration 100 µg/ml.	101
Table 3.13	Antiangiogenic effect of O.S ethosomal formulation in different concentrations on migration assay after 5 days treatment. Data shows percentage inhibition on the growth of new blood vessels.....	104
Table 3.14	Antiangiogenic effect of the samples on width and height of tubes: Analysis of images from the tube formation assay of EA.hy926 after 6 h of treatment with O.S ethosome and crude extract at the concentrations of 100 (ET100), and 50 (ET50); and 100 (EX100), and 50µg/mL (EX50), respectively. Suramin applied at a concentration of 100µg/mL (Sur100) served as the positive control; and DMSO served as the negative control. Tubule-like structures formed in the negative control; however, treatment with ET inhibited tubule formation.	106
Table 3.15	Antiangiogenic effect of the samples on area of tube like structures: Analysis of images from the tube formation assay of EA.hy926 after 6 h of treatment with O.S ethosome and crude extract at the concentrations of 100 (ET100), and 50 (ET50); and 100 (EX100), and 50 µg/mL (EX50), respectively. Suramin applied at a concentration of 100 µg/mL (Sur100) served as the positive control; and 0.1% DMSO was used as the negative control.	107
Table 3.16	Effect of treatments on colony formation of B16F10 cell line: Inhibition percentage of colony formation after treatment with O.S extract at 50 and 100 µg/mL (EX50 & EX100), ethosome at 50 and 100 µg/mL (ET50 & ET100), and rosmarinic acid at 100 µg/mL (RA100) is shown. Results are demonstrated as the means±SEM of three experiments, *** P<0.05.	109

Table 3.17	Percentage of inhibition of cell migration of B16F10 cell line: EX50 and EX100 represent the crude extract at the concentrations of 50 and 100 µg/mL, respectively. ET50 and ET100 and RA100 refer to the formulation at 50 and 100 µg/mL and rosmarinic acid 100 µg/mL, respectively. Results are demonstrated as the mean±SEM of three experiments, ****P<0.05.	112
Table 3.18	Percentage inhibition of the samples on the invasion of the B16F10 cell line was presented. Treatment was applied at 50 and 100 µg/mL for the crude extract (EX50 & EX100) and rosmarinic acid (RA100). O.S formulation was administered at the corresponding concentrations of 50 and 100µg/mL (ET50 and ET100). Results are expressed as the mean±SEM of three experiments; *P<0.05.....	114
Table 3.19	Anticancer percentage of samples in a hanging drop assay using B16F10 cell line: Inhibition percentages with different treatments were shown; ***P<0.05.	117
Table 4.1	Calibration data of the reported HPLC method.....	125
Table 4.2	Plasma extraction recovery of rosmarinic acid (RA), 3'-hydroxy-5, 6, 7, 4'-tetramethoxyflavone (TMF), sinensitin (SIN) and eupatorin (EUP).....	126
Table 4.3	LOD and LOQ of rosmarinic acid (RA), 3'-hydroxy-5, 6, 7, 4'-tetramethoxyflavone (TMF), sinensitin (SIN) and eupatorin (EUP).....	127
Table 4.4	plasma concentration vs. time profiles (mean± S.E.M, n=6) of active compounds; rosmarinic acid (RA), 3-hydroxy-5,6,7,4 tetramethoxyflavone (TMF), sinensitin (SIN) and eupatorin (EUP) after topical application of O.S extract at 500 mg/kg (EX) and topical administration of O.S-ethosomal formulation at 500 mg/kg (ET).	130
Table 4.5	Pharmacokinetic parameters of rosmarinic acid (RA), 3-hydroxy-5, 6, 7, 4 tetramethoxyflavone (TMF), sinensitin (SIN) and eupatorin (EUP) in mice plasma after topical administration of <i>O. stamineus</i> ethanolic standard extract (EX) and ethosome of <i>O. stamineus</i> ethanolic extract (ET) (n=6).	132
Table 4.6	Skin deposition of active compounds after topical application of ethosomal formulation (ET) and standardized extract (EX) of O.S, (n=6).....	136
Table 5.1	Acute dermal toxicity observations in Albino mice treated with topical ethosome gel formulation of O.S extract with 1000mg/kg.....	143

Table 5.2	Weight of organs after 30 days of topical administrations daily in repeated dose dermal toxicity study (gram).	145
Table 6.1	Antitumor percentage of ethosomal formulation in different treated and untreated animal groups.....	152
Table 6.2	Biochemistry analysis of blood evaluated in different groups. Negative: negative group treated with empty gel formulation during the test as control, EX1: group treated with 10% of not-formulated gel of O.S extract, EX2: group treated with 20% of not-formulated gel of O.S extract, EX3: group treated with 30% of not-formulated gel of O.S extract, ET1: group treated with ethosome gel formulation equivalent to 10% of O.S extract, ET2: group treated with ethosome gel formulation equivalent to 20% of O.S extract, ET3: group treated with ethosome gel formulation equivalent to 30% of O.S extract.....	162

LIST OF FIGURES

	Page
Figure 1.1 Process of angiogenesis multistep (Bryan <i>et al.</i> , 2007).....	4
Figure1.2 Tumor-neovascularization process (Conti <i>et al.</i> , 2013).....	6
Figure1.3 Schematic representation of ethosomal vesicle (Abdulbaqi <i>et al.</i> , 2016).....	26
Figure 2.1 Schematic diagram of thin film rehydration method	42
Figure 3.1 Entrapment percentage of O.S ethosome: E represents O.S crude extract in water. Ethosomes were formulated by mixing O.S ethanol-water extract with phosphatidylcholine from frozen eggs with different ratios (1-6) and from soybeans lecithin; (7).	74
Figure 3.2 Effect of acrypol concentration on yield stress obtained from flow curve test (n=3).....	76
Figure 3.3 Effect of acrypol concentration and temperature on viscosity obtained from flow curve test (n=3).	77
Figure 3.4 Effect of acrypol concentration on critical strain obtained from amplitude sweep test at 25°C (n=3).	78
Figure 3.5 Effect of acrypol concentration on elastic (G') and viscous (G'') modulus obtained in frequency sweep test at 4, 25 and 32°C (n=3).....	78
Figure 3.6 Effect of Temperature on the elastic and viscous modulus of 1 and 2% ethosomal formulations obtained from ramp test (n=3).	79
Figure 3.7 HPLC chromatograms of crude extract of O.S in formulated (ET) and non-formulated (EX) form. The chromatograms show presence of RA, TMF, EUP and SIN. A refers to <i>O.stamineus</i> extract, and B refers to <i>O.stamineus</i> ethosome. RA, TMF, SIN and EUP refer to rosmarinic acid, 3'-hydroxy-5, 6, 7, 4'-tetramethoxyflavone, sinensetin and eupatorin, respectively.	81
Figure 3.8 DSC thermogram of O.S ethosomal formulation: 1: crude extract of O.S; 2: Acrypol; 3: Phospholipid; 4: cholesterol and 5: final gel formulation.	83
Figure 3.9 FTIR spectra of O.S Ethosome: 5: Gel formulation; 4: crude extract; 3: phosphatidylcholine; 2: Acrypol; and 1: Cholesterol. Highlighted areas represent changed peaks after interaction between crude extract (4), Cholesterol (1) and Phospholipid (2).	84

Figure 3.10	Transmission electron microscopy of O.S ethosome represents round shape particles of ethosome after dissolving in water.	84
Figure 3.11	A) Penetration distribution of O.S extract in gel B) Penetration distribution of ethosome gel formulation of O.S form applied in mice for 6 hours.	85
Figure 3.12	Penetration of hydroethanolic gel of extract and ethosomal formulation after 1hour and 6 hours of topical application in Balb C albino mice.	86
Figure 3.13	Intensity of EX-gel after 1hour topical application (A) and EX-gel after 6hour topical application (B) Intensity of ET-gel after 1hour topical application (C), Intensity of ET-gel after 6hour topical application (D) in different depths of mice skin.	87
Figure 3.14	Permeation of EX and ET gel in 24 hours of topical application mice skin.	88
Figure 3.15	FT-IR spectrum of	89
Figure 3.16	(A) HPLC chromatograms of marker compounds; rosmarinic acid (1), 3'-hydroxy-5,6,7,4'-tetramethoxyflavone (2), sinensitin (3) and eupatorin (4).	90
Figure 3.17	(A) HPLC chromatograms of marker compounds rosmarinic acid (1), 3'-hydroxy-5,6,7,4'-tetramethoxyflavone (2), sinensitin (3) and eupatorin (4); Ethosomal formulation of <i>Orthosiphon stamineus</i> (ET) stored at 4°C/75% RH at month zero (B) Ethosomal formulation of <i>Orthosiphon stamineus</i> (ET) stored at 4°C/75% RH at month one; (C) Ethosomal formulation of <i>Orthosiphon stamineus</i> (ET) stored at 4°C/75% RH at month three and (D) Ethosomal formulation of <i>Orthosiphon stamineus</i> (ET) stored at 4°C/75% RH at month six	91
Figure 3.18	percentage remaining concentration of the markers in ethosomal formulation of <i>Orthosiphon stamineus</i> extract (ET) versus time in plot for first order reaction.....	94
Figure 3.19	Log of rate constant diagram versus inverse of temperature (Kelvin-1) of RA, TMF, SIN and EUP in ethosomal formulation of <i>Orthosiphon stamineus</i> extract (ET) at various temperatures, ln K (natural log of rate constant); 1/T (inverse of temperature).....	96
Figure 3.20	Percentage of EA.hy926 cell line viability after treatment. ET and EX refer to ethosomal formulation and crude extract of O.S, respectively. Statistical analysis was conducted by one-way ANOVA.	100

Figure 3.21	Percentage of B16F10 cell line viability after treatment. ET and EX refer to ethosomal formulation and crude extract of O.S, respectively. Statistical analysis was conducted by one-way ANOVA.	100
Figure 3.22	Microvessel growth in rat aortic rings treated with 0.1% DMSO (negative control); 100 µg/mL of suramin (Sur100), which was used as the positive control; 25, 50, 100, 150, 200 and 250 µg/mL of O.S ethosome-formulation (ET-25 to 250); 50 and 100 and 200 µg/mL of the crude extract (EX100 & EX200); and 50 and 100 µg/mL of empty ethosome (EE50 & EE100) significantly shows more inhibition of new vessel growth in formulation compared to crude extract.	102
Figure 3.23	Inhibition percentage of angiogenesis by the samples in different concentrations on day 5. ET, EX, EE and SUR refer to O.S ethosomal formulation, crude extract, empty ethosome and suramin, respectively. Statistical analysis was conducted by one-way ANOVA; error bars represent standard deviation; *P<0.05.	103
Figure 3.24	Anticancer effect of ET on the migration of EA.hy926 cell line demonstrates decreased area of migrated cells after treatment with ET compared to EX. The marked area of EA.hy926 cells treated with 50 and 100 µg/ml of ET and EX.; and rosmarinic acid in concentration 100µg/ml at 0 and 12h after treatment. The negative control was media. Analysis was performed using magnification 4X.	104
Figure 3.25	Inhibition percentage of EA.hy926 cell migration: ET50, ET100, EX50, EX100 and RA100 stand for ethosomal formulation of O.S in concentration 50 and 100 µg/ml, O.S crude extract with concentration 50 and 100 µg/ml and rosmarinic acid with concentration 100µg/ml, respectively. Results are revealed as mean ±SEM of three experiments, **P<0.05. The inhibition of EA.hy926 cells migration by ET compared to that treated with EX.	105
Figure 3.26	Antiangiogenic effect of the samples on tube formation assay: Images from the tube formation assay of EA.hy926 after 6 h of treatment with O.S phytosome and crude extract at the concentrations of 00 (ET100), and 50 (ET50); and 100 (EX100), and 50 µg/mL (EX50), respectively. Suramin was used at a concentration of 100 µg/mL (Sur100) served as the positive control; and 0.1% DMSO served as the negative control. Typical tubule-like structures formed in the negative control; however, treatment with ET inhibited tubule formation. Other samples of other concentrations showed incomplete networks. The positive control completely abolished the tubule structures, as shown under a 4x magnification power.	106

- Figure 3.27 Inhibition percentage of the samples in tube formation assay: tube formation assay analysis of EA.hy926 after 6 h of treatment with O.S ethosome and crude extract at the concentrations of 100 (ET100), and 50 (ET50); and 100 (EX100), and 50 $\mu\text{g/mL}$ (EX50), respectively. Suramin applied at a concentration of 100 $\mu\text{g/mL}$ (SUR100) served as the positive control; and 0.1% DMSO was used as the negative control.107
- Figure 3.28 The effect of each sample on width and length in tube formation assay: The analysis of length and width of EA.hy926 tubes were done after 6 h of treatment with O.S ethosome and crude extract at the concentrations of 100 (ET100), and 50 (ET50); and 100 (EX100), and 50 $\mu\text{g/mL}$ (EX50), respectively. Suramin applied at a concentration of 100 $\mu\text{g/mL}$ (Sur100) served as the positive control; and 0.1% DMSO was used as the negative control.....108
- Figure 3.29 Effect of samples on colony formation of B16F10 cell line: Inhibition percentage of colony formation after treatment with O.S extract at 50 and 100 $\mu\text{g/mL}$ (EX50 & EX100), ethosome at 50 and 100 $\mu\text{g/mL}$ (ET50 & ET100), and rosmarinic acid at 100 $\mu\text{g/mL}$ (RA100) is shown. Results are demonstrated as the mean \pm SEM of three experiments, *** $P < 0.05$110
- Figure 3.30 Anticancer effect of the treatments on colony formation: Survival of B16F10 cell line colonies after treatment with ET (100 and 50 $\mu\text{g/mL}$), 0.1% DMSO (negative control), rosmarinic acid (positive control) at 100 $\mu\text{g/mL}$, and O.S crude extract (EX) with equivalent concentrations to those in the formulation (50 and 100 $\mu\text{g/mL}$). Figure shows decreased survival rate of tumor cells after treatment with formulation compare with crude extract.110
- Figure 3.31 Anticancer effect of O.S ethosome on the migration of B16F10 cell line reveals decreased distance of migrated cells after treatment with formulation compared to crude extract: Wounds (marked area) of B16F10 cells were treated with 50 (ET50) and 100 $\mu\text{g/mL}$ (ET100) of the formulation; 50 (EX50) and 100 $\mu\text{g/mL}$ (EX100) of the crude extract; and rosmarinic acid at the concentration of 100 $\mu\text{g/mL}$ (RA100). Observations were made at 0 and 8 h post-treatment. The magnification power was set at 4X.....112
- Figure 3.32 Percentage of inhibition of cell migration of B16F10 cell line: EX50 and EX100 represent the crude extract at the concentrations of 50 and 100 $\mu\text{g/mL}$, respectively. ET50 and ET100 refer to the formulation at 50 and 100 $\mu\text{g/mL}$, respectively. RA100 stands for rosmarinic acid at 100 $\mu\text{g/mL}$. Results are demonstrated as the mean \pm SEM of three experiments, **** $P < 0.05$113

Figure 3.33	Effects of the samples on the invasion of the B16F10 cell line were presented as percentages of invasion inhibition. Treatment was applied at 50 and 100 µg/mL for the crude extract (EX50 & EX100) and rosmarinic acid (RA100). O.S formulation was administered at the corresponding concentrations of 50 and 100 µg/mL (ET50 and ET100). Results are expressed as the mean±SEM of three experiments; *P<0.05.....	114
Figure 3.34	Antitumor effects of the samples on the invasion of B16F10 cell line: The distribution of live cells 24 h after treatment with ET50 and ET100 (50 and 100µg/mL of O.S formulation); DMSO (untreated cells); and EX50 and EX100 (the crude extract applied at 50 and 100µg/mL) was visualized under 4x magnification power.	115
Figure 3.35	Anticancer effects of samples in a hanging drop assay using B16F10 cell line: Inhibition percentages with different treatments were shown; ***P<0.05.....	116
Figure 3.36	<i>In vitro</i> effects of O.S ethosomal formulation vs. crude extract on B16F10 tumor cells in hanging drop assay: DMSO: Negative control group (untreated cells); ET-100: ethosome (100µg/mL); ET-50: ethosome (50µg/mL); EX50: O.S crude extract at 50µg/mL, and EX100: O.S crude extract at 100µg/mL; RA100: Rosmarinic acid applied at 100µg/mL, respectively.....	117
Figure 4.1	Chromatograms of blank mice plasma (A), and mice plasma spiked with 10 µg/mL of rosmarinic acid (1), and 3'-hydroxy-5,6,7,4'-tetramethoxyflavone (2), sinensitin (3) and eupatorin (4) (B)	125
Figure 4.2	Chromatograms of rosmarinic acid (1), and 3'-hydroxy-5,6,7,4'-tetramethoxyflavone (2), sinensitin (3) and eupatorin (4) in rat plasma at 4 hour after topical administration of 500 mg/kg ethosomal formulation of <i>O. stamineus</i> ethanolic extract (A); mice plasma at 4 hours after topical administration of 500 mg/kg of <i>O. stamineus</i> ethanolic extract (B)	128
Figure 4.3	Mean plasma concentration vs. time profiles (mean± S.E.M, n=6) of active compounds; rosmarinic acid (RA), 3-hydroxy-5,6,7,4 tetramethoxyflavone (TMF), sinensitin (SIN) and eupatorin (EUP) after topical application of O.S extract at 500 mg/kg (A) and topical administration of O.S-ethosomal formulation at 500 mg/kg (B).	129

Figure 4.4	Skin deposition profiles versus times of RA, TMF, SIN, and EUP after topical administration of ethosomal formulation (ET) of O.S extract (ET) (n=6).....	136
Figure 4.5	Skin deposition profiles versus times of RA, TMF, SIN, and EUP after topical administration of not-formulated O.S extract (EX) (n=6).....	137
Figure 5.1	Body weight changes in repeated dose dermal toxicity in different days. The animals in group neg were female and male mice kept untreated as control. The animals in group F1m were male mice treated with 1000 mg/kg of ET. The animals in group F2m were male mice treated with 2000 mg/kg of ET. The animals in group F3m were male mice treated with 3000 mg/kg of ET. The animals in group F1f were female mice treated with 1000 mg/kg of ET. The animals in group F2f were female mice treated with 2000 mg/kg of ET. The animals in group F3f were female mice treated with 3000 mg/kg of ET.	144
Figure 5.2	Weight of organs after repeated dose dermal toxicity study.	145
Figure 5.3	Biochemistry analysis of group T3 (treated with 3000mg/kg body weight) and group negative (untreated as control) in repeated dose dermal toxicity.	146
Figure 5.4	Histology of organs dissected from mice after treatment with high concentration of ET compare with untreated animals. ET3- Kidney, Liver, Lung, Testis, Heart, Spleen and Adrenal stands for these organs from animals treated with ET at concentration 3000 mg/kg body weight of animals compared to Neg- Kidney, Liver, Lung, Testis, Heart, Spleen and Adrenal which stand for dissected organs from untreated animals after 30 days.	147
Figure 6.1	Shows the effect of ET in 3 dosage forms on tumor volumes compared to EX in equivalent concentrations for 21 days. ET1, ET2 and ET3 are ethosomal formulation at 10, 20 and 30 % gels.	153
Figure 6.2	Shows the tumor volume of animals after treatment with ET in 3 dosage forms on tumor volume compared to EX in 21 days. ET1, ET2 and ET3 are ethosomal formulation at 10, 20 and 30% of the gels. One way ANOVA test was used to evaluate mean of melanoma tumor size in different treated groups compare to negative untreated group. Values are demonstrated as mean± SEM, ** P<0.05, n=5.	153
Figure 6.3	Shows the antitumor percentage of ET in 3 dosage forms on tumor volume compared to EX in 21 days treatment in animals. ET1, ET2 and ET3 are ethosomal formulation at 10, 20 and 30% of animals.....	154

Figure 6.4	Day six was start of the treatment.....	154
Figure 6.5	Subcutaneous melanoma tumor in albino mice. (NEG) animals treated with empty ethosome, owing the largest size of tumor, EX1: animals after topical treatment with crude extract gel at 10%, EX2: animals after topical treatment with crude extract gel at 20%, EX3: animals after topical treatment with crude extract gel at 30%, ET1: animals after topical treatment with ethosome gel at 10%, ET2: animals after topical treatment with ethosome gel at 20%, ET3: animals after topical treatment with ethosome gel at 30%. Tumor dimensions were analyzed every three days and values are demonstrated as mean melanoma tumor size(mm ³) ±SEM.	155
Figure 6.6	Melanoma tumors harvested from albino mice. Dose dependent reduction in melanoma tumor size was observed in ET and EX treated groups in three dosage forms. EX1: tumors after topical treatment with crude extract gel at 10%, EX2: tumors after topical treatment with crude extract gel at 20%, EX3: tumors after topical treatment with crude extract gel at concentration 30% ,ET1: tumors after topical treatment with ethosome gel at 10%, ET2: tumors after topical treatment with ethosome gel at 20%, ET3: tumors after topical treatment with ethosome gel at 30%, NEG: untreated animals.	156
Figure 6.7	Average body weight of animals in different groups treated with ethosomal formulation and non-formulated of O.S extract via topical administration compare to negative control are presented as mean± SEM	157
Figure 6.8	Cross sections of melanoma tumor tissues stained with hematoxylin/eosin. The tumor cross sections were evaluated for signs of necrosis. (Neg): The viable melanoma cells and lower extent of necrosis compared to all treated groups, blood vessel and melanin distribution in negative group. (EX1) the tumor treated with 10% (EX2) 20% and (EX3) 30%not formulated gel compared to ethosomal formulation at equivalent concentrations (ET1)10%, (ET2) 20% and (ET3) 30%. V stands for live tumor cells, N sands for necrosis, BV stands for blood vessels and M stands for melanin distribution. All the images have been taken at 20x magnification.	159
Figure 6.9	Lung metastasis image in negative control group. The lung cross sections which were stained with hematoxylin/eosin, (MT) stands for metastasized cells, (BV) stands for blood vessels, (M) stands for melanin distribution excreted from melanocytes and (N) stands for normal lung cell line.	160

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
E _a	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 pemerangkapan sebanyak 80% untuk asid rosmarinic (RA) dalam vesikel etasom. 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 4°C. 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 phytochemicals 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 elastoplastic 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 4°C. 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 nutriment. 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 1mm³ 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-angiogenic 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.

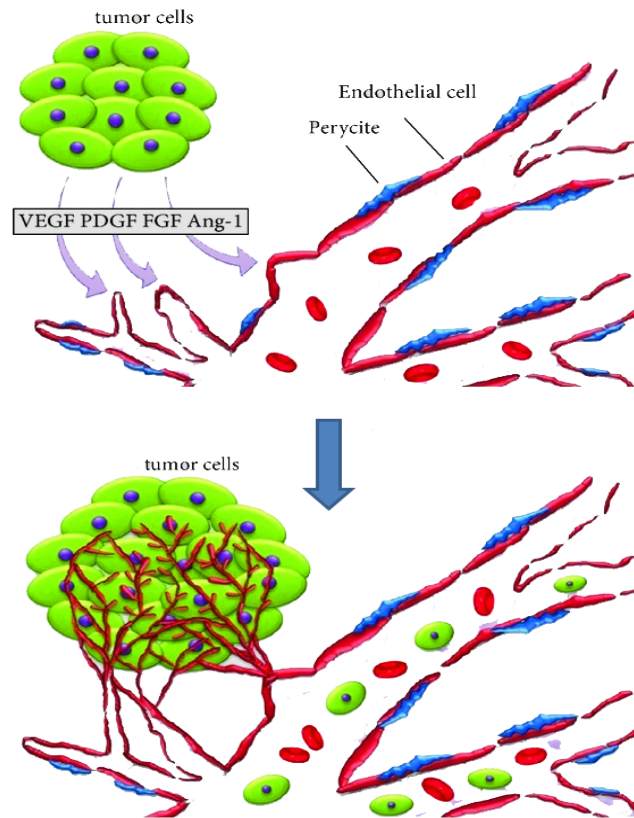


Figure1.2: Tumor-neovascularization process (Conti *et al.*, 2013)

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 (PlGF), 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 sequester 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, xanthenes 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-inflimatory 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. (Lamiales) 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 eupaporin 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* (Foad 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: eupaporin, 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* proliferation 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 smother's disease cell multiplication in organotypic 3D cell culture (Androutsopoulos *et al.*, 2008).

Tetramethoxyflavone has been shown to cause the activation of protein PKC ϵ . Down-regulation of the instigated PKC ϵ 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 nasopharyngeal 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) because 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