

**Pharmacological Characterization of Angiogenesis Effect of
Astragali Radix**

by

Guang Hu

Doctor of Philosophy in Biomedical Sciences



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Supervisor: Prof. Simon Ming-Yuen Lee

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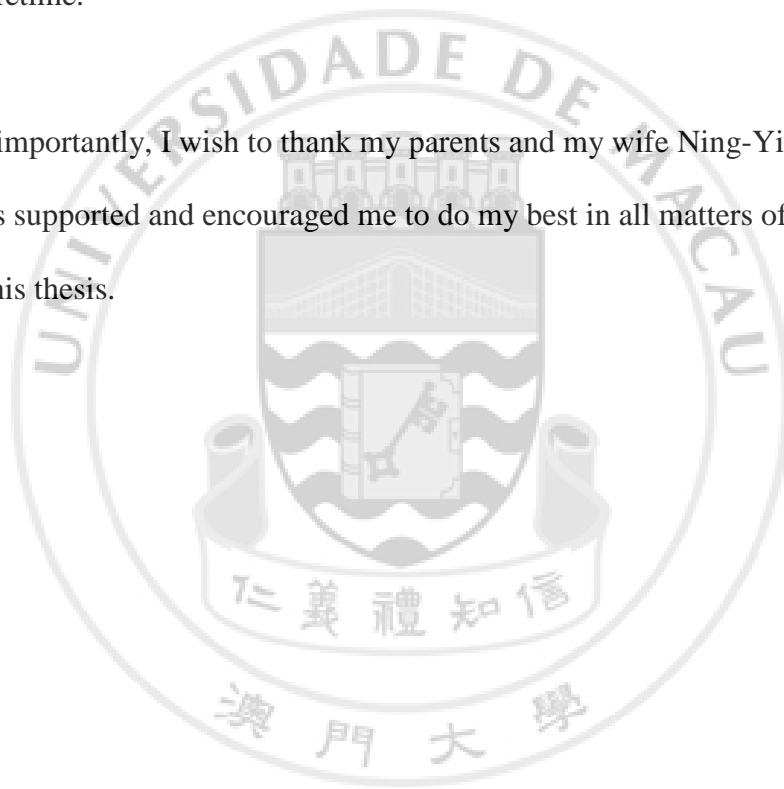
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Abstract

Astragali Radix has been used in China for more than two thousand years. The main constituents include flavonoids, saponins, polysaccharides, amino acids and trace elements. For centuries in clinical application, Astragali Radix has been prescribed for the treatment of cardiovascular and cerebrovascular diseases, and to enhance endurance and stamina in traditional Chinese medicine (TCM).

Many diseases are associated with an imbalance in the regulation of angiogenesis in which there is either excessive or insufficient blood vessel formation, such as various cancers, diabetic retinopathy, stroke and cardiovascular diseases. Astragali Radix is a rich source of novel angiogenic modulating compounds that may reveal previously unidentified angiogenic mechanisms. In an attempt to identify the biological effects of Astragali Radix on angiogenesis and to characterize its underlying mechanisms, the present study investigates the angiogenic effects of Astragali Radix in the following aspects:

1. Isolation of chemical constituents from Astragali Radix: Two Astragali Radix fractions [named Astragali Radix ethanolic fraction (ARE) and Astragali Radix water fraction], five polysaccharide fractions [P1 (MW<10000D), P2 (10000D<MW<30000D), P3 (30000D<MW<50000D), P4 (50000D<MW & DM<0.1 μ m), P5 (DM>0.1 μ m)] and three pure compounds [calycosin, formononetin and (6aR, 11aR)-9, 10-dimethoxy-3-hydroxypterocarpan were isolated from Astragali Radix crude herb.
2. Quantitative analysis of chemical constituents of Astragali Radix: An HPLC-

ELSD chemical analysis method was established to simultaneously determine six components [including calycosin, formononetin, (6aR, 11aR)-9,10-dimethoxy-3-hydroxypterocarpan, astragaloside I, II and IV] in Astragali Radix.

3. Screening of angiogenesis activity of chemical constituents of Astragali Radix: the pro-angiogenic effect of ARE, Astragaloside IV and P4 was evaluated on Human umbilical vein endothelial cells (HUVEC) *in vitro* and zebrafish *in vivo*. Also, the mechanism of action of the three components was studied.

4. In the present study, we have examined the drug biotransformation in zebrafish: biotransformation of calycosin, an isoflavone constituent naturally existing in Astragali Radix, in zebrafish *in vivo* had been examined for the first time. Ten metabolites of calycosin were identified in the zebrafish larvae treated with calycosin. Also, a time course determination of calycosin and its metabolites in zebrafish larvae by semi-quantitative analysis had been performed.

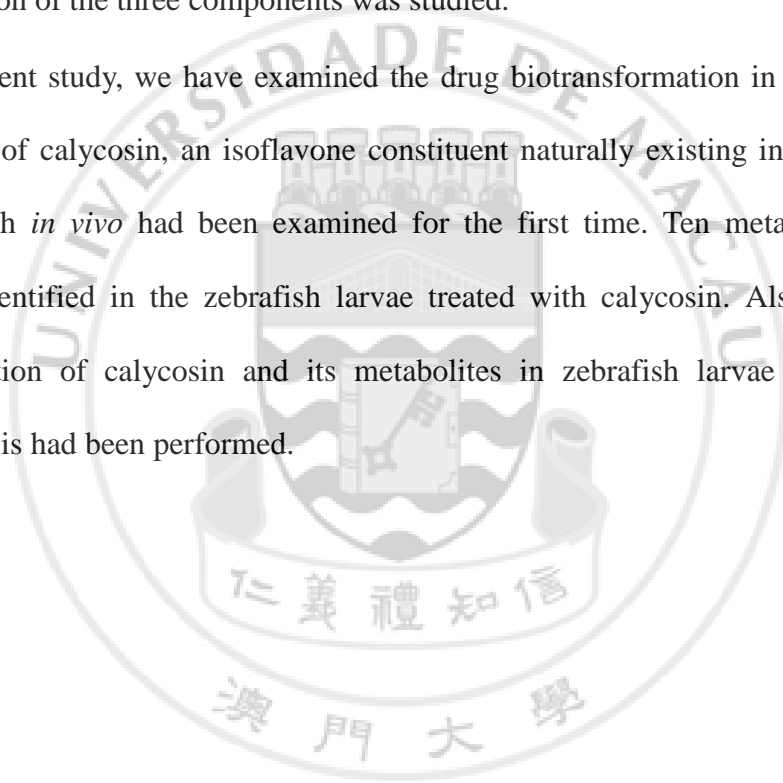


Table of contents

Acknowledgements	i
Abstract	iii
List of Figures and Tables	viii
List of Abbreviations	x
Chapter 1 General Introduction	1
1.1. General Scientific Background	1
1.2. Specific Background	4
1.2.1. Chemical constituents of Astragali Radix	4
1.2.2. Quality control of Astragali Radix	11
1.2.3. Pharmacological activities of Astragali Radix	12
1.2.4. Brief Summary of Astragali Radix	17
1.2.5. Mediators of angiogenesis.....	18
1.2.6. Cell signaling involved in angiogenesis.....	22
1.2.7. Angiogenesis assays	25
1.2.8. Brief summary of angiogenesis.....	32
1.3. Research Goals and Objectives	34
1.4. Research Methodology and Design	36
1.5. Potential Contributions	37
1.6. Organization of the thesis	38
1.7. Statement of originality	40
References	44
Chapter 2 Isolation and fractionation of compounds from Astragali Radix and chemical analysis of Astragali Radix	57
2.1. Introduction	57
2.2. Materials and methods	57
2.2.1. Samples, chemicals and reagents	58
2.2.2. Apparatus and chromatographic conditions	59
2.2.3. Sample preparation.....	60
2.2.4. Isolation of pure compounds	61
2.2.5. Optimization of HPLC-ELSD parameters	62
2.2.6. Calibration curves, limits of detection and quantification	63
2.3. Result	63
2.3.1. Result of isolation and purification of A0 part.....	63
2.3.2. Result of isolation of polysaccharide fractions	66
2.3.3. Result of HPLC separation optimization	68
2.3.4. Result of method validation	69
2.3.5. Determination of the six compounds in Astragali Radix and A0 by HPLC-ELSD	77
2.4. Conclusion	77
Chapter 3 Pro-angiogenic effect of Astragali Radix ethanolic fraction (ARE)	79
3.1. Introduction	79
3.2. Materials and methods	80
3.2.1 Chemicals and reagents.....	80
3.2.2 Preparation of ARE.	80

3.2.3 HUVEC culture.....	81
3.2.4 HUVEC's viability by XTT assay	82
3.2.5 Invasion assay on HUVEC.	82
3.2.6 Tube formation assay on HUVEC	83
3.2.7 HUVEC migration assay	84
3.2.8 Analysis of mRNA expression by real-time PCR.....	84
3.2.9. Maintenance of zebrafish (<i>Danio rerio</i>) and its embryos	85
3.2.10 Embryo collection and drug treatment.....	86
3.2.11. Morphological observation of zebrafish	86
3.2.12 Analysis of data.....	86
3.3. Results	86
3.3.1 Effect of ARE on HUVEC proliferation.....	86
3.3.2 Effect of ARE on HUVEC invasion	87
3.3.3 Effect of ARE on HUVEC Matrigel-induced tube formation	89
3.3.4 ARE enhances HUVEC migration.....	91
3.3.5 Detection of mRNA expression in ARE-treated HUVEC	92
3.3.6 ARE induces HUVEC proliferation via activation of KDR/Flk-1	93
3.3.7 ARE activates angiogenesis via the PI3K-Akt-eNOS pathway.....	94
3.3.8. ARE promotes angiogenesis in SIV region on zebrafish in vivo model.....	95
3.4. Discussion	96
3.5. Conclusion	100
Reference	101
Chapter 4 Pro-angiogenesis effect of polysaccharide extract from Astragali Radix on zebrafish model	104
4.1. Introduction.....	104
4.2. Materials and methods	105
4.2.1. Ethics statement	105
4.2.2. Chemicals and reagents	106
4.2.3. Preparation and analysis of ARP Fractions.....	106
4.2.4. Maintenance of zebrafish and its embryos	107
4.2.5. Zebrafish embryo collection and drug treatment.....	107
4.2.6. Morphological observation of zebrafish	107
4.2.7. Assessment of vascular changes	108
4.2.8. Total RNA extraction, reverse transcription, and real-time PCR.....	109
4.3. Results.....	110
4.3.1 Preparation of ARP fractions	110
4.3.2. ARP fractions rescue VRI-induced blood vessel loss in zebrafish	111
4.3.3. P4 reverses the VRI induced down regulation of Flk-1 and Flt-1 mRNA expression	117
4.4. Discussion	119
4.5. Conclusion	121
Chapter 5 The pro-angiogenesis activity of astragaloside IV in HUVEC <i>in vitro</i> and zebrafish <i>in vivo</i>.....	125
5.1. Introduction.....	125
5.2. Materials and Methods.....	126
5.2.1. Chemicals and reagents	126

5.2.2. Pro-angiogenic assay on zebrafish	127
5.2.3. Pro-angiogenic assays on HUVEC	127
5.2.4. mRNA expression analysis by real-time quantitative PCR.....	127
5.2.5. Western blotting analysis	127
5.2.6. Statistical analysis	128
5.3. Results	128
5.3.1. AS-IV promotes proliferation and tube formation in HUVEC	128
5.3.2. AS-IV enhances HUVEC migration and invasion in vitro	130
5.3.3. AS-IV increases VEGF and KDR mRNA expression and VEGF receptor tyrosine kinase inhibitor abolishes AS-IV induced HUVEC proliferation	132
5.3.4. AS-IV stimulates HUVEC proliferation via Akt activation	134
5.3.5. AS-IV rescues VRI-induced blood vessel loss in zebrafish.....	135
5.4. Discussion.....	136
5.5. Conclusion.....	138
Chapter 6. Determination of calycosin metabolites in zebrafish larvae using HPLC-MS/MS.....	142
6.1. Introduction	142
6.2. Materials and methods	144
6.2.1. Chemicals and reagents.....	144
6.2.2. Apparatus and chromatographic conditions	144
6.2.3. Metabolism of calycosin by zebrafish larvae.....	145
6.2.4. Construction of the calibration curves of calycosin in zebrafish larvae and culture medium.....	146
6.2.5. Data analysis	148
6.3. Results	148
6.3.1. Calibration curves of calycosin in zebrafish larvae and culture medium	148
6.3.2. Metabolism of calycosin in zebrafish larvae.....	150
6.3.3. Identification of calycosin metabolites in zebrafish larvae.....	152
6.4. Discussion.....	157
6.5. Conclusion.....	160
References	162
Chapter 7 General discussion and conclusion	164
7.1. Conclusions	164
7.1.1. Astragali Radix is a promising candidate herb for treatment of diseases associated with insufficient angiogenesis	164
7.1.2. Differential pro-angiogenic activity and mechanism of action exerted by different compositions of Astragali Radix	165
7.1.3. Zebrafish offers an ideal in vivo model for screening angiogenic agents and metabolic study	168
7.2. Limitation of the Current Study	171
7.3. Perspectives for Further Work	172
Reference.....	173
Appendix	177
CURRICULUM VITAE	183

List of Figures and Tables

Fig.1. 1 Structures of typical saponins in Astragali Radix.....	7
Fig.1. 2 Structures of typical saponins in Astragali Radix.....	10
Fig.1. 3 Angiogenesis signaling pathway	22
Fig.1. 4 Research methodology and design	36
Fig.2. 1 Flow chart for the extraction, fractionation and purification of Astragali Radix	64
Fig.2. 2 Structure of (6aR, 11aR)-9, 10-dimethoxy-3-hydroxypterocarpan	64
Fig.2. 3 Structure of Formononetin.....	65
Fig.2. 4 Structure of calycosin	66
Fig.2. 5 GPC chromatogram of polysaccharides in P4.....	67
Fig.2. 6 GC-MS chromatogram of acetylated-monosaccharide constituents in P4.....	68
Fig.2. 7 HPLC chromatograms of mixed standards (a) and Astragali Radix extract sample (b).	68
Table 2. 1 Gradient elution for HPLC-ELSD.	62
Table 2. 2 Effect of evaporator tube temperature on the ratio of signal to noise (S/N).	69
Table 2. 3 Effect of gas flow rate on the ratio of signal to noise (S/N).	70
Table 2. 4 Standard curves of investigated compounds.....	71
Table 2. 5 Intra-and inter-day variability for the assay of six investigated compounds.	72
Table 2. 6 Recovery of investigated components (n=3).	73
Table 2. 7 The stability test of investigated components.....	75
Table 2. 8 The content (mg/g) of investigated components in Astragali Radix samples.....	77
Fig.3. 1 Effect of ARE on HUVEC proliferation.....	87
Fig.3. 2 The effect of ARE on HUVEC invasion.	88
Fig.3. 3 Morphological changes in ARE-treated HUVEC cultured on Matrigel.....	90
Fig.3. 4 Migration assay for ARE-treated HUVEC cells.....	91
Fig.3. 5 Changes in gene expression in ARE-treated HUVEC and effect of SU5416 on ARE-induced cell proliferation.....	93
Fig.3. 6 Effects of wortmannin, SH-6 and L-NAME on ARE-induced cell proliferation.	95
Fig.3. 7 Effect of ARE on SIV region of zebrafish in vivo..	96
Fig.4. 1 GPC chromatogram of polysaccharides in P4.....	111
Fig.4. 2 GC-MS chromatogram of acetylated-monosaccharide constituents in P4.. ..	111
Fig.4. 3 The effect of ARPs on VRI-induced blood vessel loss in Tg(fli-1a:EGFP)y1 zebrafish (result of morphological observation).. ..	113
Fig.4. 4 Effects of ARPs on VRI-induced blood vessel loss in Tg(fli-1a:EGFP)y1 zebrafish (result of statistical analysis).. ..	114
Fig.4. 5 The effect of ARPs on VRI-induced blood vessel loss in Tg(fli-1a:nEGFP)y7 zebrafish (result of morphological observation).. ..	116
Fig.4. 6 Effects of ARPs on VRI-induced blood vessel loss in Tg(fli-1a:nEGFP)y7 zebrafish (result of statistical analysis).. ..	117

Fig.4. 7 Gene expression of P4 treated zebrafish.....	119
Table 4. 1 Description of ARPs extracted from Astragali Radix	111
Fig.5. 1 (A) Structure of AS-IV. (B) Effect of AS-IV on the proliferation of HUVEC compared with the control group.. ..	129
Fig.5. 2 (A) Migration assay for AS-IV-treated endothelial cells. (B) The migration ability was analyzed by averaging the mean length of the scraped area of each well compared to the control group.	130
Fig.5. 3 (A) Gene expression in AS-IV-treated HUVEC.....	132
Fig.5. 4 (A) Effects of AS-IV on Akt activation. (B) Effects of SH-6 on AS-IV-induced cell proliferation.. ..	134
Fig.5. 5 The pro-angiogenesis effects of AS-IV on damaged blood vessels in Tg(fli-1:EGFP) zebrafish embryos.. ..	135
Fig.6. 1 A typical MS/MS spectra of calycosin.....	149
Fig.6. 2 Time course of change in calycosin in the incubation medium (A), and in calycosin and its glucuronidated metabolite M2 in zebrafish larvae (B).....	151
Fig.6. 3 Time course of changes in calycosin metabolites in zebrafish larvae.. ..	152
Fig.6. 4 Identification of calycosin metabolites in calycosin-treated zebrafish larvae.. ..	153
Fig.6. 5 Proposed metabolic pathways of calycosin in zebrafish larvae.....	156
Table 6. 1 Precision and accuracy for quantification of calycosin in zebrafish larvae.	149
Table 6. 2 Precision and accuracy for quantification of calycosin in culture medium.	150
Table 6. 3 Chromatographical and mass spectral data of calycosin and its metabolites identified in calycosin-treated zebrafish larvae.....	157
Table 7. 1 Summary of pro-angiogenic activity of extract and different pure compounds in Astragali Radix	168

List of Abbreviations

DAD	Diode array detector
AR	Astragali Radix
HPLC	High Performance Liquid Chromatography
LOD	Limit of detection
LOQ	Limit of quantification
MS	Mass spectrometry
NMR	Nuclear Magnetic Resonance
PLE/ASE	Pressurized Liquid Extraction
QC	Quality Control
TLC	Thin Layer Chromatography
UV	Ultraviolet Visible
HUVEC	human umbilical vein endothelial cells
VEGF	vascular endothelial growth factor
PI3K	phosphatidylinositol 3-kinase
eNOS	endothelial nitric oxide synthase
DBT	Danggui buxue tang
ERK1/2	extracellular signal-regulated kinase 1/2
PPARalpha	peroxisome proliferatoractivated receptor α
NO	nitric oxide
F-12K	Kaighn's modification of Ham's F12 medium
FBS	fetal bovine serum
PBS	phosphate-buffered saline
EDTA	Ethylene Diamine Tetraacetic Acid
ECGS	Endothelial cell growth supplement
LN	Wortmannin and L-NAME
DMSO	dimethyl sulfoxide

TAE	Tris-acetate-EDTA
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
VPF	vascular permeability factor
dpf	day(s) post fertilization
AS-IV	Astragaloside IV
EGFP	enhanced green fluorescent protein
XTT	2,3- bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino) carbonyl] -2H-tetrazolium hydroxide
PCR	Polymerase Chain Reaction
RT-PCR	reverse transcription- Polymerase Chain Reaction
ISV	intersegmental vessels
SIV	subintestinal vessels
DLAV	dorsal longitudinal anastomotic vessels
Flt-1	fms-like tyrosine kinase
KDR	kinase-domain region
Flk-1	fetal liver kinase-1
ARP	Astragali Radix polysaccharide