

Effects of Xuefu Zhuyu Decoction on cell migration and ocular tumor invasion in *Drosophila*

Sitong Wang^{1,#}, Fanwu Wu^{1,#}, Bin Ye¹, Shiping Zhang², Xingjun Wang³, Qian Xu⁴, Guowang Li¹, Menglong Zhang¹, Shuai Wang¹, Yongsen Jia¹, Chunhua Jiang¹, Xiaojin La¹, Hong Chang¹, Zixue Zhao¹, Peng Li¹, Ji-an Li¹ and Chenxi Wu^{1*}

¹ Tangshan Key Laboratory of Traditional Chinese Medicine Pharmacology, College of Traditional Chinese Medicine, North China University of Science and Technology, 21 Bohai Road, Tangshan 063210, China

² School of Life Science and Technology, ShanghaiTech University, 393 Middle Huaxia Road, Shanghai 201210, China

³ Department of Neuroscience, Scripps Research Institute, 130 Scripps Way Jupiter, Florida 33458, USA

⁴ Collage of Integrative Medicine, Fujian University of Traditional Chinese Medicine, Fuzhou 350122, China

These authors contribute equally to this work.

*Correspondence should be addressed to: chenxi.wu@ncst.edu.cn

Supplementary materials

Wang *et al.*, Table S1

Table S1. The formulation of XFZYD (one dose)

Herb (Local name)	Medicinal parts	Amount in Application (g)
<i>Bupleurum chinense</i> DC. (Bei Chaihu)	Root	3.0
<i>Paeonia lactiflora</i> Pall. (Chi Shao)	Root	6.0
<i>Cyathula officinalis</i> Kuan (Chuan Niuxi)	Root	9.0
<i>Ligusticum chuanxiong</i> Hort. (Chuan Xiong)	Root	4.5
<i>Angelica sinensis</i> (Oliv) Diels. (Dang Gui)	Root	9.0
<i>Prunus persica</i> (L.) Batsch (Tao Ren)	Seed	12.0
<i>Glycyrrhiza uralensis</i> Fisch. (Gan Cao)	Root	6.0
<i>Carthamus tinctorius</i> L. (Hong Hua)	Flower	9.0
<i>Platycodon grandiflorum</i> (Jacq.) A. DC. (Jie Geng)	Root	4.5
<i>Rehmannia glutinosa</i> Libosch. (Sheng Dihuang)	Root	9.0
<i>Citrus aurantium</i> L. (Fuchao Zhiqiao)	Fruit	6.0

Wang *et al.*, Table S2

Table S2. Batch numbers and manufacturers of XFZYD

Herb (Local name)	Batch Number	Manufacturers
<i>Bupleurum chinense</i> DC. (Bei Chaihu)	20180610	Beijing Bencaoyuan Pharmaceutical Co.,Ltd
<i>Paeonia lactiflora</i> Pall. (Chi Shao)	20180610	Beijing Bencaoyuan Pharmaceutical Co.,Ltd
<i>Cyathula officinalis</i> Kuan (Chuan Niuxi)	20180522	Beijing Bencaoyuan Pharmaceutical Co.,Ltd
<i>Ligusticum chuanxiong</i> Hort. (Chuan Xiong)	20180523	Beijing Bencaoyuan Pharmaceutical Co.,Ltd
<i>Angelica sinensis</i> (Oliv) Diels. (Dang Gui)	20180605	Beijing Bencaoyuan Pharmaceutical Co.,Ltd
<i>Prunus persica</i> (L.) Batsch (Tao Ren)	20180330	Beijing Bencaoyuan Pharmaceutical Co.,Ltd
<i>Glycyrrhiza uralensis</i> Fisch. (Gan Cao)	17111601	Zhenxing Baicao (Beijing) Pharmaceutical Co., Ltd
<i>Carthamus tinctorius</i> L. (Hong Hua)	20180101	Beijing Tongrentang Health Pharmaceutical Co.,Ltd.
<i>Platycodon grandiflorum</i> (Jacq.) A. DC. (Jie Geng)	20180624	Beijing Bencaoyuan Pharmaceutical Co.,Ltd
<i>Rehmannia glutinosa</i> Libosch. (Sheng Dihuang)	17120315	Anhui Xiehecheng Pharmaceutical Tablet Co.,Ltd.
<i>Citrus aurantium</i> L. (Fuchao Zhiqiao)	20180613	Beijing Bencaoyuan Pharmaceutical Co.,Ltd

Wang *et al.*, Figure S1

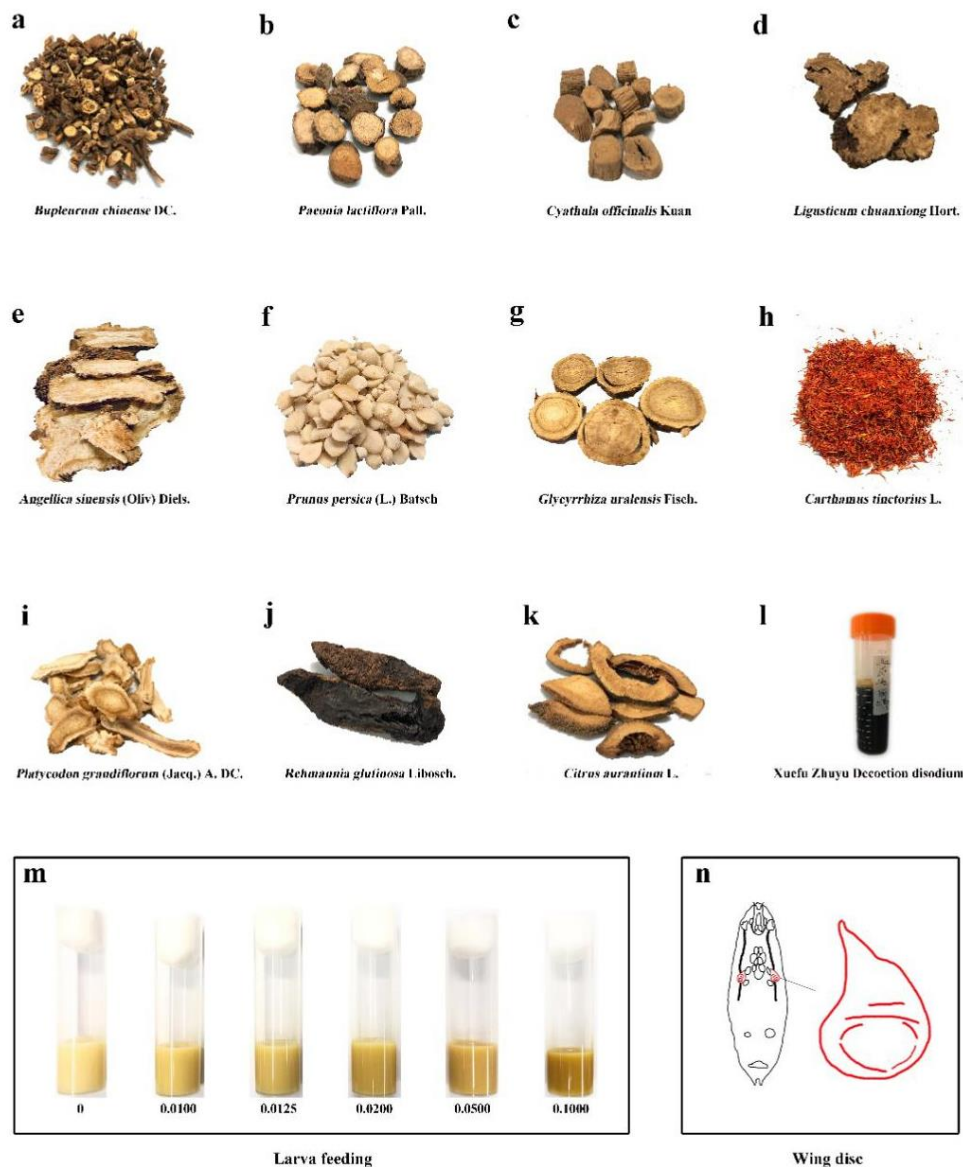


Figure S1. Composition of Xuefu Zhuyu Decoction.

(a-k) Photographs showing the composed of XFZYD: *Bupleurum chinense* (a), *Paeonia lactiflora* (b), *Cyathula officinalis* (c), *Ligusticum chuanxiong* (d), *Angelica sinensis* (e), *Prunus persica* (f), *Glycyrrhiza uralensis* (g), *Carthamus tinctorius* (h), *Platycodon grandiflorum* (i), *Rehmannia glutinosa* (j), *Citrus aurantium* (k). (l) Xuefu Zhuyu Decoction aqueous extract. (m) Photographs of XFZYD added *Drosophila* feeding medium are shown. From left to right, the final concentration is 0.0100, 0.0125, 0.0200, 0.0500 and 0.1000g/ml respectively. (n) Schematic view of 3rd instar larva wing discs location, wing discs (right) are located in the first third of the whole larva (left).

Wang *et al.*, Figure S2

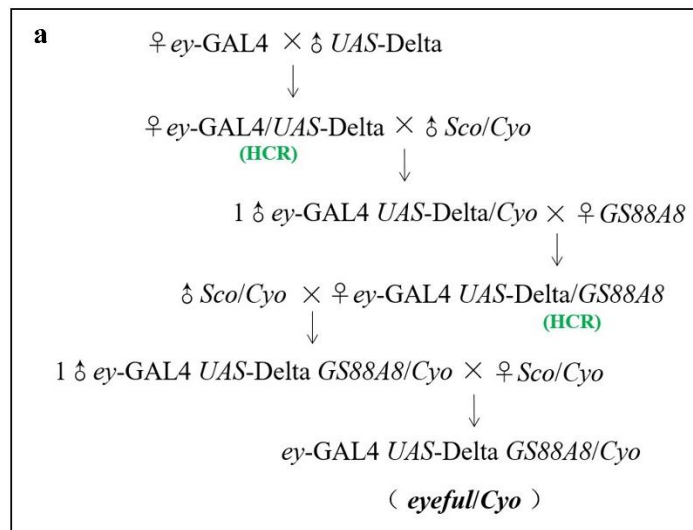


Figure S2. A flow chart for establishing the *eyeful* model.

Firstly, healthy unmated female flies with genotype *ey-GAL4* (Chromosome 2) were crossed to male flies with genotype *UAS-Delta* (Chromosome 2). From their offspring, the unmated female flies with genotype *ey-GAL4/UAS-Delta* were collected, in which the homologous chromosomes recombination (HCR) occurred, and crossed to male flies of balancer strain *Sco/Cyo*. From their offspring, only one male fly with genotype *ey-GAL4 UAS-Delta/Cyo* was crossed to female flies of *GS88A8* strain (Chromosome 2). To further recombine *ey-GAL4 UAS-Delta* with *GS88A8*, the female offspring with genotype *ey-GAL4 UAS-Delta/GS88A8* were collected and crossed to male flies of *Sco/Cyo*. Next, only one male progeny with genotype *ey-GAL4 UAS-Delta GS88A8/Cyo* was selected and crossed to female flies of *Sco/Cyo*. The chromosome *Cyo* is a balancer, which could prevent HCR. Thus, several unmated female and male offspring with genotype *ey-GAL4 UAS-Delta GS88A8/Cyo* were collected and crossed to generate the stock of *eyeful* tumor model strain.

Wang *et al.*, Figure S3

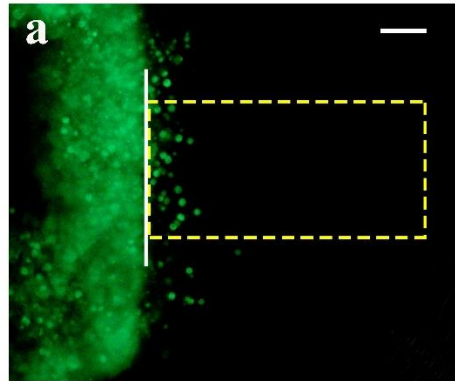


Figure S3. Measurement of migrating cell distance.

Representative example illustrating measurement of migrating cell distance. White solid line represents the baseline. Yellow dashed box indicates the specified region (90 μm high \times 180 μm wide) at the D/V boundary of wing pouches. The distance of each cell in the box to baseline was measured. Scale bar: 30 μm .

Wang *et al.*, Figure S4

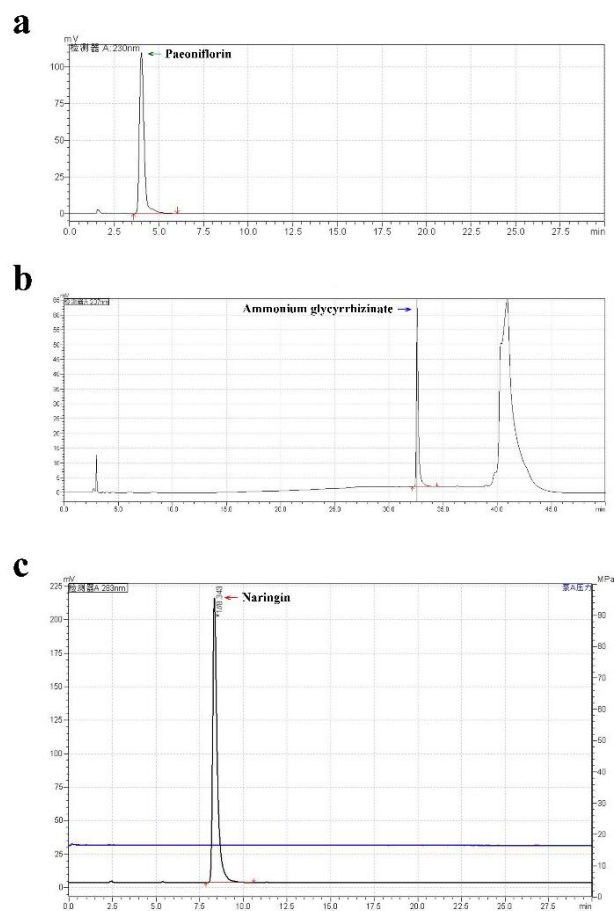


Figure S4. The HPLC chromatogram of standard controls.

The standards of paeoniflorin, ammonium glycyrrhizinate and naringin were identified by HPLC method. The detection wavelength for paeoniflorin (a), ammonium glycyrrhizinate (b) and naringin (c) is 230nm, 237nm and 283nm respectively, where their peaks indicated with colored arrows.

Wang *et al.*, Table S3

Table S3. Ocular tumor’s primary growth and invasion location in adult *Drosophila*

	Primary Growth				Invasion Location			
	I	II	III	Total	Head	Thorax	Abdomen	Total
Wild type	0	0	0	0	0	0	0	0
<i>eyeful</i>	40	27	7	74	15	2	1	18
<i>eyeful</i> +XFZYD (0.0100g/ml)	33	23	3	59	8	5	0	13
<i>eyeful</i> +XFZYD (0.0125g/ml)	29	17	6	52	7	4	1	12
<i>eyeful</i> +XFZYD (0.0200g/ml)	41	52	9	102	12	1	1	14
<i>eyeful</i> +XFZYD (0.0500g/ml)	46	25	5	76	6	1	1	8
<i>eyeful</i> +XFZYD (0.1000g/ml)	54	18	3	75	19	1	1	21