## Synergy assessments of plant extracts used in the treatment of stress and ageing related disorders

Alexander George Panossian

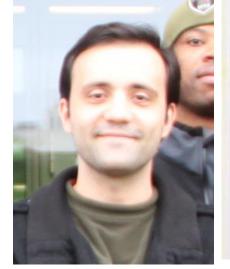
EuroPharma USA Inc., Green Bay, Wisconsin, USA

#### In collaboration with









**Onat Kadioglu** 

Ean-Jeong Seo

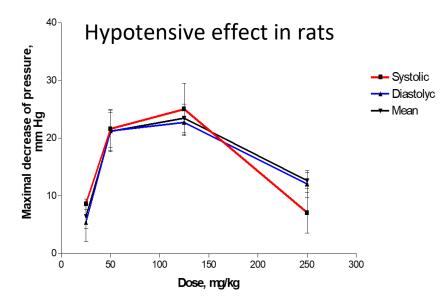


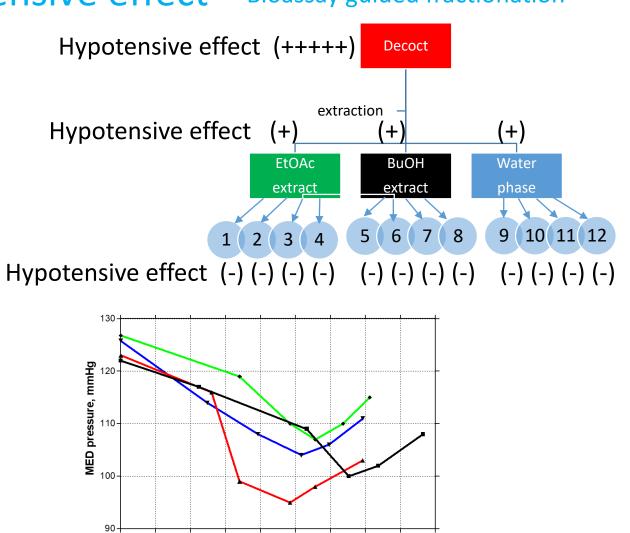
**Thomas Efferth** 

#### Synergistic interaction > hypotensive effect Bioassay guided fractionation



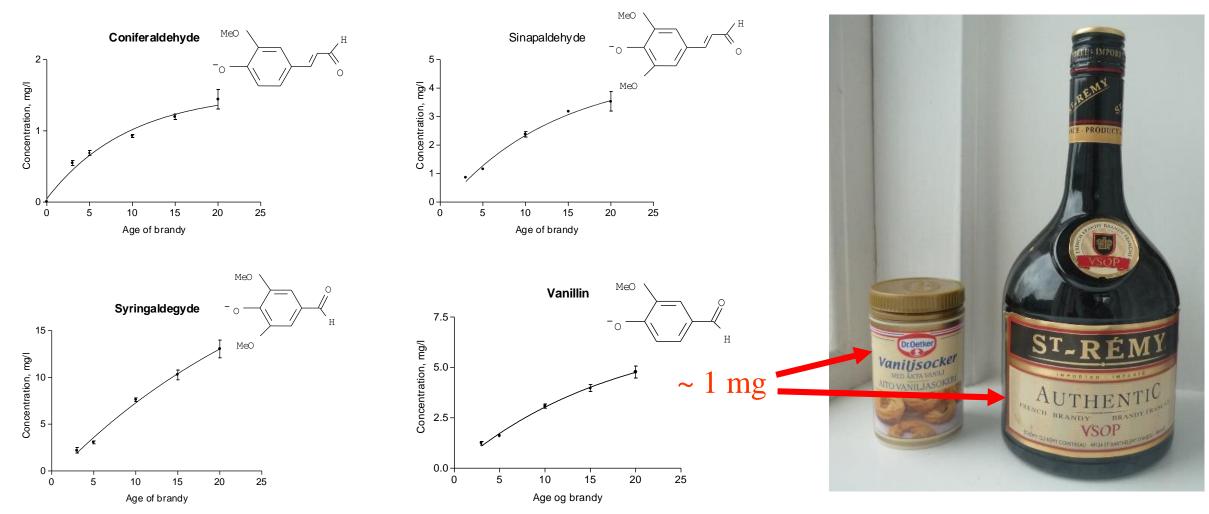
Cychorium intybus L. herb decoct





Time after Administration, min

#### Antagonistic effect – vanillin aroma



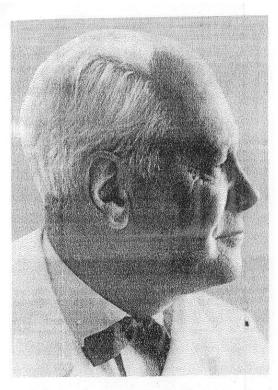
- 1. Panossian, G. Mamikonyan, M. Torosyan, E. Gabrielyan, S. Mkhitaryan. Analysis of Aromatic Aldehydes in Brandy and Wine by High Performance Capillary Electrophoresis. *Anal Chem.* 2001. Vol. 73, No 17. Pp.4379-4383.
- 2. Panossian A.G., Mamikonyan G.V., Torosyan M.A., Abrahamyan A.G., Hovanissyan A.S., Gabrielyan E.S., Grigoryants, Mkhitaryan S., Lapin B.V. Determination of phenolic aldehydes in vine and brandy by capillary electrophoresis: new markers of quality of brandy. *J. Analytical Chemistry*. 2001, v. 56, No12, pp. 11-23
- 3. Panossian A., Gabrielyan E., Gevorgyan Kh. Method of determination of age and quality of alcohol drinks stored in wood barrels. Patent of Armenia No 1137 A2, 2001.

#### Synergy and antagonism of plant extracts

## What is common in the mechanisms of action of various stress response modifiers - adaptogens?

Panossian A, Hamm R, Kadioglu O, Wikman G and Efferth T . 2013. Synergy and antagonism of active constituents of ADAPT-232 on transcriptional level of metabolic regulation of isolated neuroglial cells. *Front. Neurosci.* **7**:16. doi:10.3389/fnins.**2013**.00016

## Introduction of adaptogens: historical background



Николай Васильевич Лазарев (1895—1974)

Nicolay Lazarev

#### First definition of adaptogens

#### "Adaptogens" are compounds which increase "the state of non-specific resistance" in stress

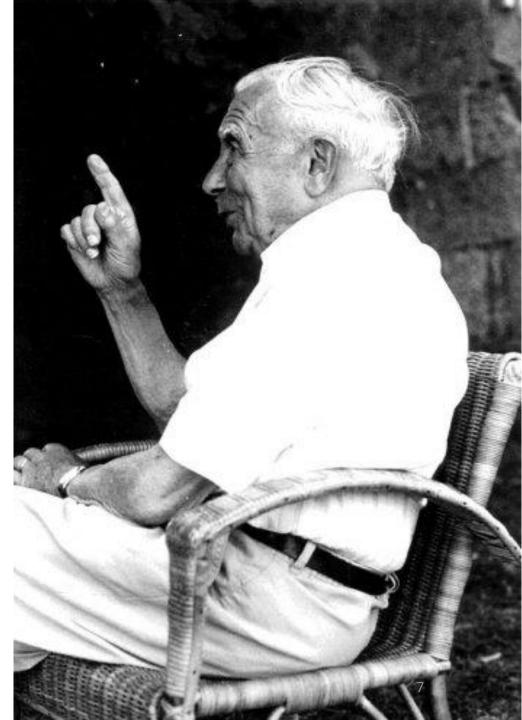
Lazarev NV. 1958. General and specific in action of pharmacological agents. Farmacol.Toxicol, 21(3): 81-86. Lazarev NV. Ljublina EI, Rozin MA. 1959. State of nonspecific resistance. Patol.Fiziol.Experim.Terapia, 3(4): 16-21.



George Canguilhem 1943

Adaptability – test of health (The Lancet, 2009: 373, 781)

Attenuation of adaptability to stress is crucial for health and survival

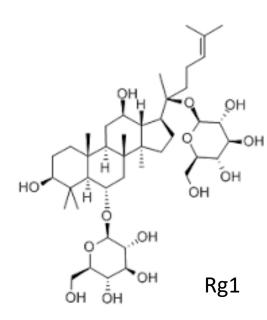


The Normal and the Pathological

Georges Canguilhem

Michel Foucault

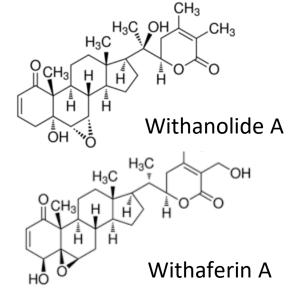
ZONE BOOKS



#### TCM and Ayurveda

*Panax ginseng* – "superior", tonic herb in TCM. used in convalescence and fatigue - Qi deficiency

Qi (Chi) - determines the state of health and lifespan



#### Withania somnifera, Ashwaganda

Prana - determines the life vital energy, activating body and mind, governing emotions, memory, and other functions



#### Adaptive stress response



STRATAKIS & CHROUSOS: THE STRESS SYSTEM

#### Neuroendorine – immune complex

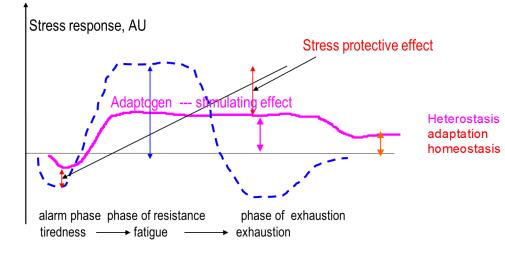


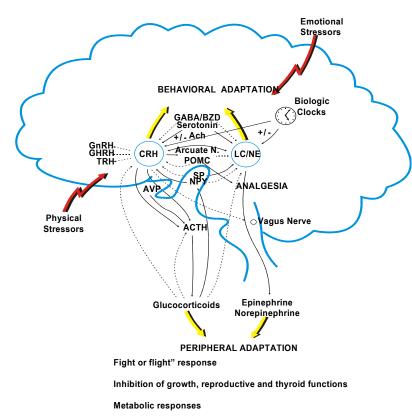
**Stress** is a state of threatened homeostasis.

Homeostasis is a complex dynamic equilibrium

Cannon, 1935: Selye, 1950,

#### Effect of adaptogens – stress response modifiers increasing the resistance to stress





Gastric stasis, increased colonic motility

# Age related disorders - complications arising from senescence - decreased ability to cope stress and to maintain homeostasis

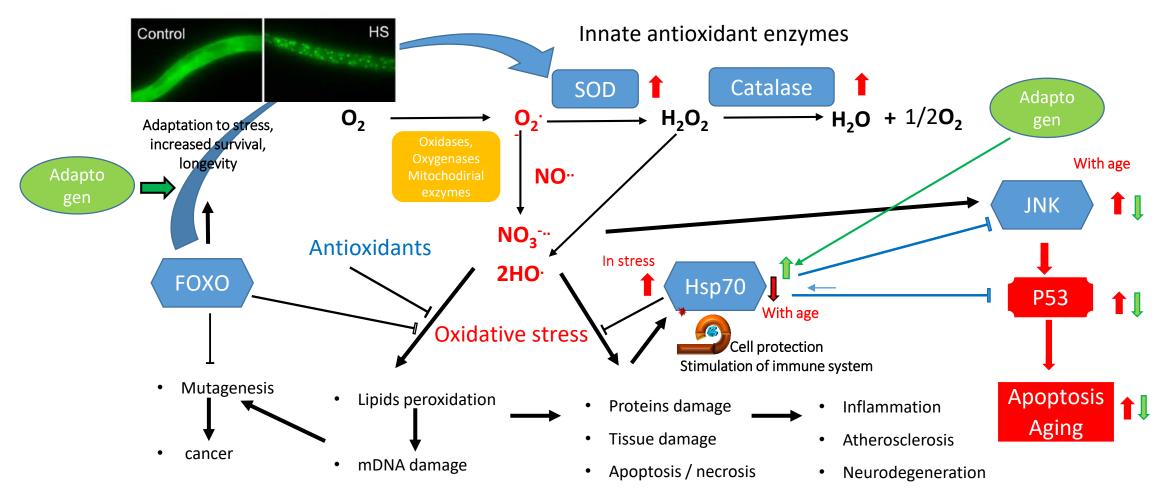
- atherosclerosis thrombosis, infarction, stroke
- cardiovascular disease and hypertension
- cancer
- degenerative joint disease (osteoarthritis)
- type 2 diabetes, obesity
- muscle degeneration (sarcopenia)
- senile dementia
- Alzheimer's disease
- Visual loss because of clouding of the lens (cataracts)



**Stress** 

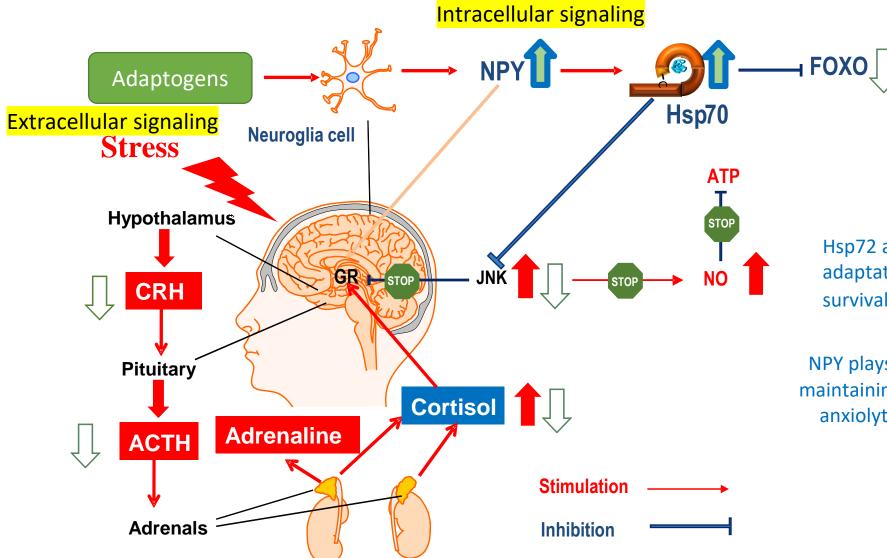
- Chronic inflammation
- Cells degeneration
- Inflammaging

# The role of Hsp70 and FOXO in current theory of oxidative stress induced inflammaging and the effects of adaptogens



Panossian A.G. 2017. Understanding adaptogenic activity: specificity of the pharmacological action of adaptogens and other phytochemicals. Ann. N.Y. Acad. Sci. 1401(1):49-64.

# Effects of adaptogens on adaptive stress response in HPA axis: FOXO, NPY and Hsp70 signaling



Activation of innate immunity, adaptation to stress, survival, longevity, cognitive function

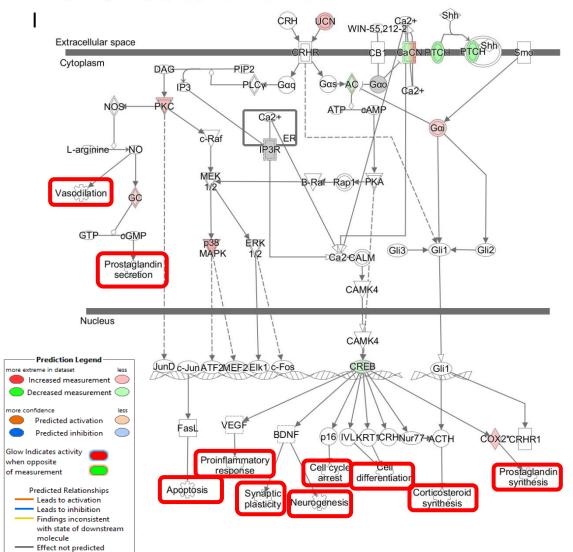
#### Immune stimulation cytoprotection

Hsp72 and NPY are directly involved in adaptation to stress, resulting in increased survival, longevity and cognitive function.

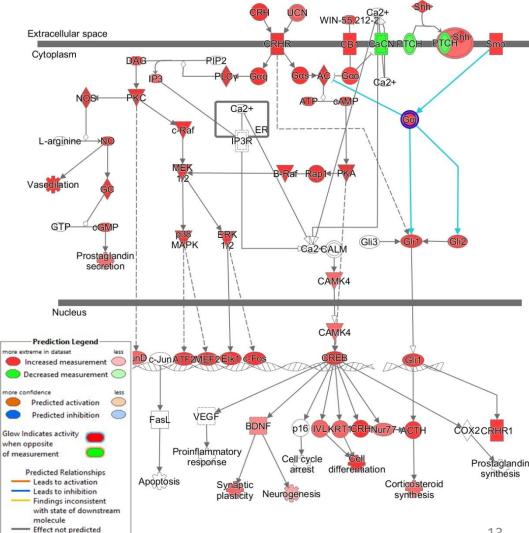
NPY plays a major role on the HPA axis and maintaining energy balance, antidepressive anxiolytic and antinarcotic effects. etc.

#### Stress induced activation of CRH Canonical Pathway

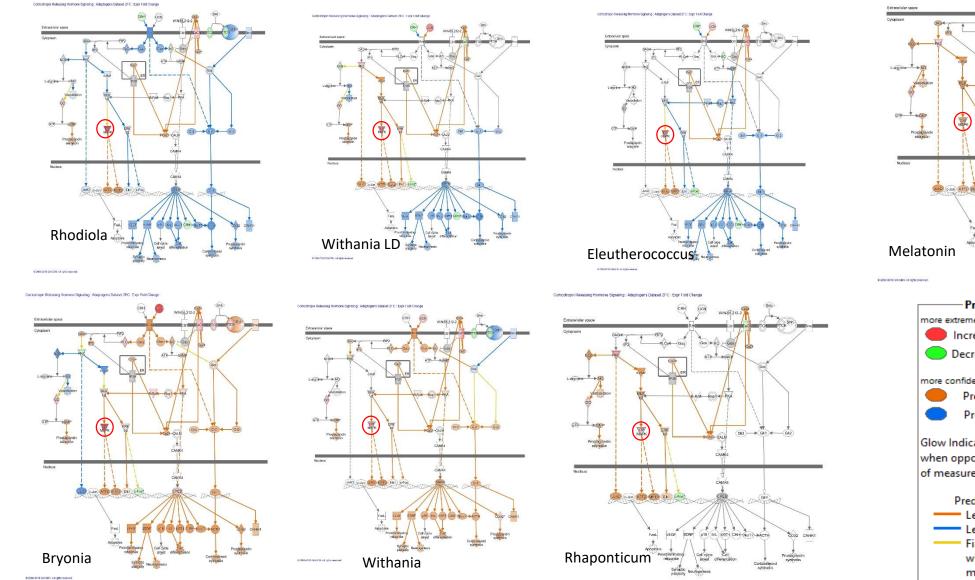
Corticotropin Releasing Hormone Signaling : Adaptogens Dataset 2FC : Expr Fold Change

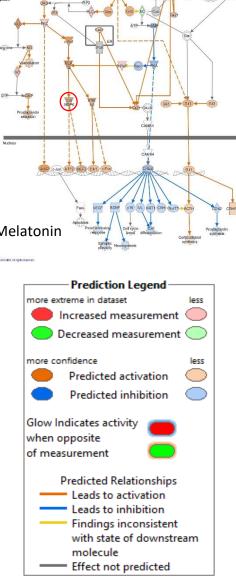


Corticotropin Releasing Hormone Signaling



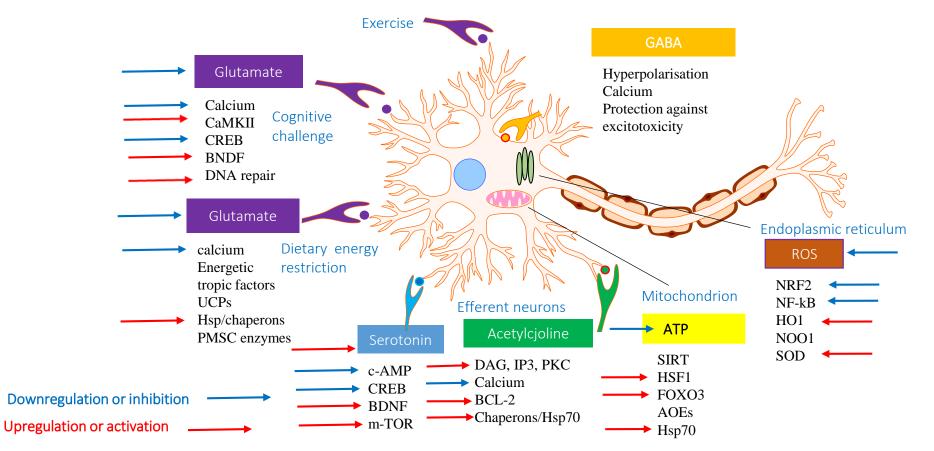
### Effects of adaptogenic plants on CRH Signaling Pathway





WIN-55,212-2

## Effects of adaptogens on adaptive stress response signaling pathways that protect neurons against degeneration and promote synaptic plasticity.



A glutamatergic neuron in the hippocampus receiving excitatory inputs from neurons activated in response to exercise, cognitive challenges and dietary energy restriction.

Panossian A.G. 2017. Understanding adaptogenic activity: specificity of the pharmacological action of adaptogens and other phytochemicals. Ann. N.Y. Acad. Sci. 1401(1):49-64.

#### Definitions of adaptive stress response and adaptogens

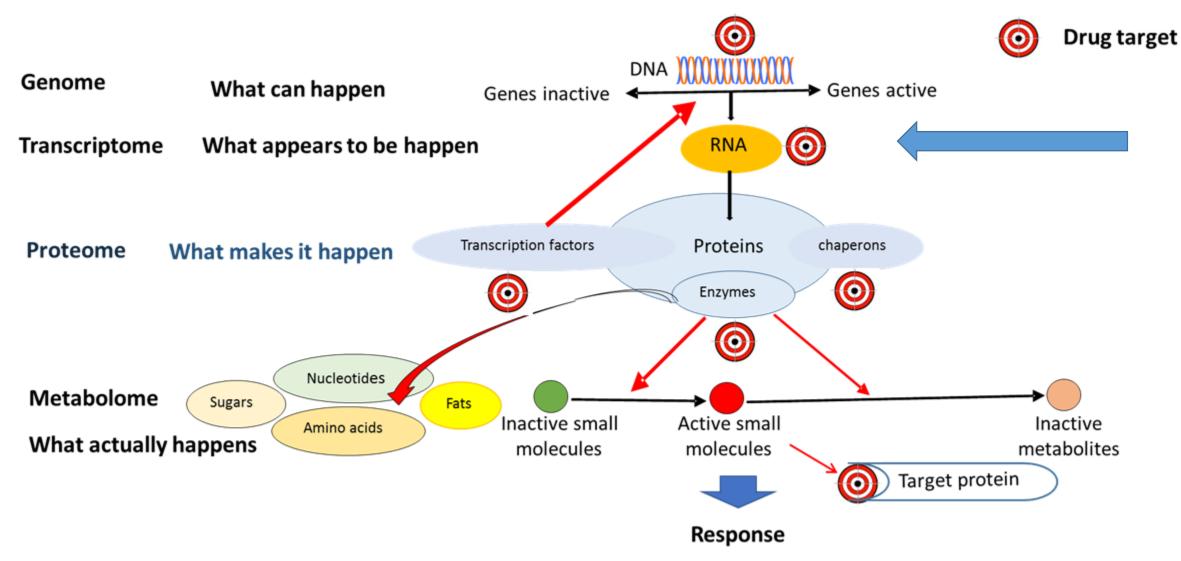
- Adaptive stress response (hormesis) involves activation of intracellular and extracellular signaling pathways and increased expression of anti-apoptotic proteins, neuropeptides, antioxidant enzymes and defense response of an organism resulting in increased survival.
- Adaptogens are adaptive stress response modifiers of cellular and organismal defense systems, activating intracellular and extracellular signaling pathways, expression of stress-activated proteins, neuropeptides, antioxidant enzymes and anti-apoptotic proteins of an organism resulting in non-specific resistance to various stressors and increased survival.
- Adaptogens, like vitamins and antioxidants constitute a separate category of nutritional and herbal medicinal products.

Panossian A.G. 2017. Understanding adaptogenic activity: specificity of the pharmacological action of adaptogens and other phytochemicals. Ann. N.Y. Acad. Sci. 1401(1):49-64.

 Which of mediators of adaptive stress response are regulated by adaptogens?
 What is common in the mechanisms of action

• What is common in the mechanisms of action of adaptogens?

#### Molecular targets of pharmacological intervention

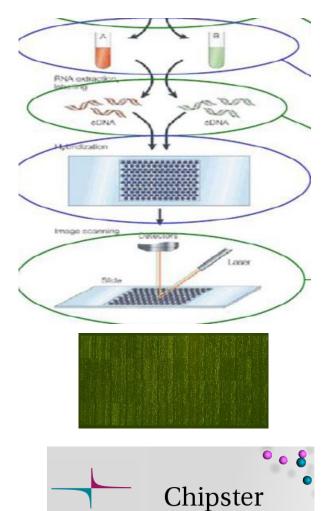


#### What we observe in our experiments? Gene expression as drug's efficacy outcome measure

- **Gene expression** is the process by which information from a gene is used in the synthesis of a functional gene product.
- **Regulation of gene expression** includes a wide range of mechanisms that are used by cells to increase or decrease the production of specific gene products protein or RNA.
- **Up-regulation** is a process that occurs within a cell triggered by an internal or external signal, which results in increased expression of genes and corresponding proteins.
- Up-regulation occurs, when a cell is deficient in some kind of receptor. In this case, more receptor
  protein is synthesized and transported to the membrane of the cell and, thus, the sensitivity of the cell
  is increased back to normal, reestablishing homeostasis.
- **Down-regulation** is a process resulting in decreased gene and corresponding protein expression.
- Down-regulation occurs, when a cell is overstimulated by a neurotransmitter, hormone, or drug for a
  prolonged period of time, and the expression of the receptor protein is decreased in order to protect
  the cell and to reestablish homeostasis.

#### Gene expression degree: RNA microarray assay

- mRNA extraction from treatment and control samples
- RNA converted to fluorescence-labeled transcripts (for detection)
- Labeled RNA fragments hybridize (bind to their matching DNA sequence) with DNA for GeneChip array. Each labelled gene "matches" with its specific "partner" DNA on the GeneChip array
- Fluorescence intensities captured/scanned on the image of Gene Chip array that reflect gene expression level: higher gene expression => higher fluorescence intensity.
- CHIPSTER Software: transformation of the intensity values to fold changes =>
   efficiently statistical analysis and differential gene expression profiling of a
   microarray data => identification of deregulated genes => deregulated gene sets.
- Venn diagram to show sets of deregulated genes
- Ingenuity Knowledge Data Base software => Interactive Pathways Analysis (IPA) downstream analysis for influenced signalling pathways and protein networks
- Validation of microarray-based mRNA expression by quantitative real-time RT-PCR.



CSC

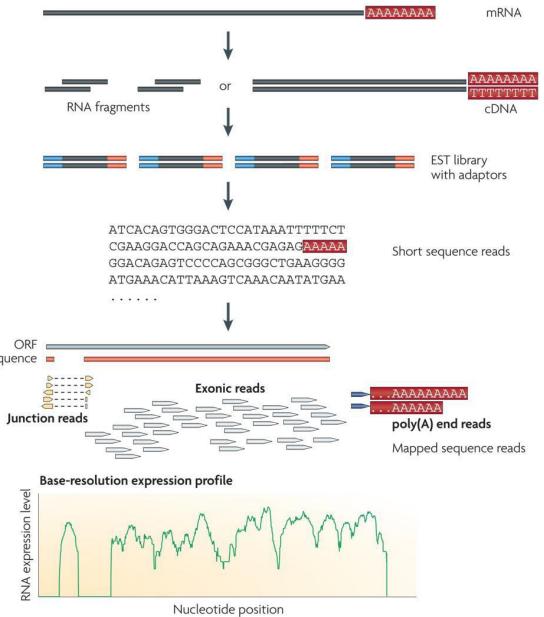
INGENU

PATHWAY ANALYSIS

Open source platform for data analysis

### Gene expression degree: RNA Sequencing

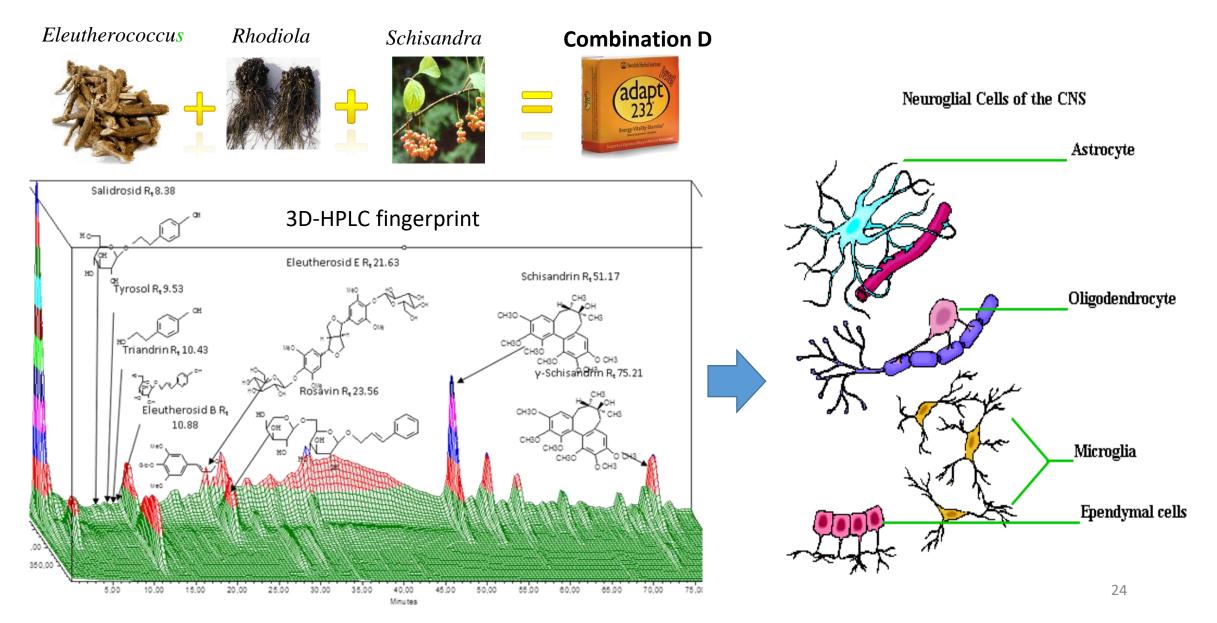
- No hybridization and labeling steps are required,
- After the alignment to reference genome, RNA read numbers determine the expression degree of each gene RPKM value (reads per<sup>Coding sequence</sup> kilobase per million mapped reads),
- Fold changes are calculated by using the ratio of each gene's RPKM values to control sample.



Complete list of plant names declared as adaptogenic in scientific literature					
Ajuga turkestanica (Regel) Briq.	Emblica officinalis Gaetrn.	Piper longum L.			
Alstonia scholaris (L.) R. Br.	Eucommia ulmoides Oliv.	Potentilla alba L.			
Anacyclus pyrethrum (L.) Lag.	Evolvulus alsinoides (L.) L.	Ptychopetalum olacoides Benth.			
Andrographis paniculata (Burm.f.) Nees	Firmiana simplex (L.) W.Wight	Rhaponticum carthamoides (Willd.) Iljin			
Aralia mandshurica Rupr. & Maxim	Gentiana pedicellata (D.Don) Wall	Rhodiola heterodonta (Hook. f. & Thomson) Boriss.			
Argyreia nervosa (Burm. f.) Bojer	Glycyrrhiza glabra L.	Rhodiola rosea L.			
Argyreia speciosa (L. f.) Sweet	Heteropterys aphrodisiaca Machado	Rostellularia diffusa (Willd.) Nees .			
Asparagus racemosus Wild	Hippophae rhamnoides L.	Salvia miltiorrhiza Bunge			
Bacopa monnieri (L.) Wettst	Holoptelea integrifolia Planch	Schisandra chinensis (Turcz.) Baill.			
Bergenia crassifolia (L.) Fritsch	Hoppea dichotoma Willd.	Scutellaria baicalensis Georgi			
Bryonia alba L.	Hypericum perforatum L.	Serratula inermis Poir			
Caesalpinia bonduc (L.) Roxb	Lepidium peruvianum/ Lepidium meyenii Walp.	Sida cordifolia L.			
Centella asiatica (L.) Urb.	Ligusticum striatum DC.	Silene italica (L.) Pers.			
Chlorophytum borivilianum Santapau & R.R.Fern.	Melilotus officinalis (L.) Pall.	Sinomenium acutum (Thunb.) Rehder & E.H.Wilson			
Chrysactinia mexicana A. Gray	Morus alba L.	Solanum torvum SW.			
Cicer arietinum L.	Mucuna pruriens (L.) DC.	Sutherlandia frutescens (L.) R.Br.			
Codonopsis pilosula (Franch.) Nannf.	Nelumbo nucifera Gaertn.	Terminalia chebula Retz.			
Convolvulus prostratus Forssk.	Ocimum sanctum L.	Tinospora cordifolia (Willd.) Miers			
Curculigo orchioides Gaertn.	Oplopanax elatus (Nakai) Nakai	Trichilia catigua A.Juss.			
Curcuma longa L., Curcumin	Panax ginseng C.A.Mey.	Trichopus zeylanicus Gaertn.			
Dioscorea deltoidea Wall. ex Griseb.	Panax pseudoginseng Wall.	Turnera diffusa Willd. ex Schult.			
Drypetes roxburghii (Wall.) Hurus.	Pandanus odoratissimus L.f.	Vitis vinifera L.			
Echinopanax elatus Nakai	Paullinia cupana Kunth	Withania somnifera (L.) Dunal			
Eleutherococcus senticosus (Rupr. & Maxim.) Maxim.	Pfaffia paniculata (Mart.) Kuntze				

Combination A	Combination B	Combination C	Combination D
Andrographis paniculata (Burm.f.)	Rhodiola rosea L.	Rhaponticum carthamoides (Willd.)	Rhodiola rosea L.
Nees	Salidroside	Iljin	Salidroside
Andrographolide	• Tyrosol		• Tyrosol
Eleutherococcus senticosus (Rupr. &	Bryonia alba L.	Eleutherococcus senticosus (Rupr. &	Eleutherococcus senticosus (Rupr.
Maxim.) Maxim.		Maxim.) Maxim.	& Maxim.) Maxim.
• Eleutheroside E		• Eleutheroside E	• Eleutheroside E
		Withania somnifera (L.) Dunal	Schisandra chinensis (Turcz.) Baill.
			Schizandrin

### Incubation of herbal extracts with neuroglial cells



Glial cells do NOT have chemical synapses. But....

Neurons HAVE synapses that use neurotransmitters...

that dendrite glial cell axon oligodendrocyte, glial cell axon oligodendrocyte, glial cell oligodendrocyte, glial

Glia contributes to the defense of the brain through:

- the expression of the innate immune response,
- promoting the clearance of neurotoxic proteins and apoptotic cells from the CNS,
- regulating the entry of inflammatory systemic cells into the brain at the blood brain barrier.

This stimulates both tissue repair and the rapid restoration of tissue homeostasis.

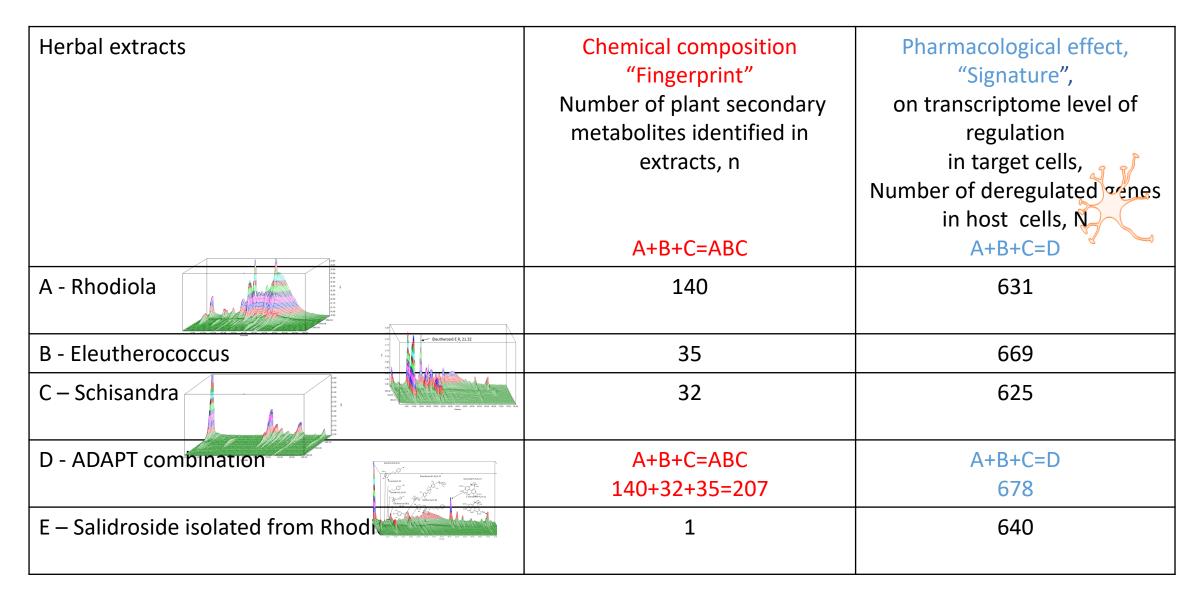
An important physiological function of neuroglial cells is metabolic supply of energy and other substances, maintaining brain homeostasis.

Astrocytes, but not neurons, prevent macrophage and T-Cell inflammation in the CNS, to attenuate axonal loss and gliosis resulting in neuroprotection.

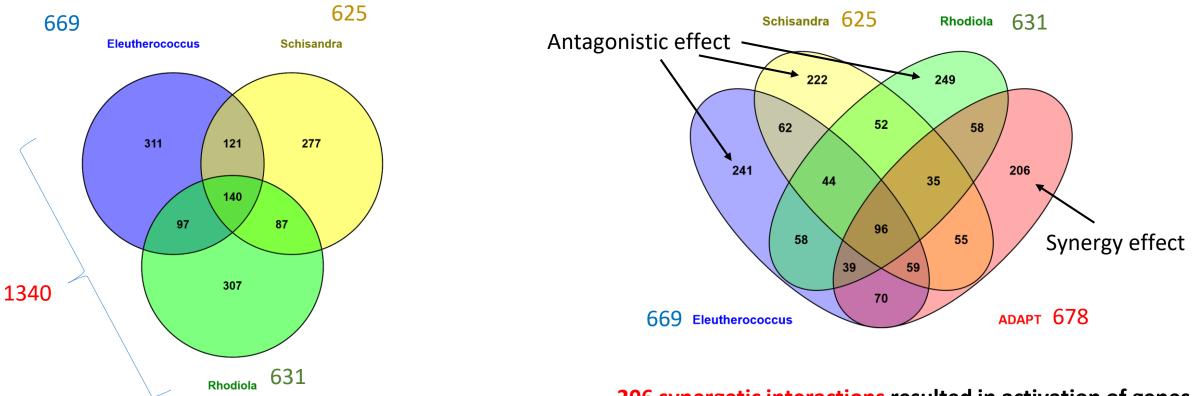
Glia has been shown to have several functions, including:

- serving as a transportation link between the bloodstream and neurons, uptake of neurotransmitters,
- synthesis and release of neurotrophic factors,
- immune regulation, and
- modulation of synaptic activity .

#### How many targets has one purified active compound?



### Venn diagram to show sets of deregulated genes



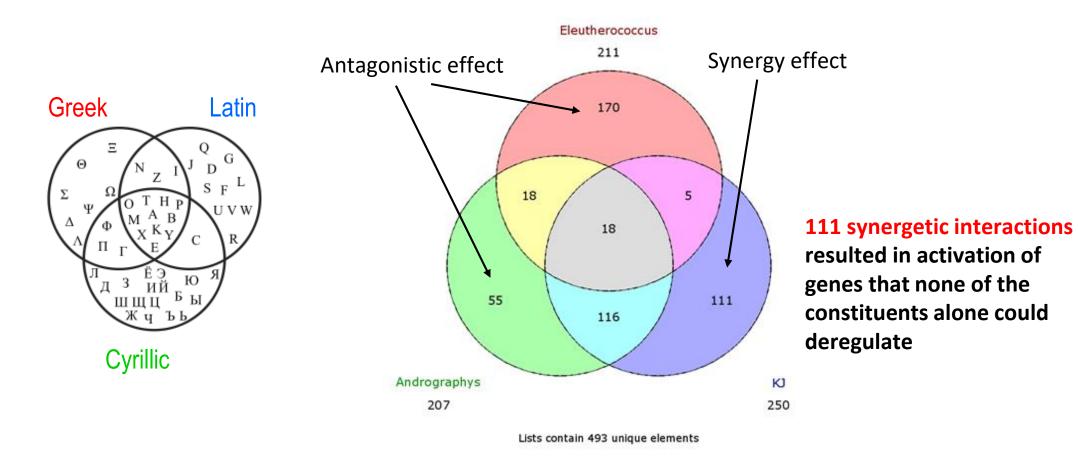
#### **206 synergetic interactions** resulted in activation of genes that none of the constituents alone could deregulate

Panossian, A., Hamm, R., Kadioglu, O., Wikman, G., Efferth, T., 2013. Synergy and antagonism of active constituents of ADAPT-232 on transcriptional level of metabolic regulation of isolated neuroglial cells. Front Neurosci. 7:16. http://journal.frontiersin.org/article/10.3389/fnins.2013.00016/abstract

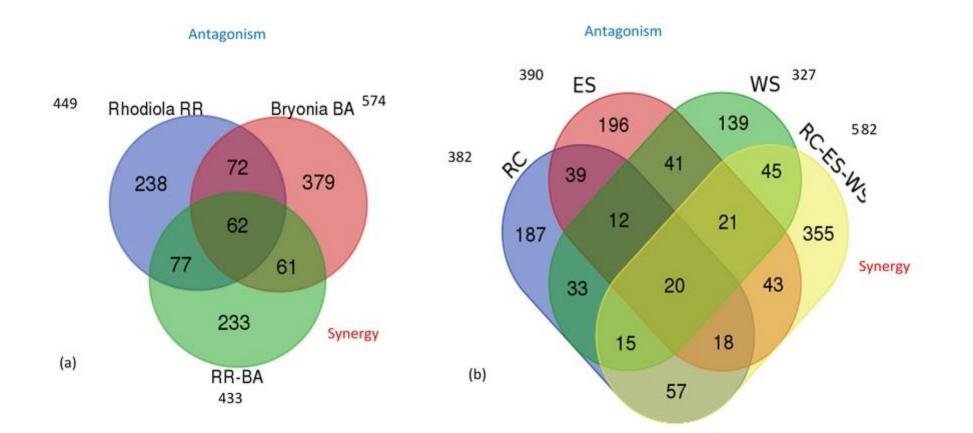
Herbal extracts	Chemical composition "Fingerprint" Number of plant secondary metabolites identified in extracts, n	Pharmacological effect, "Signature", on transcriptome level of regulation in target cells,
	A+B=AB	Number of deregulated genes in host cells, N A+B=C
A - Andrographis	39	207
B - Eleutherococcus	35	211
C – A+B combination	39+35=74	250
D – Andrographolide from Andrographis	1	626

#### Synergy and antagonism

#### Venn diagram to show sets of deregulated genes



#### Venn diagram to show sets of deregulated genes



### What is synergism? Current Definition # 1

- "Synergy and antagonism imply that the different constituents affect each other's actions" assuming that "If it is synergistic, the drugs will be more effective in combination than separately" (Goldin and Mantel, 1957).
- "A combination of agents that is more effective than is expected from the effectiveness of its constituents is said to show synergy" (Berenbaum, 1977).
- This definition of synergy is related to effectiveness of the combinations, which is associated with the concentration of an agonist needed to elicit half of the maximum biological response of the agonist. The effectiveness of a drug usually is considered relative to its safety (therapeutic index).
- This definition of synergy is not associated with the efficacy of the combination, where maximum possible effect relative to the ingredients is considered, particularly when the effect is 0 or negative for the ingredients of the combination.

## Definitions of effectiveness and efficacy

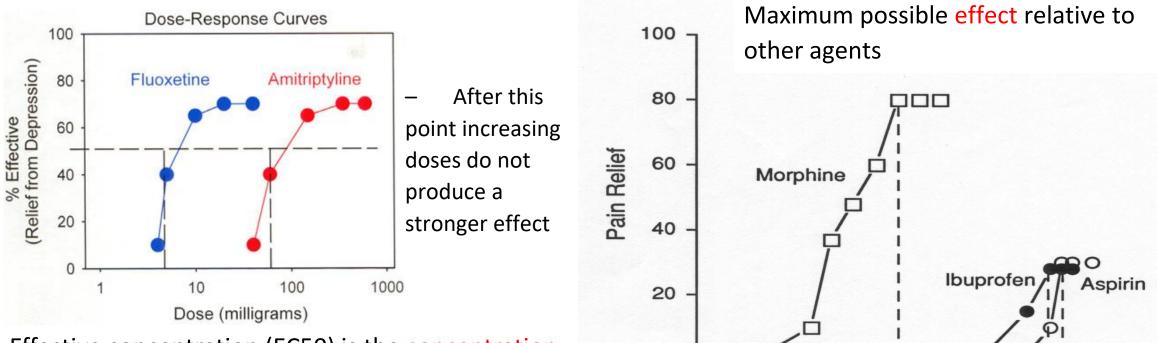
#### Effectiveness



10

100

1000



0

0.1

- Effective concentration (EC50) is the concentration of an agonist needed to elicit half of the maximum biological response of the agonist
- The effectiveness of a drug is considered relative to its safety (therapeutic index)

### Definition # 1 Update

In this context, it would be reasonable to distinguish among definitions of synergy and implement more relevant definitions for assessing efficacy and effectiveness of fixed combinations of active agents, such as:

- Amplification, if 1+1>2, or synergistic amplification instead of a synergy
- Attenuation, if 1+1<2
- Addition, if 1+1=2
- Potentiation, if 0+1>1
- **Bionetwork synergy**, If 0+0>0

### Limitation of Definition # 1

• The models of assessment of synergy, e.g., the isobole method (Berenbaum, 1977; Greco et al., 1995; Chow, 2010; Roel; et al., 2017), are not suitable for assessing complex interactions of molecular networks involved in drug-induced synergistic or antagonistic response. They cannot be used to predict the effects of multi-target interactions or homeostatic feedback on the pharmacological response.

#### What is synergism? Current Definition # 2

"Two or more agents working together to produce a result not obtainable by any of the agents independently"

(Skirven et.al., 2011; <u>https://www.theopedia.com/synergism;</u> http://googledictionary.freecollocation.com/meaning?word=synergy).

can be interpreted as

Generation of new pharmacological activity, which is specific only for the combination of two or more agents

### Definition # 2 Update

- The term **bionetwork synergy** is more suitable for network interactions of two or more agents resulting in qualitatively new pharmacological effects that cannot be obtained by any single constituent independently, regardless of dose.
- Similarly, antagonism results from bionetwork interactions of several constituents in combination, leading to lack, reduction, or prevention of effects that any individual ingredient in that combination yields.

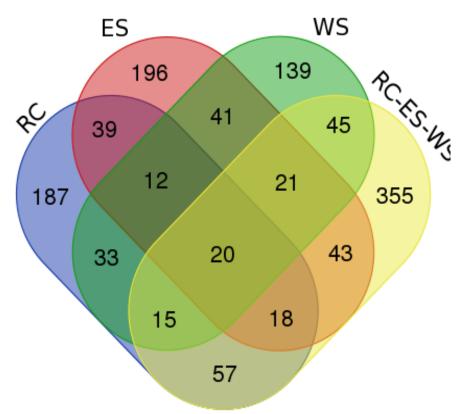
In other words:

- **Bionetwork synergy** results from synergistic interaction of ingredients A and B of the combination AB, inducing new pharmacological activity c: a+b=c;
  - where (a) is the activity of substance A, (b) is the activity of substance B, and (c) is bionetwork synergy—induced activity of the combination AB = the hybrid substance C.
- Bionetwork antagonism results from antagonistic interaction of ingredients A and B of the combination AB (the hybrid substance C), resulting in suppression of (a) and (b): – a+b=0

### **Applications**

 This definition is suitable and relevant to pharmacological studies of combinations of plant extracts where the focus is the discovery of unexpected potential indications and toxic effects of the combinations from complex intracellular and extracellular interactions of molecular networks with many players at several phases of development of final pharmacological outcomes.

#### Further evidences from IPA analysis



**RC** - *Rhaponticum carthamoides* (Willd.) Iljin

ES - Eleutherococcus senticosus (Rupr. & Maxim.) Maxim.

WS - Withania somnifera (L.) Dunal

**RC-ES-WS** – Their Combination

#### **Comparison effects on Canonical Pathways**

Activation z-score -1.342	2.449	-log(p-value)	0.00 E00	3	Activation z-s	core	2.449
way	RC RC RC		way	RC	Expr Fold Cha	ange -15.462	11.792
(e) Canonical Pathway	Observation 6 F Observation 7 V Observation 8 F Observation 9 F	(b)	Canonical Pathway	Observation 6 F Observation 7 V Observation 8 E Observation 9 F	(c)	enes in the Dendriti	Observation 6 R Observation 7 WS Observation 8 ES Observation 9 RC
Dendritic Cell Maturation Role of NFAT in Cardiac He ErbB Signaling Opioid Signaling Pathway Synaptic Long Term Depre cAMP-mediated signaling GP6 Signaling Pathway Calcium Signaling GPCR-Mediated Nutrient S HMGB1 Signaling Production of Nitric Oxide Renin-Angiotensin Signaling GDNF Family Ligand-Rece IL-6 Signaling Toll-like Receptor Signaling	ss Sei a nc pt	GPCR-Me Renin-And Toll-like R cAMP-me Opioid Sig Role of NF GDNF Fam HMGB1 Si Synaptic L GP6 Signa IL-6 Signal	ignaling Cell Maturatio diated Nutrien aiotensin Signal diated signalin diated signalin naling Pathwa AT in Cardiac hily Ligand-Re gnaling ong Term Dep ling Pathway	nt Sei alinc ling ng av Hyp scept	MAPK13 FCGR2C TLR9 FSCN2 IL-23p19 NGFR MAPK10 IL1A PLCB1 ICSBP HLA-DRB1 FGFR4 PLCL1	Genes	Obs Obs

#### **Effects on CRH Canonical Pathway**

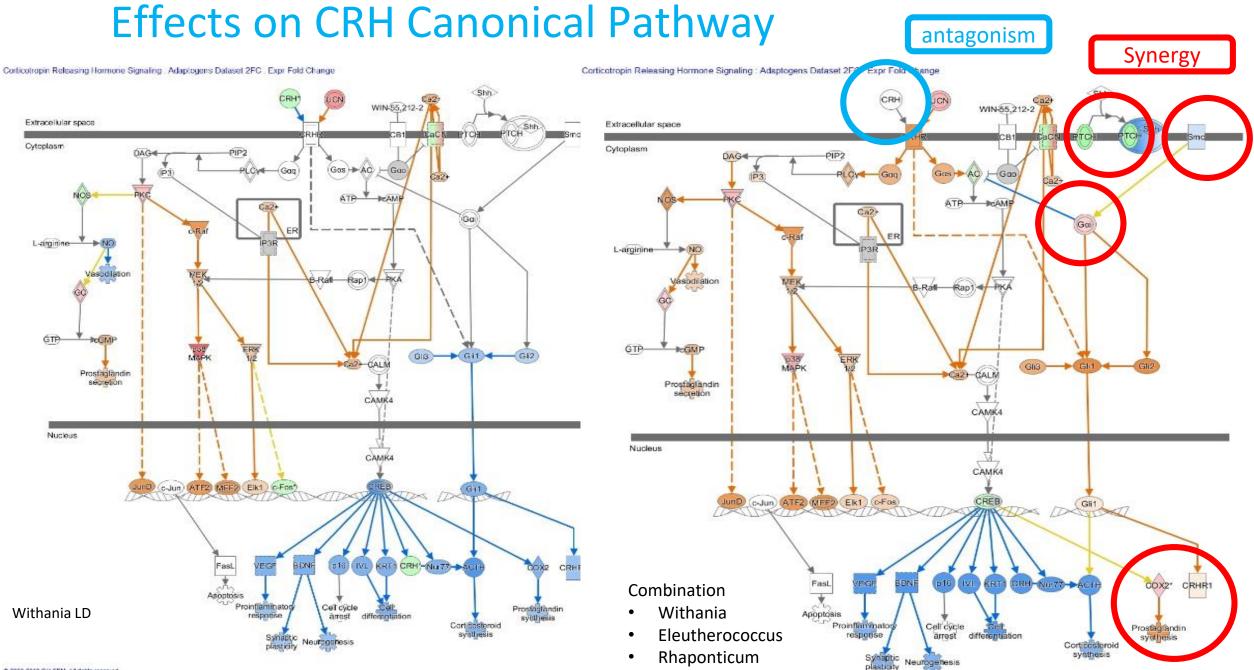
WIN-55,212-2 Extracellular space Shh PTCH CaC& PICH Smo Cytoplasm DA/ Gos AC Gog Ga (IP? NOS ATP--PEAM Ga24 Gai L-arginine--NO asodiation/ Rap1 B-Raff ÌRK. CGMF ĠŦŔ-ERK GIB)-- Gil 4 Ca2+ CALM Prostaglandin secretion CAMK Nucleus CAME C-Jun ATF2 MEF2 Elk1 C-Fos (Gi1 (p18) (IVL) KRT CRH -Nu 77->ACT EDNE FasL VEGI COX2 CREE Asoptosis Proinfilamithator Cell cýcle arrest Withania LD response differentiation synthesis Continuationoid svattesis Synaptic Neurogenesis plasticity

Conticotropin Releasing Hormonie Signaling . Adaptogens Dataset 2FC . Expr Fold Change

**UCN** WIN-55,212-2 Extracellular space Shh TCH ACN PTCH Small Cytoplasm Gos AC Gao IP3 ATP V -MAME Ca2-(Gai) L-arginine -NO Vasodilation B-Raff Rap1 GTP-CGM ERK D38 GII3) → Gli1) Gi2 CALIN Prostaglandin secretion CAMK Nucleus CAMK4 c-Jun ATF2 MEF2 Ek1 C-Fos CREB Gli1 (p16)(IVL) KRT1 (CRH)-Nur77-MACTH VEGE BONE FasL COX2 CRHR1 Apoptosis Proinflämmator Cell'cýcle Cell Prostaglandin differentiation Rhaponticum response arrest synthesis Corticosteroid synthesis Synaptic Neurogenesis

plasticity

Corticotropin Releasing Hormone Signaling : Adaptogens Dataset 2FC : Expr Fold Change



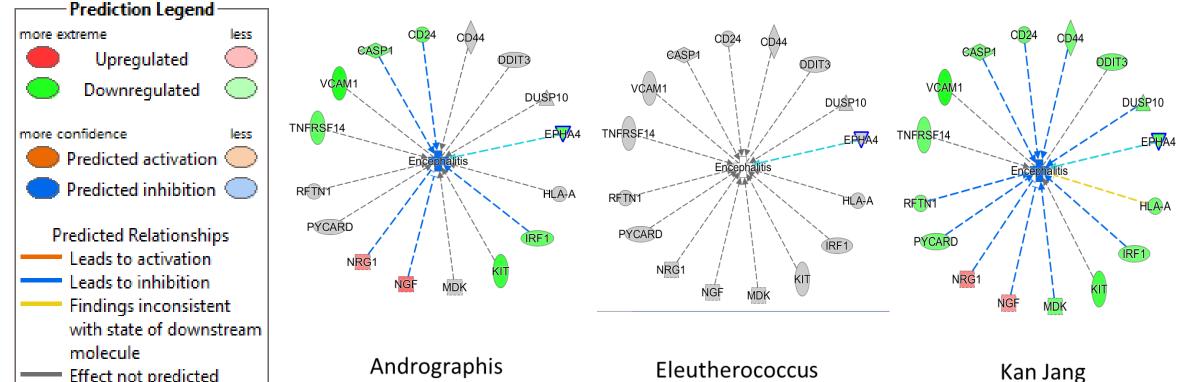
#### Comparison of effects on biological and cellular functions

Activation z-score -2.425	2.035	Activation z-score -2.425	2.035
Diseases and Bio	Observation 6 RC Observation 7 WS Observation 8 ES Observation 9 RC	Diseases and Bio	Observation 6 RC Observation 7 WS Observation 8 ES Observation 9 RC
Stimulation of cells Cell movement Synthesis of fatty acid Synthesis of prostaglandin Release of L-glutamic acid Proliferation of pheochrom Production of reactive oxyo Synthesis of eicosanoid Migration of cells Organization of cytoskeleto Microtubule dynamics Release of amino acids Transport of ion Concentration of cyclic AW Secretion of molecule Quantity of vitamin Peroxidation of lipid Removal of cells Proliferation of blood cells Extension of cellular protru Colony formation of tumor Secretion of neurotransmith Transport of cation Transmigration of cells Release of steroid hormone Mobilization of Ca2+ Maturation of cells Colony formation Oxidation of fatty acid Apoptosis of phagocytes Cellular homeostasis I-kappaB kinase/NF-kappaB Binding of guanosine 5'-O-	IF	Neurotransmission Growth of bacteria Differentiation of liver Differentiation of liver cells Transmigration of mononuc Transmigration of leukocyte Differentiation of naive T lym Differentiation of naive T lym Differentiation of cD8+ T lymp Proliferation of epithelial cel Concentration of hormone Development of epithelial cel Differentiation of helper T lym Lipolysis of adipocytes Differentiation of peithelial t Proliferation of lymphatic sy Synaptic transmission Synaptic transmission of cell Homing of T lymphocytes Neurotransmission of synap Differentiation of leukocytes Proliferation of leukocytes Proliferation of lymphocytes Migration of mononuclear les Binding of hematopoietic pr Leukocyte migration Maturation of phagocytes Secretion of aldosterone Cell movement of T lympho	

#### **Comparison of effects on diseases**

Activation z-score		-log(p-value)	Expression Analysis - Observation 6 RC-ES-WS							
-2.425	2.035	1.7	17	Summary \ Can	onical Pat	hways 🗍 Upstream An	alysis Diseases & Fur	nctions \ Regulator Ef	fects $\overline{\setminus}$ Networks $\overline{\setminus}$ Lists	
Bio	ပိုလ္လလုပ	and	ion 6 RC ion 7 WS ion 8 ES ion 9 RC	Diseases and Bio Functions \         Downstream Effects Analysis: Evidence for Effects         Male genital neoplasm predicted to be decreased (z-score -2.230).						
and B	£ К ≶ Ш £ Б									
(0	ation ation ation									
Disease	Observation Observation Observation Observation	Disease	Male genital neoplasm predicted to be decreased (z-score -2.230). 5 of 159 genes have measurement direction consistent with decrease in ADD TO MY PATHWAY ADD TO MY LIST CUSTOMIZE TABLE CREATE DATASET						Male genital neoplas	
Abdominal cancer		Abdominal cancer				Genes in dataset	∧ Prediction (bas	. Expr Fold Change	Findings	
Digestive system cancer Male genital neoplasm	Gastrointestinal neoplasia Cancer of secretory structur		GNRH1		GNRH1	Decreased	<b>↑</b> 3.841	Decreases (59)		
Tumorigenesis of genital orc		Digestive system cancer		Findings: Male genital neoplasm						
Genital tumor		Genital tract cancer       Review the information that supports the gene-to-function relationship. Click the plus icon to view the reference information.         Tumorigenesis of genital orc       PlainText v EXPORT REFERENCES							ce information.	
Cancer of secretory structure										
Synthesis of fatty acid		Genital tumor		Findings 1 to 20 of 59 << Previous 20   Next 20 >> Show Findings 1 to 20						
Degranulation of mast cells		Pelvic tumor								
Growth of bacteria Synthesis of prostaglandin E	_	Malignant neoplasm of mal Male genital neoplasm	le -			or metastatic prostate can		part of the combination of	ug bicalutannue and	
Prostate Cancer and Tumors		Prostate Cancer and Tumor	are	0323018009 Mosby's Drug Consult, 13th Edition.						
Pelvic tumor Gastrointestinal neoplasia		Allergy	>	NCT00255268 Longitudinal, Randomized, Open and Prospective Clinical Trial to Evaluate the Efficacy of Continuous vs Intermittent Maximum Androgen Blockade (CMAB vs IMAB) With Goserelin-Bicalutamide Combination in the Treatment of Hormonal naïve With Metastatic Prostate Cancer ClinicalTrials.gov.						
Carditis		Release of L-glutamic acid Degranulation of mast cells		Source: Ingenuity Expert Findings						
Genital tract cancer Malignant neoplasm of male		Transmigration of mononuo		Triptorelin, an agonist of human GNRH1 protein, is in Phase 3 clinical trial as a part of the combination drug flutamide and triptorelin as components of a treatment for prostate cancer in human.						
Release of L-glutamic acid		Differentiation of liver Synthesis of fatty acid		NCT00003734		mised Comparison of Sho k Localized Prostate Cance		uvant Hormonal Therapy Prior to Radiation Therapy of		
Differentiation of liver Differentiation of liver cells		Carditis Differentiation of liver cells		16595220       Giampietro F, Sancilio S, Tiboni GM, Rana RA, Di Pietro R. Levels of apoptosis in human granulosa cells seem to be comparable after therapy with a gonadotropin-releasing hormone agonist or antagonist. Fertil Steril. 2006         Source: Ingenuity Expert Findings         Goserelin, an agonist of human GNRH1 protein, is in Phase 3 clinical trial as a part of the combination drug bicalutamide and goserelin acetate [goserelin] as components of a treatment for prostate cancer in human.         0323018009       Mosby's Drug Consult, 13th Edition.						
Allergy		Proliferation of pheochrom	c							
Transmigration of mononuc Proliferation of pheochrome	and the second se	Production of reactive oxyg Retinal degeneration	IE .							
Retinal degeneration		Synthesis of prostaglandin E	E.							
Production of reactive oxyge		Growth of bacteria		NCT00014586	Asympto		udy On Initial Antiandrogen leason) NO or Nx M0 Prosta		son With Watchful Waiting In ut Local Treatment With	

#### Product specific predictable effects on molecular network associated with encephalitis



6 of 9 deregulated genes lead to predicted inhibition of encephalitis

Effect not predicted

#### Eleutherococcus

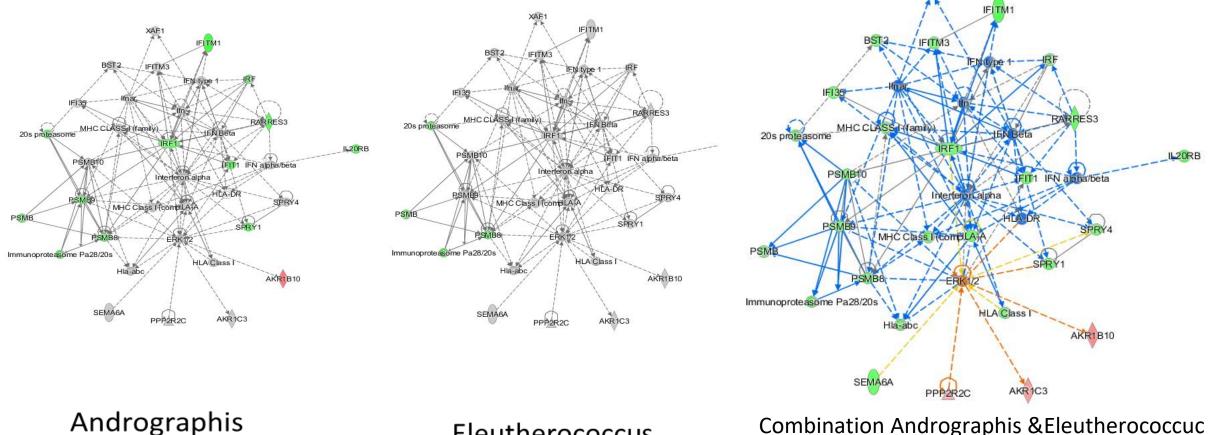
Only 1 gene is deregulated presumably with predicted inhibition of encephalitis

11 of 16 deregulated

genes lead to predicted

inhibition of encephalitis

Inhibitory effects of Andrographis, Eleutherococcus extracts and their combination (AE) on molecular network associated with anti-inflammatory response

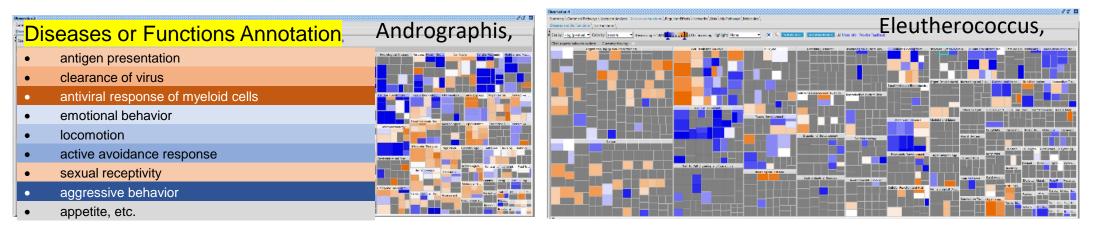


Combination Andrographis & Eleutherococcuc

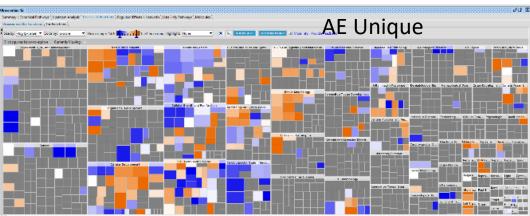
Eleutherococcus

#### Conditional "biological signatures" of Andrographis, Eleutherococcus and their combination (AE)

the color-coded heat-maps identify **functions and diseases** that are expected to increase or decrease.







#### Unique signature

- Unlike to chemical reactions, when the chemical properties of purified compounds remain the same as in their mixture with other chemicals, biological activity/signature of their combination differs from signatures of ingredients.
- Combination of two or more plant extracts in one provides a qualitatively new substance, quite similar but different from its constituents.
- In fact, it is a "offspring" unique biologically active substance, a hybrid of "parent" ingredients.
- The expectations to have a sum of biological activities from two ingredients are illusive. The biological activity of their combination could be qualitatively different.

## Conclusions related to observed synergistic and antagonistic interactions

- The specificity of a complex biological activity does not necessarily arise from the specificity of individual molecules, as these may act in different processes.
- Biological specificity results from the way in which these components assemble and function collectively.
- Component interactions, as well as the environment, gives rise to new features, such as network behavior, which are absent when considering the components.

# Potential effects of adaptogens in ageing and stress induced disorders

#### Inflammation – atherosclerosis

- Down regulation of CETP,
- Deregulation of GPCR,
- Neurodegeneration impaired cognitive functions (learning, memory, abstract thinking, planning, organizing)
  - Down regulation of cAMP
  - Down regulation of ESR1
  - Up-regulation of PKC
  - Up-regulation of serpine
  - Deregulation of GPCR,

- Metabolic disorders and energy metabolism—
  - Down regulation of cAMP
  - Inhibition of ATP metabolism
- Impaired apoptosis Cancer
  - Down regulation of ESR1, OLFM
  - Up regulation of MAPK, IP3, PLC, DAG, PI3K
  - Deregulation of GPCR
- Stress-induced mental and behavioral disorders
  - Down-regulation of serotonin 5-HT3 GPCR
  - Up regulation of IP3
  - Down regulation of ESR1

### New method of assessment of synergy of combinations of herbal extracts by transcriptome-wide microarray profiling

- Analysis of RNA microarray data from isolated cells and the comparison of the number of genes regulated by plant extracts and their fixed herbal formulation might be a useful tool/method for assessment of synergistic and antagonistic interactions of herbal extracts in human organism.
- Panossian A, Seo E-J, Wikman G. Efferth T. 2015. Synergy assessment of fixed combinations of Herba Andrographidis and Radix Eleutherococci extracts by transcriptome-wide microarray profiling. Phytomedicine, 22: 981-992
- Panossian, A., Hamm, R., Kadioglu, O., Wikman, G., Efferth, T., 2013. Synergy and antagonism of active constituents of ADAPT-232 on transcriptional level of metabolic regulation of isolated neuroglial cells. Front Neurosci. 7:16. http://journal.frontiersin.org/article/10.3389/fnins.2013.00016/abstract

#### Conclusions

- Analysis of effects of herbal extracts on RNA microarray profiles of isolated cell lines is a valuable tool of drug discovery, understanding the mechanisms of action of herbal drugs and their possible clinical benefits.
- Core Comparison Analysis, using:
  - the RNA sequencing or microarray data obtained in vitro experiments on isolated cells incubated with various herbal extracts, their combinations and purified compounds,
  - Integrative OMICS profiling database related to physiological functions and diseases, allows to predict their pharmacological activity and potential indications in medicine.