

Traditional Knowledge Base For Drug Discovery

EU – India Partnering Event

05.11.2009

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Strength of ISM in India

Particulars	Modern System	Indian System
Practitioners	2,40,000	5,64,476
Indoor Hospitals	5,767	2,607
Graduate medical Schools	110	284
Manufacturing Units	16,000	9,000
Turnover	8 billion US\$	1billion US\$

Recent Indian Initiatives

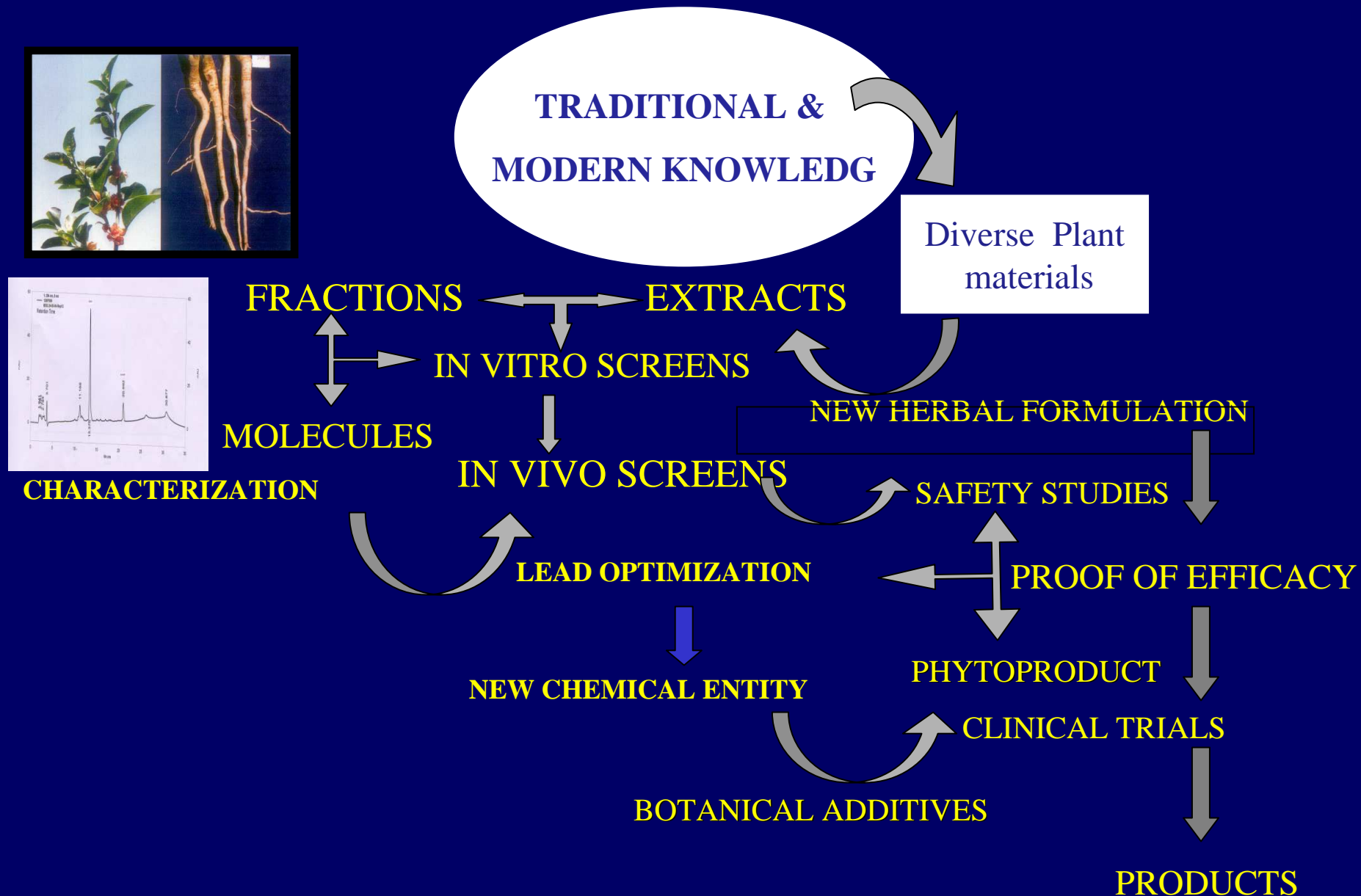
- TKDL (collection of millions of documents)
- GTP (Integrative Approach : Traditional doctors + Modern practitioners + Contemporary Scientists)
- Premier Product Certification (QCI)
- New Drug Delivery Approach for TM
- Reverse Pharmacology approach for clinical validation of modified but conceptually traditional formulations
- ICMR registry for clinical trials of TM and new botanicals

Quest of the time

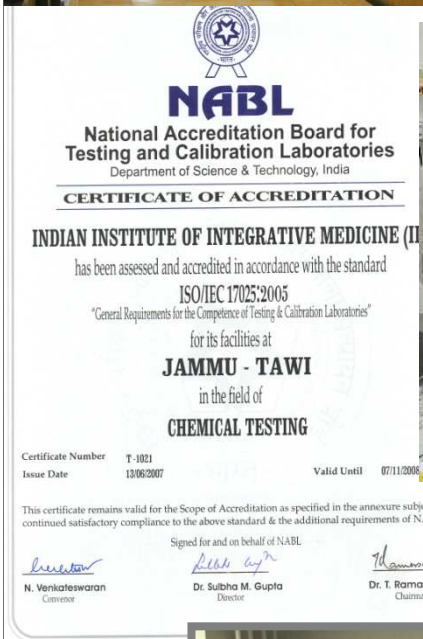
Products that are:

- Alternate/Complimentary
- Adjunct/Adjuvant
- Better (Validated Faster)
- Safer
- Affordable

Standardization & Discovery Platform



Analytical Tools

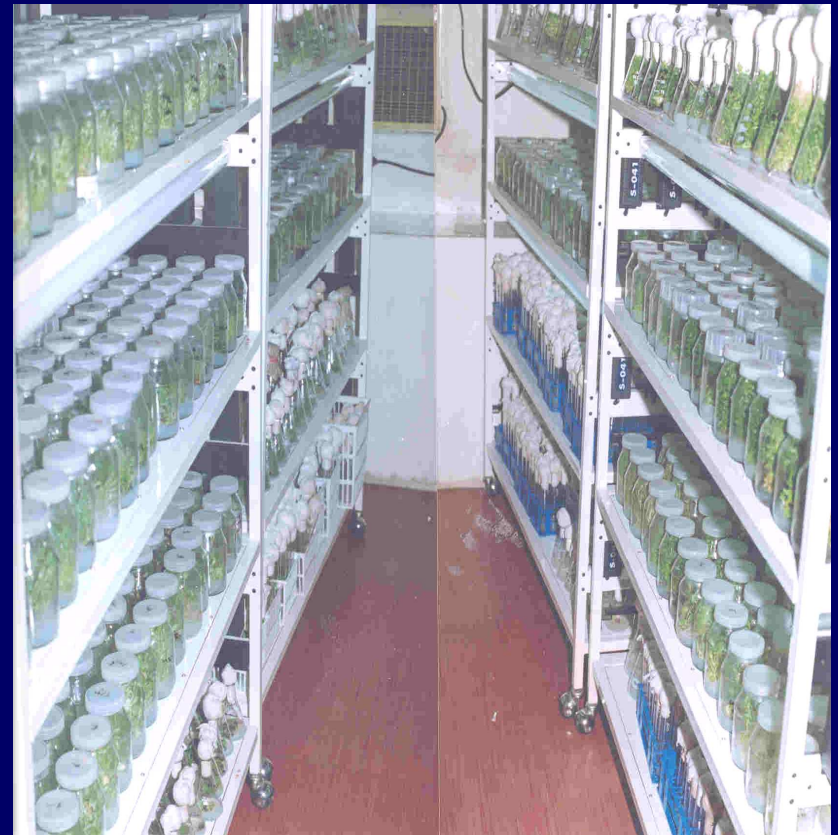


Characterization of Plant Product (Drug Substance)

- Chemical classes of the mixture
 - Polar, Medium Polar and Non-polar Compounds
- Isolation of Individual chemical constituents in each class
 - fractionation
 - Prep. HPLC
- Analytical tools
 - GC, LC, HPTLC, LC-MS, UPLC-MS, Hyphenated LC-NMR- MS
- *in vitro* and *in vivo* biological activity assays

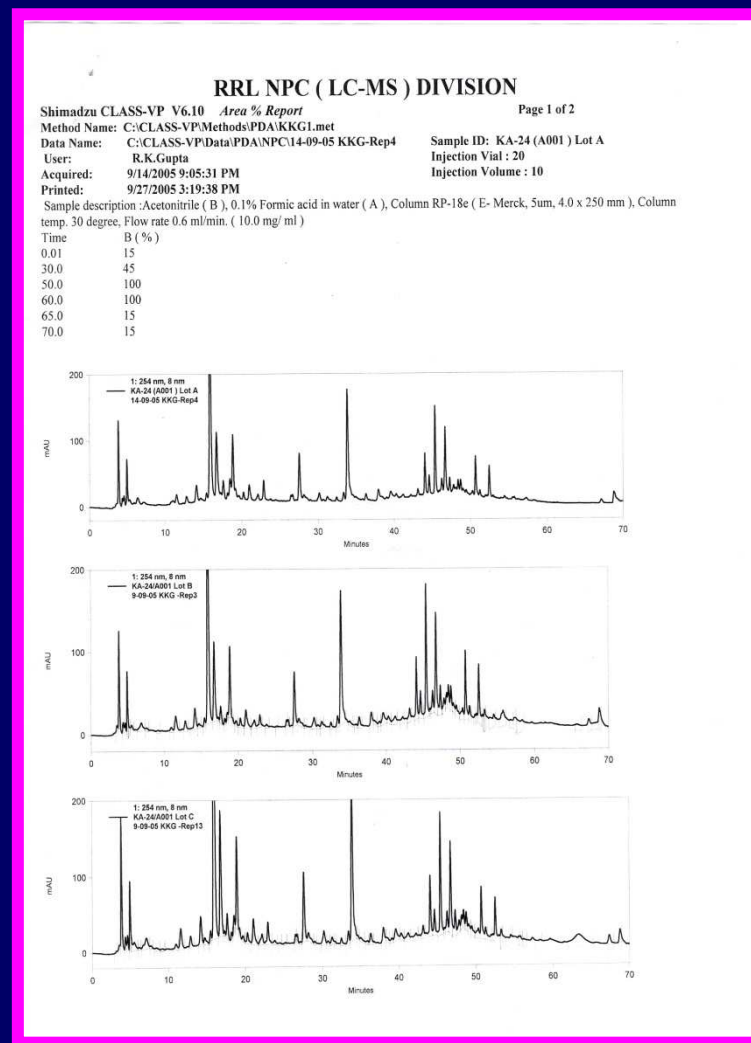
Lot-to-Lot Consistency

- Equivalence between material used for clinical trials and product for the market
- Marker based equivalence before and after manufacturing process
 - Physical Characteristics
 - Chemical finger print
 - Bio-assay
 - Formulation



Critical Elements to Assure Quality and Consistency

- Strict quality controls for (Chemistry and manufacture) of each plant raw material
- In-process controls
- Sound process validation
- Appropriate and adequate testing of final product and dosage form using validated test methods





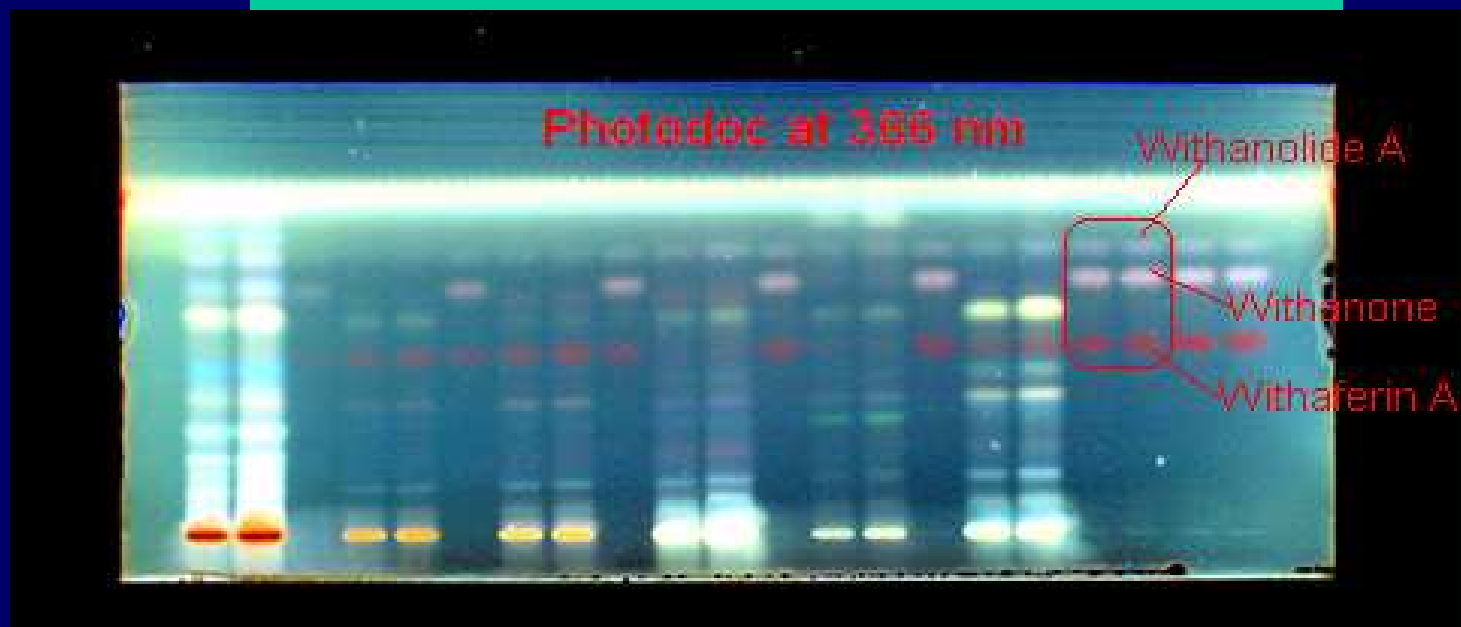
Withania somnifera

ACB002

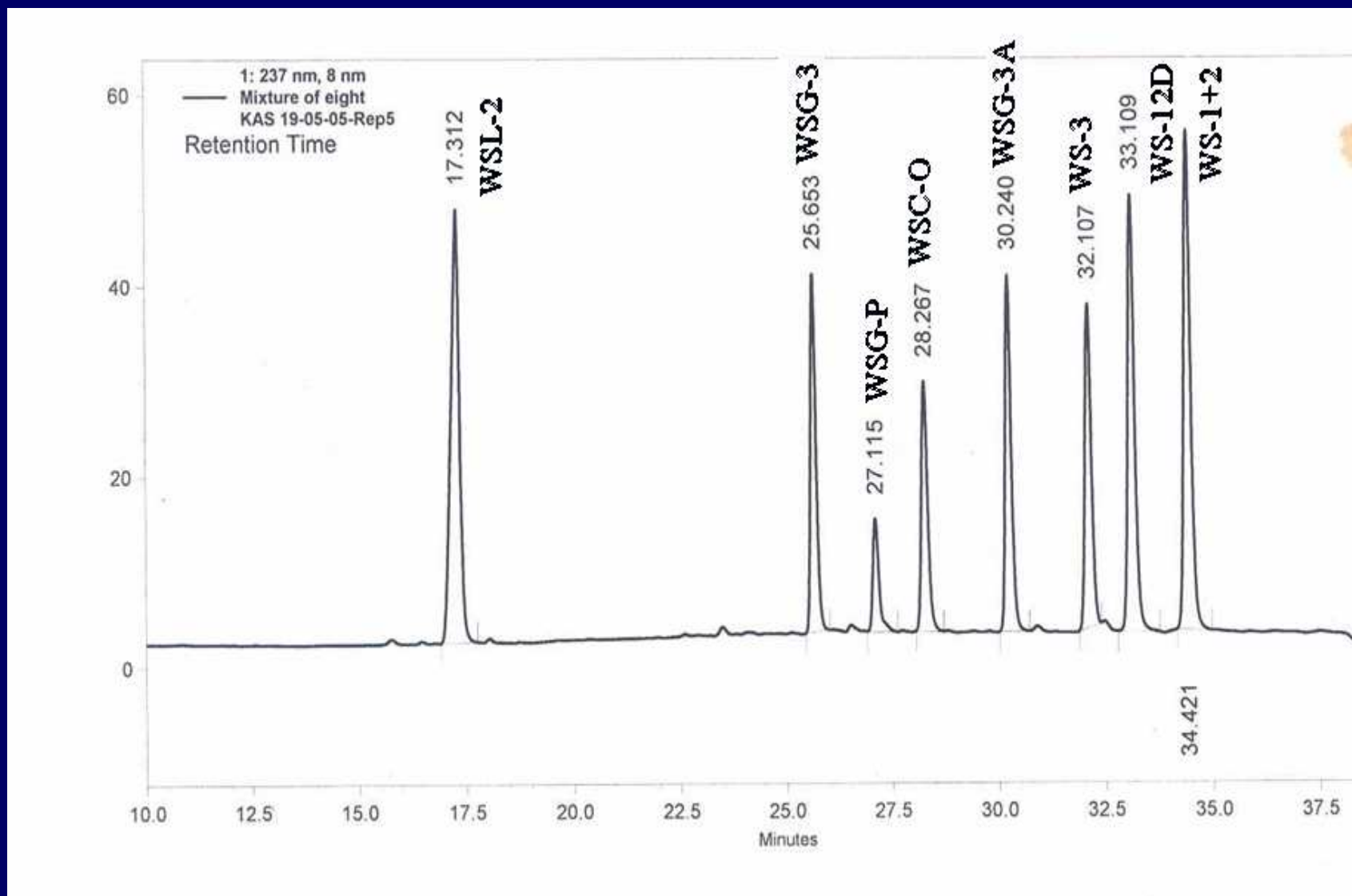


- a) a flowering plant
- b) flower (close up)
- c) flower and fruiting calyx
- d) inflated fruiting calyx (close up)
- e) plant in fruiting
- f) ripe berries
- g) seed

Withania somnifera A002 root extract

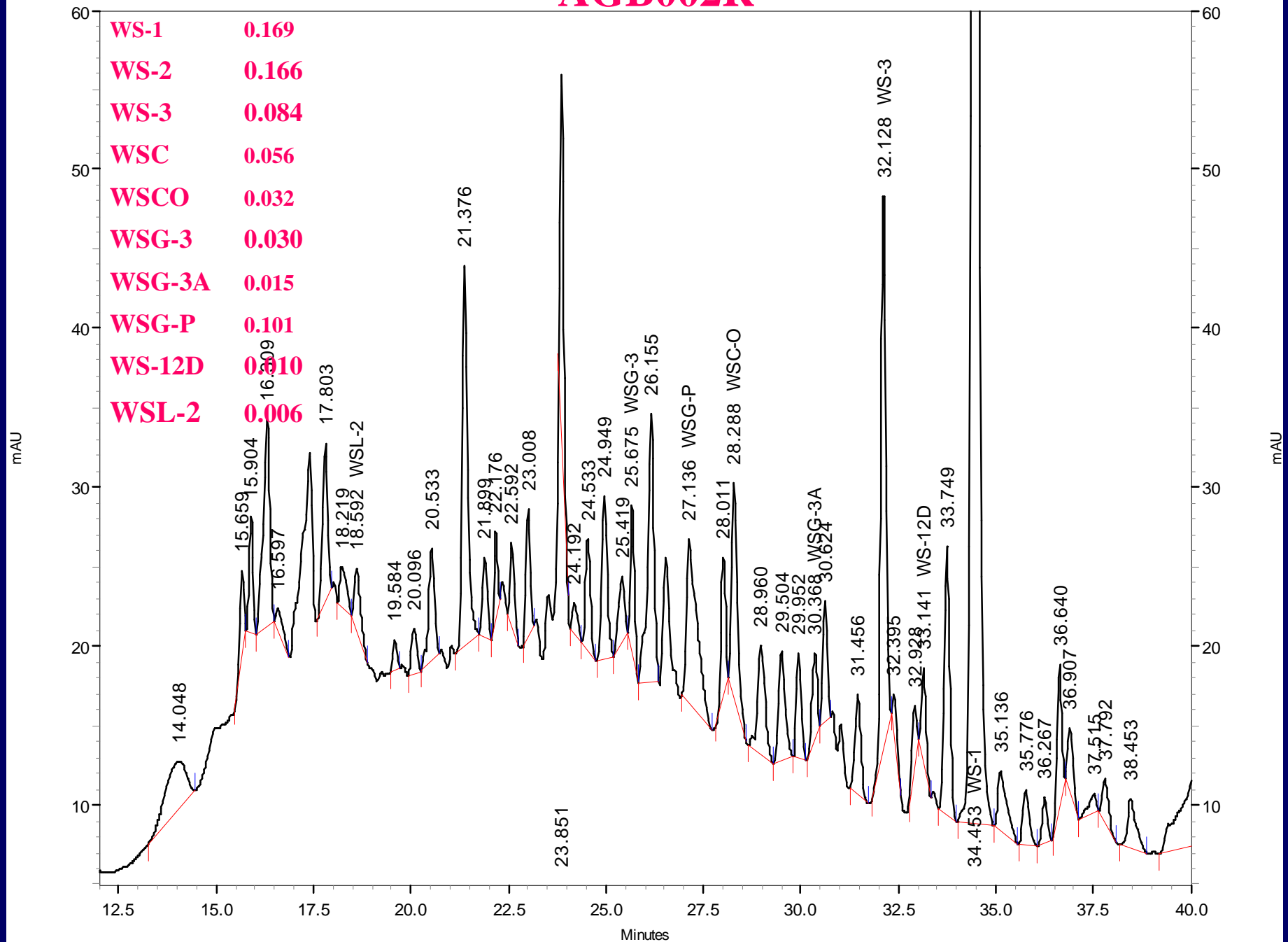


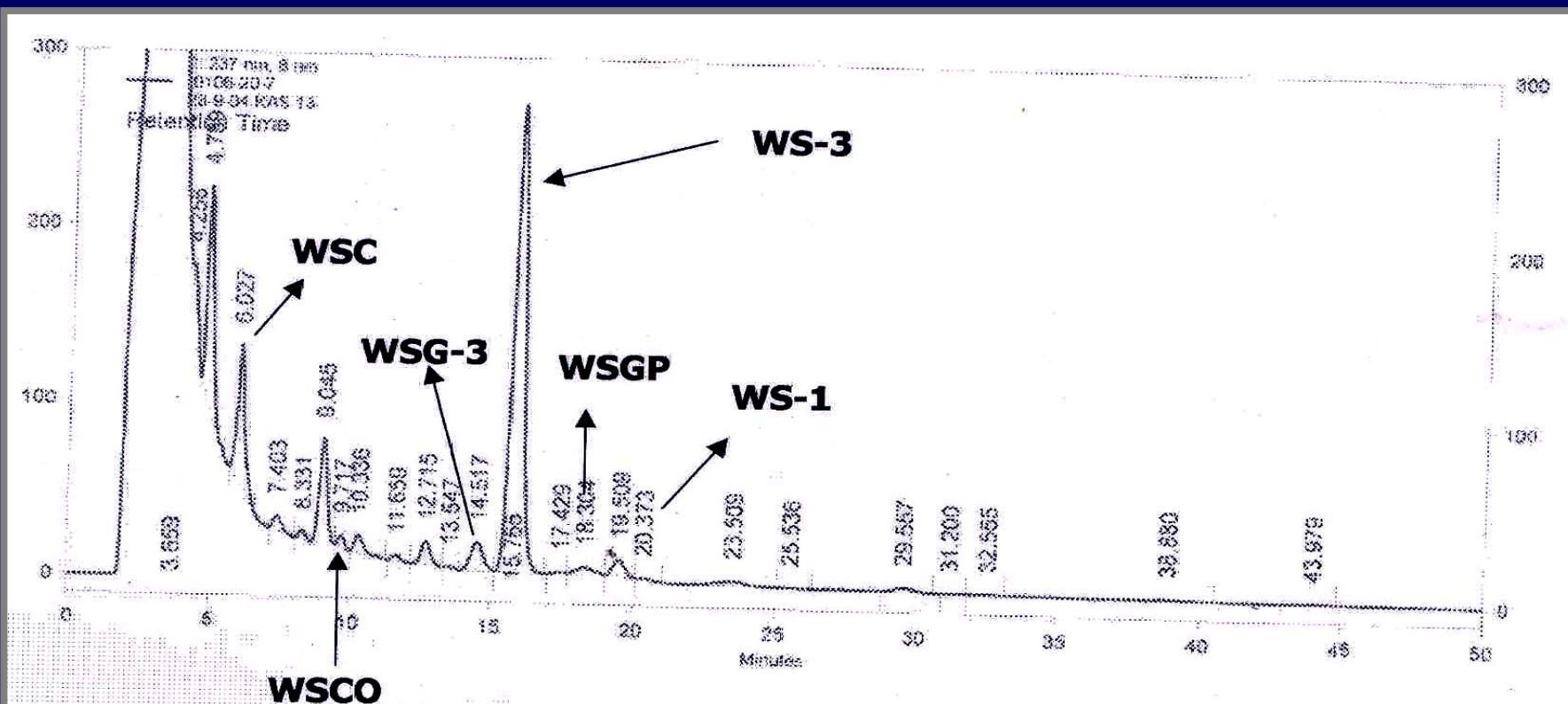
- Sample is applied in duplicate
- Mobile phase: Chloroform: Methanol(19:1)
- Plate is derivatized by Vanillin –boric acid reagent at 120°C for 10 minutes
- AGR1 PT/070806
- AGR2 PC/WS/RRL/06
- AGR3 Zandu Pharma
- AGR4 Ixoreal Biomedical Pvt.Ltd.
- AGR5 Om Pharma
- AGR6 Udaipur, Rajasthan



**HPLC-UV(DAD) Chromatogram (237nm) of withanolides,
Glycowithanolides in gradient conditions.**

AGB002R





HPLC profile of AGB055 L

CHEMICAL ANALYSIS OF ASHWAGANDHA SAMPLES ON THE
BASES OF WITHANOLIDES (FIVE) AND GLYCOWITHANOLIDES
(TWO)

Sample code	WS-1	WS-2	WS-3	WSC	WSCO	WSG-3	WSG-P
<u>AGB002L</u>	-	<u>0.043</u>	<u>0.164</u>	<u>0.026</u>	<u>0.046</u>	<u>0.016</u>	<u>0.003</u>
AGB002R	<u>0.013</u>	<u>0.013</u>	<u>0.008</u>	<u>0.007</u>	-	<u>0.008</u>	-
AGB009L	0.025	0.126	0.217	0.013	0.028	0.050	0.025
AGB009R	0.117	0.005	0.007	0.009	0.001	0.010	0.012
AGB015L	0.005	-	0.567	-	0.020	0.059	0.021
AGB015R	0.015	-	0.044	0.004	0.001	0.042	0.017
AGB025L	-	-	0.174	0.002	-	0.012	-
AGB025R	0.019	-	0.007	-	-	0.003	0.017
AGB030L	0.009	0.127	0.248	0.125	0.147	0.084	0.007
AGB030R	0.029	0.011	0.002	0.002	-	-	0.006

AGB002 (Root Extract, 50% alcoholic)

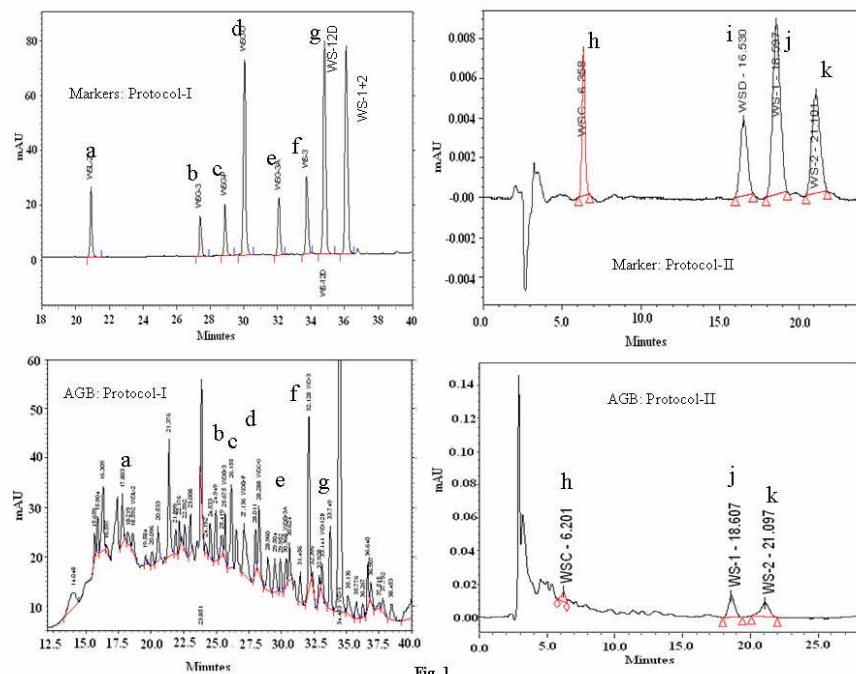
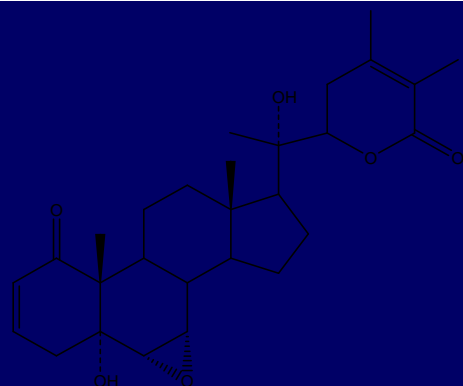


Fig.1



Withanolide-A (WS-1)

Chemical composition of AGB002R

Marker	(g/100g)
a. Unidentified withanolide (WSL-2)	0.076±0.005
b. Withanoside-IV (WSG-3)	0.079±0.005
c. Physagulin (WSGP)	0.103±0.003
d. 27-hydroxy withanone (WSCO)	0.022±0.002
e. Withanoside-VI (WSG-3A)	0.024±0.003
f. Withaferin-A (WS-3)	0.017±0.004
g. 12-deoxywithastramonolide(WS-12D)	0.023±0.003
h. Withastramonolide (WSC)	0.017±0.002
i. Withanolide-D (WSD)	ND
j. Withanolide-A (WS-1)	1.340±0.006
k. Withanone (WS-2)	0.315±0.005

**Acceptance / Rejection Criterion
For
some of the selected Ayurvedic plants
Based on estimation of active constituents**

Sr. No.	Plant name	Extractive Value 50 % Aq. Alc. Extract	Markers	Acceptable range of Markers (in plant material based on HPLC analysis of 50 % Alc. Ext.)
1	Ashwagandha <i>Withania sominifera</i>, Roots)	16.5 - 18 %	Withaferin A Withanolide A Withanone	0.005 – 0.020 % 0.020 – 0.101 % 0.002 – 0.010 %
2	Amalaki <i>(Phyllanthus emblica</i>, Fruit coat)	30.35 %	Gallic acid Ellagic acid	1.05 - 2.02 % 0.75 – 1.60 %
3	Haldi <i>(Curcuma longa</i> Rhizomes)	10.0 – 12%	Curcumin Demethoxy-curcumin Bisdemethoxy- curcumin	0.40 – 1.52 % 0.02 – 0.36 % 0.05 – 0.35 %
4	Brahmi <i>(Bacopa moneri</i> Whole herb)	12 – 14 %	Bacoside A₂ Bacoside A₃	0.42 – 0.85 %

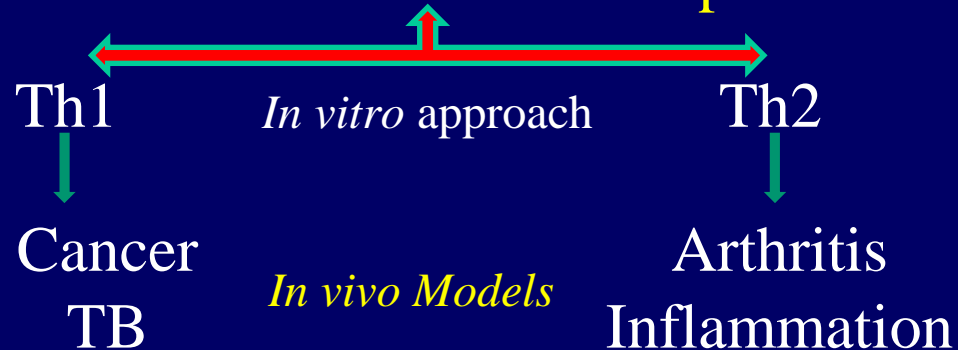
Converting traditional knowledge into evidence based medicines

Chemical Standardization & the mechanism of action of a therapeutics formulation

Based on *Withania somnifera* (Ashwagandha)

Study Design

Mechanism of immune response



Cancer cell cytotoxicity



Withania somnifera, Biological Mechanism and therapeutic application



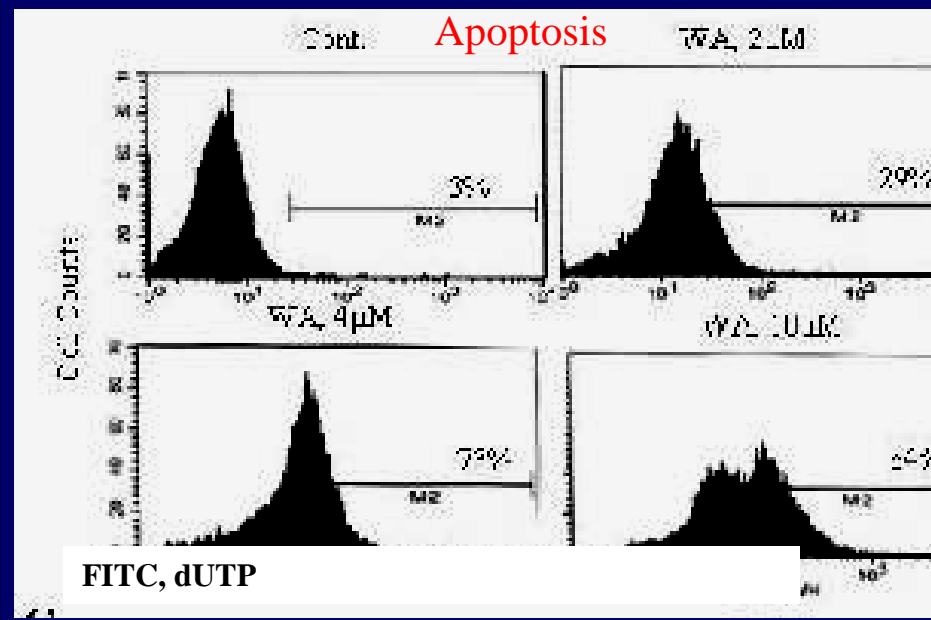
- ❖ Potential Th1 immune stimulator
- ❖ Withanolide A, identified
- ❖ Reduced microbial load mTB infected mice models
- ❖ Currently under patient trials

- ❖ Potential anticancer agent
- ❖ Withaferin A, Identified
- ❖ Reduced tumor growth in mice tumor models
- ❖ Chemically standardized herbal formulation under clinical trials

Isolation, Identification and Mechanism of action of *Withaferin A* (WA) in cancer cell death

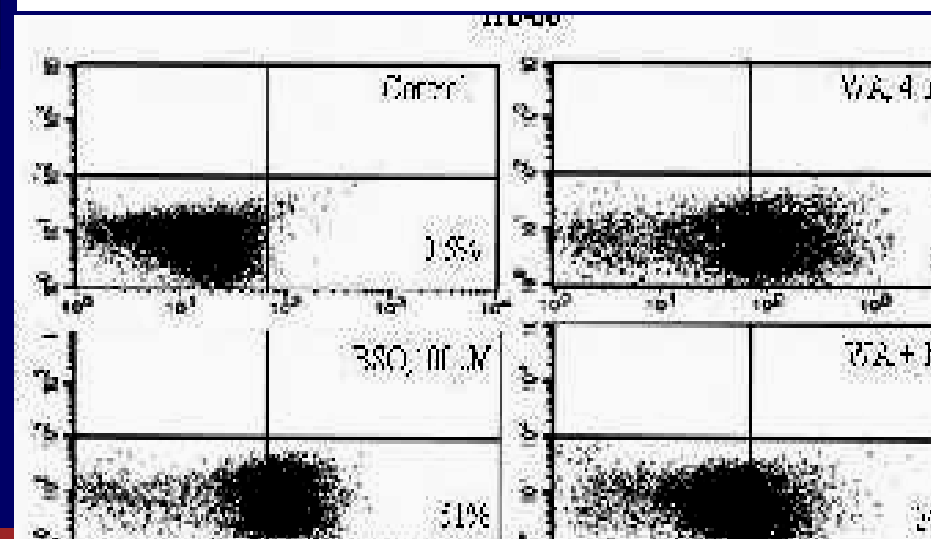
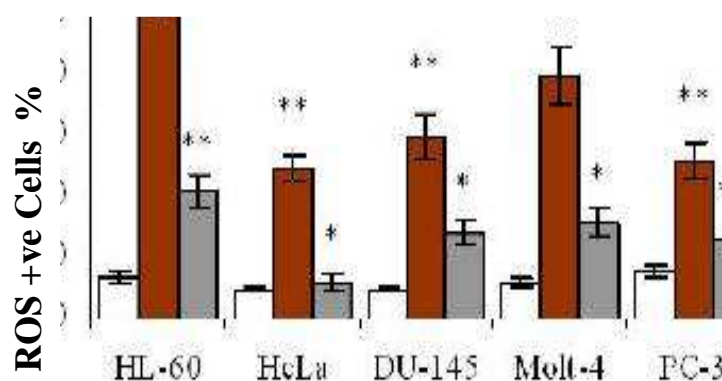
Leaf Extract (1:1, Aqu:Alcoh)

Markers	X(-)%	IC ₅₀
Withanolide-A (WS-1)	0.060	>10μM
Withanone (WS-2)	1.282	>10μM
Withaferin-A (WS-3)	1.910	0.5μM
Withastramonolide (WSC)	0.406	>10μM
27-hydroxy withanone (WSCO)	0.079	>10μM
Withanolide-D (WSD)	0.073	>10μM
Withanoside-IV (WSG-3)	0.671	>10μM
Withanoside-VI (WSG-3A)	0.213	>10μM
Physagulin (WSGP)	0.204	>10μM
12-deoxywithastramonolide (WS-12D)	0.019	>10μM
Unidentified withanolide (WSL-2)	3.253	>10μM



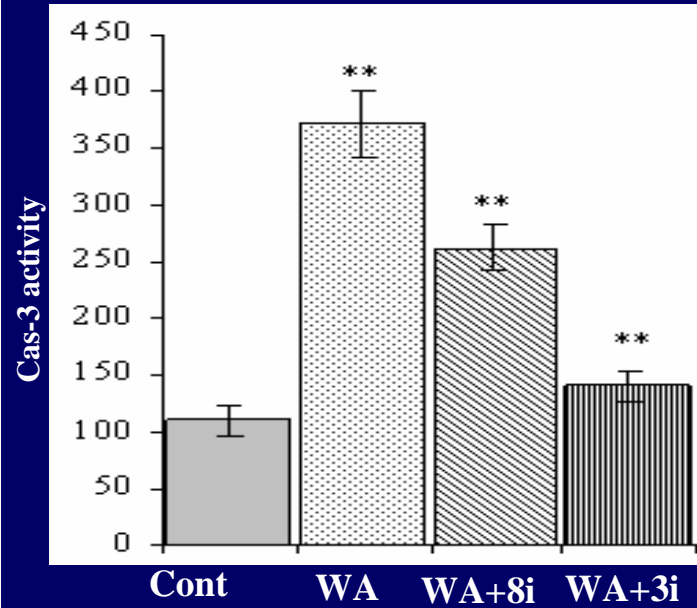
WA induce oxidative burst in cancer cells

NAC rescue WA induced ROS

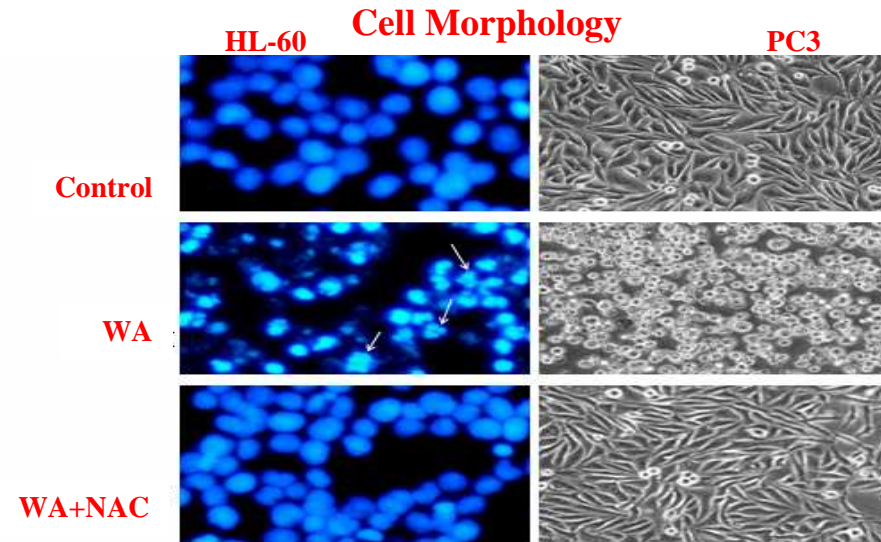
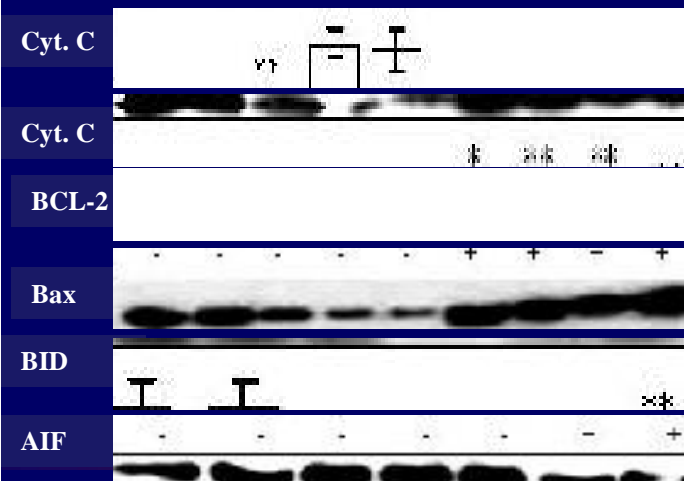


Withaferin A

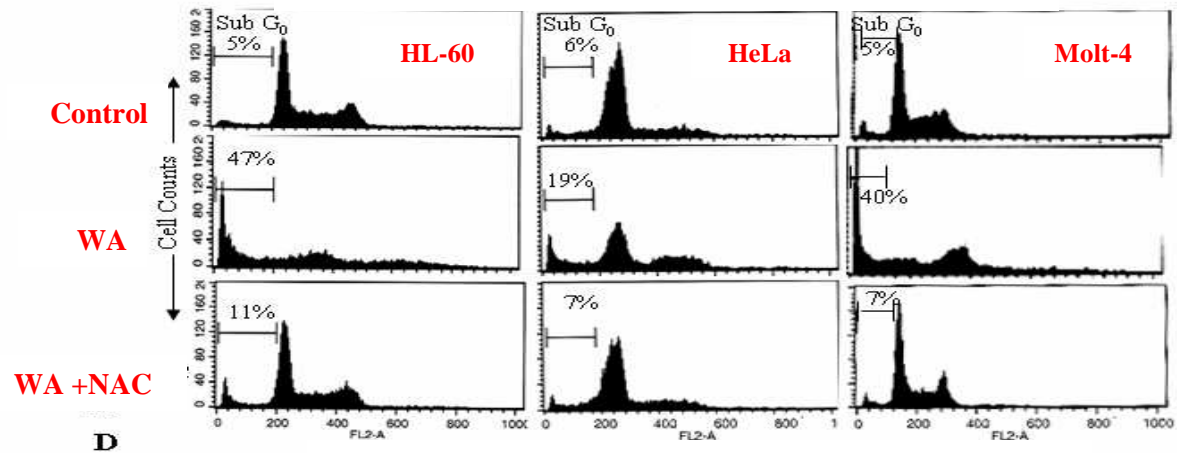
Oxidative stress mediated cell death by WA is rescued by NAC



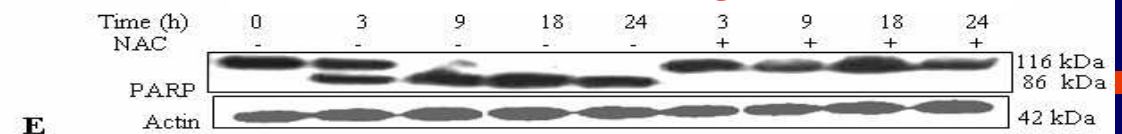
Pro apoptotic proteins



Sub G1 phase

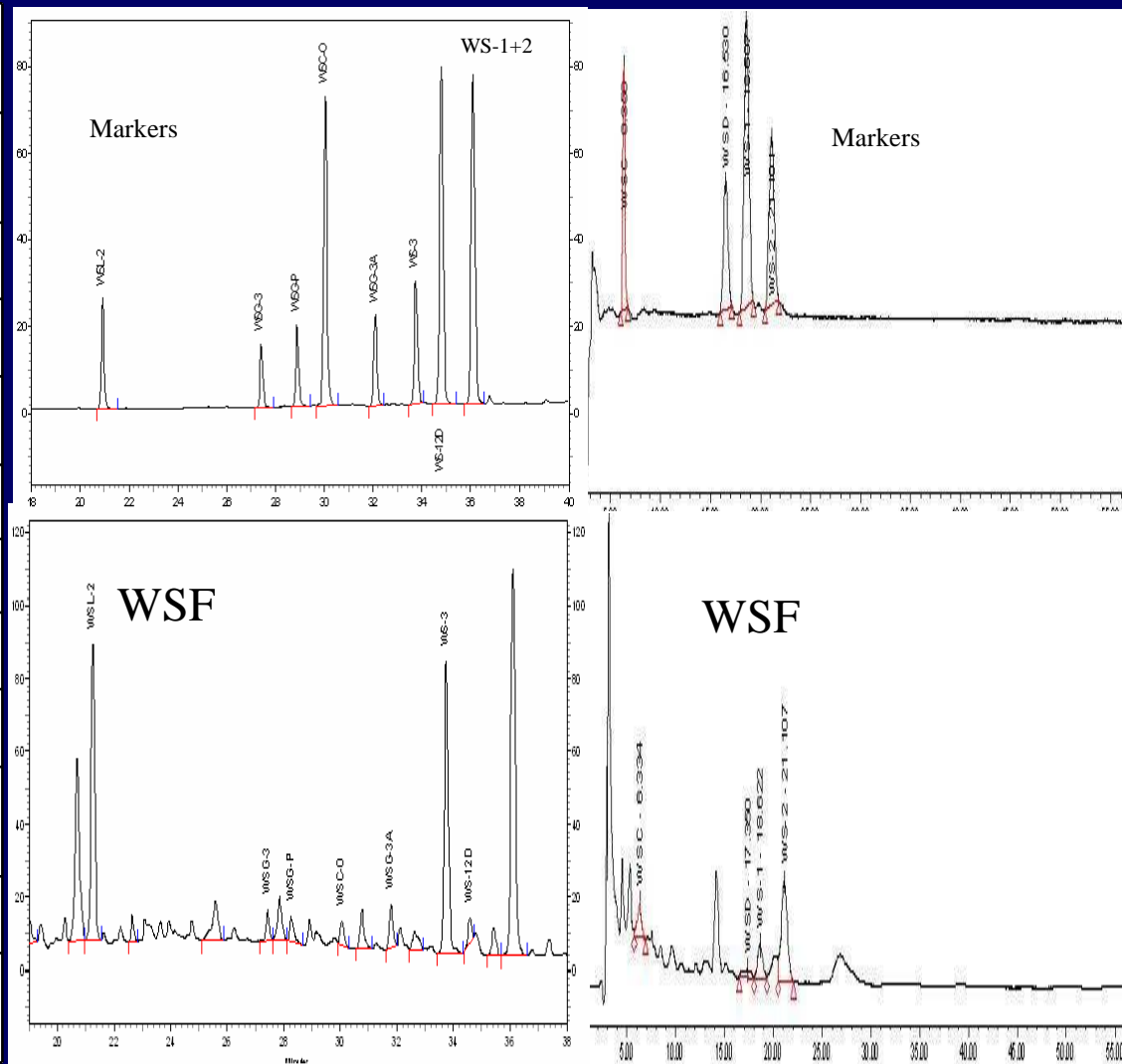


PARP Cleavage



Chemical Standardization of WSF

Chemical composition of the formulation, % dry weight of the extract		
Markers used for chemoprofiling	g/100g	S.D.
Withanolide A (WS – 1)	0.689	0.006
Withanone (WS – 2)	0.815	0.006
Withaferin-A (WS – 3)	0.608	0.005
Withastramonolide (WSC)	0.333	0.005
27-Hydroxy withanone (WSCO)	0.032	0.003
Withanolide-D (WSD)	0.041	0.003
Withanoside-IV (WSG-3)	0.267	0.004
Withanoside – VI (WSG- 3A)	0.075	0.003
Physagulin-D (WSGP)	0.098	0.004
12-Deoxywithastramonolide (WS-12D)	0.011	0.001

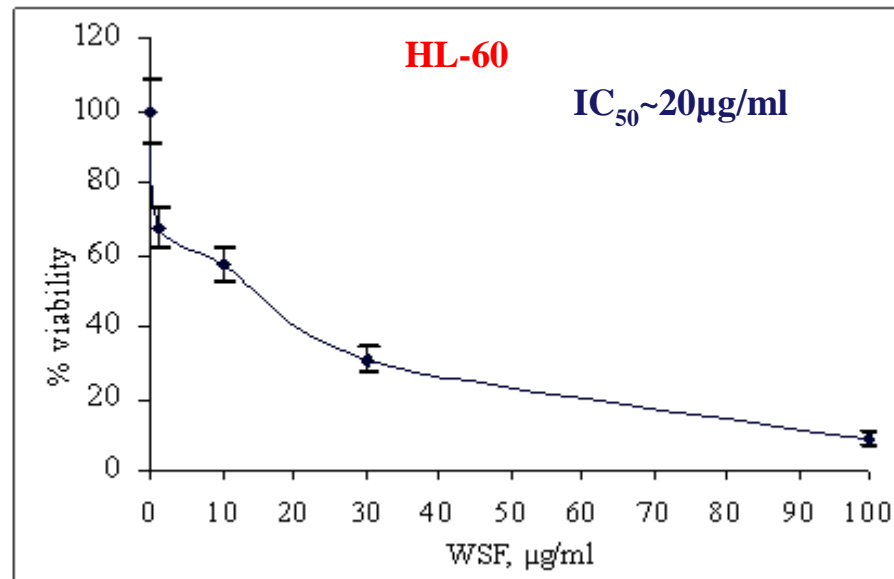
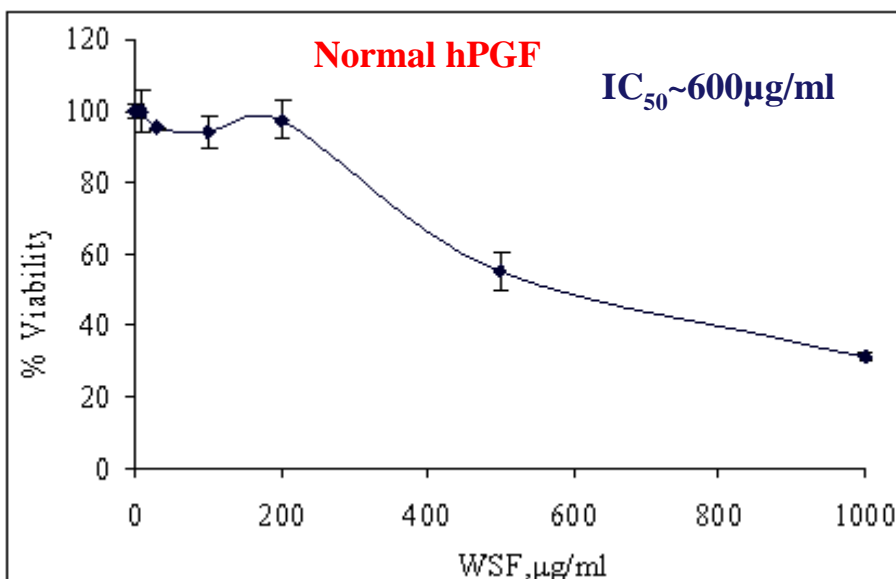


HPLC protocol-I

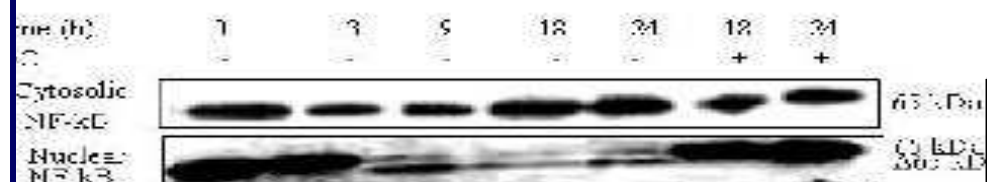
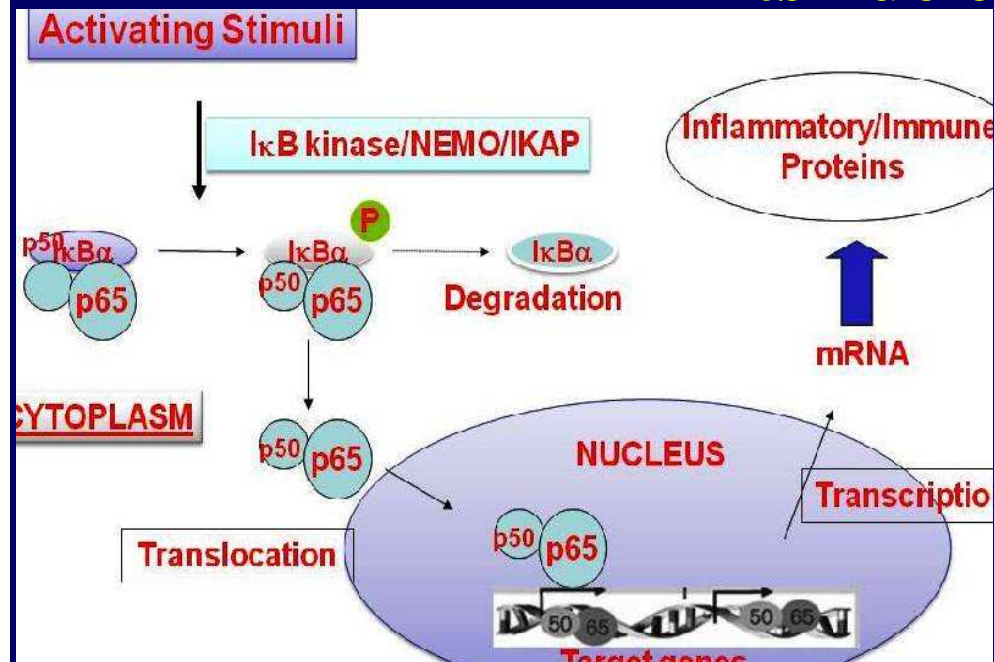
HPLC protocol-II

In vitro cytotoxicity of WSF against a panel of human cancer cell lines

WSF μg/ml	Colon					Liver	Lung		Neuroblas -toma	CNS	Prostate	
	HCT- 15	SW- 620	502 713	COL O-205	HT- 29	Hep- G2	HOP -62	A- 549	IMR-32	SK- N-SH	DU- 145	PC-3
1		36	0	6	8	61	6	7	29	37	3	5
3		68	0	9	9	77	10	10	63	54	11	21
10	81	80	0	22	34	91	35	38	69	87	38	44
30	97	92	69	36	56	91	56	77	90	94	80	87
100	100	92	88	96	90	100	77	85	99	95	99	99



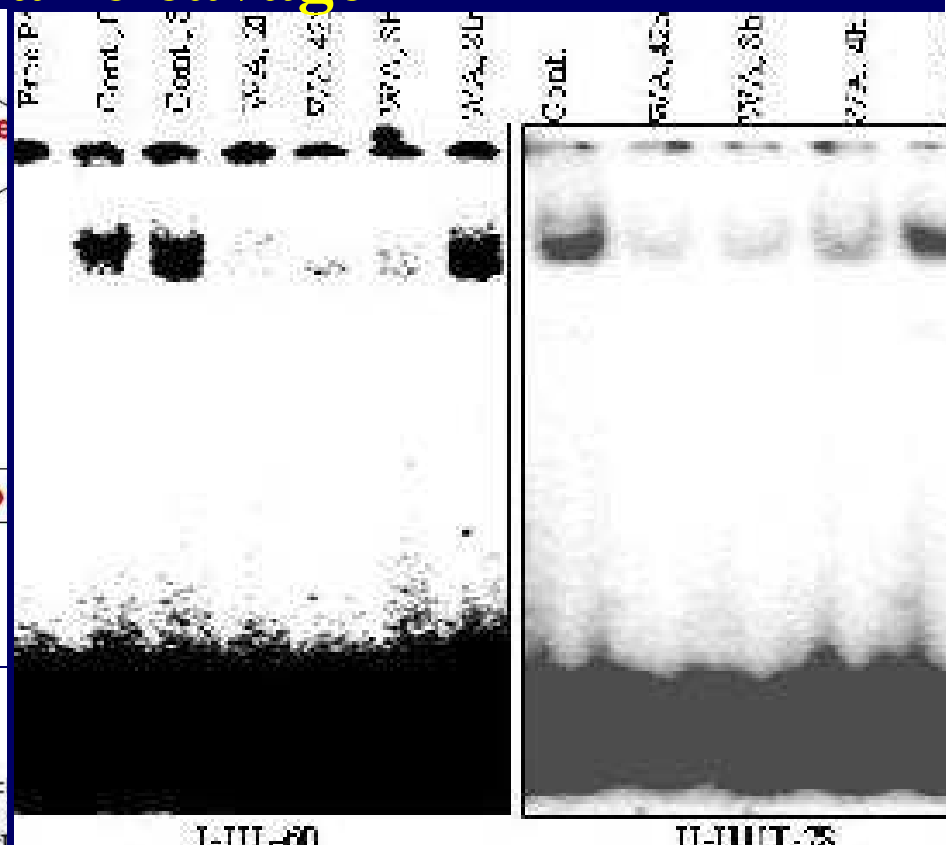
WA Inhibit translocation of NFk-B/p65 and cause its nuclear cleavage



0.4 μM

0 - -

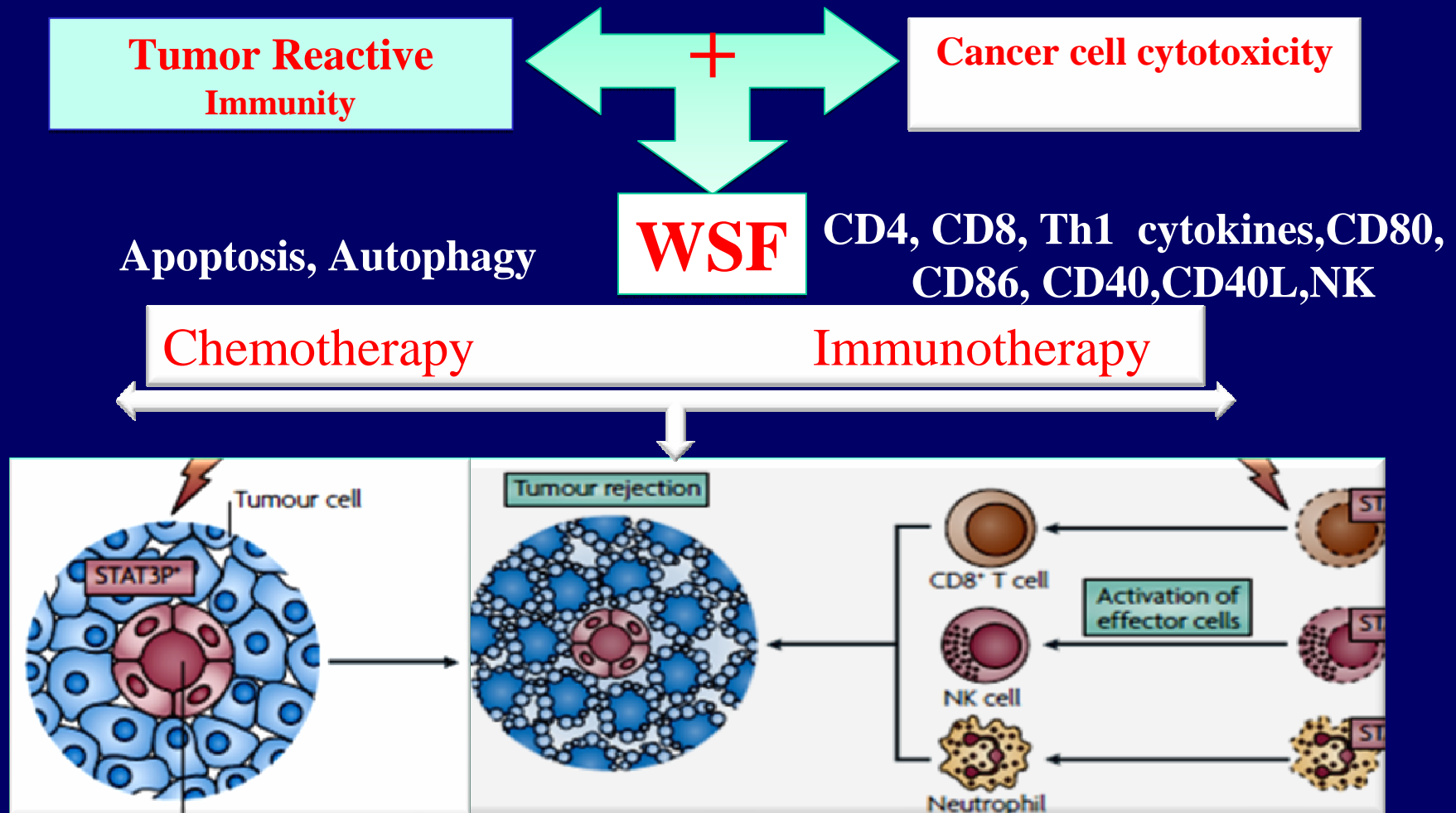
+



Tumor growth inhibition in Mice tumor models

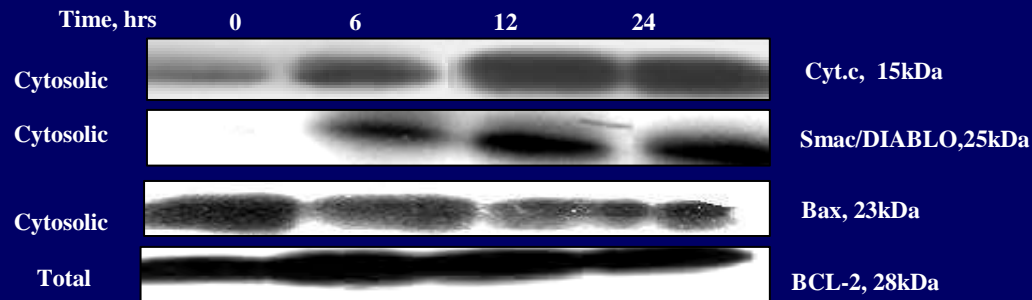
Withaferin A (i,p) (mg/kg)	Sarcoma-180 (ascites)	12	49*
	Sarcoma-180 (solid)	12	41

WSF as chemo-immunotherapeutic agent for the treatment of cancer: Multi target approach

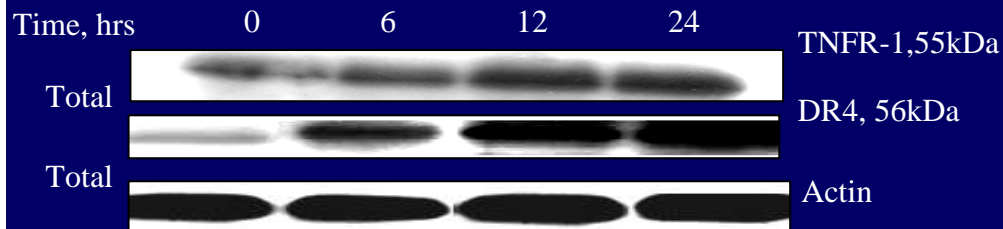


Schematic representation of mechanism of apoptosis induced by WSF in HL-60 cells

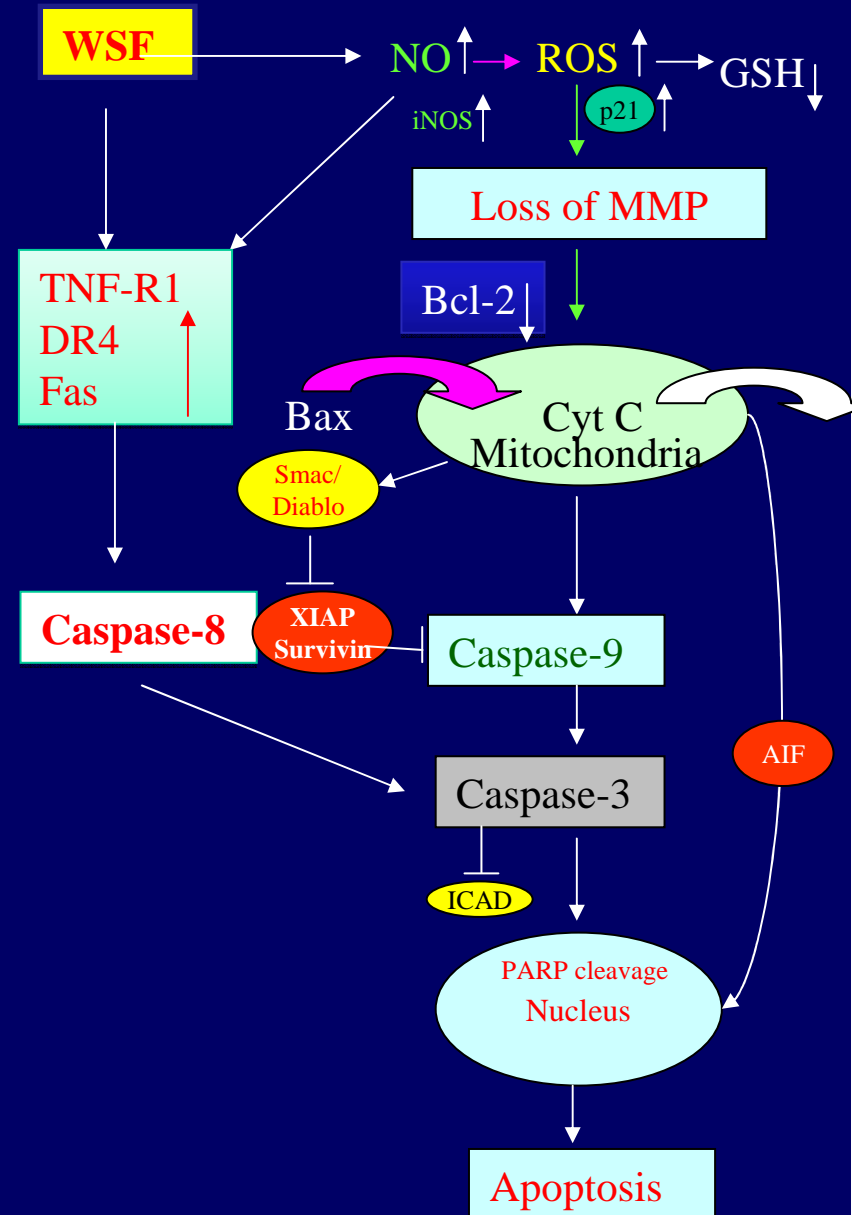
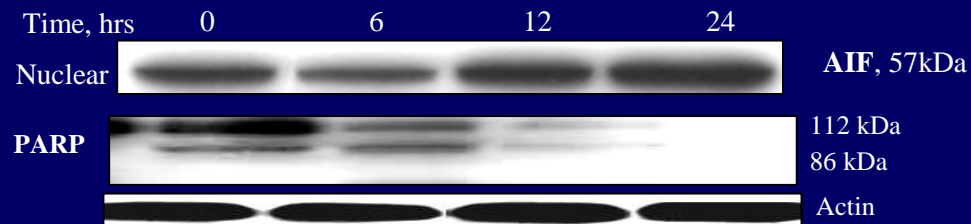
Mitochondrial Pathway



Receptor Mediated Pathway

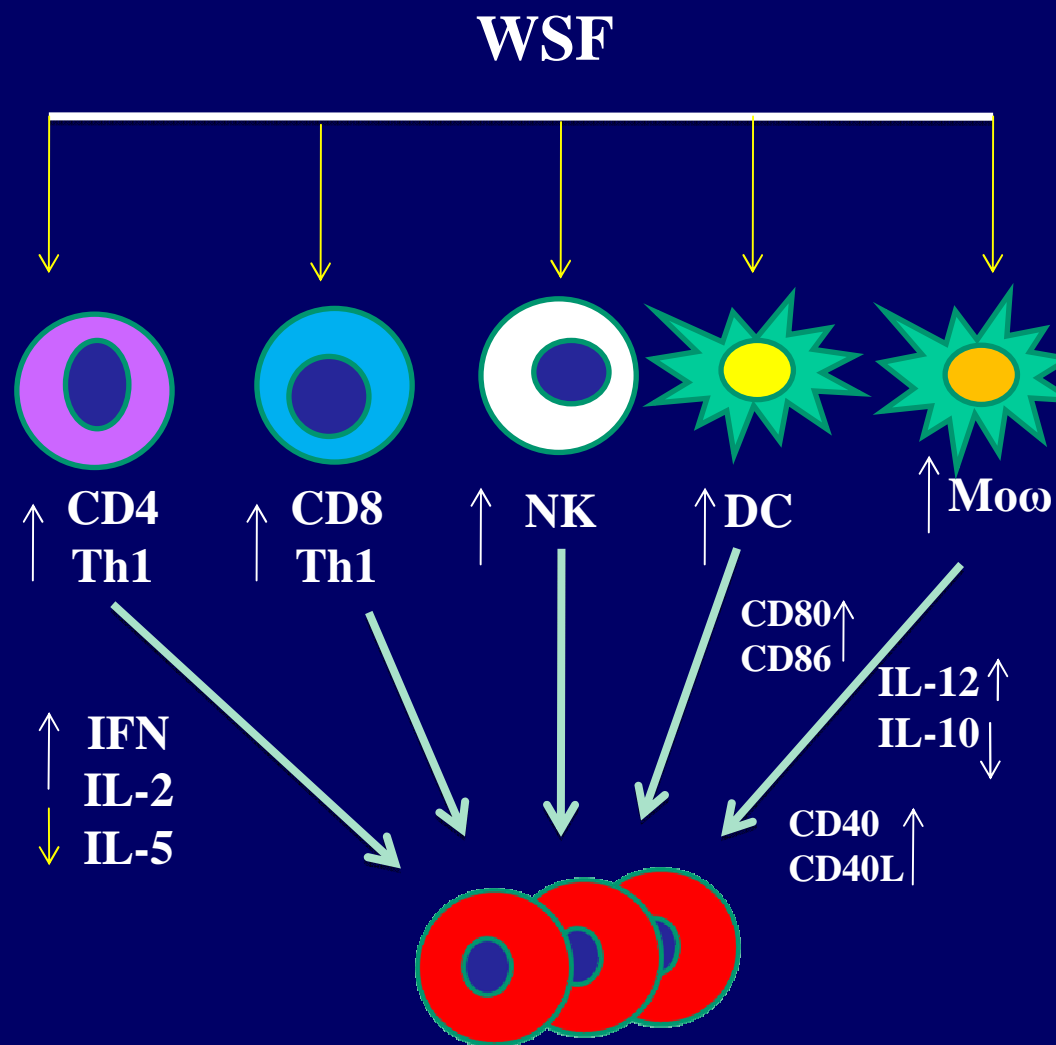


Execution



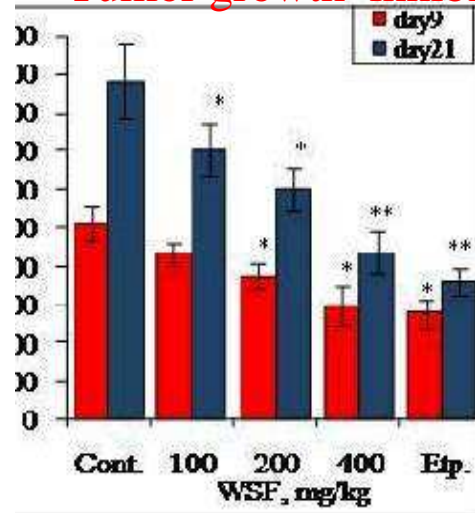
WSF inhibits tumor growth in mice with simultaneous activation of tumor reactive immunity

WSF	Dose-mg/kg (i,p)	Tumor growth inhibition (%)
<u>Ehrlich Ascitic Tumor (solid)</u>		
Control	-	
WSF	100	41
5FU	22	62
<u>Sarcoma 180 (Solid)</u>		
Control	-	
WSF	150	52
5FU	22	51
<u>Ehrlich Ascitic Carcinoma (suspension)</u>		
Control	-	
WSF	150	76
WSF	350 (oral)	61
5FU	22	95%

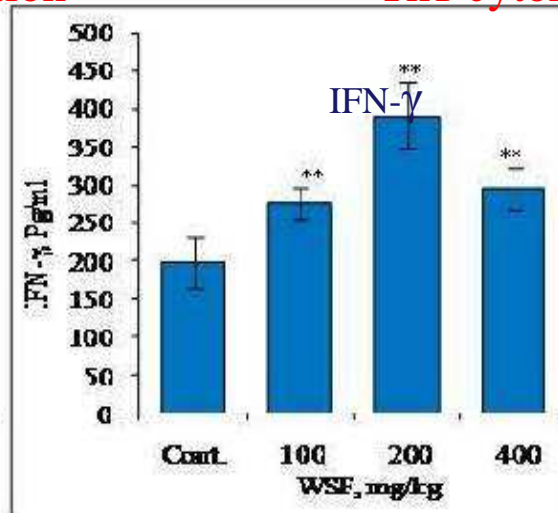


Tumor reactive Immune activation by WSF in Tumor bearing Mice

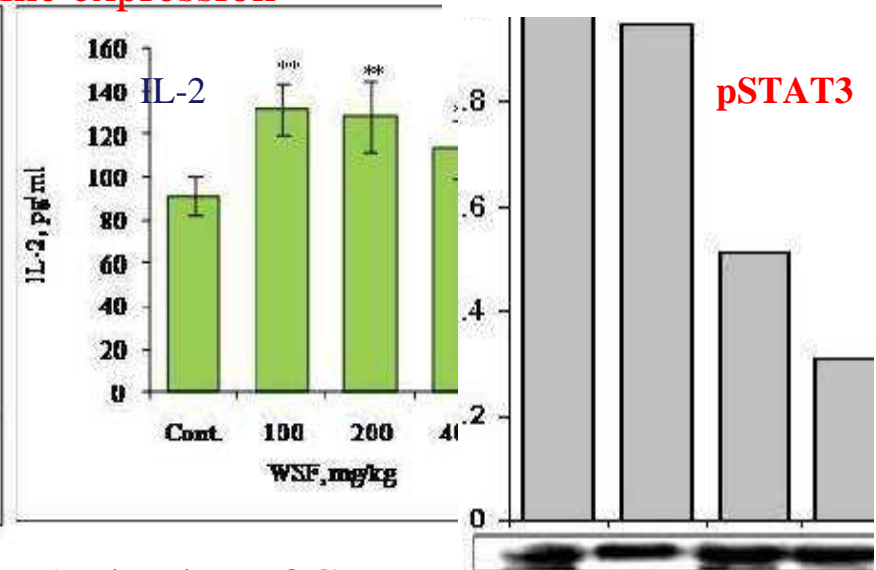
Tumor growth inhibition



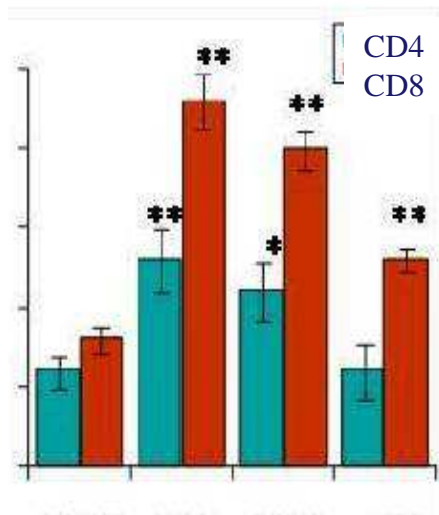
Th1 cytokine expression



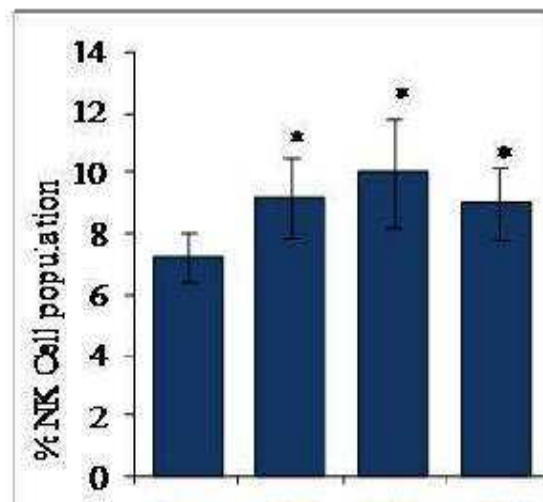
Cont 100 200 400



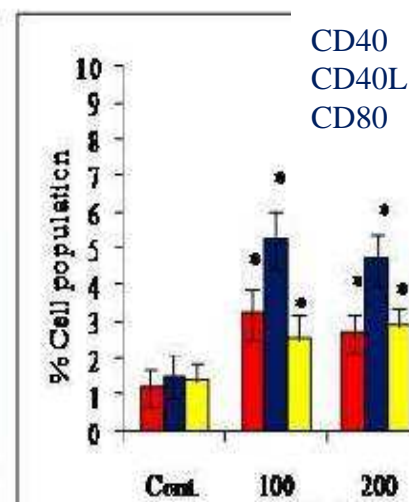
T cell proliferation



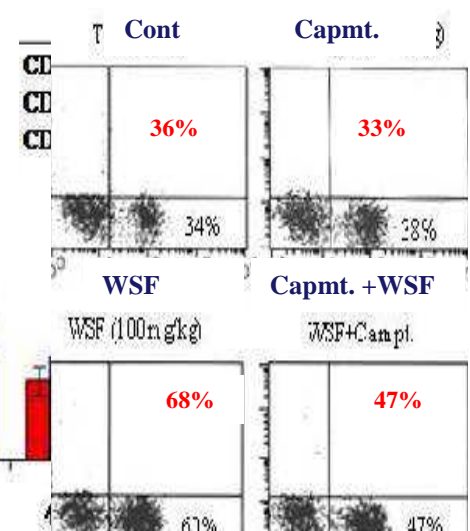
NK Cell activation



Activation of Co-stimulatory Signals



CD3⁺cells; Capmt+WSF



IPR:

A synergistic non-toxic herbal formulation extracted from Withania Somnifera useful for anti-cancer and Th1-dominant immune up regulating activities. Qazi, G.N.; Singh, Jaswant; Malik, Fayaz; et al., [0202NF2006/IN Dt 06/09/2006; 1321DEL 2007 Dt. 19/06/2007]

Publication:

European Journal of Cancer (2009) 45(8):1494-509. Fayaz Malik, et al.,
Immune modulation and apoptosis induction:
Two sides of anti tumoral activity of a
standardized herbal formulation of *Withania
somnifera*

Publications

Authors	Title of the paper	Journal
<u>Fayaz Malik</u> * <u>Sheema Khan</u> , Jaswant singh	Molecular insight into the immune up-regulatory properties of the leaf extract of Ashwagandha and identification of Th1 immunostimulatory chemical entity	<i>Vaccine</i> (2009) In press
Shashi Bhushan, <u>Fayaz Malik</u> Ajay Kumar, e Jaswant singh t al.	Activation of p53/p21/PUMA alliance and disruption of PI-3/Akt in multimodal targeting of apoptotic signaling cascades in cervical cancer cells by a pentacyclic triterpenediol from <i>Boswellia serrata</i>	<i>Molecular Carcinogenesis</i> (2009) (In press)
<u>Fayaz Malik</u> , Ajay Kumar, Shashi Bhushan, Dilip Mondhe, and Jaswant Singh et al.,	Immune modulation and apoptosis induction: Two sides of anti tumoral activity of a standardized herbal formulation of <i>Withania somnifera</i>	<i>European Journal of Cancer</i> (2009) 45(8):1494-509.
<u>Fayaz Malik</u> , Ajay Kumar, Shashi Bhushan, Sheema Khan, Jaswant Singh et al.,	Reactive oxygen species generation and mitochondrial dysfunction in the apoptotic cell death of human myeloid leukemia HL-60 cells by a dietary compound withaferin A with concomitant protection by <i>N</i> -acetyl cysteine	<i>Apoptosis</i> (2007) 12:2115–2133
<u>Fayaz Malik</u> , Jaswant Singh ,Anamika Khajuria , Krishan A. Suri et al.,	A standardized root extract of <i>Withania somnifera</i> and its major constituent withanolide-A elicit humoral and cell-mediated immune responses by up regulation of Th1-dominant polarization in BALB/c mice.	<i>Life sciences</i> (2007) 80:1525-1538
Shashi Bhushan, Ajay Kumar, <u>Fayaz Malik</u> , Samar Singh et al	A triterpenediol from <i>Boswellia serrata</i> induces apoptosis through both the intrinsic and extrinsic apoptotic pathways in human leukemia HL-60 cells	<i>Apoptosis</i> (2007) 12:1911–1926
Ajay Kumara, <u>Fayaz Malik</u> , Shashi Bhushan, Vijay K. Sethi, Ashok K. Shahi, et al.,	An essential oil and its major constituent isointermedeol induce apoptosis by increased expression of mitochondrial cytochrome c and apical death receptors in human leukaemia HL-60 cells	<i>Chemico Biological Interactions</i> (2008) 171(3):332-347

Publications

Authors	Title of the paper	Journal
Anamika Khajuria, Amit Gupta, <u>Fayaz Malik</u> , Surjeet Singh, G Qazi	A new vaccine adjuvant (BOS 2000) a potent enhancer mixed Th1/Th2 immune responses in mice immunized with HBsAg.	<i>Vaccine</i> (2007) 25: 4586–4594
Anamika Khajuria, Amit Gupta, Surjeet Singh, <u>Fayaz Malik</u> , Jaswant Singh, G Qazi	RLJ-NE-299A: A new plant based vaccine adjuvant.	<i>Vaccine</i> (2007) 25: 2706-2715
Anamika Khajuria, Amit Gupta, <u>Fayaz Malik</u> , Jaswant Singh, G Qazi	Immunomodulatory activity of biopolymeric fraction BOS 2000 from <i>Boswellia serrata</i>	<i>Phototherapy Research</i> (2008) 3:340-348.
<u>Fayaz Malik</u> , Jaswant Singh, A Bhatia and G Qazi	Multiple therapeutic target based novel herbal formulation with anticancer and immunostimulatory activities (Abstract)	<i>European Journal of Cancer, Supplements</i> (2007) Vol.5(4) pg. 89

Abstracts

<u>Fayaz Malik</u> , et al.	A Novel standardized plant based formulation bearing anticancer and Th1 immune up regulatory properties: Promising cancer therapeutics	EMBO work shop at Zaragoza (2008).
<u>Fayaz Malik</u> , et al.	Withanolide induces apoptosis in HL-60 through mitochondrial dysfunction, caspase activation and NFκ-B suppression	15th ECDO at SLOVENIA 2007
<u>Fayaz Malik</u> , et al.	A standardized extract of Ashwagandha induce apoptosis in HL-60 cells through ROS generation and stimulate Th1-response in mice	14th ECDO at ITALY 2006
<u>Fayaz Malik</u> , et al.,	Withaferin A Induced Oxidative Stress Requires the Intrinsic Pathway of Apoptosis in the Death of Human Myeloid Leukemia HL-60 cells, and Protection by N-acetyl Cysteine	ICSB USA 2007

Patents

1.A Plant based vaccine adjuvant. A. Khajuria, A. Gupta, S.Singh, F. Malik, Jaswant Singh, KL Bedi, KA Suri, NK Satti, OP Suri, GN Qazi, VK Srinivas, Gopinathan, K Ella. EP 1 837 027 A1

2.Induction of apoptosis in cancer cells by a natural product from *Boswellia* species for its usefulness as anti-cancer agent. G.N.Qazi,S.C.Taneja, Jaswant Singh, A.K. Saxena, V.K.Sethi, D.M.Mondhe, B.K.Kapahi, S.Bhushan, S.S.Andotra, Samar.Singh, B.Shah, S.Singh, H.C.Pal, F. Malik, A.Kumar, M. Sharma. [0151NF2006 Dt.31/05/2006] (0570 DEL2007 Dt. 16/03/07)

3.Semi-synthetic sesquiterpene lactone parthenin compound useful for anticancer activity. Qazi, Ghulam Nabi, Taneja Subash Chandra.; Singh, Jaswant; Saxena Ajit Kumar; Sethi Vijay Kumar; Shah Bhahwal Ali, Kumar Ajay, Andotra Samar Singh, Malik, Fayaz; Muthiah Shanmugavel, Agarwal, Satyam Kumar [(0207NF-2006/IN Dt. 11/09/2006; 0839 DEL2008 Dt. 31/03/2008)]

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Thanks