Kastrat de Geneeskunde
Fakultas Kedokteran Universitas Sebelas Maret
Surakarta

6 SKP

# One Day National Symposium

Solo Paragon | Minggu, 11 November 2012

MedsMotion 2012
Medical Sebelas Maret Scientific Competition

# Actual Comprehensive Database: Invention, Therapies, and Regulation on HERB MEDICINES

Indonesia memiliki 30.000 jenis tanaman dan 7.000 di antaranya memiliki khasiat sebagai obat. Pemanfaatan tanaman untuk mengobati penyakit bukan menjadi rahasia lagi. Akan tetapi para dokter, apoteker, dan farmasi belum banyak ikut andil dalam mengembangkan potensi ini. Bagaimana sebenarnya aplikasi herbal di berbagai aspek bidang kesehatan? Temukan jawabannya di Simposium MedsMotion 2012!

# *Aplication of Molecular Biology to support Herbal Medicine*

# Aplikasi Biologi Molekuler dalam Mendukung Penggunaan Obat Hebal

Prof. Sismindari Apt. SU.,PhD
Faculty of Pharmacy UGM

# OUTLINE

- INTRODUCTION
- APPLICATIONS ON
  - Drug Discovery
  - Activity and Mechanism of Action
- CONCLUSIONS

# Introduction

- "**Herbal Medicines**" →plant remedies which have medicinal uses.

- Discovery by trial and error
- Passed on from generation to generation through word of mouth

# Phytochemicals


- No magic, diets high in fruits, grains, legumes reduce the risk of a number of diseases, including cancer, diabetes, high blood pressure.
- Phytochemicals are the biologically active substances in plants that are responsible for giving them natural disease resistance, color, and flavor

# Urgent need to study medicinal plants

The utility of plants in current therapy

The rapid destruction of our tropical rainforests threatens the development of potentially useful drugs

There has been a rush to develop synthetic medicines based on plant medicines, but often the synthetic medicines don't work as well as the original plant medicines.

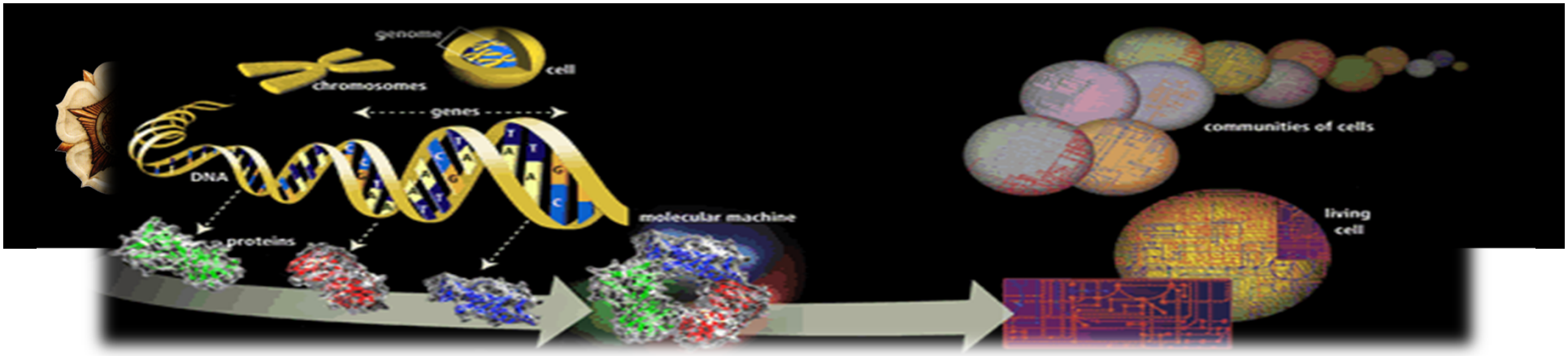
For example – quinine and malaria

# Herbal Medicines

| No | Plant | Drugs | Used for |
|---|---|---|---|
| 1 | *Ephedra sinica* | Efedrin | Anti asthma |
| 2 | *Cinchona ledgeriana* | Quinine | Malaria |
| 3 | *Digitalis purpurea* | Digoxin | Cardiac |
| 4 | Kulit batang | Aspirin | Analgetik, antipyretik |
| 5 | Catharanthus roseus | Vincristine | anticancer |
| 6 | *Taxus brevifolia* | Taxol | anticancer |
| 7 | *Trichosanthes kirilowii* | Thrichosantin | anticancer |
| 8 | Curcuma longa | Curcumin | antioxidant |

# *Digitalis purpurea*

- *Healing the Heart*
- Effective in treating heart problems
- Digoxin – cardiac glycoside
- Have strong effect on cardiac muscle
- Improve blood circulation
  - Relieve fluid retention
  - Kidney function

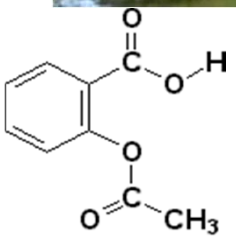


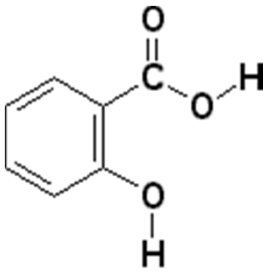

**Molecular biology** → concerns on understanding the interactions between the various systems of a cell, including **DNA, RNA and protein synthesis** and learning how these interactions are regulated.

***Human Genome Project* has been completed**

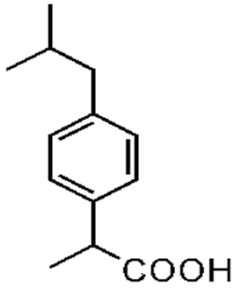
*Identification of Molecule Target,*
*Analysis of activity & mechanism of action*

# Traditional Methods





Aspirin



Ibuprofen

natural (plant-derived) treatment for illness

↓

isolation of active compound (small, organic)

⇓

Synthesis of compound

⇓

Structure manipulation to get better drug

(greater efficacy, fewer side effects)

Melissa Passino,Structural Bioinformatics in Drug Discovery

# Modern Methods of Drug Discovery

What's different?


- Drug discovery process begins with a *disease* (rather than a treatment)
- Use disease model to pinpoint relevant genetic/biological components (i.e. possible drug targets)

# Modern Drug Discovery

Melissa Passino,Structural Bioinformatics in Drug Discovery

*disease* → genetic/biological target

↓

discovery of a "lead" molecule

**- design assay to measure function of target**

**- use assay to look for modulators of target's function**

↓

high throughput screen (HTS)

**- to identify "hits" (compounds with binding in low nM to low µM range)**

# Biological Research in 21st Century

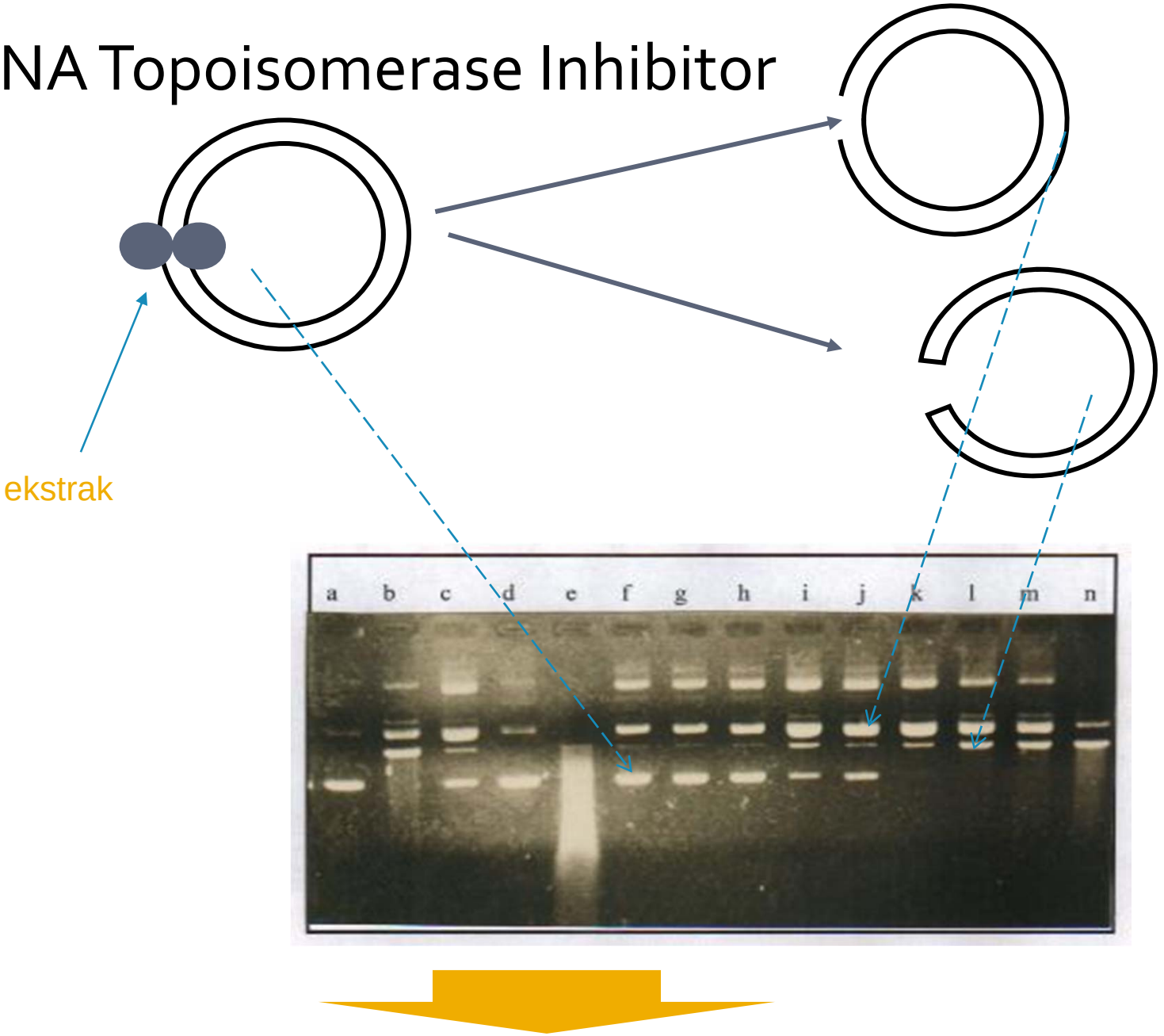
" The new paradigm, now emerging is that all the 'genes' will be known (in the sense of being resident in databases available electronically), and that the starting "point of a biological investigation will be theoretical."

**- Walter Gilbert**

# Screening of Herbal Medicine

- DNA Replication Inhibitor
  - DNA Topoisomerase : enzymes that control DNA topology and perform essential functions at several different steps in replication
- Protein Synthesis Inhibitor
  - RNA-N-glycosidase activity

# DNA Topoisomerase Inhibitor



- the bark and stem of *Camptotheca acuminata*

→ Camptothecin

(Pommier et al, 2003)

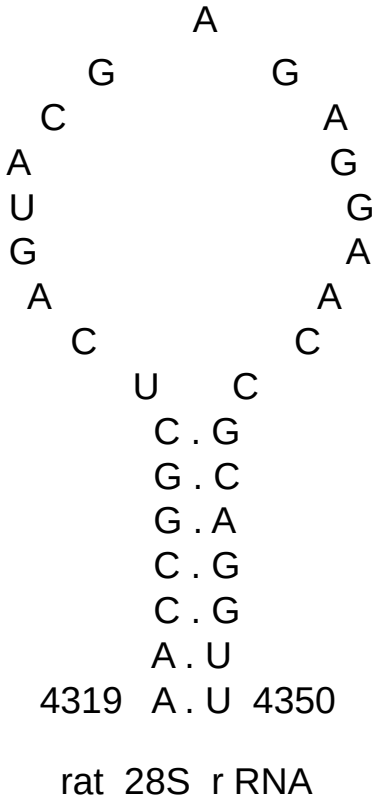
- sambiloto (*Andrographis paniculata* Nees)
- benalu teh (*Scurula arthopurpurea)*

(Sukardiman et al.,2002)

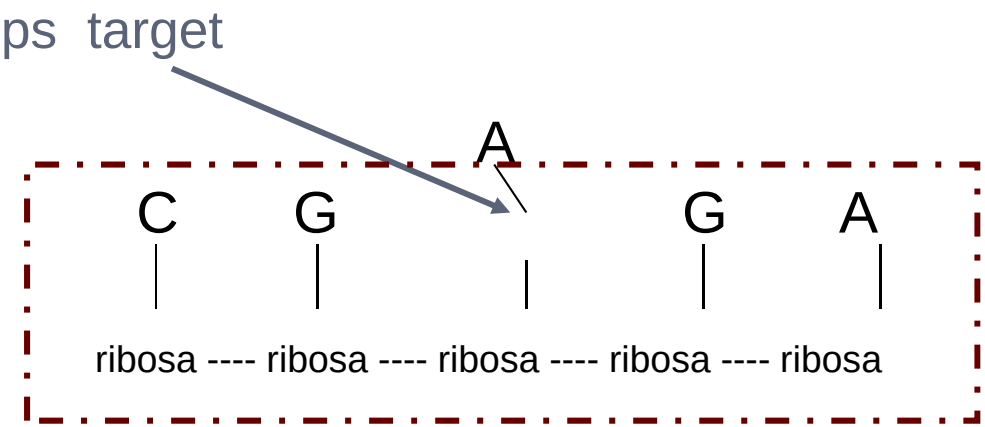
- *Erythrina fusca*

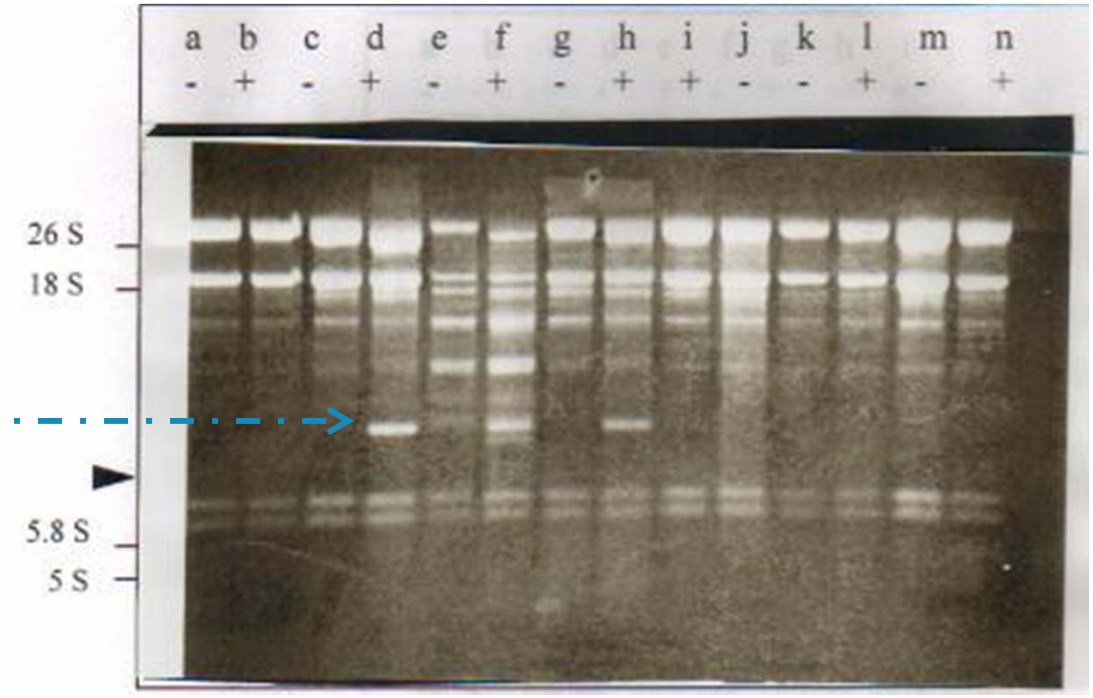
*(Sismindari dkk., 2002)*

# Protein Synthesis Inhibitor




rat 28S r RNA




Sismindari and Lord, 2000

# Saponin (from soapwort)

**Soapwort**

*Saponaria officinalis*

- Saporin - 29.5 kD Protein
- Seeds a rich source
- N-glycosidase activity
- Inactivates 28s rRNA
- Inhibits protein synthesis

potent
single chain
ribosome
inactivating
protein

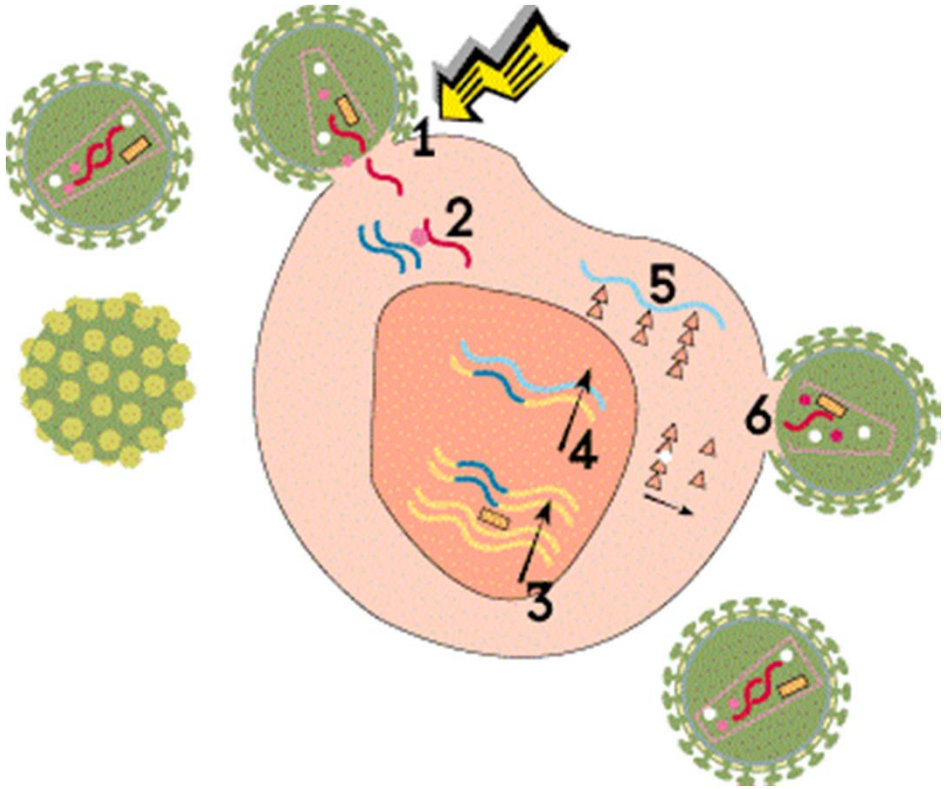
Goal:
treatment of
leukemias
and lymphomas

For T-cell leukemia could be targeted via CD7

: www.som.soton.ac.uk/.../members/DFlavell/djf2.htm

# Target for Antiviral molecule

**The HIV Life Cycle**




## Step 1: Binding

A virus consists of an outer envelope of protein, fat and sugar wrapped around a set of genes (in the case of HIV, genetic information is carried as RNA instead of DNA) and special enzymes.

HIV has proteins on its envelope that are strongly attracted to the CD4+ surface receptor on the outside of the T4-cell. When HIV binds to a CD4+ surface receptor, it activates other proteins on the cell's surface, allowing the HIV envelope to fuse to the outside of the cell.

## Step 2: Reverse Transcription

## Step 3: Integration

## Step 5: Translation

## Step 6: Viral Assembly

Herbal Materia Medica

# Astragalus membranaceus

*www.pitt.edu/~super7/3011-4001/3561.ppt*

- The antiviral action of Astragalus is most likely due to **increased immunity** and **interferon production**.

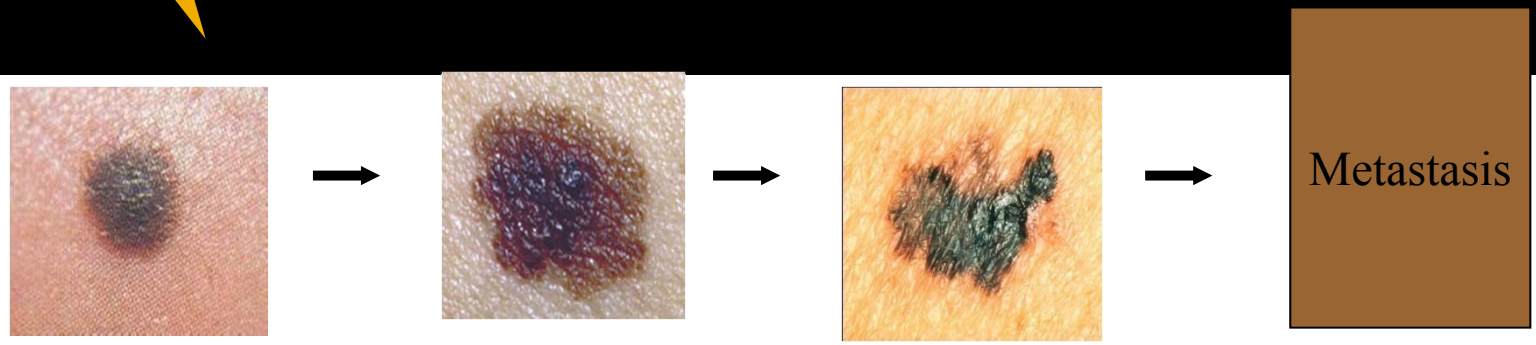
# Study on Activity & Mechanism of action

# Anticancer Medicines

- ANTI CANCER
  - inhibiting the abnormal cells growth or
  - killing the cancer cells

- Cancer →rapid and uncontrolled formation of abnormal cells which by mass together to form a growth or tumor, or proliferate through the body initiating abnormal growth at other site

# Melanoma Carcinogenesis




Transformation → Proliferation → Invasion

ROS

NF kappa B
AP-1
MAPK

COX-2
LOX
NOS
VEGF

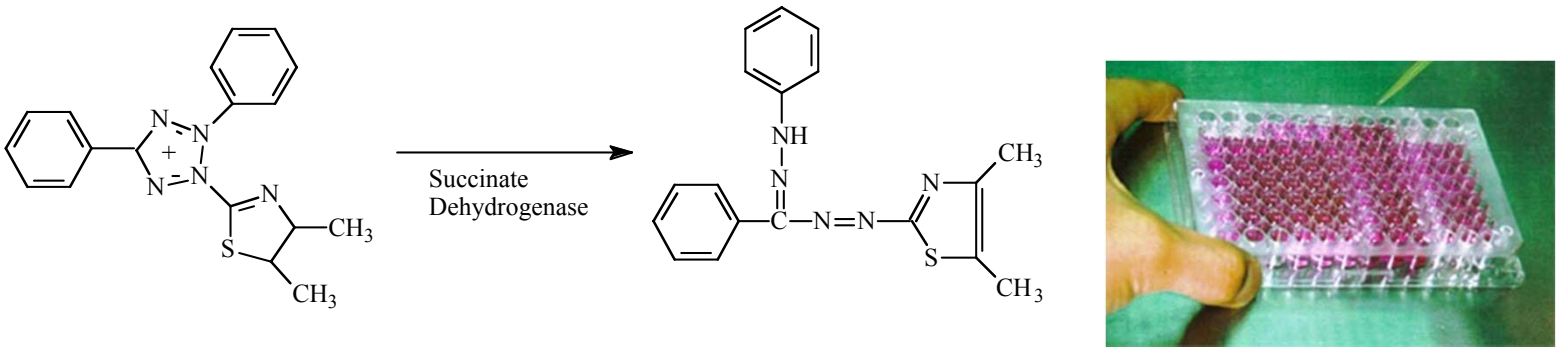
BRAF
RAS
CDKN2A

**IDEAL AGENT**

- **Anti-proliferative**
- **Anti-oxidant**
- **Enhance DNA repair**
- **Induce apoptosis of damaged cells**
- **Inhibit angiogenesis**
- **Restore/enhance immune system**

# Proliferative Assays

- MTT Assay
  - *Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxcity assays**




**MTT** **Formazan**

*Mossman T. J Immunol Meth 1983;65:55-63.

## Cytotoxic effect to cancer cell lines

# MJ Protein → isolated from *M. jalapa*

## Ribosome-inactivating protein

**Supercoiled DNA CLEAVAGE**
(Sismindari,*et.al.*, 1997)

**Cytotoxic effect**
(Ikawati,*et.al.*, 2003)

**RNA N-Glikosidase activity**
(Sismindari andLord, 2000)

**HeLa, (0,39 mg/ml)**

**Myeloma (1,86 mg/ml)**

**Raji, (1,68 mg/ml)**

**mechanism of HeLa cell death via apoptosis**

# Protein dari *Mirabilis jalapa*

## MJ30
(CM-Sepharose)
(Sudjadi,dkk., 2003)

- Sel T47D, LC50 0,36 mg/ml (Ikawati,dkk.,2006)
- Sel Myeloma, LC50 1,12 mg/ml (Sudjadi,dkk., 2003)
- Sel Hela, LC50 7,058 mg/ml (Sudjadi,dkk., 2003)

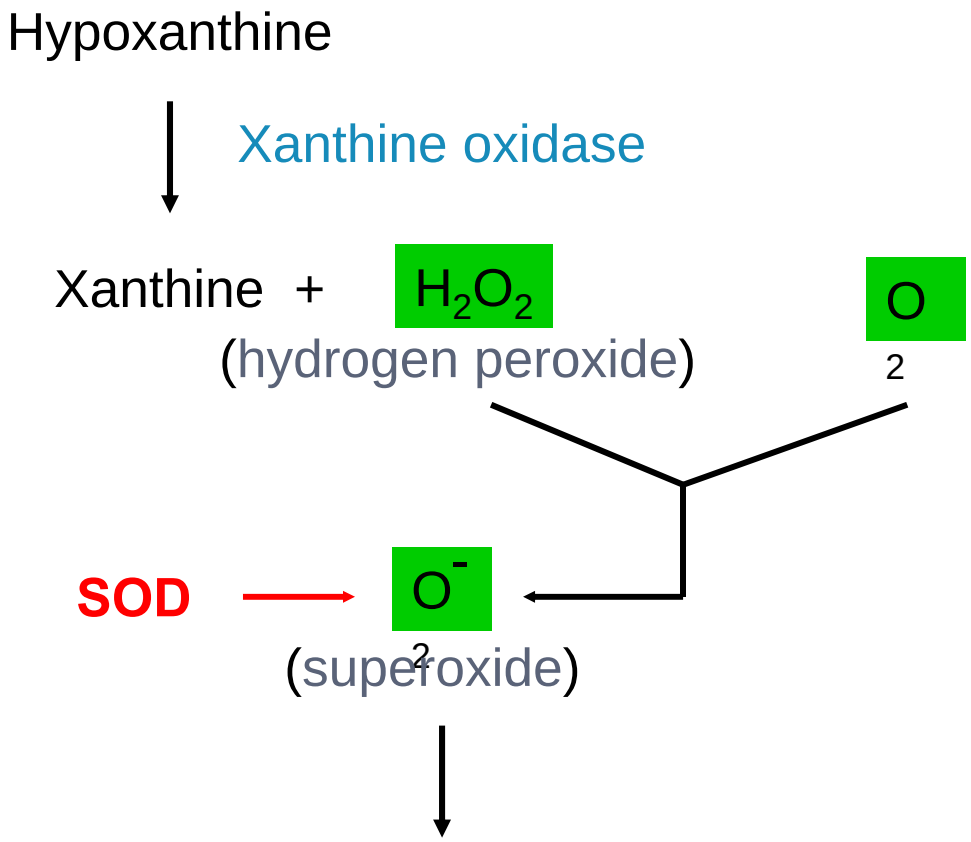
## Fraksi prot. dgn MJC
(Ionenaustauscher type II)
(Sudjadi,dkk., 2007)

- Sel T47D, LC50 0,28mg/ml
- Sel Myeloma, LC50 0,007 mg/ml
- Sel Hela, LC50 0,014 mg/ml

# Anti-Oxidants

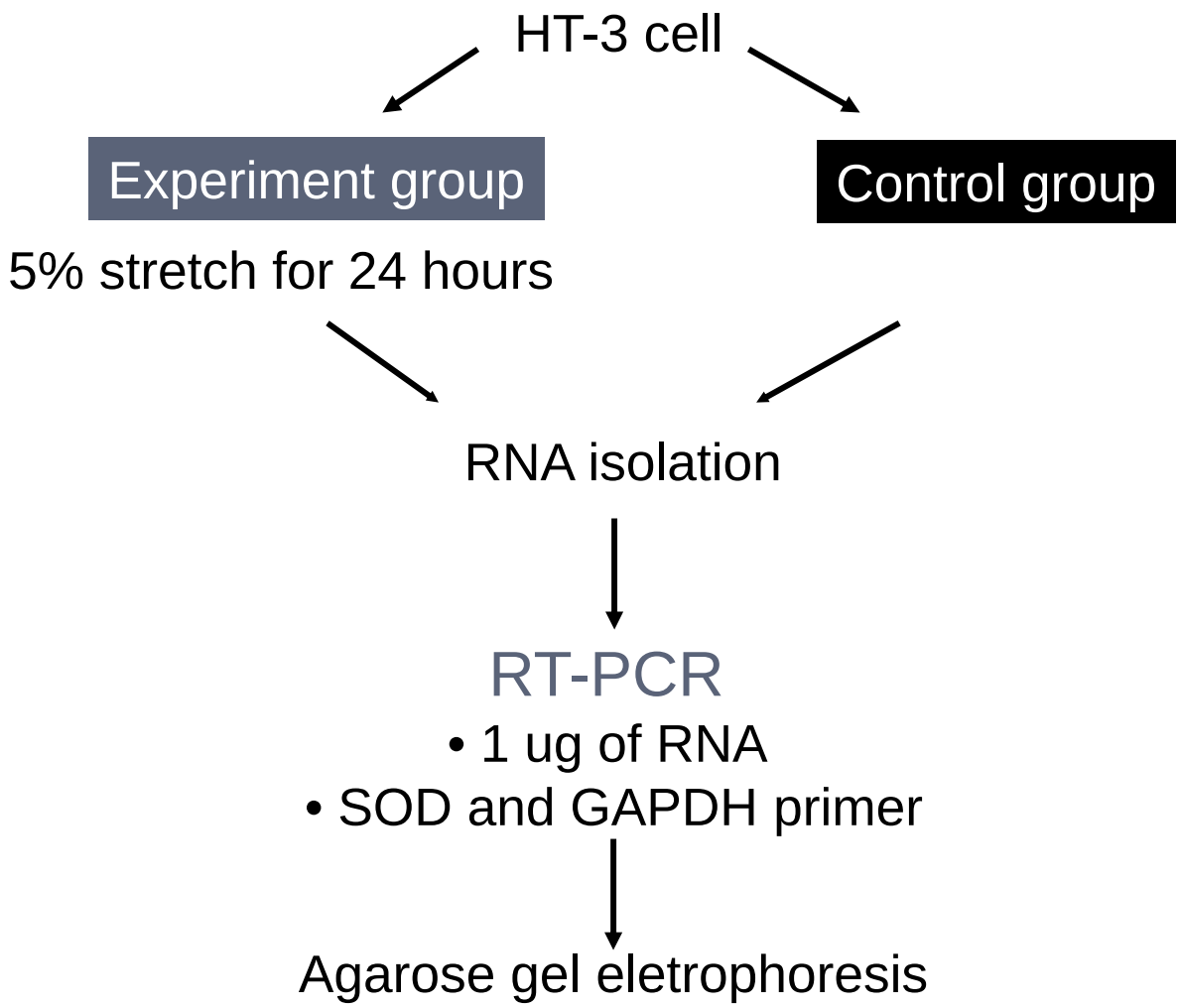
- Cellular enzymes
  - Glutathione reductase (Se)
  - Superoxide dismutase (Zn, Mg, Cu)
- Small molecules
  - Vitamin C & E, uric acid, bilirubin (chain-breaking antioxidants)

# SOD activity assay




Detect by water soluble tetrazolium
(Yield a coloured insoluble formazan compound)

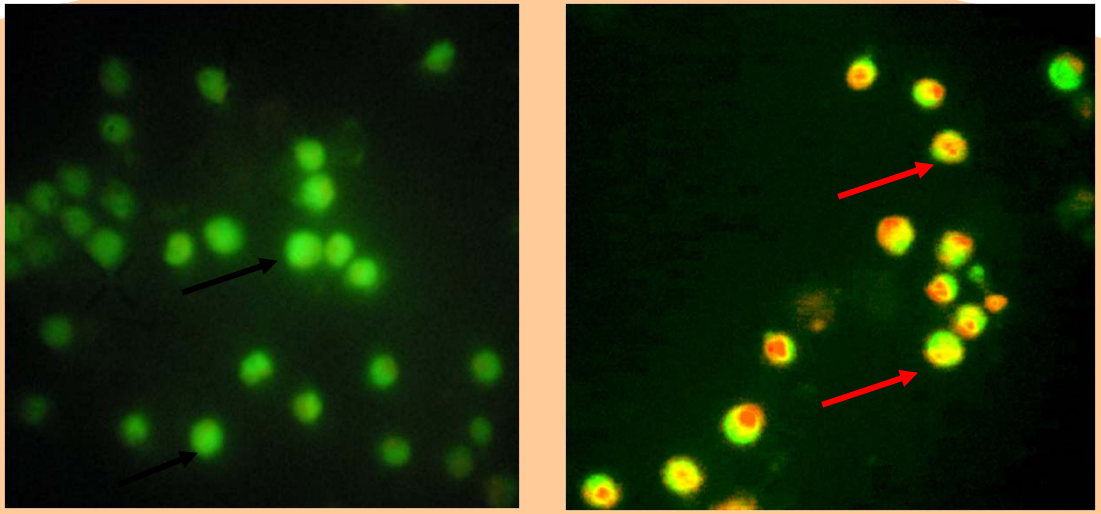
# Expression of SOD
## Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)



# Enhance DNA Repair : Gadd45
# Induce Apoptosis : p53, caspase

- RNA Expression : RT-PCR
- Protein Expression: Immunocytochemistry (ICC)

# MORFOLOGI SEL SETELAH PENGECATAN DENGAN ACRIDIN ORANYE-ETIDIUM BROMID

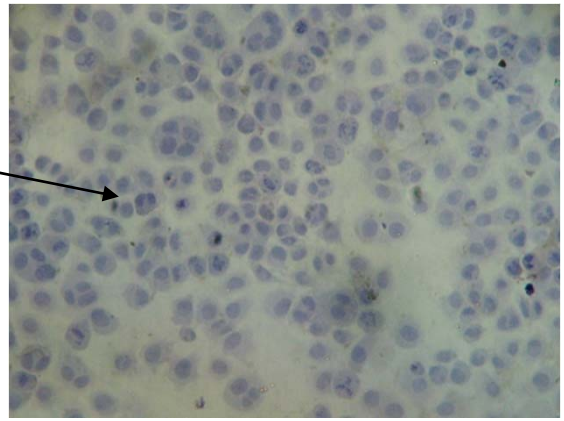



A. Untreated cells, B. Treated cells with MJ protein

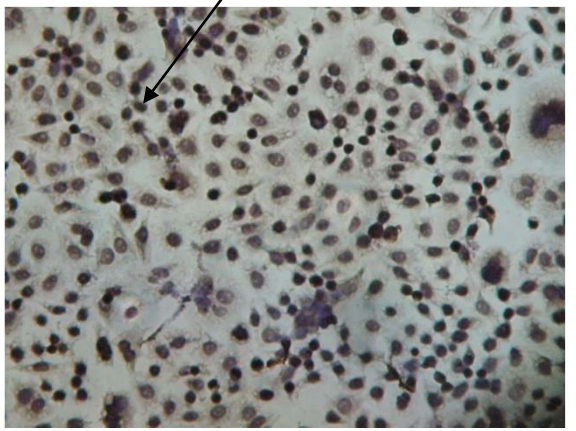
# Effect of MJ-C on Ras Expression

Sismindari *et al.*, 2008

Ras -



Ras +



Sel HeLa → treated with MJ-C protein concentration 2,7 µg/300µl .

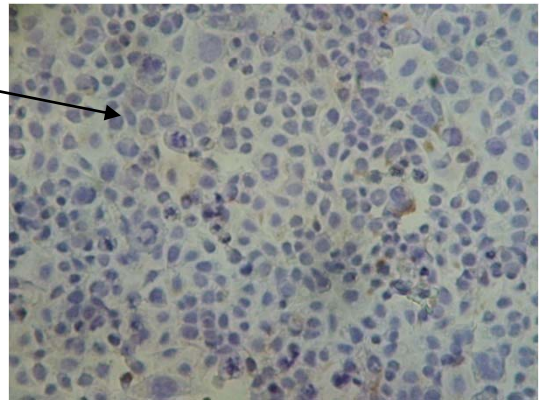
Sel HeLa → treated with MJ protein concentration of 7,2 µg/300µl .

MJ protein 7,2 µg/300µl
consist of
MJC 0,0034 µg/300µl

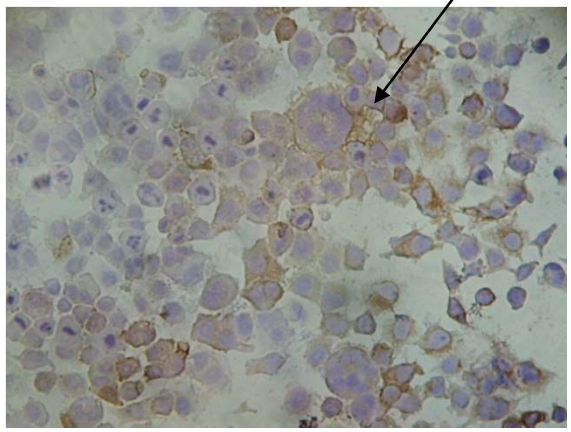
# Effect of MJ-C on COX2 Expression

Sismindari *et al.*, 2008

Cox2 -



Cox2 +



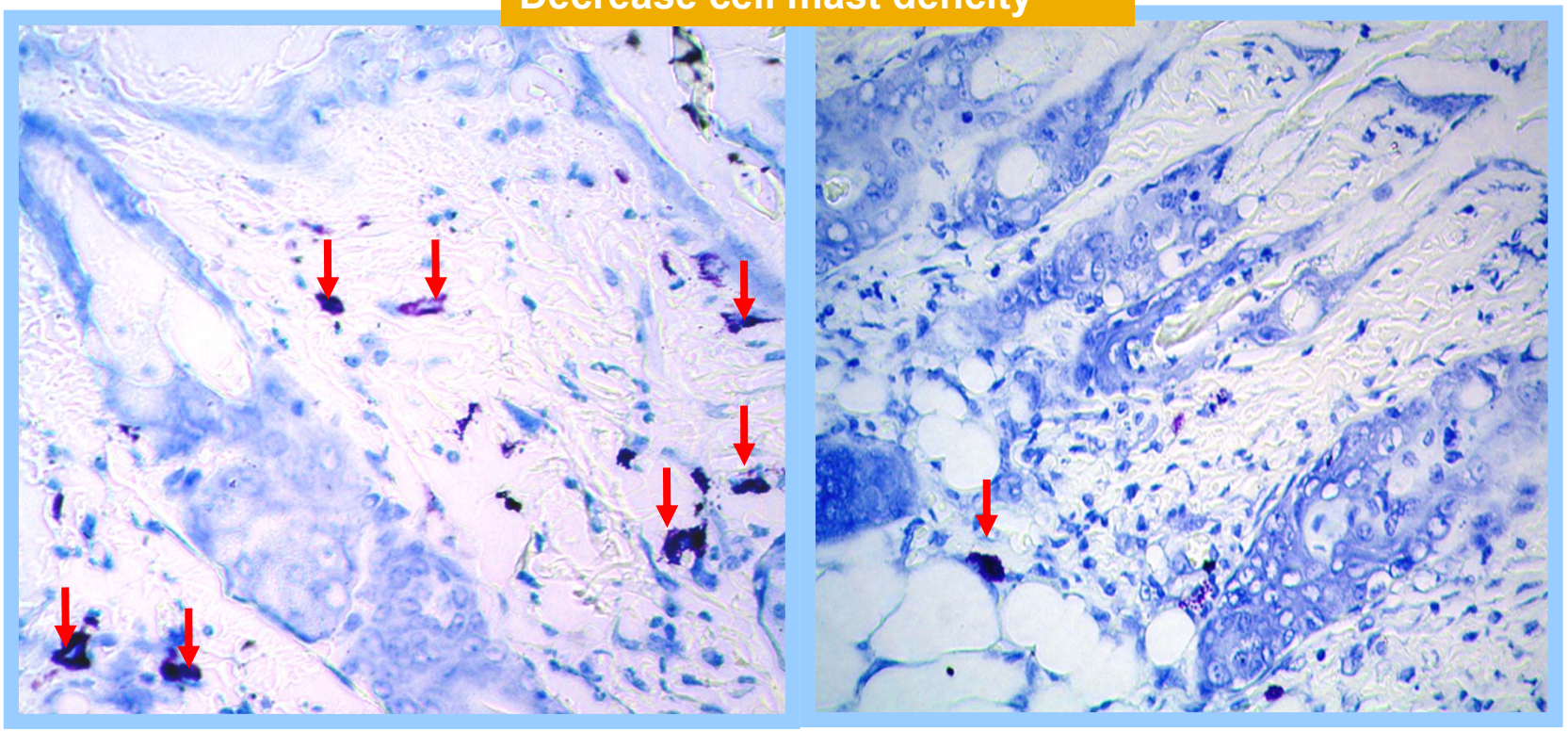
Sel HeLa → treated with protein MJ-C protein concentration 2,7 µg/300µl .

Sel HeLa → treated with Protein MJ protein concentration of 7,2 µg/300µl .

# Enhance Immun Respons

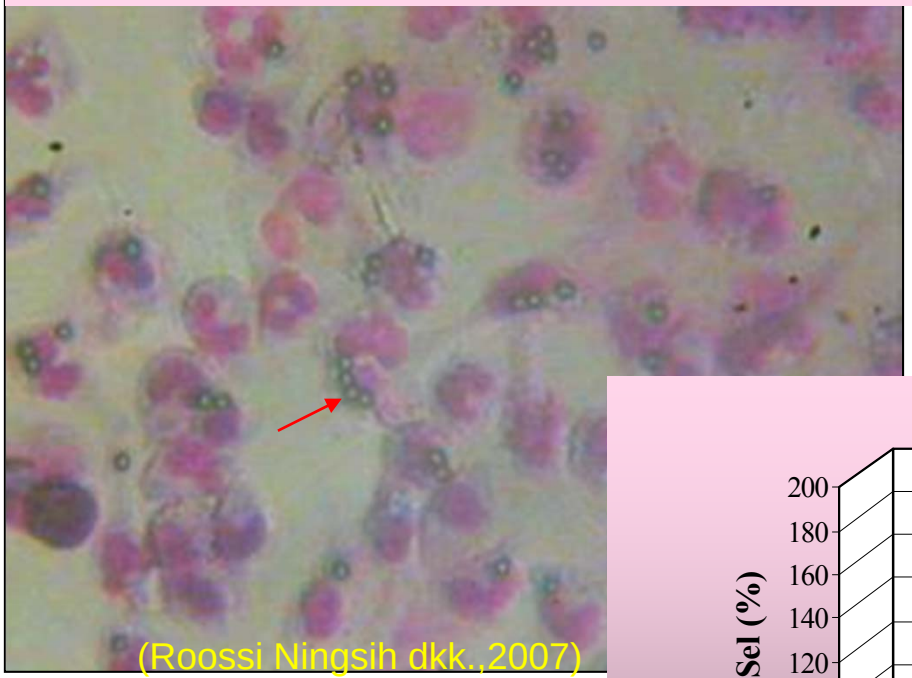
Sismindari et al, 2012

**Decrease cell mast dencity**

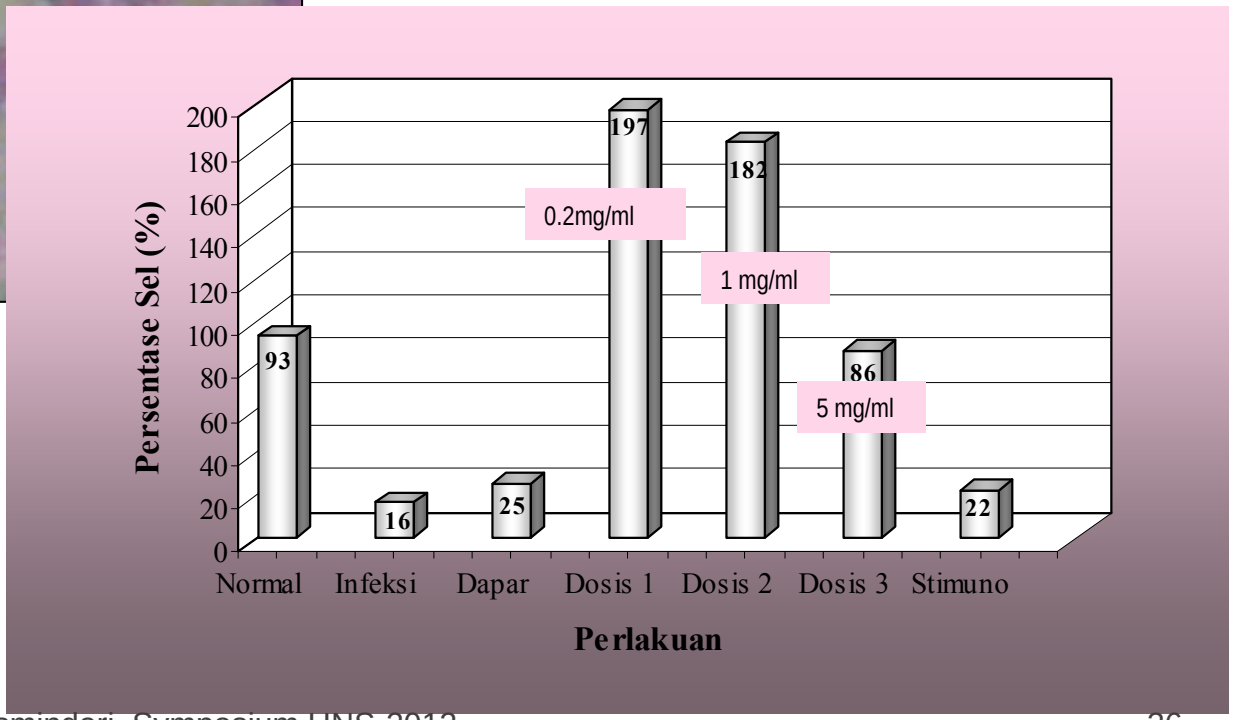


# Enhance Immun Respons

Increase macrophage phagocytotic activity



(Roossi Ningsih dkk.,2007)



Anti proliferation → ↓ RAS

- Anti inflamation → ↓ COX2

- Apoptotic induction → ↑ p53

→ MJ-C potential as anticancer agent

# *Ginkgo biloba*

In China → herbal tea of ginkgo has been used for 4,000 years to treat breathing problems (asthma, bronchitis).

In modern western medicine, flavenoids in ginkgo extracts →
improved microcirculation and
**nootropic effects** (enhances memory and brain function),
anti-oxidant function.

# Garlic (Allium sativum)
# Onion (Allium cepa)

- Organo-sulfur compounds from leaves
- Anti-carcinogenic and anti-microbial
- Anti-atherosclerosis and anti-hypertensive
- Toxic in high amounts

# Green Tea (Camellia sinensis)

- Polyphenols from leaves
- anti-cancer inhibiting tumor initiation and cell proliferation
- Enhance DNA repair activity
- anti-oxidant

# Cyclooxygenase inhibitory and antioxidant cyanidin glycosides in cherries and berries.

Seeram NP, Momin RA, Nair MG, Bourquin LD.,
Phytomedicine 2001 Sep;8(5):362-9

Anthocyanins from tart cherries, Prunus cerasus L. (Rosaceae) cv. Balaton and Montmorency; sweet cherries, Prunus avium L. (Rosaceae); **bilberries, Vaccinum myrtillus L**. (Ericaceae); blackberries, Rubus sp. (Rosaceae); blueberries var. Jersey, Vaccinium corymbosum L. (Ericaceae); cranberries var. Early Black, Vaccinium macrocarpon Ait. (Ericaceae); elderberries, Sambucus canadensis (Caprifoliaceae); raspberries, Rubus idaeus (Rosaceae); and strawberries var. Honeoye, Fragaria x ananassa Duch. (Rosaceae), were investigated for cyclooxygenase inhibitory and antioxidant activities……

# Anti-angiogenic property of edible berries

Roy S, Khanna S, Alessio HM, Vider J, Bagchi D, Bagchi M, Sen CK.
Free Radic Res 2002 Sep;36(9):1023-31

Recent studies show that edible berries may have potent chemopreventive properties. Anti-angiogenic approaches to prevent and treat cancer represent a priority area in investigative tumor biology. Vascular endothelial growth factor (VEGF) plays a crucial role for the vascularization of tumors. The vasculature in adult skin remains normally quiescent. However, skin retains the capacity for brisk initiation of angiogenesis during inflammatory skin diseases such as psoriasis and skin cancers. We sought to test the effects of multiple berry extracts on inducible VEGF expression by human HaCaT keratinocytes. ....

# *Taxus brevifolia*.

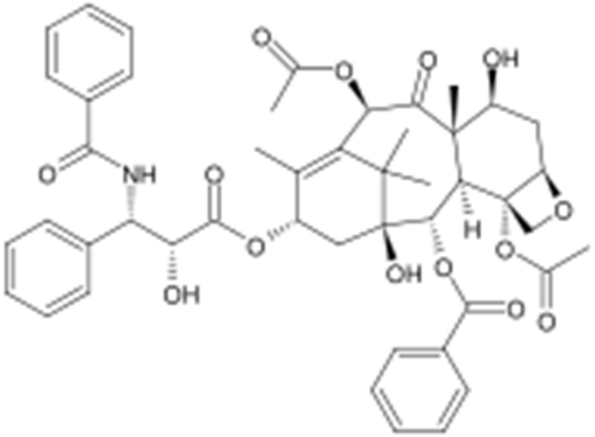
→ Taxol (paclitaxel) is yet another example of a drug found ‘just in time’.

taxol is extracted from the bark

The drug yield is very small, and plant grows very slowly.

It requires the yield of 6 100-year old trees to treat one patient. The yew was on the edge of becoming endangered. What to do?

Taxol has a complicated structure, and lab synthesis is still not possible. However, a number of alternate means of production have been developed.




# Production of Taxol

1. Taxol can be “semi-synthesized” using a much more abundant chemical found in a number of other yews. Cell culture has also been used to produce that starting chemical, *deacetylbaccatin*.
2. Fermentation (combined with a little genetic engineering) technology can be used to produce taxol-like chemicals from actinobacteria, or paclitaxel itself from *Nodulisporium sylviforme* culture.
3. Plant cell fermentation is now used to produce most taxol using a specific callus tissue from *Taxus*. The drug is extracted from the culture, separated by chromatography, and purified by crystallization.

# Co-chemotherapy

- *Areca catechu* L – doxorubicin
  - Synergic effects
  - Decrease the doses of doxorubicin

- **Ginseng** (Panax ginseng) lowers blood concentrations of alcohol and warfarin, and induces mania if used concomitantly with phenelzine.
  **Garlic** (Allium sativum) changes pharmacokinetic variables of paracetamol, decreases blood concentrations of warfarin and produces hypoglycaemia when taken with chlorpropamide.
- ***Areca catechu* L**: Synergic effects with doxorubicin, decrease the doses of doxorubicin

# Conclussions

- The advent of molecular biology and, in particular of genomic sciences → profound impact on the use of herbal medicine
- Study of the activity and the mechanism of action , Identification of the adverse side effects

→ **could develope safe combination of herbal medicine**

# POISONOUS PLANT AND SAFE MEDICINE

- "poisonous plant".

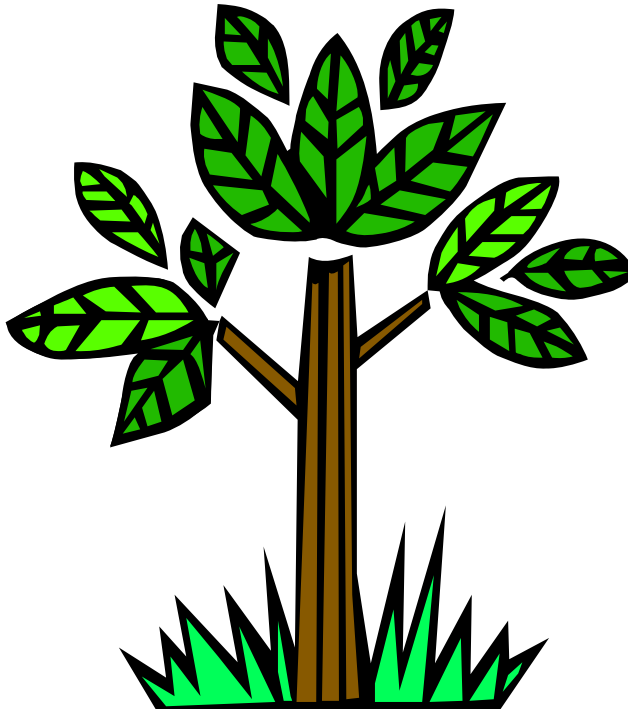
*The major poisonous principles found among plants are organic compound.

***Herbal medicines are to be prescribed with greatest care**.

# Terimakasih

# Thankyou

# Medicinal Herbs
# The Whole better than part.



- Powerful ingredients.
- Only 15% of estimated plant species on earth have been investigated for possible Medicinal uses.
- The world Health organization estimates that 80% of the earth population today depends on plants to treat common ailments.

*www.pitt.edu/~super7/3011-4001/3561.ppt*

# Drug Discovery

- Where do new medicinal drugs come from?
- More precisely: where do **drug families** come from, since once a useful pharmaceutical drug has been discovered, it gets modified in thousands of ways by chemists trying to improve it.
- In the past, two sources: compounds suggested by traditional herbal medicine, and serendipitous (random chance) discoveries.
- Today, rational drug design is becoming important: understanding of how the disease works and where it might be intervened with, coupled with knowledge of the physical structure of enzymes involved allows the design of completely new drug molecules.
- Also, combinatorial chemistry: start with a useful compound, make a large library of modified versions, then test them all against a target.
- The whole process of discovering and testing a new drug is very expensive and laborious: let's say $1 billion and 10 years to get from discovering a new drug to getting it on the market.