Evaluation of health function of spices by global cooperation between Indonesia and Japan.
Spices and herbs with powerful health benefits

➢ The use of spices and herbs has been incredibly important throughout history.

➢ Many spices were celebrated for their medicinal properties, well before culinary use.

➢ Modern science has now shown that many of them do indeed carry remarkable health benefits.

We empirically know the health benefits of spices. However, scientific evaluation of their health functions is not enough.
Name: *Cuminum cyminum* L.

Family: Apiaceae

Use: Spice

Cumin seed is one of popular spices in the world. Several functions of cumin seed have been reported.

**Lipid-soluble components** (Essential oil)
- *p*-menthe-1,4-dien-7-al, cumin aldehyde, γ-terpinene
  - Anti-inflammatory, anti-oxidant, anti-bacterial effects

**Water-soluble components**
- Little is known about the health functions.

We focused on the functions of water-soluble substances in cumin.
Immunostimulatory effect of cumin seed aqueous extract
Macrophage

- Receptors
- Cytokines
- Chemokines

Antigen presentation
Sample preparation

- **Cumin seed powder**
- **Extraction** 0.1 g/mL in 10 mM sodium phosphate buffer (NaPB), 24 h
- **Centrifugation** 200,000 \( \times g \), 30 min
- **Dialysis** Dialyzed with MWCO 14,000 membrane
- **pH adjustment** pH 7.4
- **Sterilization** 0.45 \( \mu \)m filter

Sample (CAE)
Experimental method

Cells
- Mouse macrophage cell line; RAW264.7 cells
- Mouse peritoneal macrophages (P-Mac)

Media
- 10% Fetal bovine serum (FBS)-DMEM medium
- 10% FBS-RPMI 1640 medium

Evaluations
- Cell viability
- Cytokine production
- Gene expression
- Intracellular signal transduction
- Phagocytotic activity
Cytotoxicity of CAE on RAW264.7 cells

*P < 0.05 vs. control
Effect of CAE on cytokine production by RAW264.7 cells

IL-6 production (pg/mL) vs. CAE conc. (µg/mL)

TNF-α production (pg/mL) vs. CAE conc. (µg/mL)

ET: EndoTrap treated
Effect of CAE on mRNA expression in RAW264.7 cells

- **IL-6**
  - Relative mRNA expression
  - Ctrl vs. CAE: *P < 0.05, **P < 0.01 vs. control

- **TNF-α**
  - Relative mRNA expression
  - Ctrl vs. CAE: *P < 0.05 vs. control

* P < 0.05, ** P < 0.01 vs. control
Effect of CAE on cytokine production by P-Mac

**IL-6**

- CAE conc. (µg/mL) vs. IL-6 production (pg/mL)
- ctrl

**TNF-α**

- CAE conc. (µg/mL) vs. TNF-α production (pg/mL)
- ctrl

* P < 0.05, ** P < 0.01 vs. Ctrl
Effect of CAE on mRNA expression in P-Mac

**IL-6**

Relative IL-6 mRNA expression

**TNF-α**

Relative TNF-α mRNA expression

* P < 0.05, ** P < 0.01 vs. control
Macrophage-activating signaling

Bacterial substances

Receptors

MAPKK

IKK

JNK

p38

ERK

Cytosol

Nucleus

NFκB

AP-1

Cytokines

Chemokines
Effect of CAE on MAPK and NF-κB pathways

**NF-κB**

Bar graph showing the expression levels of NF-κB and cNF-κB proteins in control (Ctrl) and CAE-treated groups.

**JNK**

Bar graph showing the expression levels of JNK and p-JNK proteins in control (Ctrl) and CAE-treated groups.

**p38**

Bar graph showing the expression levels of p38 and p-p38 proteins in control (Ctrl) and CAE-treated groups.

**ERK**

Bar graph showing the expression levels of ERK and p-ERK proteins in control (Ctrl) and CAE-treated groups.

**cNF-κB** (cytosol) and **nNF-κB** (nucleus) are also shown in the image.
Mode of action of CAE

Bacterial substances

Receptors

IKK → P

NF-κB → P

MAPKK → P

JNK → P

p38 → P

ERK → P

Cytosol

Nucleus

NF-κB → P

AP-1

Cytokines

Chemokines
Effect of CAE on cytokine production under TLR4 inhibition

IL-6 production (pg/mL)

CAE conc. (µg/mL)

TNF-α production (pg/mL)

CAE conc. (µg/mL)

Ctrl
Ctrl (TAK)
LPS
LPS (TAK)
CAE
CAE (TAK)

TAK: TLR4 inhibitor, TAK-242
Effect of CAE on phagocytosis of RAW264.7 cells

Control

CAE

Cell count

24.0 ± 8.2%

33.8 ± 6.2%

Texas Red-labeled Zymosan A

p=0.1246
Evaluation of function of Indonesian food stuffs

Arrowroot  Bengkoang
Snake fruit  Kedawung
Pornag  Red fruits
Oncom  Hakoi

Immunostimulation
Anti-allergy
Anti-inflammation
Ant-obesity etc....

Gadjah Mada Univ.  Univ. of Papua  Jember Univ.

- Cytotechnology, 64, 131-137, 2012
- J. Funct. Foods, 5, 582-589, 2013
- Cytotechnology, 66, 75-85, 2014
- Food Funct., 5, 1403-1408, 2014
- Cytotechnology, 68, 1737-1745, 2016
- Molecules, 22, E1187, 2017
- Biodiversités, 19, 318-322, 2018
- Biodiversitas, 19, 330-335, 2018
- Canrea Journal, 2, 136-142, 2018
Collaborative development of functional foods both in Indonesia and Japan using same evidences

**Academia**

- Evaluation of health function of spices
- Evaluation of the mode of action

- Academic papers
  - *Cytotechnology*, 71, 599-609, 2019

**Spices**

**Herbs**

**International patents**

**Development of functional foods both in Indonesia and Japan using same evidences**

**Food industry**
➢ Cytokine production-promoting effect of CAE was not derived from contamination of endotoxin.

➢ CAE activates NF-κB and MAPK signaling pathways to stimulate cytokine gene expression.

➢ CAE tended to enhance phagocytosis activity of RAW264.7 cells.

➢ CAE may interact with TLR4 to induce macrophage activation.

Conclusion

Cumin seed also has potential as Immuno-stimulatory functional food.
Terima Kasih !