“Indonesian Herbs and Spices: From Traditional to Modern Food and Herbal Medicine”.

Muhammad Sasmito Djati
Division of Animal and Human Cell Culture
Laboratory of Animal Physiology
Department of Biology, Faculty of Science
Brawijaya University
Indonesia

- Population: 237,556,363
- Flag: ![Indonesia Flag]
- Archipelago of 17,508 islands (6,000 inhabited)
- Languages: 700 locals and Indonesian National (bahasa)
- Unity in diversity
- Public University: 576
- Private University: 4,017
East Java

- About 48,000 km²
- People: 38 million
Malang Hinduism temple’s
Malang Chinese temple’s
Malang Churches
Malang Mosques
Malang City

- Population: 1 million
- 89% suny’s muslem (minority Christians, Hinduism, budhism, confusion)
- 500 – 1000 m from sea level; 17 °C – 28 °C; RH 65 - 98
- From Jakarta: about 1000 km, by Plane (1 hour), by train (14 hours)
- From Surabaya: about 100 km (2 hours by car or by train)
“Raja Brawijaya”
Indonesia
at the Campus

Mojopahit temple
at the Campus
At campus
Students

- UB Students come from all provinces in Indonesia and foreigner.
- Now carrying 54,000 – 65,000 undergraduate students, master degree and doctoral programs
### Faculties

<table>
<thead>
<tr>
<th></th>
<th>Faculties</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Law</td>
</tr>
<tr>
<td>2</td>
<td>Economics and Business</td>
</tr>
<tr>
<td>3</td>
<td>Administration Science</td>
</tr>
<tr>
<td>4</td>
<td>Agricultural</td>
</tr>
<tr>
<td>5</td>
<td>Animal Husbandary</td>
</tr>
<tr>
<td>6</td>
<td>Engineering</td>
</tr>
<tr>
<td>7</td>
<td>Medical</td>
</tr>
<tr>
<td>8</td>
<td>Fishery and Marine Sciences</td>
</tr>
<tr>
<td>9</td>
<td>Mathematics and Natural Sciences</td>
</tr>
<tr>
<td>10</td>
<td>Agricultural Technology</td>
</tr>
<tr>
<td>11</td>
<td>Social and Political Sciences</td>
</tr>
<tr>
<td>12</td>
<td>Culture Studies</td>
</tr>
<tr>
<td>13</td>
<td>Veterinary Medicine</td>
</tr>
<tr>
<td>14</td>
<td>Technology Information and Computer Science</td>
</tr>
<tr>
<td>15</td>
<td>Dentistry</td>
</tr>
<tr>
<td>16</td>
<td>Vocational Program</td>
</tr>
<tr>
<td>17</td>
<td>Graduate Program</td>
</tr>
</tbody>
</table>
## Study Programs/departments

<table>
<thead>
<tr>
<th>No</th>
<th>SP/departments</th>
<th>2019</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vocational (S-0)</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>Undergraduate (S-1)</td>
<td>64</td>
</tr>
<tr>
<td>3</td>
<td>Specialist (Sp-1)</td>
<td>15</td>
</tr>
<tr>
<td>4</td>
<td>Master (S-2)</td>
<td>39</td>
</tr>
<tr>
<td>5</td>
<td>Doctor (S-3)</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td><strong>Total</strong></td>
<td><strong>136</strong></td>
</tr>
</tbody>
</table>
Foreign Students

• We also educate foreign students from various countries.
• Also we had a joint degree / double degree programs with overseas universities
National and International Achievements of Students
At PIMNAS 27th

At KJI & KBGI 2017

At ASHRAE Chicago

At Bandung Marchingband Championship 2019

National Student Writing Competition in Makassar
Rempah-rempah

INDONESIAN TRADITIONAL SPICES
Indonesia’a mega-biodiversity

Despite covering only 1.4% of the world’s surface area, Indonesia has:

• 25,000 species flowering plants (10%, world total)
• 1,530 species birds (17%), of which 380 species (24%) endemic
• 515 species mammals (12%), of which 36% endemic
• 16% of all reptiles and amphibians
• 20% of all fish species
INDONESIAN TRADITIONAL FOOD

all Indonesian traditional food are spiced with rempah-rempah
INDONESIAN TRADITIONAL DRINK
Indonesian Traditional Market
Traditional restaurant
Modern Indonesian style Restaurant
"JAMU" INDONESIAN TRADITIONAL DRUGS
According to National Social economic survey (2001), 57.7% of Indonesian treat their body without medical help, but they are choose traditional medicine. Jamu is a traditional herbal medicine that have been used by the Indonesian community for hundreds of years (Elfahmi, et al., 2012).
Jamu is made from the part of the plant (leaves, roots, fruits) or animal extract, which has the active compound to healing certain diseases (Sarimole, et al., 2014).
Definition: small or high dose

Rempah or “Spices”

• Bumbu or “Herb” come from leaves of aromatic plants.

Flavor

The sensation: combination of smell, taste, and vision

Taste

Sense of taste

✓ natural sources of antioxidant
✓ used for traditional medicine
Source of spice and herb

**Bark**
- Serai / lemongrass (*Cymbopogon citratus*)
- Kayu manis / cinnamon (*Cinnamomum verum*)
- Secang (*Caesalpinia sappan L.)*

**Flower**
- Cengkeh (*Syzygium aromaticum*)
- Kecombrang (*Nicolai speciosa Horan*)
- Star anise / pekak (*Illicium verum*)

- Lemongrass Essential oil
- Kayu manis / cinnamon
- Secang (*Caesalpinia sappan L.)*
- Cengkeh (*Syzygium aromaticum*)
- Kecombrang (*Nicolai speciosa Horan*)
- Star anise / pekak (*Illicium verum*)

- Cengkeh Essential oil
- Pekak Powder
Fruit

Asam Jawa (tamarind)  
*Tamarindus indica*

Pala / nutmeg  
*Myristica fragrans*

Chili  
*Capsicum sp.*

Seed

Jintan  
*(Nigella sativa Linn.)*

Ketumbar / Coriander  
*Coriandrum sativum*

Tamarind Essential oil
Nutmeg Essential oil
Chili Essential oil
Habbatussauda Essential oil
Coriander Essential oil

10 Health Benefits of Tamarind

Nutmeg Powder for increase serotonin

Health supplement
### Leaves
- Kaffir Lime: *Citrus hystrix*
- Seledri / celery: *Apium graveolens*

### Root
- Ginger / Jahe: *Zingiber officinale*
- Turmeric: *Curcuma sp.*

### Bulb
- Onion: *Allium cepa*
- Garlic: *Allium sativum*

---

**Kaffir leaf Essential oil**

**Celery Seed**
- Common Uses:
  - Arthritis
  - Rheumatism
  - Digestive Problems
  - Liver Problems
  - Hepatitis

**Ginger Essential oil**

**Turmeric Essential oil**

**Onion Essential oil**

**Garlic Essential oil**
Antioxidant compounds in spices and herbs

<table>
<thead>
<tr>
<th>Spice</th>
<th>Antioxidant compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clove</td>
<td>Phenolic acids (gallic acid), flavonol glucosides, phenolic, volatile oils (eugenol, acetyl eugenol), tannins</td>
</tr>
<tr>
<td>Ginger</td>
<td>Shogoal, gingerol</td>
</tr>
<tr>
<td>Mace</td>
<td>Myristphenone</td>
</tr>
<tr>
<td>Marjoram</td>
<td>Beta-carotene, beta-sitosterol, caffeic acid, carvacrol, eugenol, hydroquinone, linalool-1-acetate plant, 3-17, myrcene, rosmarinic-acid, terpenen-4-ol</td>
</tr>
<tr>
<td>Nutmeg</td>
<td>Myristphenone, phenolic volatile oils, phenolic acid (caffeic acid), flavanoids (catechin)</td>
</tr>
<tr>
<td>Oregano</td>
<td>Caffeic acid, p-coumaric acid, rosmarinic acid, caffeoyl derivatives, cavaclrol, flavonoids</td>
</tr>
<tr>
<td>Red pepper</td>
<td>Beta-carotene fruit, beta-sitosterol plant, caffeic acid campesterol, camphene fruit, capsaicin fruit, capsanthin fruit, chlorogenic-acid fruit, eugenol fruit, gamma-terpinene fruit, hesperidin fruit, myristic acid</td>
</tr>
<tr>
<td>Rosemary</td>
<td>Carnosol, 12-O-methylcarnosic, rosmanol, caffeic acid, rosmarinic acid, caffeoyl derivatives, phenolic diterpenes (carnosic acid), caramosol, epirosmanol, flavonoids</td>
</tr>
<tr>
<td>Sage</td>
<td>Rosmanol, epirosmanol, phenolic acids (rosmarinic acid), phenolic diterpenes (carnosic acid), flavonoids</td>
</tr>
<tr>
<td>Sesame seed</td>
<td>Sesaminol, a-tocopherol, sesamol</td>
</tr>
<tr>
<td>Turmeric</td>
<td>Curcumin, 4-hydroxycinnamoylmethane</td>
</tr>
<tr>
<td>Thyme</td>
<td>Phenolic acids (gallic acid, caffeic acid, rosmarinic acid), thymol, phenolic diterpenes, flavonoids</td>
</tr>
</tbody>
</table>
• Herbs contain unique anti-oxidants, essential oils, vitamins, phyto-sterols and many other plants derived nutrient substances, which help equip our body to fight against germs, toxins and to boost immunity level.

“Herbs are, in fact, medicines in smaller dosages”
Herbs and spices as Medicines

**DIscoveR The MEDICInal Power of 7 SuPER HERB & SPICES**

1) **CLOVES**
   - Found to have the highest antioxidant content of all spices and can be used as a painkiller and has been used for centuries to treat toothaches and gum pain.
   - Eases cold and allergies, and oil of cloves is useful as antiseptic in mouthwash.

2) **OREGANO**
   - 1/2 tsp has the same amount of antioxidants as a quarter cup of almonds and four times the antioxidant activity of blueberries.
   - GoGreek make a greek salad and sprinkle on the oregano!
   - Oregano is rich in Vitamin K, iron, magnesium, and kills e.coli, salmonella, and virtually all food-borne pathogens.

3) **GINGER**
   - Over 50 antioxidants have been found in ginger. It helps increase circulation, calms digestive problems.
   - Ginger has also been used to treat food poisoning, shown to lower cholesterol, treat arthritis, reduce inflammation, and can be used to help increase insulin sensitivity in diabetes.

4) **CINNAMON**
   - Plays an important role in regulating blood sugar in people with diabetes.
   - Clinical studies have shown a consistent intake of cinnamon daily help reduce glucose, triglyceride, and LDL cholesterol with type II diabetes.

5) **TURMERIC**
   - The bright yellow color comes from the phytochemical Curcumin and can eliminate cancer cells, help reduce obesity, and metabolic diseases.
   - Scientists have found by creating a new molecule from curcumin, called CNB-002, this molecule triggers the mechanisms that safeguard and restore brain cells after a stroke.

6) **ROSEMARY**
   - Blocks HCA inhibitors or carcinogenic compounds found in your favorite grilled meats.
   - Rosemary oil can improve cognitive performance and fight off free radicals that cause Alzheimer’s, strokes, and dementia.

7) **MUSTARD**
   - The compound ATC found in mustard seed is known to be an anti-cancer compound - this plant-compound is also found in wasabi & horseradish.
   - Studies show that ATC, stopped the growth of bladder cancer by 33%.
Study | Research outcomes
--- | ---
**Anti-cancer activity** | 6-gingerol exhibited anti-cytotoxicity in human epidermoid carcinoma cells by inducing apoptosis [5]. Ginger compound are effective against the various cancer such as colorectal, gastric, ovarian, liver, skin, breast, and prostate cancers [6].

**Anti-coagulant activity** | Ginger reduce the production of platelet thromboxane in vitro and platelet aggregation [7].

**Anti-emetic activity** | Gingerols, shogaols, and galanolactone, a diterpenoid of ginger have an antiemetic effect and importante to modulate the nausea and vomiting during pregnancy [4].

**Anti-inflammatory effect** | Ginger reduce the elevated expression of NFκB and TNF-α in rats with liver cancer [8].

**Antioxidant effects** | 6-shogaol of ginger is the most potent antioxidant and 6-gingerol also showed antioxidant activity against HL-60 cells from oxidative stress [9].
KUNYIT or Turmeric (*Curcuma* sp.)

<table>
<thead>
<tr>
<th>Study</th>
<th>Research outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Antioxidant activity</strong></td>
<td>Curcumin in <em>Curcuma</em> sp. could increase superoxide dismutase (SOD) serum activities [81] and inhibit ROS-generating enzymes such as lipoygenase/cyclooxygenase and xanthine hydrogenase/oxidase [82].</td>
</tr>
<tr>
<td><strong>Anti-inflammatory activity</strong></td>
<td>Curcumin block NF-κB activation increased by several different inflammatory stimuli [83].</td>
</tr>
<tr>
<td><strong>Anti-arthritis</strong></td>
<td>Anti-arthritic effects of curcumin in humans with osteoarthritis and rheumatoid arthritis (RA) [84].</td>
</tr>
<tr>
<td><strong>Anti-metabolic syndrome</strong></td>
<td>Curcumin attenuate metabolic syndrome by improving insulin sensitivity, suppressing adipogenesis and reducing elevated blood pressure, inflammation and oxidative stress [85, 86].</td>
</tr>
</tbody>
</table>

High antioxidant (curcumin)  
For skin and cosmetic

Jamu kunyit asam  
Turmeric powder
## ANDALIMAN (Zanthoxylum acanthopodicum)

<table>
<thead>
<tr>
<th>Study</th>
<th>Research outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anti-bacterial activity</strong></td>
<td>Three kinds of Andaliman extract had antibacterial activity against Bacillus cereus with MIC and MBC value 0.2% and 0.8% [24].</td>
</tr>
<tr>
<td><strong>Antioxidant activity</strong></td>
<td>Andaliman ethanolic extract had singlet oxygen quenching effect in light-induced lipid oxidation. Andaliman hexane and acetone extracts had a stronger lipid peroxidation effect than the antioxidative effect of phenolic substances [25]. Andaliman fruit extract has the highest antioxidant activity on aqueous form [26].</td>
</tr>
<tr>
<td><strong>Locomotor activity</strong></td>
<td>The locomotor activity test using modified Wheel cage method showed that inhalation of essential andaliman fruits essential oils affected the locomotor activity of mice depended given doses [19].</td>
</tr>
<tr>
<td><strong>Anti-migration activity</strong></td>
<td>Ethylacetate fraction of Andaliman has an antimigration activity in the 4T1 breast cancer cell line through inhibition of cell cycle phase [27].</td>
</tr>
</tbody>
</table>
# ATTARASA (*Litsea cubeba* Pers)

<table>
<thead>
<tr>
<th>Study</th>
<th>Research outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-bacterial activity and Kinetic</td>
<td>Attarasa oil penetrate and destroy the outer and inner membrane of <em>E. coli</em> cells. The antibacterial properties of Attarasa mainly caused to the presence of aldehydes in this plant [31].</td>
</tr>
<tr>
<td>Biological response of common carp</td>
<td>This plant increased nonspecific immunity of carp (<em>Cyprinus carpio</em>) after infection with Aeromonas hydrophila. Attarasa leaf powder supplementation diet can be used in aquaculture to reduce antibiotic administration [32].</td>
</tr>
<tr>
<td>Anti-microbial activity on cariogenic bacteria</td>
<td>The essential oil of <em>Litsea cubeba</em> could inactive &gt;90% of the cariogenic bacterial population (Streptococcus mutans, Streptococcus sobrinus and Streptococcus sanguinis) within 10 minutes. The best antimicrobial effect of this plant is applicable in food system [33].</td>
</tr>
<tr>
<td>Anti-microbial synergy in aquaculture</td>
<td>There were an antimicrobial synergy of <em>Litsea cubeba</em> essential oil and antibiotic that often used in the treatment of bacteria activity in aquaculture. <em>Litsea cubeba</em> and the antibiotic could enhance efficacy, reduce toxicity, and reduced side effect of bacteria in aquaculture [34].</td>
</tr>
</tbody>
</table>
# MENGKUDU / Noni (*Morinda citrifolia*)

<table>
<thead>
<tr>
<th>Study</th>
<th>Research outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anti-diabetic and anti-hyperlipidemia effect</strong></td>
<td>Blood glucose of diabetic rats returned to normal level after oral administration with <em>M. citrifolia</em> and <em>C. indica</em> at dose of 300 mg/kg BW. This effects preserved the islets of Langerhans β-cells function, resulted in significant increase in insulin [54]. This plants regulate elevated blood sugar level and could serve as a suitable supplement for diabetic patients [55].</td>
</tr>
<tr>
<td><strong>Anti-bacterial activity</strong></td>
<td>Ethanol and hexane extract of <em>M. citrifolia</em> inhibited the formation of over 80% of <em>Mycobacterium</em>. Significant antibacterial effects of this plants on various strains of <em>Salmonella</em>, <em>Siggella</em> and <em>E. coli</em> [56].</td>
</tr>
<tr>
<td><strong>Anti-cancer effect</strong></td>
<td><em>M. citrifolia</em> fruit extract exhibited chemopreventive effect by inhibiting growth of mouse epidermal cells. This effect is contributed by 6-o(beta-oglucopyronosyl)-1-o-octanoyl-betaD-glucopyranose and Asperulosidic acid [57,58].</td>
</tr>
<tr>
<td><strong>Antioxidant activity</strong></td>
<td><em>M. citrifolia</em> juice had significantly higher radical scavenging effect than vitamin C and pycnogenol [59].</td>
</tr>
</tbody>
</table>
### Study

<table>
<thead>
<tr>
<th>Study</th>
<th>Research outcomes</th>
</tr>
</thead>
</table>
| **Anticancer activity**| - Ethanol extract of *A. muricata* leaves prevent the DMBA-induced DNA damage in breast tissue of mice [71]. The leaves boiled water inhibit the metastatic breast cancer [72].  
- Ethanol extract of *A. muricata* leaves induced the apoptosis of K562 chronic myeloid leukemia cells [73] and reduction the ACF formation in DMH induced colon cancer [74].  
- Ethyl acetate extract of *A. muricata* leaves induce mitochondrial-mediated apoptosis and cell cycle arrest at G1 phase [75]. |
<p>| <strong>Antioxidant</strong>         | The stem bark extract (200 mg/kg) showed protective effects against oxidative stress induced by carbon tetrachloride in rats and significantly increased the oxidant levels [76]. |
| <strong>Antiparasitic activity</strong>| Ethyl acetate extract of <em>A. muricata</em> pericarp exhibit the toxicity effect against <em>L. braziliensis</em> and <em>L. panamensis</em> the higher than Glucantine as a positive control [77]. |
| <strong>Antihypertensive activity</strong> | Aqueous leaf extract of <em>A. muricata</em> decreased blood pressure in a dose dependent manner without affecting heart rates in normotensive Sprague–Dawley rats [78]. |</p>
<table>
<thead>
<tr>
<th>Study</th>
<th>Research outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-inflammatory activity</td>
<td>The essential oil of Cymbopogon flexuosus inhibit skin inflammatory response in mice [63]. Lemongrass showed anti-inflammatory activity in human skin cells and this plant is a good therapeutic candidate for treating inflammatory condition of the skin [62].</td>
</tr>
<tr>
<td>Antioxidant activity</td>
<td>Protected from oxidative stress and DNA damage in human embryonic lung fibroblast cell [64].</td>
</tr>
<tr>
<td>Anticancer activity</td>
<td>An essential oil from Cymbopogon flexuosus exhibited anticancer activity. It is indicated by the morphological studies of Sarcoma-180 solid tumor cells from animal treated with the essential oil of Cymbopogon flexuosus that revealed condensation and fragmentation of nuclei typical of apoptosis. This plant oil also causes loss in tumor cell viability by enhancing apoptosis process [65].</td>
</tr>
<tr>
<td>Antibacterial activity</td>
<td>The essential oil of lemongrass and citral were effective to inhibit and kill Acinetobacter baumannii strains that confirmed as multi-drug-resistant (MDR). This evidence make the lemongrass as a possible alternative antibiotic to kill both MDR gram negative and positive bacteria [66].</td>
</tr>
</tbody>
</table>
Herbal drugs

Jamu
drugs

Continuity from plants to drugs

Pharmacological potency

Salicylic acid

Aspirin

Low-MW

Drugs

Willow bark
(Salicin)

Functional

foods

Plants
foods

Anis
(Shikimic acid)

Chemical purity

Tamiflu
(oseltamivir)

Anti-viral drug

2016-16
Traditional systemic approach?

- Mutual nourishment
- Mutual restraint

Liver
Gall bladder

Kidney
Urinary bladder

Heart
Small intestine

Lung
Large intestine

Spleen
Stomach

Yin Yang
Synergy

• Most of the effective phytomedicine in market are as whole extracts of plants
• Practitioners believe that synergistic interactions between the components of individual or mixture of herbs are a vital part of therapeutic efficacy
• Mechanism of action of many phytomedicine is still unknown and there are several instance where total extract shows better activity than an equivalent dose of isolated compound chemical and pharmacological evidence to demonstrate conclusively the concept of synergism
Multi-target approach

- Combinations of herbs are normal and are based on empirical observation and reasoning based on a particular patient.
- These herbal combinations may not be targeted to a particular organ (multi-targeted), cell, tissue or any biochemical system, making this synergy even more difficult to identify. Possibility of drug interactions and the adverse reactions arising out of these have to be checked before coming out with the drug.
An Effort Scenario

Functional food Reproductive Local Knowledge
IMMUNE SYSTEM
Neutrophils
Monocytes
Eosinophils

Nonspecific Phagocytosis
Mechanism of Phagocytosis

1. Microbe adheres to phagocyte
2. Phagocyte forms pseudopods that eventually engulf the particle
3. Phagocytic vesicle is fused with a lysosome
   - Phagolysosome
4. Microbe in fused vesicle is killed and digested by lysosomal enzymes within the phagolysosome, leaving a residual body
5. Indigestible and residual material is removed by exocytosis

(a) Macrophage
(b) Lysosome
- Phagocytic vesicle containing antigen (phagosome)
- Acid hydrolase enzymes
- Residual body
Inflammatory Response

1. Histamine & prostaglandins released
2. Capillaries dilate, clotting begins
3. Chemotactic factors attract phagocytic cells
4. Phagocytes consume pathogens & cell debris
Haematopoiesis is systemic approach?

Hematopoiesis (formation of blood cells) has served as a classic experimental model for studying how cells form and differentiate to acquire their unique, specialized functions.
antigen

Th0

Th1, IFN-γ, TNF-α

Th1

Th0

MHC II

TCR

Th2

IL-4, IL-5, IL-6, IL-9, IL-10, IL-13, GM-CSF

B

IgE

Treg

IL-10, TGF-β, FoxP3
Background

- Iron for blood Plasm, Hb, Placenta, fetus, and parturition
- Prolactine, progesterone
- Immune system, partus and post partus
- B lymphocyte cells, T, CD4, CD8, and TER 199
Indonesian social problem

• More than 2 million Pregnant women Fe Deficiency (Pavord et al 2011)
• Abortion
• Low birth weight
• Lactation
• Immune system
• Paracytes
• Epidemy
Feed intake?
1. Improving immune system
2. Fetus growth and development
3. Parturation
4. Lactation
**Elephantopus scaber (Tapak Liman)**

Contain epifrieelinol, lupeol, stigmasterol, triacontan-1-ol, dotriacontan-1-ol, lupeol acetate, deoxyelephantopin, isodeoxyelephantopin, flavonoid, polifenol luteolin-7, glucoside
Flavonoid → **imunomodulator**

---

**Sauropus androgynous (Katuk)**

1,14 tetradecanediol → **antimicrobial**
Phytol → **cancer prevention, autoimmune response**
Octadecyne, hexadecyne, pyrene, azulene → **anti-inflammatory, antibacterial, antitumor, anticancer, anti-allergy**
• The anatomical barrier between baby and mother, through separate circulatory systems within the placenta
  • The antigenic immaturity of the fetus. Major histocompatibility (MHC) antigen expression is reduced on trophoblast cells on the foetal side of the placenta.
  • Development of an immunosuppressive environment within the uterus
Mother is consuming and delivering oxygen for two!
HORMONE LEVELS

INTERACTION OF PITUITARY AND FOLLICLE

GROWTH OF ENDOMETRIAL LINING

DAYS OF CYCLE

FSH, LH, PROGESTERONE, ESTROGEN

DEVELOPING FOLLCLE, MATURE FOLLICLE, EGG, CORPUS LUTEUM

RISING PROGESTERONE, LH SURGE

1 7 14 21 28
Our Research

- Experimental animal preparation
- Extraction process
- Bacterial infection
- Oral Administration of *E. scaber* and *S. androgynus*
  - P1 (100% of *E. scaber*)
  - P2 (75% of *E. scaber* and 25% of *S. androgynus*)
  - P3 (50% of *E. scaber* and 50% of *S. androgynus*)
  - P4 (25% of *E. scaber* and 75% of *S. androgynus*)
  - P5 (100% of *S. androgynus*)
- Lymphocyte Isolation
- FACS Analysis
- Statistical Analysis
Bone Marrow Cells Lymphocyte Activity of Pregnant Mice with Therapy of *E. scaber* and *S. androgynus* Post Infection *Salmonella typhimurium*

**Figure 1.** Expression of B220+ cells  
*Description:* ES= *Elephantopus scaber*, SA= *Sauropus androgynus*, I= infected by *Salmonella typhimurium*  
a. day surgery to 12th; b. day surgery to 18th; c. mean relative number of B220+ cells (%)

(Basyaruddin et al., 2015)
The level of TER119 and GR-1

➢ The combination of *E. scaber* and *S. androgynus* for *S. thypimurium* infected mice restored into their normal conditions.

➢ The best combination for B220+ cells is P2 treatment (150 mg.kg-1BW *E. scaber* and 37.5 mg.kg-1BW *S. androgynus*).

➢ While the best dose for increasing TER119+ cells is P5 treatment (*S. androgynus* 150 mg.kg-1BW, while the GR-1+ cells affected by the surgery.

➢ These dose formulations can be used to optimize the number of lymphocytes and not harmful for the developing fetus.

(Basyaruddin et al., 2015)
Herbal Supplement Formula of *E. scaber* and *S. androgynus* Promotes IL-2 Cytokine Production of CD4\(^+\) T Cells in Pregnant Mice with Typhoid Fever

**RESULTS**

Figure 1 The intracellular expression levels of IL-2 cytokine produced by CD4\(^+\) T cells in all treatments.

P1 = 100% *E. scaber*; P2 = 75% *E. scaber* : 25% *S. androgynus*; P3 = 50% *E. scaber* : 50% *S. androgynus*; P4 = 25% *E. scaber* : 75% *S. androgynus*; P5 = 100% *S. androgynus*.

On day 18, mice were dissected, splenocyte cells were stained by intracellular staining then analyzed by flowcytometry. Data were mean ± SD values of 5 mice in each group with p value < 0.05.

*Djati et al. 2016; Fuadah et al. 2015*
Figure 2 The relative number of CD4+ T lymphocyte cells in all treatments. P1 = 100% *E. scaber*; P2 = 75% *E. scaber*: 25% *S. androgynus*; P3 = 50% *E. scaber*: 50% *S. androgynus*; P4 = 25% *E. scaber*: 75% *S. androgynus*; P5 = 100% *S. androgynus*. On day 18, mice were dissected, splenocyte cells were stained by extracellular staining then analyzed by flowcytometry. Data presented is mean ± SD values (0.78, 2.74, 1.44, 0.48, 3.35, 6.23, 3.96) of 5 mice in each group with p value < 0.05. Notation letters printed on each bar showed the significant difference between the investigated groups.

Djati et al. 2016

Figure 3. The intracellular expression levels of IFN-γ cytokine produced by CD4+ T cells in all treatments. P1 = 100% *E. scaber*; P2 = 75% *E. scaber*: 25% *S. androgynus*; P3 = 50% *E. scaber*: 50% *S. androgynus*; P4 = 25% *E. scaber*: 75% *S. androgynus*; P5 = 100% *S. androgynus*. On day 18, mice were dissected, splenocyte cells were stained by intracellular staining and then analyzed by flowcytometry. Data presented is mean ± SD values of 5 mice in each group with p value < 0.05.

Djati et al. 2016
The intracellular expression levels of IL-4 cytokine produced by CD4+ T cells in all treatments. P1 = 100% E. scaber; P2 = 75% E. scaber : 25% S. androgynus; P3 = 50% E. scaber : 50% S. androgynus; P4 = 25% E. scaber : 75% S. androgynus; P5 = 100% S. androgynus. On day 18, mice were dissected, splenocyte cells were stained by intracellular staining and then analyzed by flowcytometry. Data were mean ± SD values of 5 mice in each group with p value < 0.05.

Djati et al. 2016; Fuadah et al. 2015

The relatives number of B220+ T lymphocyte cells in all treatments. P1 = 100% E. scaber; P2 = 75% E. scaber : 25% S. androgynus; P3 = 50% E. scaber : 50% S. androgynus; P4 = 25% E. scaber : 75% S. androgynus; P5 = 100% S. androgynus. On day 18, mice were dissected, splenocyte cells were stained by extracellular staining then analyzed by flowcytometry. Data presented is mean ± SD values (2.39, 20.45, 0.63, 0, 2.08, 3.42, 0.75) of 5 mice in each group with p value < 0.05. Notation letters printed on each bar showed the significant difference between the investigated groups.

Djati et al. 2016
Findings

✓ Typhoid fever decreased the amount of CD4 T cells but rather increased the amount of B cells.

✓ The formulation of *E. scaber* and *S. androgynus* was able to modulate the immune system via promoting IL-2 cytokines in pregnancy during typhoid fever.

✓ This condition made the relative number of CD4+ T cells and the production of IFN-γ cytokines increase while the expression level of IL-4+ cytokine and B220+ lymphocyte cells decreased.

✓ The formulation of *E. scaber* and *S. androgynus* that gave the optimum effect in all investigated parameters was P3 (50% *E. scaber* : 50% *S. androgynus*) which was not significantly different compared to healthy pregnant mice.
Modulating hormonal system in pregnant mice with *S. typhi* infection

A. 9 days pregnancy after *S. typhi* infection and administrated with combination *E. scaber* and *S. androgyunus* leaves extract.
B. 13 days pregnancy after *S. typhi* infection and administrated with combination *E. scaber* and *S. androgyunus* leaves extract.
C. 17 days pregnancy after *S. typhi* infection and administrated with combination *E. scaber* and *S. androgyunus* leaves extract.

*Djati et al. 2017*
E. scaber and S. androgynus modulated erythrocytes levels

Figure 2: Effects of different formulations of ethanol extract from E. scaber and S. androgynus leaves on erythrocyte (TER119+) numbers.
T1, control;
T2, Salmonella typhi infection;
T3, Salmonella typhi infection with 100 % E. scaber;
T4, Salmonella typhi infection with 75 % E. scaber and 25 % S. androgynus;
T5, Salmonella typhi infection with 50 % E. scaber and 50 % S. androgynus;
T6, Salmonella typhi infection with 25 % E. scaber and 75 % S. androgynus;
T7, Salmonella typhi infection with 100 % S. androgynus.

Djati et al. 2017
Figure 3: Effect of different formulations of ethanol extracts from *E. scaber* and *S. androgynus* leaves on the numbers of erythrocyte precursors (TER119.VLA4+) and mature erythrocytes (TER119.VLA4-).

T1, control;  
T2, *Salmonella typhi* infection;  
T3, *Salmonella typhi* infection with 100 % *E. scaber*;  
T4, *Salmonella typhi* infection with 75 % *E. scaber* and 25 % *S. androgynus*;  
T5, *Salmonella typhi* infection with 50 % *E. scaber* and 50 % *S. androgynus*;  
T6, *Salmonella typhi* infection with 25 % *E. scaber* and 75 % *S. androgynus*;  
T7, *Salmonella typhi* infection with 100 % *S. androgynus*.

Djati et al. 2017
Findings

✓ *E. scaber* and *S. androgynus* modulate erythropoiesis and prolactin levels in pregnant typhoid mice.

✓ Administration of *E. scaber* extract increases the number of erythrocyte precursors, whereas administration of *S. androgynus* extract increases the number of mature erythrocytes and prolactin levels.

✓ Administration of the combination of 50 % *E. scaber*/50 % *S. androgynus* results in balanced numbers of precursor and mature erythrocytes.
HORMONAL BALANCE
Recall from Chapter 3 that G protein signaling mechanisms are like a molecular relay race.

1. **Hormone (1st messenger) binds receptor.**

2. **Receptor activates G protein (G_s).**

3. **G protein activates adenylate cyclase.**

4. **Adenylate cyclase converts ATP to cAMP (2nd messenger).**

5. **cAMP activates protein kinases.**

These processes trigger responses of the target cell (activates enzymes, stimulates cellular secretion, opens ion channel, etc.)
Figure 16.3  Direct gene activation mechanism of lipid-soluble hormones.

1. The steroid hormone diffuses through the plasma membrane and binds an intracellular receptor.

2. The receptor-hormone complex enters the nucleus.

3. The receptor-hormone complex binds a specific DNA region.

4. Binding initiates transcription of the gene to mRNA.

5. The mRNA directs protein synthesis.
HERBAL FORMULA
Feed supplement
Methodology

Leaf extract
Elephanttopus scaber & Sauropus Androgynus

Formulation

Pregnant mice

Salmonella sp

Treatments

ES 100%
ES 75%
ES 50%
SA 75%
SA 100%

sectio

timus
PGST
PRL

Ssm tl

llimpa

CD 4, CD8, TERR 199
CD 4, CD8+, TERR 199
CD 4, CD8+, TERR 199
PDST
PRL
Result and discussion
GRAFIK KONSENTRASI HORMON ESTRADIOL (ESTROGEN)
Ho: \( P_2 = P_3 = P_4 = P_5 \)
H1: \( P_2 \neq P_3 \neq P_4 \neq P_5 \)

\[ Y = BX + C \]

Intercept test:
Ho: \( = Bp_2 = Bp_3 = Bp_4 = Bp_5 \)
H1: \( = Bp_2 \neq Bp_3 \neq Bp_4 \neq Bp_5 \)

Constanta test
Ho: \( Cp_2 = Cp_3 = Cp_4 = Cp_5 \)
H1: \( Cp_2 \neq Cp_3 \neq Cp_4 \neq Cp_5 \)
FSH (Follicle stimulating hormone)

H₀: P₂=P₃=P₄=P₅
H₁: P₂=P₃=P₄=P₅
GRAFIK KONSENTRASI HORMON LH
LH (Luteinizing hormone)
GRAFIK KONSENTRASI HORMON PROGESTERON
progesteron
GRAFIK KONSENTRASI HORMON PROLAKTIN
prolactin
Profil respon imunitas *M. musculus* bunting diinfeksi *S. typhimurium* terhadap dosis kombinasi ekstrak *E. scaber* dan *S. androgynus*. Tanda panah warna hijau ( ) menunjukkan adanya pengaruh dengan nilai koefisien. Tanda panah ( ) menunjukkan adanya pengaruh lain. Perlakuan dan hari pembedahan memberikan pengaruh terhadap jumlah sel T CD4⁺ dan sel T CD8⁺.
<table>
<thead>
<tr>
<th>Treatment</th>
<th>CD4+ Day-12</th>
<th>CD4+ Day-18</th>
<th>CD4+IL2+</th>
<th>B220+</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCMC</td>
<td>16.61 ± 0.16a</td>
<td>19.73 ± 4.75ab</td>
<td>12.93 ± 3.62abc</td>
<td>1.64 ± 0.30bc</td>
</tr>
<tr>
<td>NaCMC + I</td>
<td>36.81 ± 18.15</td>
<td>17.98 ± 1.35a</td>
<td>9.29 ± 3.28a</td>
<td>1.44 ± 0.19abc</td>
</tr>
<tr>
<td>ES 200 + I</td>
<td>37.55 ± 14.83</td>
<td>25.21 ± 4.92ab</td>
<td>15.65 ± 2.54bc</td>
<td>1.33 ± 0.29ab</td>
</tr>
<tr>
<td>ES 150 + SA 37.5 + I</td>
<td>15.05 ± 1.91ab</td>
<td>17.33 ± 5.17ab</td>
<td>20.88 ± 3.37ab</td>
<td>1.25 ± 0.30a</td>
</tr>
<tr>
<td>ES 100 + SA 75 + I</td>
<td>24.32 ± 4.22b</td>
<td>34.35 ± 18.68</td>
<td>15.82 ± 5.67a</td>
<td>1.33 ± 0.17ab</td>
</tr>
<tr>
<td>ES 50 + SA 112.5 + I</td>
<td>31.31 ± 14.60</td>
<td>20.39 ± 2.11ab</td>
<td>30.85 ± 10.20b</td>
<td>1.71 ± 0.42c</td>
</tr>
<tr>
<td>SA 150 + I</td>
<td>23.07 ± 7.69b</td>
<td>33.06 ± 12.77</td>
<td>30.54 ± 8.79b</td>
<td>1.55 ± 0.14abc</td>
</tr>
<tr>
<td>Total Rerata ± SD</td>
<td>33.71 ± 13.51a</td>
<td>31.31 ± 14.60</td>
<td>30.54 ± 8.79b</td>
<td>1.55 ± 0.14abc</td>
</tr>
</tbody>
</table>

**Diagram**

- **Stem cell**
- 0.193
- 0.945
- 0.945
- P<0.05

**Legend**

- Infeksi
- E. scabir
- S. androginus
- Infeksi+ES 150+SA 37.5
- Infeksi+ES 100+SA 75
- Infeksi+ES 50+SA 112.5
Antibacterial activity of *E. scaber* and *S. androgynous* leaves and whole plants extract combination against *E. coli*

by:
Muhammad Sasmito Djati

Biology Department
Faculty of Mathematics and Natural Sciences,
Brawijaya University, Malang 65145, East Java, Indonesia
Antibacterial Activities

Shiga toxin type 2

pregnancy loss and maternal morbidity in rats in the early stage of pregnancy (Sacerdoti et al., 2014).

antioxidant, antimicrobial (Hayati et al., 2016)

anti-bacterial, anti-inflammatory, wound healing and antibiotics (Ariharan et al., 2013).

Extract combination

Leaves

Whole plant extract

No report about antibacterial activity
Methods

Antibacterial activity test

Plant extraction

E. coli inoculums preparation

In vitro Antibacterial Assay

disc diffusion method (Bauer et al. 1966).

1. Leaves extract combination
2. Whole plant extract (Leaves, root, stem) combination

various ratio doses of E. scaber (ES) and S. androgynous (SA) extract:
1. 100:0 %,
2. 75:25 %,
3. 50:50 %,
4. 25:75 %, and
5. 0:100 %.
Results
Antibacterial activity of *E. scaber* and *S. androgynus* leaves extract combination

![Figure 1. Antibacterial activity of *Elephantopus scaber* and *Sauropus androgynus* L. leaves extract combination](image-url)
Antibacterial activity of *E. scaber* and *S. androgynus* whole plant extract combination

![Graph showing inhibition zone of whole plant extract combination](image)

Figure 2. Antibacterial activity of *Elephantopus scaber* and *Sauropus androgynus* L. whole plant extract combination
Figure 1. Zone of inhibition of *E. scaber* and *S. androgynus* leaves extract combination.

Figure 2. Zone of inhibition of *E. scaber* and *S. androgynus* whole plant extract combination.

**P3** = ES(50%) : SA(50%) 

The highest zone of inhibition in leaves extract combination treatment

The highest zone of inhibition in whole plant extract combination treatment
Roles of cells in the liver: Nitric oxide (NO), a pro-inflammatory mediator

*Bacteria*

Bacterial endotoxins (LPS)

*Innate immunity:*
Anti-bacterial and anti-viral effects

*Kupffer cells (macrophages)*

IL-1β

*Hepatocytes*

NO

TNF-α

*When excessive:*
Cell injury and death
Septic shock (NO)

**2016-5**
Discussion

• We used the combination of *E. scaber* and *S. androgynous* extract to know the best combination for inhibit the bacterial growth. As we know that many research has been proved that the antibacterial activity of each plant extract. Thus, the use of the extract combination of both plants extract may be generate the most potent herbal to treat *E. coli* infection.

• The antibacterial action in leaves extract of *S. androgynus* is due to the existence of vitamin, peptide, glycoside, alkaloid, saponin, terpenoid, and flavonoid (Ariharan et al., 2013).

• The tannins found in *S. androgynus* extracts may be responsible for the antibacterial activity.

• *E. scaber* has chemical substance like phenol, saponin, steroids, tanin, and xanthoprotein where the saponin has ability as antibacterial agents of plants and also the steroid.

• The use of the extract combination of both plants extract may be generate the most potent herbal to treat *E. coli* infection.
Conclusion

- *E. scaber* and *S. androgynous* both leaves and whole extract combination were potentially effective against *E. coli* at dose ratio of ES: SA = 50:50.
- Hence, this study confirms the extract combination of *E. scaber* and *S. androgynous* can be used as natural antimicrobial alternative and suggests the possibility of employing them for treatment of infectious disease caused by *E. coli*. 
Histopathological Study on Kidney
Histopathological study on Liver and Kidney

Fig 6. The effects of combination ethanol extracts from *E. scaber* and *S. androgynus* leaves on average number of necrotic of hepatocyte cell and ephitelia cell of cortex kidney.
• Kidney histopathology structure (400 x magnification). (A) Normal group (K-);

• (B) *S. typhimurium* infection without treatment (K+);

• (C) *S. typhimurium* infection with 100 % *E. scaber* (P1);

• (D) *S. typhimurium* infection with 75 % *E. scaber* and 25 % *S. androgynous* (P2); (E) *S. typhimurium* infection with 50 % *E. scaber* and 50 % *S. androgynous* (P3);

• (F) *S. typhimurium* infection with 25 % *E. scaber* and 75 % *S. androgynous* (P4);

• (G) *S. typhimurium* infection with 100 % *S. androgynous* (P5). Black circle indicating normal hepatocyte cell, red circle indicating necrotic hepatocyte cell.
A). Histological structure of kidney cortex from k-group, Red circle is indicate of necrotic ephitel cell. Black circle is indincate of normal ephitel cell.
• B). Histological structure of kidney cortex from K+ group, Red circle is indicate of necrotic ephitel cell. Black circle is indincate of normal ephitel cell.
• C). Histological structure of kidney cortex from P1 group, Red circle is indicate of necrotic epithel cell. Black circle is indicate of normal epithel cell.
• D). Histological structure of kidney cortex from P2 group, Red circle is indicate of necrotic epithel cell. Black circle is indincate of normal epithel cell.
• E). Histological structure of kidney cortex from P3 group, Red circle is indicate of necrotic epithel cell. Black circle is indincate of normal epithel cell.
• F). Histological structure of kidney cortex from P4 group, Red circle is indicate of necrotic epithel cell. Black circle is indicate of normal epithel cell.
• G). Histological structure of kidney cortex from P5 group, Red circle is indicate of necrotic epithel cell. Black circle is indincate of normal epithel cell.
• Histophatological study on the liver showed that the infection of *Salmonella typhimurium* caused hepatic necrotic.

• This mechanism was encode by *Salmonella* phatogenicity island 1 (SPI-1). SPI-1 secretion system was shown to induced invasion of macrophages that caused rapid apoptosis in host. SPI-1 will be activates SipB, a translocated effector, is a mediator to form a hole in the host plasma membrane. SipB binds and activates caspase-1, which is has role as pro-apoptotic protease.
Salmonella typhimurium invasion into epithelium. S. Typhimurium escapes into the cytosol in a fraction of epithelial cells, thereby triggering proinflammatory cell death (pyroptosis)(Winter and Baumler, 2011).
• The mechanism of pathogenesis of S. typhimurium as initiated by S. typhimurium bacterial penetration into the epithelial cells before inducing disease.

• Upon contact with the epithelium, S. typhimurium induces cell followed by damaging the inner membrane as a place of contact between the bacterial cell and the host cell. When bacteria invade perfectly, S. typhimurium located and multiply inside the endosome.
• The combination extract of *E. scaber* and *S. androgynus* could decreased the average percentage of necrotic cell in kidney and liver.
• Both plant contain deidzein isoflavone.
• deidzein was showed to significantly suppress LPS-induced mRNA expression of pro-inflammatory in murine microgial cell \(^{18}\). Another study showed that the administration of deidzein was induced the decreasing of TNF-\(\alpha\) level
Conclusion

• The combination of *E. scaber* and *S. androgynus* leaf extract have efficacy for:
  
  • 1. Stabilizing progesterone and estrogen in pregnant mice infected with *S. typhimurium* at a dose P4.
  
  • 2. The histopathological study showed that all treatments were significantly decreased the percentage of necrotic cell on kidney.
HERBAL FORMULA as Feed supplement
Thank you
PENCAK SILAT IS INDONESIAN ENDEMIC CULTURE

TERIMA KASIH
Traditional Medicine Research Group

Dr. M. S. Djati (Prof)

Dr. N. Widodo (Prof)

Dr. M. Rifai (Prof)

Y. A. Rahma

J. I. Cristhina

Dwijayanti