

CHEMOPREVENTION OF BAWANG DAYAK/SABRANG (*Eleutherine palmifolia* (L.) Merr.) EXTRACTS TO COLITIS-ASSOCIATED COLON CANCER MODEL AS THE APPLICATION OF AL QUR'AN SURAH AL-BAQOROH VERSE 61

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Burhan Ma'arif, S.Farm., M.Farm., Apt

Collabration with:
Prof. Dr. Nobuo Kawahara From NIBIOHN (*National Institute Biomedical Health
and Nutrition*) Japan

**31 JULI-2 AGUSTUS 2017
HOTEL AMOROSSA BEKASI**



BACKGROUND

Table 1. Leading Causes of Death Worldwide by Income Level, 2012 (Thousands)

	Worldwide			Low- and Middle-income			High-income		
	Rank	Deaths	%	Rank	Deaths	%	Rank	Deaths	%
Cardiovascular diseases	1	17,513	31%	1	13,075	30%	1	4,438	38%
Malignant neoplasms	2	8,204	15%	3	5,310	12%	2	2,894	25%
Infectious and parasitic diseases	3	6,431	12%	2	6,128	14%	7	303	3%
Respiratory diseases	4	4,040	7%	4	3,395	8%	3	645	6%
Unintentional injuries	5	3,716	7%	5	3,212	7%	5	504	4%
Respiratory infections	6	3,060	5%	6	2,664	6%	6	396	3%
Digestive diseases	7	2,263	4%	7	1,748	4%	4	515	4%
Diabetes mellitus	8	1,497	3%	8	1,243	3%	9	254	2%
Intentional injuries	9	1,428	3%	9	1,185	3%	10	243	2%
Genitourinary diseases	10	1,195	2%	10	935	2%	8	260	2%
Nutritional deficiencies	11	559	1%	11	534	1%	14	25	0%
Congenital anomalies	12	556	1%	12	515	1%	13	42	0%
Maternal conditions	13	296	1%	13	293	1%	16	3	0%
Musculoskeletal diseases	14	216	0%	14	158	0%	12	58	1%
Other neoplasms	15	193	0%	15	116	0%	11	77	1%
All causes		55,843			44,172			11,671	

Source: World Health Organization Global Health Observatory Data Repository, Mortality and Global Health Estimates 2012. apps.who.int/gho/data/?theme=main. Accessed August 24, 2014.

American Cancer Society, Inc., Surveillance Research, 2015



WORLDHEALTHRANKINGS
LIVE LONGER LIVE BETTER



Indonesia

Cancer Statistics

Population in 2012:	244.8m
People newly diagnosed with cancer (excluding NMSC) / yr:	299,700
Age-standardised rate, incidence per 100,000 people/yr:	133.5
Risk of getting cancer before age 75:	14.0%
People dying from cancer /yr:	194,500

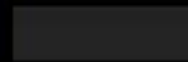
Data from IARC GlobalCan (2012)



INDONESIA CANCER RANK BY TYPE AGE-STANDARDIZED DEATH RATE

PER 100,000 POPULATION

GOOD



POOR

	Rate	World Rank		Rate	World Rank
1. Breast Cancer	19.02	61	9. Oral Cancer	5.74	28
2. Lung Cancers	17.16	65	10. Leukemia	4.12	71
3. Prostate Cancer	14.01	105	11. Pancreas Cancer	3.22	90
4. Colon-Rectum Cancers	10.34	65	12. Stomach Cancer	3.12	136
5. Liver Cancer	9.73	38	13. Bladder Cancer	2.18	83
6. Cervical Cancer	9.20	75	14. Uterin Cancer	2.04	84
7. Ovary Cancer	6.86	24	15. Oesophagus Cancer	1.12	137
8. Lymphomas	6.37	43	16. Skin Cancers	0.84	135

WHAT SHOULD WE DO?

Finding a new specific
drug as anticancer to
solve this problem

SOLUTION

وَإِذْ قُلْتُمْ يَا مُوسَىٰ لَنْ نَصْبِرَ عَلَىٰ طَعَامٍ وَاحِدٍ فَادْعُ لَنَا رَبَّكَ يُخْرِجْ لَنَا مِمَّا تُنْبِتُ الْأَرْضُ مِنْ بَقْلِهَا وَقِثَّائِهَا وَفُومِهَا وَعَدَسِيهَا وَبَصَلِهَا قَالَ أَتَسْتَبْدِلُونَ الَّذِي هُوَ أَدْنَىٰ بِالَّذِي هُوَ خَيْرٌ اهْبِطُوا مِصْرًا فَإِنَّ لَكُمْ مَّا سَأَلْتُمْ وَضُرِبَتْ عَلَيْهِمُ الذِّلَّةُ وَالْمَسْكَنَةُ وَبَاءُوا بِغَضَبٍ مِنَ اللَّهِ ذَٰلِكَ بِأَنَّهُمْ كَانُوا يَكْفُرُونَ بِآيَاتِ اللَّهِ وَيَقْتُلُونَ النَّبِيِّينَ بِغَيْرِ الْحَقِّ ذَٰلِكَ بِمَا عَصَوْا وَكَانُوا يَعْتَدُونَ

1. Onion is Special plant that mentioned in the Quran, one kind of the species of bawang merah (onion) is *Eleuterine palmifolia* (bawang dayak)
2. Special plant origin from Indonesia especially in Kalimantan
3. Dayak Tribe usually used bawang dayak as traditional herb for all disease including cancer
4. Bawang dayak have been explore, but we need explore specific potency for cancer disease.



BAWANG DAYAK (*Eleutherine palmifolia*)

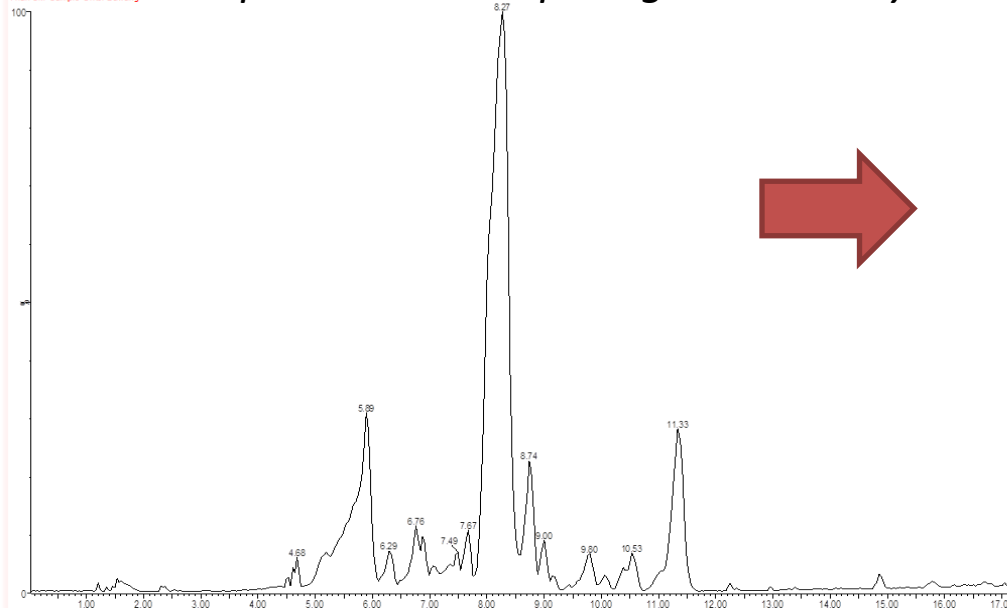
1. *Eleuterine palmifolia* have hight potency againt cervix cancer



Fraction	IC ₅₀ Value (µg/mL)
N-hexane	199,38
Chloform	107,842
Ethyl acetate	44,494
Water	1339

2. We found 28 compuond from bawang dayak, the mayor active compound are isoliquiritigenin and oxyresveratrol

UNIPOL
Titrasi sample Umbi Bawang

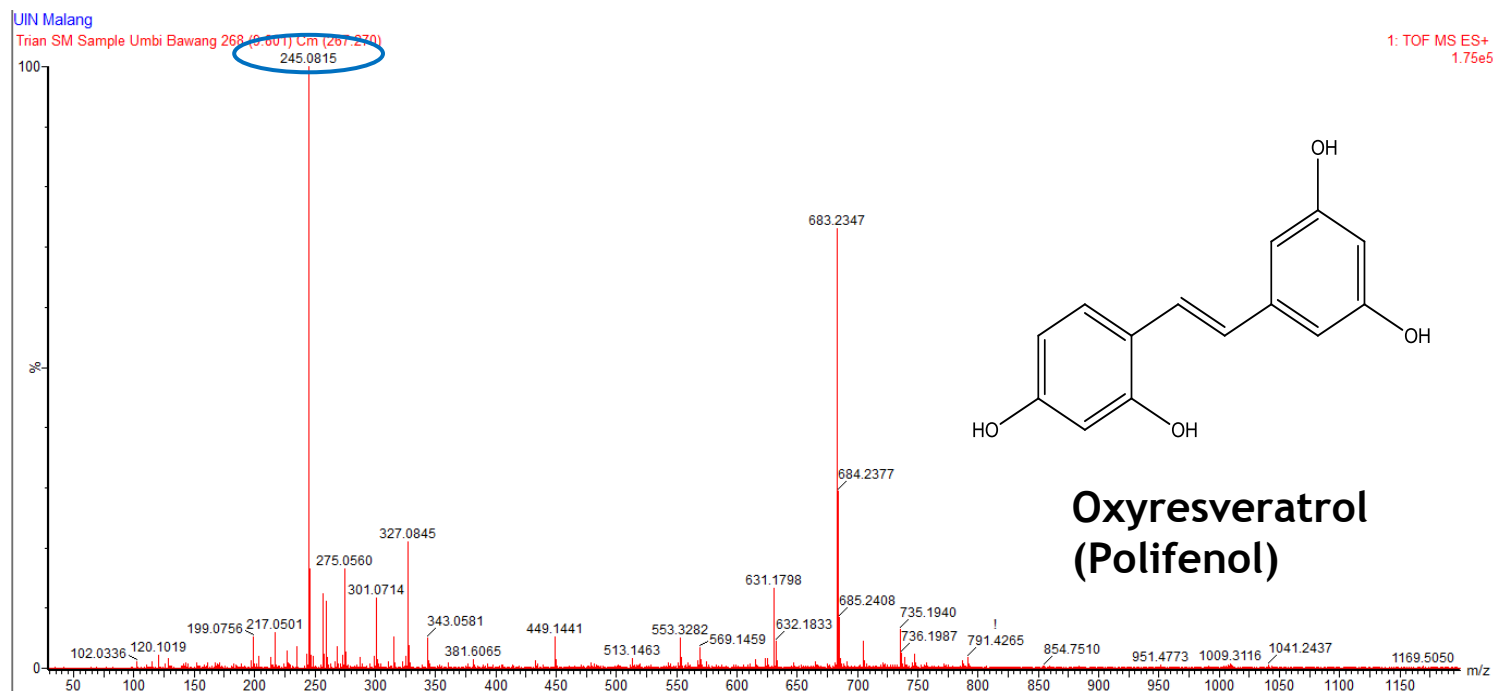
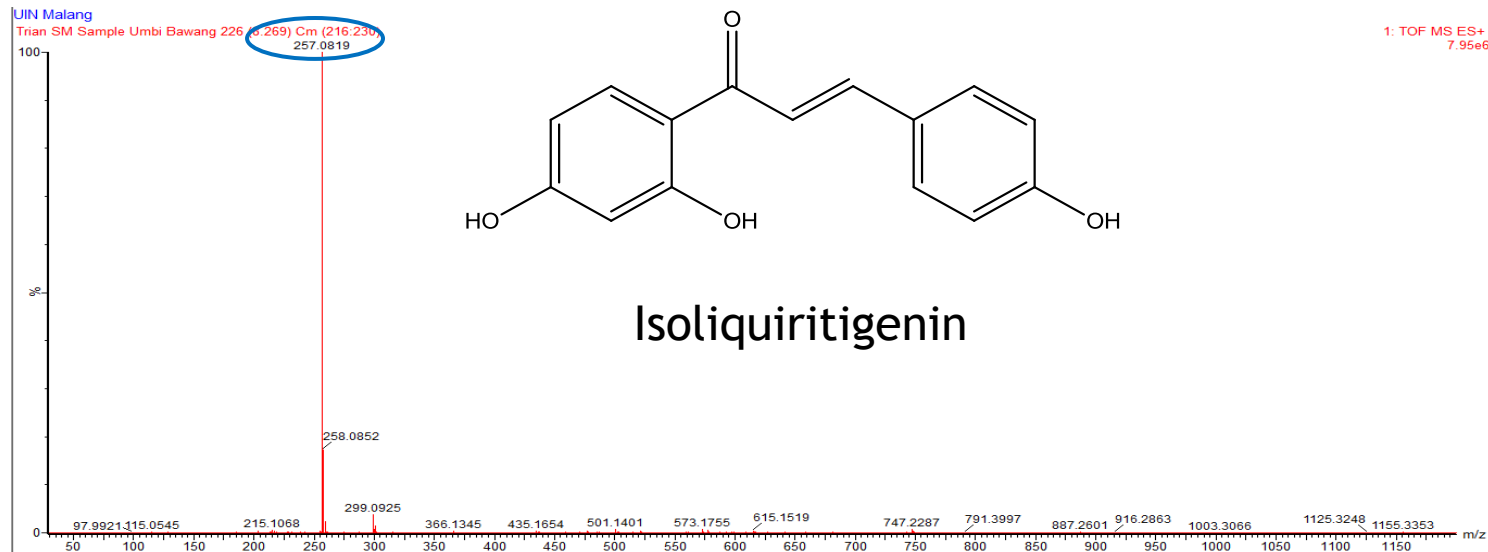


1-TOF MS ES+
BP1
7.88e5

6.291	244,0736	244,0736	1,161828	C ₁₄ H ₁₂ O ₄ Oxyresveratrol	Polifenol (stilbene)
6.760	234,0892	234,0892	2,81839	C ₁₅ H ₁₄ O ₄ 4-(1,3-Benzodioxol-5-yl)-4-hydroxycyclohexanone	Terpenoid
7.057	256,11	256,1099	0,246105	C ₁₆ H ₁₄ O ₃ Pterostilbene / (3,5-dimethoxy-4'-hydroxystilbene)	Polifenol (stilbene)
7.492	258,0892	258,0892	0,753431	C ₁₅ H ₁₄ O ₄ Menbutone / 4-(4-Methoxy-1-naphthyl)-4-oxobutanoic acid	Naphtalene
7.674	434,1577	434,1577	1,294673	C ₂₂ H ₂₆ O ₉ 3,3'-[Oxybis(2,1-ethanedioxy-2,1-ethanedioxy)]dibenzoic acid	Terpenoid
8.269	256,0736	256,0736	51,52892	C ₁₅ H ₁₁ O ₄ Isoliquiritigenin / (2E)-1-(2,4-Dihydroxyphenyl)-3-	Flavonoid (khalkon)

BACKGROUND

**Mayor
Active
Compound**



THE AIM OF OUR RESEARCH:

1. To proof the anticancer activity of extract bawang dayak against colon cancer cell
2. To find the antiproliferation effect of bawang dayak by exemine MPO level
3. To Find the potency of bawang sabrang as immunomodulator by exemine IL4, IL 10.

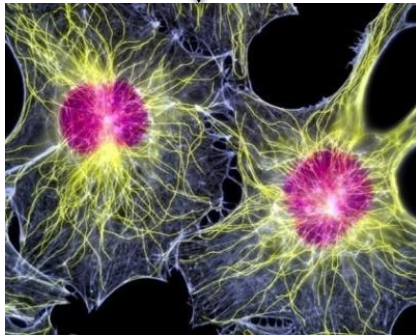


ROAD MAP



Eleutherine palmifolia

Drug Formulation



In Vitro Assay



In Vivo Assay



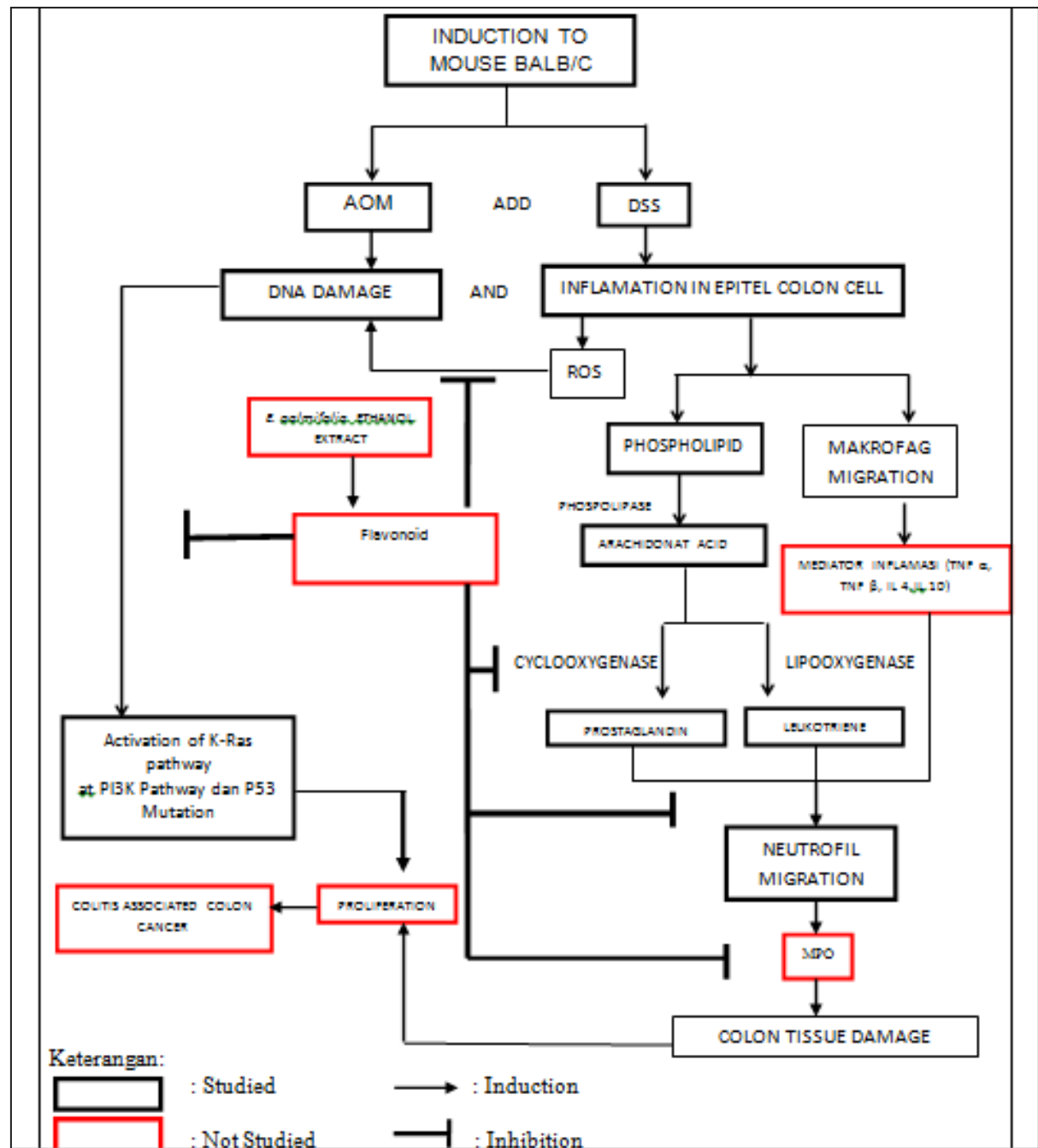
Clinical Study



**PHYTOHARMACA
FOR CANCER**



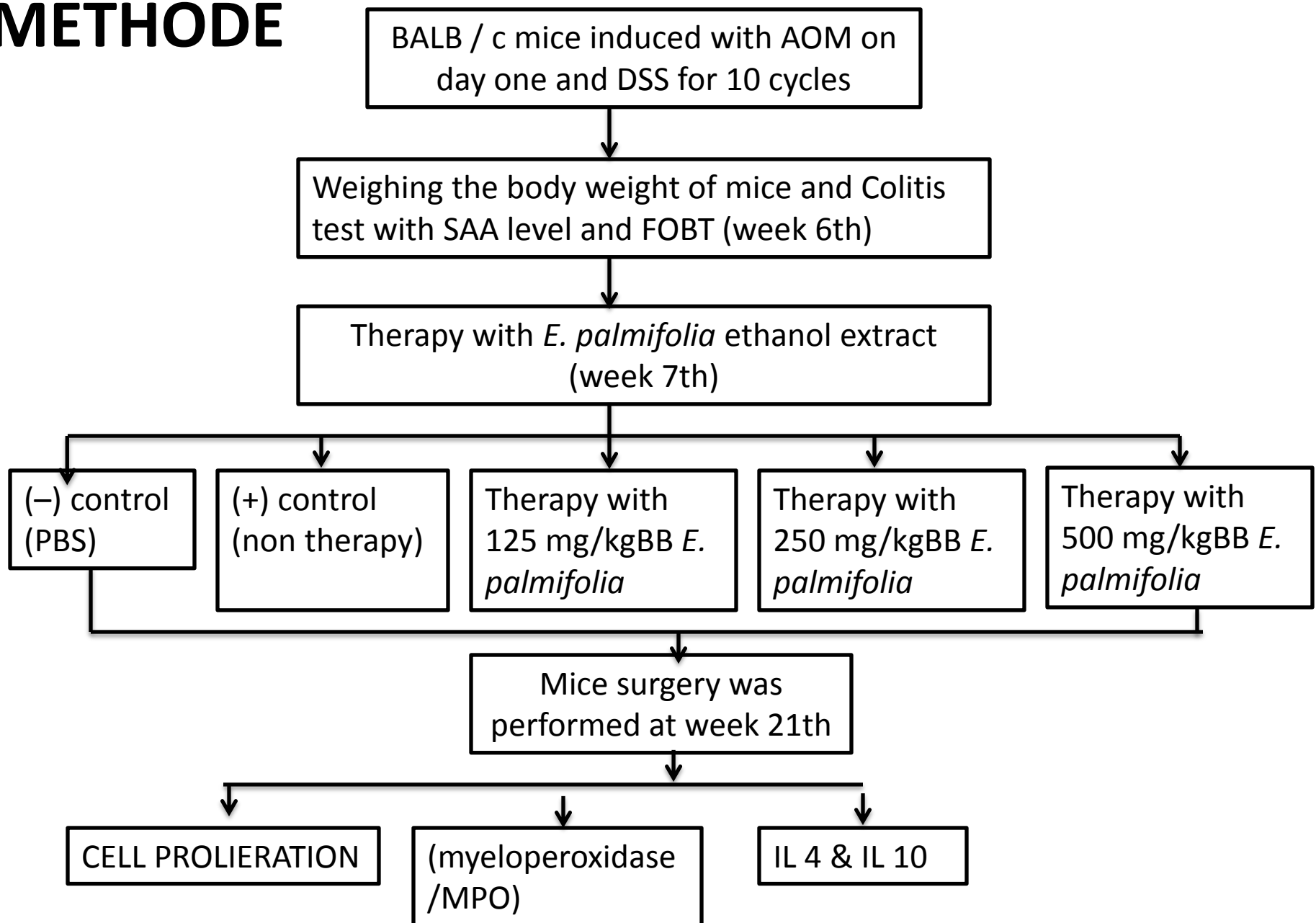
Conceptual Framework



METHODE



METHODE



April 8, 2017

To : Whom it may concern

LETTER OF ACCEPTANCE

I am Dr. Nobuo Kawahara, The Director of the Research Center for Medicinal Plant Resources, National Institutes of Biomedical Innovation, Health and Nutrition (NIBIOHN), agreed to accept the person below:

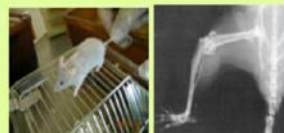
Name : Dr. Roihatul Mutiah, S.F., MKes., Apt
Date of Birth : February 3rd, 1980
Place of Birth : Malang
Nationality : Indonesia
Occupation : Lecturer of FKIK UIN Maulana Malik Ibrahim Malang, Indonesia. As an International Collaborative Research Program at my laboratory.

Sincerely yours,

Dr. Nobuo Kawahara,

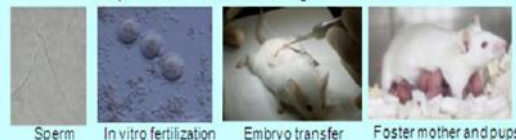
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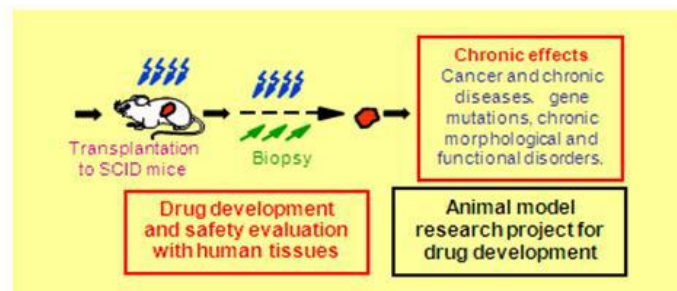
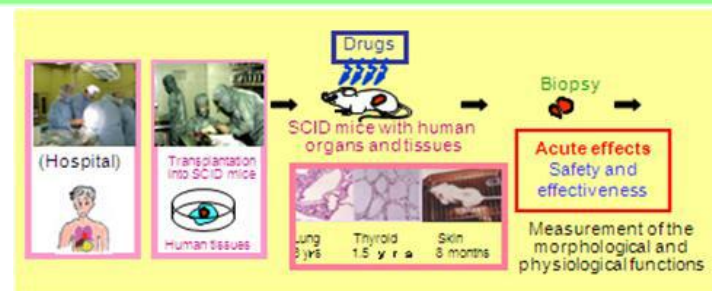
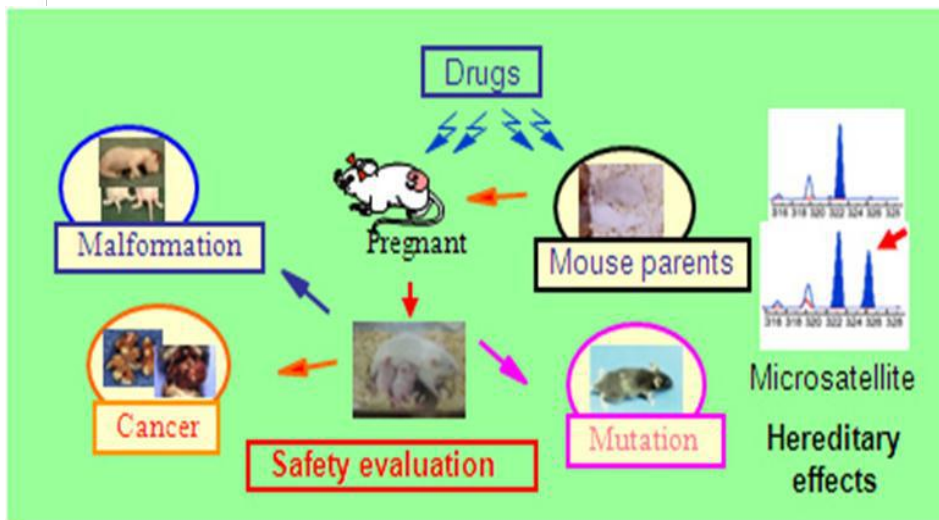
Osteoarthritis mice

Reproductive biotechnologies in mice



Sperm In vitro fertilization Embryo transfer Foster mother and pups


NIBIOHN has *laboratory animal model for human disease*



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Nobuo Kawahara article



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PMCID: PMC4820610

Pterisin B prevents chondrocyte hypertrophy and osteoarthritis in mice by inhibiting Sik3

Yasuhiro Yahara,^{1,2} Hiroshi Takemori,³ Minoru Okada,¹ Azuma Kosai,¹ Akihiro Yamashita,¹ Tomohito Kobayashi,¹ Kaori Fujita,¹ Yumi Itoh,³ Masahiro Nakamura,⁴ Hiroyuki Fuchino,⁵ Nobuo Kawahara,⁵ Naoshi Fukui,⁶ Akira Watanabe,⁴ Tomoatsu Kimura,² and Noriyuki Tsumaki^{2,1}

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Abstract



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Identification of New Diterpenes as Putative Marker Compounds Distinguishing *Agnus Castus* Fruit (Chaste Tree) from Shrub Chaste Tree Fruit (*Viticis Fructus*)

Naohiro Oshima^{1,2}, Sayaka Masada^{1,2}, Ryuta Suzuki¹, Kanae Yagi¹, Hiroshi Matsufuji², Emi Suenaga¹, Yutaka Takahashi¹, Tadahiro Yahagi¹, Masato Watanabe¹, Shoji Yahara¹, Osamu Iida¹, Nobuo Kawahara¹, Takuro Maruyama¹, Yukihiko Goda¹, Takashi Hakamatsuka¹

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Journal of Natural Medicines
January 2016, Volume 70, Issue 1, pp 28-35

Callicarpa longissima extract, carnosol-rich, potently inhibits melanogenesis in B16F10 melanoma cells

Authors Authors and affiliations

Minori Yamahara, Koji Sugimura, Ayako Kumagai, Hiroyuki Fuchino, Azusa Kuroi, Mai Kagawa, Yumi Itoh, Hidehisa Kawahara, Yasuo Nagaoka, Osamu Iida, Nobuo Kawahara, Hiroshi Takemori, Hideto Watanabe

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本文

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前の記事 次の記事

本文

既存添加物カンゾウ油性抽出物の成分組成の多変量解析に基づく基原植物種の検討

多田 敦子¹, 石附 京子¹, 杉本 直樹¹, 吉松 高代², 川原 佳夫¹, 末松 孝子¹, 有福 和記³, 深井 俊夫⁴, 田村 幸吉⁵, 大橋 崇¹, 田原 麻衣子¹, 山崎 社¹, 嶋山 浩¹

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Effect of Bean Extract of *Yabumame* (*Amphicarpaea bracteata* (L.) Fernald subsp. *edgeworthii* (Benth.) H. Ohashi) on Low-Density Lipoprotein Oxidation *In Vitro*

Lifeng Yang¹, Junichi Kikuchi², Shogo Sakamoto², Mitsuhiro Takaseguchi², Kenji Fukumaga², Ryota Hosomi², Atsuyuki Hishida², Nobuo Kawahara¹, Takeshi Yamaguchi², Hirofumi Arai²

1) Department of Biotechnology and Environmental Chemistry, Kitami Institute of Technology 2) Department of Applied Chemistry and Biochemistry, Faculty of Engineering, Kyushu Sangyo University 3) Faculty of Chemistry, Materials and Bioengineering, Kansai University 4) Division of Hokkaido, Research Center for Medicinal Plant Resources, National Institute of Biomedical Innovation 5) Applied Research Center, Kitami Institute of Technology
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Ethyl acetate fraction of *Calotropis gigantea* roots induce apoptosis through increased G2/M and increased expression of caspase-8 in colon cancer WiDr cell line

Roihatul Mutiah, Aty Widyawaruyanti, Sukardiman Sukardiman
Universitas Islam Negeri Maulana Malik Ibrahim, Malang Indonesia
Universitas Airlangga

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Calotropis gigantea, ethyl acetate fraction, apoptosis, cell cycle, caspase-8, WiDr cell

ABSTRACT

Objectives: Cell apoptosis is one of important mechanisms and used as target for anticancer drugs. This study aimed at determining the mechanism of apoptosis induced by the most active fraction of *Calotropis gigantea* root extract in colon cancer cells WiDr. **Methods:** *Calotropis gigantea* root extract (CGRE) was fractionated using solvents including water, dichloromethane, ethyl acetate and butanol. All four fractions were tested for cytotoxicity using MTT method and the absorbance was measured at wavelength of 595 nm. Further, the mechanisms of cell cycle and apoptosis induced by the most active fraction were analyzed using Fluorescence-Activated Cell Sorting with marker (probe) propidium iodide (PI) and annexin V. **Results:** The results showed that the cytotoxicity of CGRE on WiDr cell line was 44.2 µg/ml, F1 (IC₅₀ 0.367 µg/ml), F2 (IC₅₀ 0.063 µg/ml), F3 (IC₅₀ 0.18 µg/ml), and F4 (IC₅₀ 2.24 µg/ml). WiDr cells treated with F2 caused changes in the cell cycle profile through an increased G2/M phase (38.18%), increased cell apoptosis (20.05%) and increased expression of caspase-8 (27.4%). **Conclusion:** F2 of CGRE exhibited anticancer activity against WiDr cell through cell cycle arrest G2/M phase enhancement and increased expression of caspase-8, that resulted in an increased cell apoptosis.

INTRODUCTION

Treatment of colon cancer has been intensified with surgery, chemotherapy and radiotherapy. However, such treatment is not able to effectively cope with cancer. Failure in the treatment of cancer by chemotherapy, for instance is due to low selectivity of anticancer drugs to normal cells, causing serious side effects in patients. Moreover, the failure of

problems. In general, selection of therapeutic targets in cancer cells is based on changes in the molecular regulation of cancer cells. Therefore, the present study investigated two main targets i.e. induction of cell apoptosis and cell cycle regulation. Apoptosis is programmed cell death and plays an important role in maintaining homeostasis of human body. Failure of apoptosis is the main factor of malignancy of cancer cells. Cancer treatment through induction of apoptosis has been known to prevent promotion, progression



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Original Article

CYTOTOXIC EFFECT OF CRUDE EXTRACT AND FRACTION FROM *CALOTROPIS GIGANTEA* LEAVES ON HUMAN COLON CANCER WIDR CELL LINES

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Received: 22 Sep 2015 Revised and Accepted: 08 Nov 2016

ABSTRACT

Objectives: This paper sought to understand and determine the cytotoxic effects of crude extract and its fraction from *Calotropis gigantea* leaves on human colon cancer WiDr cell lines.

Methods: The ethanolic extract was fractionated gradually with certain substances to yield four fractions. The substances were dichloromethane, ethyl acetate, and butanol. The four fractions resulted in dichloromethane fraction, ethyl acetate fraction, butanol fraction, and a water fraction. These fractions were then investigated for their cytotoxic effects on WiDr cells. The cell viability was assessed using MTT colorimetric assay.

Results: The result indicated that the cytotoxic effects of the ethanolic extract (IC₅₀ 48.5 µg/ml), ethyl acetate fraction (IC₅₀ 41.79 µg/ml), and dichloromethane fraction (IC₅₀ 40.57 µg/ml) produced a much more potent effect than the butanol fraction (IC₅₀ 737.74 µg/ml) and water fraction (IC₅₀ 8493 µg/ml).

Conclusion: The ethanolic extract, ethyl acetate fraction and dichloromethane fraction exhibited a potent cytotoxic effect on human colon cancer WiDr cell line. The crude extract and fractions are potential to be developed as an anticancer agent in colon cancer therapy.

Keywords: Cytotoxic, *Calotropis gigantea*, WiDr cells, Colon cancer.

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Title	Authors	Status
[Anticancer activity and apoptosis induction of ethyl acetate fraction of <i>Calotropis gigantea</i> roots in colon cancer WiDr cell line]	Roihatul Mutiah, Aty Widyawaruyanti, Sukardiman Sukardiman	Acceptance letter in PDF
		Your article is ready for publishing

THANK YOU

Procedure of AOM and DSS Induction

• Group of Research Subject

Group of Research Subject

Mgg	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
(+)	●	▲	○	▲	○	▲	○	▲	○	▲	○	▲	○	▲	○	▲	○	▲	○	▲	○	◇
125 mg/kgBB	●	▲	○	▲	○	▲	○	▲	○	▲	○	▲	○	▲	○	▲	○	▲	○	▲	○	◇
Therapy with 125 mg/kgBB E. palmifolia ethanol extract																						
250 mg/kgBB	●	▲	○	▲	○	▲	○	▲	○	▲	○	▲	○	▲	○	▲	○	▲	○	▲	○	◇
Therapy with 250 mg/kgBB E. palmifolia ethanol extract																						
500 mg/kgBB	●	▲	○	▲	○	▲	○	▲	○	▲	○	▲	○	▲	○	▲	○	▲	○	▲	○	◇
Therapy with 500 mg/kgBB E. palmifolia ethanol extract																						
(-)	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	◇

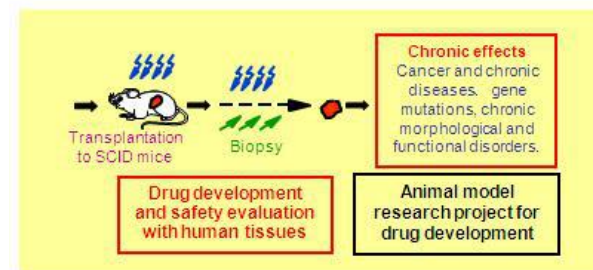
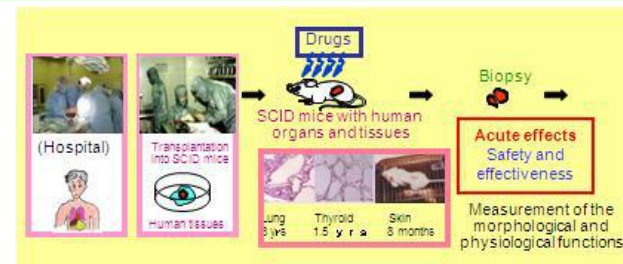
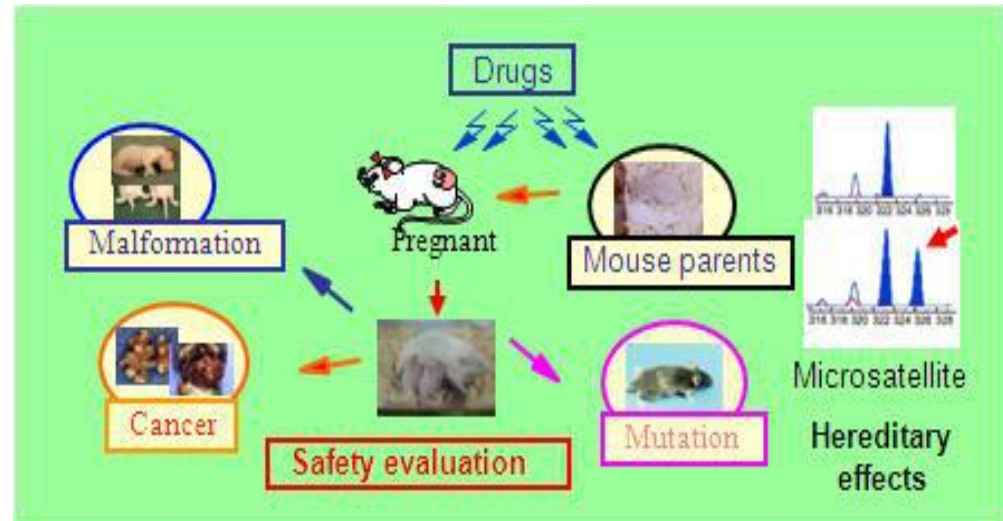
Info :

- : AOM intraperitoneal
- ▲ : DSS 5% + H₂O per oral
- : H₂O
- ◇ : Surgery

The Purpose collaboration with Nobuo Kawahara

1. To learn making animal model of Colitis Associated Colon Cancer
2. To Learn How to write & Publish our article to International journal Q1.
3. -Nobuo Kawahara wrote 168 publication , 2012 citation in some Q1 journal for example:
 - a. Nature communication
 - b. Planta Medica
 - c. Journal of Natural Medicine
 - d. Chemical and Pharmaceutical Bulletin
 - e. Journal of Biological Chemistry etc

NIBIOHN has *laboratory animal model for human disease*



<i>No</i>	<i>Activities</i>	<i>Place of research implementation</i>
1	Literature review	Biology Pharmaceutical Laboratory of UIN Maulana Malik Ibrahim, Malang Indonesia
2	Preparation of test materials	
3	Extraction	
4	Induction of carcinogens	
5	Testing on experimental animals	
6	Test MOP	Research Center for Medicinal Plant Resources, National Institute of Biomedical Innovation, Health and Nutrition (NIBIOHN), Hachimandai, Tsukuba, Ibaraki, Japan
7	Test cell proliferation	
8	Test IL-4 dan IL-10	
9	Induction Test of apoptosis	
10	Publication	International journal scopus index : <ol style="list-style-type: none"> 1. Asian Pacific Journal of Cancer Prevention (APJCP). 2. Journal of Applied Pharmaceutical Science JAPS 3. Saudi Pharmaceutical Journal (Elsevier)