

# Isolation and Cytotoxicity Test of Dichloromethane Extract of *Syzygium malaccense*

Novirlin Yosephin Daely, Tukiran\*  
Chemistry Departement  
Universitas Negeri Surabaya  
Surabaya, Indonesia  
tukiran@unesa.ac.id

**Abstract**—*Syzygium malaccense* or also known as Malay Apple is one of Myrtaceous plants. It is originally found in India and Malaysia. This plant has strong potential which can be traditional medicine. In the present study, it has been done an isolation the chemical components from dichloromethane extract of the plant and followed by identification of molecular structure on the isolated compound. Based on FT-IR analysis and by comparison with literature data, the isolated compound can be suggested as methyl ester. Cytotoxicity in BHK-21 cells using MTT-assay showed that the isolated compound is non toxic which has %living cell more than 60%.

**Keywords**—*S. malaccense*, ester, MTT-assay, BHK-21

## I. INTRODUCTION

Tropical plants can live well in harsh conditions due to climatic factors, herbivorous, insect or disease pests. Therefore, tropical plants are able to engineer many chemical compounds with various bioactives such as insecticides, antibacterials and cytotoxics. Natural chemical compounds in plants are secondary metabolites such as alkaloids, terpenoids, lignans, phenolic, flavonoids, and polyketides depending on the type of plants [1].

The genus *Syzygium* is considered one of the largest genera belonging to family Myrtaceae with about 156 species such as *S. aqueum*, *S. aromaticum*, *S. cumini*, *S. polyccephallum*, *S. malaccense*, *S. polyanthum*, *S. jambos*, and *S. pycnanthum* [2].

*S. malaccense* falls under the family of Myrtaceae and known as “Malay apple”. Some parts of the plant have been applied in traditional medicine. The plant extract of *S. malaccense* has potential to be developed as antimicrobial preservative agent against *S. aureus* and *C. albicans* [3]. Its bark extract has been shown to effectively serve a hypoglycemic agent that improved the fasting blood-sugar level and the liver-glycogen depletion [4]. According to its potential, *S. malaccense* can be the material of medicine or dental product.

The requirements of medicine or dental product are non irritant, biocompatible, non toxic and should not have negative effect against local, systemic or biological environment [5]. Cytotoxicity test use MTT-assay method. MTT is yellow tetrazolium bromide [3-(4,5-Dimethylthiazol-2-yl) 2,5-diphenyltetrazolium bromide].

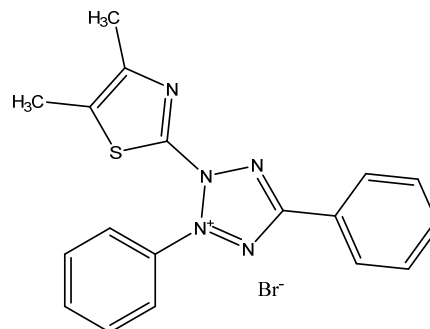


Fig. 1. MTT structure

MTT measure the living of cell based on mitochondria activity which reduce yellow tetrazolium bromide into insoluble formazan purple sediment. The darker color of purple of solution it means higher the number of living cell.

The present study is designed to isolate the bark extract of *S. malaccense* and evaluate cytotoxicity potentially. Identify of *S. malaccense* dichloromethane extract using different chromatographic techniques. Cytotoxicity test will be carried out by MTT method on BHK-21.

## II. MATERIAL AND METHOD

### A. Extraction

The bark of *Syzygium malaccense* was collected from Gondang Manis, Jombang, East Java, Indonesia. The sample was washed with tap water, dried overnight. It was dried and ground. Dried bark was macerated with methanol then partitioned with hexane and dichloromethane. Dichloromethane was evaporated to dryness in a vacuum rotary evaporator.

### B. Isolation

The dichloromethane extract of bark of *Syzygium malaccense* was isolated with some chromatography method use eluent hexane : ethyl acetate (5:1). Then it was identified with FT-IR Spectrophotometry.

### C. Cytotoxicity Test

*Baby Hamster Kidney (BHK-21)* cells were cultivated in a roux bottle and harvested with trypsin versene solution. BHK-21 was put into each 96 well microplate that has added D-MEM media which contain 10% fetal bovine serum albumin and incubated in 37°C for 24 hours. Isolated compound added into 96 well with concentration 12.5%, 25%, 50% and 100%. Microplate well was incubated for 24

hours. After 24 hours, each well that will be read were added with solution containing MTT reagent in PBS, and then reincubated for 4 hours. Then, each well was added with DMSO and shaken with plate shaker. The wells were read at Elisa reader in 620 wave length. Percentages of the living cells were calculated with the following formula, according to experimental study [6].

$$\% \text{ Percentage of the living} = \frac{\text{Treatment} + \text{media}}{\text{cell} + \text{media}} \times 100\%$$

### III. RESULT AND DISCUSSION

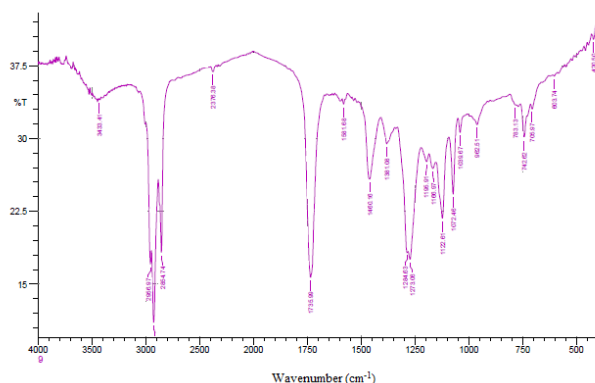


Fig. 2. FT-IR spectrum of isolated compound of *S.malaccense*

Isolated of dichloromethane extract of bark of *Syzygium malaccense* is yellow liquid. Identification with FT-IR Spectrophotometry could be seen on Table I.

Figure 1 shown FT-IR spectrum has peak at 1273 and 1284 which are strong C-O possibility as alcohol, ether, carboxylic acid or ester. Peak at 1700 is strong C=O possibility as aldehyde, ketone, carboxylic acid or ester

TABLE I. FT-IR OF ISOLATED OF SYZYGIUM MALACCENSE

Wavenumber (cm <sup>-1</sup> )			Type of Bond
Isolated compound	Methyl Ester [7]	Oleic acid[8]	
3433.41	3471.87	-	O-H, N-H
2956-2854	2924-2852	2924-2854	C-H (alkanes)
2376.38	2400-2300	-	C=C
1753.99	1747.51	1701	C=O (Ester) strong
1581.68	1625.99	-	C=C
1460.16	1462-1375	-	C-H
1284-1273	1228.66	1282	C-O strong
1122.61	1111		C-O
962.51	964.41		HC=CH (trans)
742-705	723.31		HC=CH (cis)

Based on the form of the isolated compound in the form of liquid and the result of FTIR according to Tabel II, isolated compound are thought to be esters. This compound is identified in the wavenumber region 1753 cm<sup>-1</sup> which is the absorption of C=O carbonyl esters and supported in the region of infrared spectrum 1284-1273 cm<sup>-1</sup> which is the absorption of O=C-O. This assumption is reinforced by the metyl ester spectrum that has region 1747cm<sup>-1</sup> and 1228 cm<sup>-1</sup> also oleic acid at 1701 cm<sup>-1</sup> and 1282 cm<sup>-1</sup>. It's typical of esters. There are two strong peak from 2956-2854 cm<sup>-1</sup> shown asymmetric CH<sub>2</sub> dan symmetrical CH<sub>2</sub>. The peak at 1460 cm<sup>-1</sup> corressond to asymmetric stretching of -CH<sub>3</sub>.

Saturated fatty acid is identified at 2956 cm<sup>-1</sup> and 2854cm<sup>-1</sup>. Whereas unsaturated fatty acids at 1581 cm<sup>-1</sup>, and supported at the region 742-705 cm<sup>-1</sup> which is the absoption of HC=CH *cis* and also HC=CH *trans* at 962.51 cm<sup>-1</sup>. The stretching of free OH, represented by the absorbance at 3433.41 cm<sup>-1</sup>. According to the result, indicate strongly that isolated compound of dichloromethane extract of bark of *Syzygium malaccense* is a methyl ester.

Study of cytotoxicity using MTT assay (in vitro) are used due to well maintained cell integrity and cell is capable of multiplying in toxicity test for medicine or dental material. The result of this study can be applied as accurate basic test [9]. MTT reagen is a yellow tetrazolium salt which can be evaluated cellular enzymatic activity. Mitochondria of living cell can produce dehydrogenase and formed formazan. Formazan that solute in DMSO were read by Elisa Reader is Optical Density [10].

TABLE II. MEAN OF OPTIC FORMAZAN DENSITY AND %LIVING CELL

Concentration of isolated	Number of Sample	Mean of optic formazan density	% Living cell
12.5%	8	0.274	80.36%
25%	8	0.248	74.23%
50%	8	0.236	71.29%
100%	8	0.227	69.12%
Control cell	8	0.355	100%
Control media	8	0.06	0%

In Table 1 shown the group test has significant decrease. Percentage of living cell which is the optic density of dehydrogenase mytochondria enzyme in cells culture of BHK-21 has shown in Table II.

If dehydrogenase is not active due to cytotoxicity effect, formazan will not be formed. The number of formed formazan is equal to enzymatic activity of ling cell. Cell culture BHK-21 is used because it comes from fibroblast cell which are important cell in the pulp, ligament, periodontal, and gingival components [11]



Fig. 3. Baby Hamster Kidney cells

The result show that group 12.5%=80.36%, goup 25%=74.23%, group 50%=71.29%, and group100%=69.12%. Cytotoxicity test of isolated shown %living of cells are >60%. It means that isolated is non toxic to BHK-21 cells and safe due to good biocompatibility for medicine and dental material.

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