

# In Silico Anticancer Activity and *In Vitro* Antioxidant of Flavonoids in *Plectranthus amboinicus*

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## ABSTRACT

**Background:** *Plectranthus amboinicus* (Lour.) Spreng is a plant that has a high flavonoid content. The leaves of *Plectranthus amboinicus* (Lour.) Spreng contain many flavonoids Chrysoeriol, Cirsimaritin, Eriodictyol, Luteolin, Rutin, Salvigenin, Thymoquinone, Quercetin, Apigenin, and 5-O-Methyl-Luteolin. **Objectives:** To determine the antioxidant activity and anticancer activity of flavonoid compounds contained in *Plectranthus amboinicus* (Lour.) Spreng. **Methods:** Anticancer activity testing was carried out by in silico against several cancer receptors and antioxidant activity testing was carried out by in vitro using the 1,1-Diphenyl-2-Picrylhydrazil method. The results showed that the flavonoid compounds contained in *Plectranthus amboinicus* (Lour.) Spreng have similar anticancer activity to the reference molecule at the P-Glycoprotein-1, Cyclin Dependent Kinase-2, and Phosphoinositide-3-Kinase receptors, as well as better anticancer activity than the reference molecule for the Cyclooxygenase-2 and Phosphoenolpyruvate Carboxykinase receptors. **Results:** The antioxidant activity of the extract gave an Inhibitory Concentration 50% value of 9.77 µg/mL, the flavonoid compounds contained in *Plectranthus amboinicus* (Lour.) Spreng gave an Inhibitory Concentration 50% value that lower than the extract, which ranged from 6.92 µg/mL to 8.50 µg/mL. Flavonoids in *Plectranthus amboinicus* (Lour.) Spreng anticancer activity by in silico molecular docking and antioxidant activity by in vitro 1,1-Diphenyl-2-Picrylhydrazil method. **Conclusions:** All the flavonoid compounds contained in the ethanolic extract of *Plectranthus amboinicus* (Lour.) Spreng leaves exhibit very strong anti-cancer and antioxidant activity, which results in ethanolic extract of *Plectranthus amboinicus* (Lour.) Spreng leaves have very strong antioxidant activity. **Key words:** Anticancer, Antioxidant, In Silico, *In Vitro*, Flavonoid.

## INTRODUCTION

Exposure to carcinogenic substances will damage Deoxyribose Nucleic Acid (DNA). If the Deoxyribose Nucleic Acid (DNA) repair fails, it will lead to cancer.<sup>1</sup> Based on research results from the International Agency for Research on Cancer, the most common cancers in Indonesia are breast, cervical, lung, ovarian, rectum, thyroid, colon, liver and nasopharyngeal cancer.<sup>2</sup> The main strategy of choice in the treatment of colon cancer is chemotherapy with 5-fluorouracil (5-FU) but it is very toxic to other normal tissues.<sup>3-4</sup>

The diversity of plants in Indonesia is one of the important opportunities in developing Indonesia potential in the era of globalization.<sup>5</sup> Natural sources of antioxidants that come from food ingredients are found in spices, leaves, seeds, vegetables.<sup>6</sup> Most sources of natural antioxidants are plants, which are generally phenolic compounds, including the flavonoid group. Flavonoids that are active as antioxidant and anticancer compounds. The leaves of *Plectranthus amboinicus* (Lour.) Spreng contain many flavonoids Chrysoeriol, Cirsimaritin, Eriodictyol, Luteolin, Rutin, Salvigenin, Thymoquinone, Quercetin, Apigenin, and 5-O-Methyl-Luteolin.<sup>7</sup>

The ability of flavonoids as antioxidants has been widely researched recently, because flavonoids have

the ability to reduce free radicals. This study aims to determine the antioxidant activity of the ethanol extract of *Plectranthus amboinicus* (Lour.) Spreng leaves and 10 pure flavonoid compounds contained in the leaves of *Plectranthus amboinicus* (Lour.) Spreng by in vitro. The research was also continued by testing the anticancer activity of 5-fluorouracil and 10 pure flavonoid compounds contained in the leaves of *Plectranthus amboinicus* (Lour.) Spreng against several cancer receptor by in silico.

## MATERIALS AND METHODS

### Materials

Sample, Methanol (Merck), Chrysoeriol (Sigma Aldrich), Cirsimaritin (Sigma Aldrich), Eriodictyol (Sigma Aldrich), Luteolin (Sigma Aldrich), Rutin (Sigma Aldrich), Salvigenin (Sigma Aldrich), Thymoquinone (Sigma Aldrich), Quercetin (Sigma Aldrich), Apigenin (Sigma Aldrich), and 5-O-Methyl-Luteolin (Sigma Aldrich), 1,1-Diphenyl-2-Picrylhydrazil (Sigma Aldrich).

### Tools

Personal Computer Asus Core i9 RAM 16 GB ROM 1 TB, Software PLANTS, Software MARVIN SKETCH, Software Statistical Package for Social Sciences version 26 year 2019, Drying Cabinet, Glassware (Iwaki).

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## Preparation of Extract

Leaves of *Plectranthus amboinicus* (Lour.) Spreng were obtained from Dolok Marlawan Village, Jorlang Hataran District, Simalungun Regency, North Sumatra Province, Republic of Indonesia, Postal Code 21172. Determination of plant materials was carried out at Herbarium Medanense, Faculty of Mathematics and Natural Sciences, University of North Sumatera. The samples used were collected, washed, drained, sliced, dried, blended, and sieved. A total of 500 grams of powder were added 3.75 L of 96% ethanol, left for 5 days while stirring frequently, filtered the extract, readded 1.25 L of 96% ethanol, left for 5 days while stirring frequently, filtered the extract, concentrated with a rotary evaporator, evaporated in a water bath until a crude extract was obtained.<sup>8</sup>

## In Silico Anticancer Test

The molecule obtained from the Protein Data Bank is a combination of the native ligand and the binding pocket molecule. Native ligand molecule ATP and binding pocket molecule P-Glycoprotein-1 with protein code 1MV5; native ligand molecule IMN and binding pocket molecule Cyclooxygenase-2 with protein code 4COX; native ligand molecule 1YG and binding pocket molecule Cyclin Dependent Kinase-2 with protein code 4LYN; native ligand molecule 1UK and binding pocket molecule Phosphoinositide-3-Kinase with protein code 4KZC; native ligand molecule GCP and binding pocket molecule Phosphoenolpyruvate Carboxykinase with protein code 1KHB. The three dimensional conformation of native ligand molecules were redocking into the each binding pocket molecule then calculated as the Root Mean Square Deviation value is valid if it is less than 2 Å. The three dimensional conformation of the test molecule and the reference molecule were docking to binding pocket molecule, and the binding energy value of each conformation of the molecule of the test molecule and the reference molecule were obtained in the various binding pocket molecule.<sup>9</sup>

## In Vitro Antioxidant Test

Free radical solution 1,1-diphenyl-2-picrylhydrazil with a concentration of 200 µg/mL was fresh prepared, pipetted as much as 5 mL, inserted in a 25 mL volumetric flask, added a certain volume of the extract solution in methanol or flavonoid compound solution in methanol with a concentration of 100 µg/mL (obtained a concentration of 2.5 µg/mL to 20 µg/mL), diluted with methanol, allowed for 60 minutes, measured at a wavelength of 516 nm, calculated the Inhibitory Concentration 50% with Statistical Package for Social Sciences version 26 year 2019.<sup>10</sup>

## RESULTS AND DISCUSSIONS

### In Silico Anticancer Test

The native ligand molecule was extracted from the protein obtained from the protein data bank and redocking native ligand molecule against the binding pocket molecule to determine the validity of the binding pocket molecule. The results of validation of the binding pocket molecule validation can be seen in Table 1.

The in silico molecular docking process of all native ligand compounds in each binding pocket molecule was valid, because the value of Root Mean Square Deviation obtained was less than 2 Å.<sup>11</sup> So that the entire binding pocket molecule can be used to docking the test compound molecule by in silico. The binding energy of the test molecule and reference molecule as a result of the molecular docking test can be seen in Table 2.

The binding energy value by in silico docking result of the whole test molecule were lower than the reference molecule against the Cyclooxygenase-2 and Phosphoenolpyruvate Carboxykinase. This means that all the test molecules have a stronger interaction than the reference molecules against the Cyclooxygenase-2 and Phosphoenolpyruvate Carboxykinase. On the P-Glycoprotein-1, Cyclin Dependent Kinase-2, and Phosphoinositide-3-Kinase, the test molecule gave results that were not significantly different from the reference. This means that all the test molecules have a similar interaction to the reference molecules against the P-Glycoprotein-1, Cyclin Dependent Kinase-2, and Phosphoinositide-3-Kinase. The binding energy resulted by in silico molecular docking is reflection of the affinity between the test molecule and the binding pocket molecule.<sup>12</sup> The lower the binding energy value, the stronger the interaction between the test molecule and the binding pocket molecule.<sup>13</sup>

The in silico molecular docking results of flavonoid compounds in the glycoside form gives a lower bond energy yield than flavonoid compounds in the non glycoside form. This occurs because in silico molecular docking testing of glycoside compounds has bigger molecule (steric barrier effect), making it difficult to enter the binding pockets and difficult to bind with receptors. However, in reality in the body, most of the ether and ester after penetrate in the cell will undergo hydrolysis. So that in this study the 5-O-Methyl-Luteolin molecule is undergoes hydrolysis to become a Luteolin molecule which has better bonding energy (lower) and better bond affinity (higher).<sup>14</sup>

### In Vitro Antioxidant Test

The test for the antioxidant activity of the ethanol extract of *Plectranthus amboinicus* (Lour.) Spreng leaves and pure flavonoid compounds contained in the ethanol extract of *Plectranthus amboinicus* (Lour.) Spreng leaves was carried out by the free radical scavenging method. The use of the compound 1,1-diphenyl-2-picrylhydrazil is a free radical that has an unpaired nitrogen atom. The reaction between 1,1-diphenyl-2-picrylhydrazil with hydrogen atoms in the antioxidant will cause a color change from purple to yellow. The results of antioxidant activity of the extract and pure flavonoid compounds can be seen in Table 3.

The results showed that the Inhibitory Concentration 50% value of the extract was higher than the pure compound, but this difference was not significantly different from the Inhibitory Concentration 50% value compared to pure compounds. These results indicate that the antioxidant activity of the extracts and pure compounds is similar. So that the use of extracts is very effective and efficient in terms of acquisition and price, because pure compounds have a higher price because the isolation process is longer.<sup>15</sup>

**Table 1: The results of validation of the binding pocket molecule validation.**

Protein Code	Native Ligand	Binding Pocket	Root Mean Square Deviation
1MV5	ATP	P-Glycoprotein-1	1.4634 Å
4COX	IMN	Cyclooxygenase-2	1.3948 Å
4LYN	1YG	Cyclin Dependent Kinase-2	1.6391 Å
4KZC	1UK	Phosphoinositide-3-Kinase	1.2475 Å
1KHB	GCP	Phosphoenolpyruvate Carboxykinase	0.9942 Å

**Table 2: The binding energy of the test molecule and reference molecule as a result of the molecular docking test.**

Molecule	Binding Energy				
	1MV5	4COX	4LYN	4KZC	1KHB
Verapamil	-84.68	-	-	-	-
Celecoxib	-	-83.18	-	-	-
Flavopiridol	-	-	-85.32	-	-
Idelalisib	-	-	-	-85.21	-
Metformin	-	-	-	-	-61.45
Chrysoeriol	-82.95	-89.24	-84.24	-84.45	-84.25
Cirsimaritin	-83.61	-88.15	-85.12	-83.82	-88.15
Eriodyctol	-83.42	-88.36	-84.84	-84.65	-89.24
Luteolin	-85.06	-89.72	-86.09	-85.96	-85.14
Rutin	-85.84	-88.65	-86.55	-85.84	-84.64
Salvigenin	-83.92	-87.26	-85.64	-84.51	-87.15
Thymoquinone	-83.57	-86.34	-84.34	-84.35	-84.15
Quercetin	-86.34	-90.35	-88.64	-87.23	-89.45
Apigenin	-85.36	-88.92	-86.02	-86.34	-84.45
5-O-Methyl-Luteolin	-81.36	-85.34	-81.33	-81.34	-82.84

**Table 3: The results of antioxidant activity of the extract and pure flavonoid compounds.**

Test Solution	Regression Equation	Inhibitory Concentration 50%
Extract	$Y = 4.9911 \times X + 1.2437$	9.77 µg/mL
Chrysoeriol	$Y = 5.8942 \times X + 1.3412$	8.26 µg/mL
Cirsimaritin	$Y = 6.0246 \times X + 1.4427$	8.06 µg/mL
Eriodyctol	$Y = 6.3148 \times X + 1.4521$	7.69 µg/mL
Luteolin	$Y = 6.4127 \times X + 1.4684$	7.57 µg/mL
Rutin	$Y = 6.2517 \times X + 1.4321$	7.77 µg/mL
Salvigenin	$Y = 6.1413 \times X + 1.3718$	7.92 µg/mL
Thymoquinone	$Y = 5.7245 \times X + 1.3248$	8.50 µg/mL
Quercetin	$Y = 6.9934 \times X + 1.6045$	6.92 µg/mL
Apigenin	$Y = 6.8042 \times X + 1.5521$	7.12 µg/mL
5-O-Methyl-Luteolin	$Y = 5.7511 \times X + 1.3154$	8.47 µg/mL

Inhibitory Concentration 50% is a number that can indicate a concentration that can inhibit 50% of free radical activity.<sup>15</sup> Inhibitory Concentration 50% is used to compare the antioxidant activity so that the Inhibitory Concentration 50% value is inversely proportional to the ability of antioxidants to reduce free radicals. The lower the Inhibitory Concentration 50% value, the higher the antioxidant activity. The higher the Inhibitory Concentration 50% value, the lower the antioxidant activity.<sup>17</sup>

A substance has antioxidant properties when the Inhibitory Concentration 50% value is less than 200 µg/mL. Antioxidants play a role in preventing free radical tissue damage by minimizing the formation of radicals, reducing or increasing breakdown. Specifically, a compound is categorized to be a very strong antioxidant for Inhibitory Concentration 50% values less than 50 µg/mL, strong antioxidants for Inhibitory Concentration 50% values between 50 µg/mL to 100 µg/mL, moderate antioxidants for Inhibitory Concentration 50% values between 100 µg/mL to 150 µg/mL, and weak antioxidants for Inhibitory Concentration 50% values between 151 µg/mL to 200 µg/mL.<sup>18</sup>

## CONCLUSIONS

All the flavonoid compounds contained in the ethanolic extract of *Plectranthus amboinicus* (Lour.) Spreng leaves exhibit very strong anti-cancer and antioxidant activity, which results in ethanolic extract of *Plectranthus amboinicus* (Lour.) Spreng leaves have very strong antioxidant activity.

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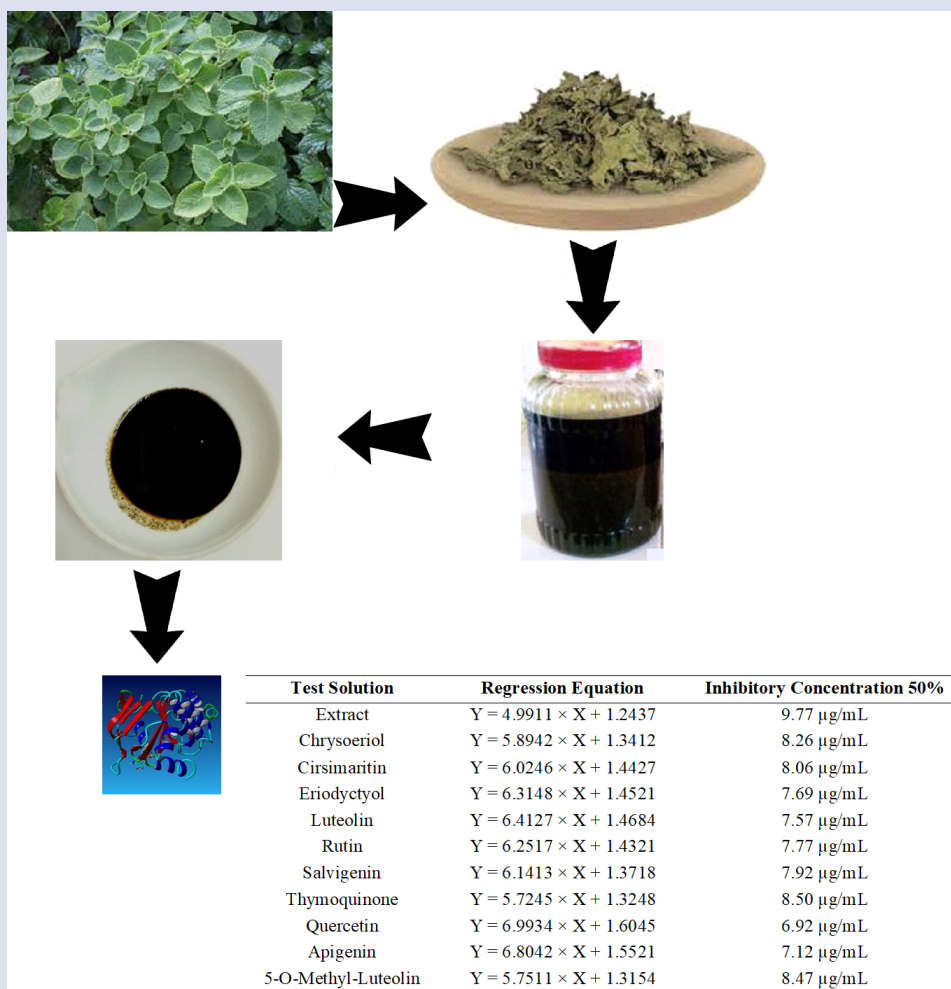
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## GRAPHICAL ABSTRACT



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