

## ANTIOXIDANT AND ANTICANCER ACTIVITIES OF MURBEI (*Morus alba L.*) STEM EXTRACT ON IN VITRO WiDr CANCER CELLS

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### ABSTRACT

Mulberry is considered as an important plant in traditional Chinese medicine, due to its various compounds, including phenols and flavonoids. These flavonoids have antioxidant activities so that can be a potential anticancer candidate. The aims of this study were to determining antioxidant activity, phenolic content, and potential anticancer activity in Mulberry stem extract. The extraction was carried out by maceration using ethanol as the solvent, antioxidant activity test using ABTS (*2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)*) method, phenolic content determination using Folin-Ciocalteu reagents, and anticancer activity test using the MTT (*3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromida*) method on WiDr cancer cells and Vero cells. The result of total phenolic Mulberry stem extract was 35.9%, the antioxidant activity value was 83.18  $\mu\text{g} / \text{mL}$ , the  $\text{IC}_{50}$  value for anticancer activity for WiDr cells was 71.24  $\mu\text{g} / \text{mL}$  and Vero cells  $\text{IC}_{50}$  value was 154.241  $\mu\text{g} / \text{mL}$ . It could be concluded that the Mulberry stem ethanol extract had strong antioxidant activity and had the potential anticancer selectively against cancer cells WiDr.

**Keywords:** anticancer; antioxidant;  $\text{IC}_{50}$ ; mulberry stem extract

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### INTRODUCTION

Cancer is one of the leading causes of death in the worldwide. The number of cancer patients is predicted to increase each year. Cancer patients reach 23.6 million cases per year in 2030 (Ministry of Health RI, 2016). One of the plants used by the community as medicine is Mulberry (*Morus Alba L.*) which has many potentials, including reduce blood cholesterol, antidiabetes, and antihypertension (Mallaleng *et al.*, 2011).

The plants generally contain phenolic compounds such as flavanoids, synamic acid derivatives, coumarins, and tocopherols (Gupita and Rahayuni, 2012). Antioxidant compounds produced as vitamin C, vitamin E, carotene, groups of phenolic compounds- especially polyphenols and flavonoids-are known to have the potential to reduce the risk

of degenerative diseases (Kuntorini and Astuti, 2010).

Antioxidant activity is thought to inhibit the growth of cancer cells, due to the similarity of the mechanism of resistance at the cellular level (Anam *et al.*, 2014). Compounds that act as antioxidants include flavonoids, alkaloids, tannins, and also phenolic. The content is thought to have anticancer activities such as flavonoids which work by inhibiting carcinogenesis inactivation, cell cycle inhibition, inhibition of angiogenesis, cell proliferation and apoptotic mechanisms (Ahmad *et al.*, 2014; Meiyanto, *et al.*, 2008).

Burhan A. and Aisyah (2018), studied about the toxicity test of mulberry stem extract with various solvents, found that the  $\text{IC}_{50}$  values for ethanol extract was 2.8045  $\mu\text{g} / \text{mL}$ .

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This result showed the potential of mulberry stems as anti-cancer.

Based on all of those studies above, The aim of this study was to determine antioxidant activity by the method of ABTS (2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) and to determine anti-cancer activity using WIDR cells and vero cells.

## METHODS

### Instrument

UV-Vis spectrophotometer (Shimadzu<sup>®</sup>), ELISA reader (Thermo fisher Scientific<sup>®</sup>), rotary evaporator (Buchi<sup>®</sup>), microplate (Iwaki<sup>®</sup>).

### Materials

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) (Sigma<sup>®</sup>), ABTS (2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (Merck<sup>®</sup>), n-hexane (Brataco Chemica<sup>®</sup>), ethanol (Onemed<sup>®</sup>), Follin-Ciocalteu (Merck<sup>®</sup>), Phosphate Buffer-Saline (PBS) (Gibco<sup>®</sup>), gallic acid (Sigma<sup>®</sup>), Sodium dodecyl sulfate (SDS) (Merck<sup>®</sup>).

### Extraction of mulberry steam

The preparation of the material begins with taking mulberry stems which is 60-90 days old in The Mountainous Area of Sidenreng Rappang, South Sulawesi. Extraction was carried out by maceration method using ethanol solvent, then extract was applied with rotary evaporator until it got thick extract.

### Phytochemical screening

Phytochemical screening includes examination of alkaloids, flavonoids, tannins, phenols and saponins.

**Quantitative testing of antioxidant activity** using the ABTS method. Samples were made in series with concentrations of 40 ppm, 80

ppm, 120 ppm, 160 ppm, each concentration mixed with ABTS solution and then let stand for 12 hours, then measured by UV-Vis spectrophotometry with a wavelength of 750 nm, the comparison used in this experiment was vitamins C. Total phenol was tested using the UV-Vis spectrophotometer method with Follin-Ciocalteu reagent with a wavelength of 714 nm and gallic acid as a control.

### In Vitro Test of Anticancer Activity

In vitro test of anticancer was carried out using WiDr cells and vero cells, cells were inserted into the wellplate, then the cells were incubated for 24 hours, after the cell was ready, the well plate was taken from the incubator then discarded the cell media, then inserted 100 µL PBS into the well cells have been filled, then discard PBS. The concentration of the sample was included in the well (triplo) then incubated in a 5% CO<sub>2</sub> incubator for 24 hours at 37°C. The MTT 100 µL reagent was added to the well. Cell suspension was incubated again for 2-4 hours in a CO<sub>2</sub> incubator. SDS 100 µL was added when formazan was clearly formed, then wrapped in paper and stored overnight at room temperature in a dark place, the color intensity that occurred was read by ELISA reader at a wavelength of 595 nm.

## RESULTS AND DISCUSSION

The extraction process was carried out by maceration using ethanol solvents with a yield value of 4.91%. The study which was carried out by Burhan A. and Aisyah (2018), showing the yield obtained in the extraction of mulberry stems in 3 solvents namely non-melt, ethyl 70% acetate and ethanol, ie 0.34%, 0.859% and 1.51% of the yield obtained showed that the larger polar solvents produced yields, but with the amount of yield obtained not necessarily proportional to the quality of the extract obtained.

**Table I.** Results of phytochemical screening of mulberry stem extract

No.	Compounds	Result
1	Alkaloid	+
2	Fenol	+
3	Flavanoid	+
4	Tanin	-
5	Saponin	+

**Table II.** Results of the antioxidant activity of stem extract using the ABTS method

Sample concentrations (ppm)	Absorbance	% inhibition	Probit	IC <sub>50</sub> Value (ppm)	Equation linear regression
40	0.550	18.599	4.12	83,18	$y = 2.8979x - 0.559$
80	0.351	48.002	4.95		
120	0.270	60.089	5.25		
160	0.111	83.572	5.99		

Screening of phytochemical compounds aimed to determine the class of compounds contained in mulberry stem extracts. The results of phytochemical screening of mulberry stem extract can be seen in Table I. The results of phytochemical screening Mulberry stem extract showed that the plant contained flavonoid compounds, alkaloids, phenols and saponins. The presence of flavonoid compounds causes mulberry stem extract to have antioxidant potential. Flavonoid compounds have many hydroxyl (OH) groups, hydroxyl atoms can be donated to unstable radical compounds so that radical compounds can be stable, alkaloid compounds also have OH groups so that they can also donate to radical compounds as well as phenol compounds.

Total phenolic test with quantitative test using spectrophotometric method with Folin–Ciocalteu reagent and comparison of gallic acid. Gallic acid is used because gallic acid is a derivative of benzoate hydroxyl acid which is classified as simple phenolic acid and also as a stable and pure standard (Rahmawati, 2009). From the total phenolic test, mulberry stem extract was obtained by 35.9%. According to several studies it is known that the phenolic component has high antioxidant activity, therefore to determine the potential of compounds that act as antioxidants from

mulberry stem extract, activity tests were carried out using the ABTS method.

Test for antioxidant activity using the ABTS method. Antioxidant activity testing using the ABTS method, antioxidant activity is known by looking at how much IC<sub>50</sub> produced by mulberry stem extract in reducing ABTS free radical compounds, IC<sub>50</sub> shows 50% reduction of free radicals, smaller the IC<sub>50</sub> is obtained the greater the potential of the extract as an antioxidant. The comparison used is vitamin C as it is known that vitamin C is a compound that is used as one of the natural antioxidants. The result of antioxidant activity can be seen in Table II.

Obtaining IC<sub>50</sub> from the extract can be calculated using a linear regression equation. The results of IC<sub>50</sub> obtained at 83.18 µg / mL. From the results of mulberry stem extract measurements IC<sub>50</sub> results were 83.18 µg / mL, according to Blois (1985), a compound has a very strong antioxidant if the IC<sub>50</sub> value is <50 ppm, strong if the IC<sub>50</sub> is worth 50-100 ppm, if the IC<sub>50</sub> is worth 101-150 ppm, and weak if the IC<sub>50</sub> is worth 151-200 ppm, according to the classification above the mulberry stem mulberry extract is in the strong category.

**Table III.** Results of cytotoxic activity of mulberry stem extract on WiDr cancer cells and normal vero cells

Sample concentration (ppm)	% death		IC <sub>50</sub> value (µg/ml)		Equation linear regression	
	WiDr cells	vero cells	WiDr cells	vero cells	WiDr cells	vero cells
7,8	22,90	1,73				
15,6	30,49	8,36				
31,2	36,08	18,30				
62,5	44,04	35,30	71,24	154,24	$y = 1,6724x + 1,9015$	$y = 1,4603x + 1,8045$
125	58,41	43,84				
250	75,03	66,21				
500	93,45	70,21				
1000	97,66	88,35				

One way to prevent the formation of free radicals is to use nutrients that can act as antioxidants such as vitamin E, carotene, vitamin C and other drugs that can capture these free radicals. Free radicals are considered dangerous because they become very reactive in an effort to get an electron pair, the damage that can be caused by free radicals includes damage to cell membranes, proteins, DNA and lipids. The damage can cause various kinds of degenerative diseases, one of which is cancer (Auroma, 1994). To find out the anticancer activity in extracts there are various methods used, one of which is the MTT method.

Anticancer activity test. Anticancer activity test using MTT method using WiDr cells and Vero cells. Anticancer activity is known by looking at IC<sub>50</sub> values, IC<sub>50</sub> obtained using probit analysis, probit analysis is usually used to determine the response of the subject under study. The calculation results obtained in testing the anticancer activity of mulberry extract with WIDR cells and Vero cells can be seen in Table III. The toxicity test results of mulberry stem extract on WiDr cells and vero cells can be seen in Table II, showing IC<sub>50</sub> values for WiDr cells 71,24 µg / ml and for vero cells having IC<sub>50</sub> values 154,241 µg/mL, toxic boundary determination of this study using National criteria Cancer Institute (NCI) 2009, stated that an extract was

declared to have active activity having an IC<sub>50</sub> value <30 µg/mL, moderate active if it had an IC<sub>50</sub> value ≥ 30 µg /mL and was said to be inactive if IC<sub>50</sub>> 100 µg /mL, from the calculation results IC<sub>50</sub> value obtained from mulberry stem extract against WiDr cancer cells has a value of 71.24 µg/mL, the value obtained is in the moderate active range. In this study it was found that mulberry stem extract was moderate active, but did not work selectively because of the results of the selectivity comparison between WiDr cancer cells and vero cell 2.16. A chemotherapy agent is said to have a high selectivity if the SI value is ≥ 3, and is said to be less selective if the SI value is <3 (Rahmawaty, I, 2016).

Compounds that are thought to play an anticancer role in mulberry plants are quercetin and anthocyanin which are potential substances as chemo preventive agents. The anthocyanin type which has the effect of being a chemo preventive agent is cyanidin-3-O-glucoside. In vitro, cyanidin-3-O-glucoside is known to be able to reduce invasion of A549 lung cancer cells and reduce cell motility (Chen, 2006) and quercetin is known to significantly inhibit HL-60 cell growth and can induce differentiation HL-60 cells to express CD 66B and CD 14 antigens (Kim *et al.*, 2000). Quercetin is also known to be able to inhibit development, adhesion and

migration of hela cells and can trigger apoptosis in hela cell cultures.

## CONCLUSION

It can be concluded that mulberry stem extract has the value of total phenolic was 35.9 %, while the value of antioxidant activity was 83.18 µg / mL. The value of anticancer activity for WiDr cells IC<sub>50</sub> value 71.24 µg / mL and for vero cells has IC<sub>50</sub> value 154.241 µg / mL.

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