

Evaluation of Antioxidant and Cytotoxicity Activity of *Cyperus rotundus* L. Rhizome Extract and Fractions

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Abstract: Excess oxygen as free radicals and unbalanced antioxidant protection capabilities result in many diseases such as breast cancer. The purpose of this study was to evaluate the radical activity, total phenolic and cytotoxicity on 4T1 cell lines extracted from ethanol (EE), n-hexane (nHF), ethylacetate (EAF) and residual (RF) fractions of *Cyperus rotundus* L. rhizomes. maceration method to obtain ethanol extract and fractionated with ethylacetate and n-hexane. DPPH method to measure antioxidant activity, colorimetric method to measure total phenolic content, and MTT assay to measure cytotoxicity activity. Antioxidant activity from DPPH assay measured as IC₅₀ were 41.12 ± 0.03; 212.43 ± 0.10; 57.34 ± 0.03; 194.08 ± 0.05 µg/mL respectively. Total phenolic content measure were 138.25 ± 0.73; 51.93 ± 1.71; 213.01 ± 2.83; 111.07 ± 0.68 mg/GAE respectively. Cytotoxic activity were found to have IC₅₀ of 139.92 ± 0.91; 125.71 ± 0.57; 3685.36 ± 201.93; 3999.98 ± 437.39 µg/mL respectively. The results reveal that *Cyperus rotundus* L. rhizomes have antioxidant and cytotoxicity activities. Our further study is to assess the anticancer mechanism of *Cyperus rotundus* L. rhizomes.

1 INTRODUCTION

Reactive oxygen species (ROS) such as superoxide anions, singlet oxygen, hydrogen peroxide and hydroxyl are always produced in biological reactions or from the surrounding environment (Nagulendra, 2007). Spesies reaktif adalah molekul yang memiliki fluiditas elektronik dan sangat reaktif. Produksi radikal bebas oksigen yang berlebihan dan mekanisme pertahanan antioksidan yang tidak seimbang menyebabkan banyak penyakit, terutama penebaran dan kanker (Yang, 2004) (Nagmoti, 2012) (Jamuna, 2012) (Rosidah, 2008).

Breast cancer is the most common in the 1st estimated incidence of cancers, and 2nd highest incidence of death occurs in women who have cancer in the USA published in recent research (Siegel, 2015).

Cyperus rotundus L. has been traditionally used as a medicine for abdominal pain, menstrual irregularities, and intestinal disorders, (Peerzada, 2015). Its extract is used as antimutagenic, radical scavenging, insecticide and volatile oils as antibacterial and cytotoxic based on the results of

bioassay (Hadi, 2007) (Memariani, 2017) (Vitaglione, 2004) (Lanciotti, 2004) (Kilani, 2008) (Tenore, 2011) (Nam, 2016) (Heman, 2014) (Sonwa and Konig, 2001) (Liu, 2016) (Kilani, 2007) (Jirovetz, 2004) (Liu, 2016). The aim of our research was to assess the antiproliferative and antioxidant effect of ethanol extract from *Cyperus rotundus* L. rhizome.

2 MATERIALS AND METHODS

2.1 Plant and Chemicals Material

Extract and fractions were obtained from Masfria. The chemicals used are sodium bicarbonate (Merck), Dymethylsulfoxide (Sigma), DPPH (TCI), MTT salt (Sigma), folin ciocalteu (Sigma), and distilled water.

2.2 Preparation of Extract

Extraction with ethanol 96% and fractionation were followed by previous study (Satria, *et al.*, 2015; Anggraeni, *et al.*, 2014; Hasibuan, *et al.*, 2015).

2.2.1 Free Radical Scavenging Activity Assay

Free radical scavenging activity was determined based on (Satria, 2017) procedure and IC₅₀ value was described with regression analysis

2.2.2 Determination of Total Phenol Concentration

Briefly, 0.1 mL extract and fraction (500 µg / ml) were used in analysis and the procedure based on (Rosidah, *et al.*, 2008). The concentration is expressed as GAE in mg per gram of extract. (Jamuna, 2012) (Sitorus, 2017).

2.3 Cytotoxicity Assay

The cells were exposed with ethanol extract, n-hexane, ethylacetate, residual fraction. In this test, 4T1 cell lines were seeded in DMEM medium, and the procedure was followed by previous study (Harahap, 2018). The data absorbed from each well is converted into viable percentage of cells (Satria, 2014) (Dalimunthe, 2018).

2.4 Statistical Analysis

Load data as mean ± SD. SPSS 22 software is used to analyze all statistics.

3 RESULTS AND DISCUSSION

3.1 Radical Scavenging Activity

DPPH, which is a stable free radical, is centered on nitrogen and produces a deep purple color in methanol solution so that it can be used to test the antiradical effect of plant samples determined in terms of the ability of the active compound to contribute to hydrogen. Both antioxidants transfer electrons or hydrogen atoms to DPPH, thus neutralizing the freeradical character. The DPPH test has been widely used as a fast, accurate and

reproducible parameter for finding high antioxidant activity from pure compounds from plant extracts (Koleva, 2002) (Meir, 1995). Low IC₅₀ values reflect high fraction antioxidant activity, because concentrations are important for inhibiting radical oxidation at 50% low. IC₅₀ for ethanol extract, n-hexane, ethylacetate, the residual fraction in DPPH test was 41.12 ± 0.03; 212.43 ± 0.10; 57.34 ± 0.03; 194.08 ± 0.05 µg / mL respectively.

3.2 Total Phenolicand Total Flavonoid Contents

The Folin Ciocalteau method for measuring total phenolic content (TPC) claimed by based on the reduction of the phosphomolybdic-phosphotungstate to the blue colour in alkaline solution (Cicco, 2009). Ethanol, n-hexane, ethylacetate, residual fraction of *Cyperus rotundus* L. were found to contain various levels of phenolic content. 138.25 ± 0.73; 51.93 ± 1.71; 213.01 ± 2.83; 111.07 ± 0.68 mg GAE / g respectively.

Phenolic compounds are recognised as antioxidant (Shahidi and Wana Sundara, 1992) and they are compounds in plants which important cause of their radical scavenging ability due to their hydroxyl groups. (Hatano, 1989) (Diab, Reham and Shin, 2015) (Dai and Mumper, 2010) (Heim, Tagliaferro and Bobilya., 2002).

3.3 Inhibitory Concentration 50% (IC₅₀)

The percentage of cell viability was measured by the MTT method after being stored for 24 hours. In each n-hexane treatment, ethanol extract, ethylacetate, residual fraction represents inhibition of cell growth. IC₅₀ value of ethanol extract, n-hexane, ethyl acetate, residual fraction was 139.92 ± 0.91; 125.71 ± 0.57; 3685.36 ± 201.93; 3999.98 ± 437.39 µg / mL, respectively. Cytotoxicity estimates of natural products related to the level of active compounds in this plant include *Cyperus rotundus* L.

These plants contain coumarin flavonoids, monoterpene, sesquiterpenes, saponins, and steroids which can be measured as active substances (Nidulaga, 2017) (Nidigula, 2016) (Yadav, 2010).

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