THE STUDY OF ANTIOXIDANT ACTIVITY OF ETHANOL FRACTION FROM BLACK CUMIN (*Nigella sativa*, L.)

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ABSTRACT

Black cumin (Nigella sativa, L.) is a plant with a lot of advantages. Many research have been conducted on this plant and most of them use nonpolar, such as petroleum ether and chloroform, as a solvent in extraction process. In this research, extraction of black cumin using ethanol was applied.Black cumin extraction started with maceration using ethanol p.a. and partition using chloroform (1:1). The antioxidant activity of ethanol fraction of black cumin was examined by DPPH method. To identify the composition, phytochemical screening, including test for terpenoid, flavonoid, saponin, tannin and alkaloid, was also conducted.The result showed that the scavenging ability on DPPH method was 22.483% with IC50 2743.59. Phytochemical screening on ethanol fraction showed the presence of flavonoid, tannin and alkaloid. The study concluded that polar fraction of black cumin has small antioxidant activity.

Key words: black cumin, ethanol, DPPH

I. INTRODUCTION

Researchers have been founding that free radical has been theculprit of many diseases. Free radical, a chemical species with one or more unshared electrons, has a high reactivity and able to reaction with protein, lipid, carbohydrate, or DNA to cause human diseases. However, the diseases could be recovered with antioxidant.

Synthetic antioxidant such as tert-butyl hydroxyl anisole (BHA), tert-butyl hydroxyl toluene (BHT), and tert-hydroquinone (TBHQ)have been widely used to retard lipid oxidation, however, such antioxidant are not preferred due to toxicological concerns. For this reason, there has been increasing interest in the exploration of natural antioxidant especially from fruits and vegetables (Rababah et.al, 2004). One of them is black cumin (Nigella sativa, L.).

Black cumin is an annual herbaceous plant belonging to the Ranuncuaceae family, native to southwest Asia and originally a seed in East Mediterranean countries (Best, 2009). Based on haditsshahih and scientific research which has been conducted by lots of medical scientist over the world, black cumin was qualified tcure all of diseases.

Major compounds of black cumin, i.ethymoquinone (TQ), dihidrothymoquinone (DTQ), thymol (THY) and carvacrole, have non polar properties and have function as antioxidant. In the other side, the polar compound of black cumin have been reported and showed antioxidant potency too. However, still limited research has been conducted in this subject. The information of polar compounds was not completely known. This study was trying to fulfill it by measuring antioxidant activity and identifying polar compound (ethanol fraction) of black cumin.

II. EXPERIMENTAL PROCEDURES

1. Preparation

Black cumin seed (*Nigella sativa*, L.) were collected from MateriaMedica Research Center, Batu,Malang. The sample was dried under the sunlight and oven at 30-37°C before powdering process.

2. Extraction of Black Cumin

Black cumin powder was extracted by maceration and partition

extraction.Twenty five gram of powder was soak in ethanol 100 mL and was shake 200 rpm for 3 hours. Maceration repeated until the extract colorless. All of the extract was concentrated under vacuum on a rotary evaporator to yield crude extract. Partition on crude extract was applied using chloroform p.a. (1:1) followed by evaporating the solvent.

3. Antioxidant Assay

Antioxidant activity was examined by DPPH method (Hanani, 2005). The extract was dissolved in the ethanol in various kind of concentration are 5, 50, 200, 400, 800, 1000 and 1200 ppm. Two mL of filtrate in the tube were added 0.5 mL DPPH 1 mM and 2.5 mL distillated water then incubated at 37°C for 30 minutes. The absorbance was measured at 517 nm.

The percentage of antioxidant activity was calculated based on the following equation (Molyneux, 2003):

$$\% \text{ Antioxidant} = \frac{(Ao - Ac)}{Ao}$$

Where, Ao = Absorbance of control
Ac = Absorbance of sample

A percent inhibition to concentration was plotted. The concentration of sample required for 50% inhibition was determined and represented as IC_{50} value.

4. Phytochemical screening

Identification of secondary metabolite in sample was conducted by phytochemical screening including test of terpenoid, flavonoid, saponin, tannin and alkaloid.

5. Data analysis

Analysis of data measured the percentage of inhibition (antioxidant activity) of the sample and control. Absorbance of ascorbic acid (Asc) and BHT were the control. IC_{50} value was got by regression equation.

III. RESULT AND DISCUSSION

DPPH is one of the tests to measure antioxidant activity in inhibiting free radicals. DPPH compound have unpaired electron in outsider orbital. It gives purple color and has maximum absorbance at 517 nm. When the electron paired, the color will turn from purple to yellow. The resulting decolorization is stoichiometric with respect to number of electrons captured. It indicated that increasing capability of antioxidant to retard free radicals compound (Prakash, 2001).

Table1 Decolorization of the extract, Asc and BHT

No	Sample	Incubation time	
		Before	After
1	Black cumin extract (BCE)	Purple	Purple reddish
2	Ascorbic acid	Purple reddish	Yellow pale
3	BHT	Yellow reddish	Yellow pale

Parameters used to determine antioxidant potency were the percentage of antioxidant and IC_{50} (Inhibition concentration). Antioxidant activity value shows capability antioxidant to inhibit freeradicals, while IC_{50} reveals concentration of substrate that causes 50% loss free radicals. Percentage of antioxidant activity of black cumin extract, Asc and BHT displayed in Table 2 and Figure 1.

Table 2 Percentage data of antioxidant activity in various concentration

(x) Concentration (ppm)	(y) Antioxidant activity (%)		
	BCE	Asc	BHT
5	0.967	12.274	20.755
50	4.184	23.853	30.709
200	8.551	32.481	58.495
400	11.307	33.397	85.327
800	18.514	34.365	93.935
1000	21.608	33.172	94.920
1200	22.483	31.655	94.387

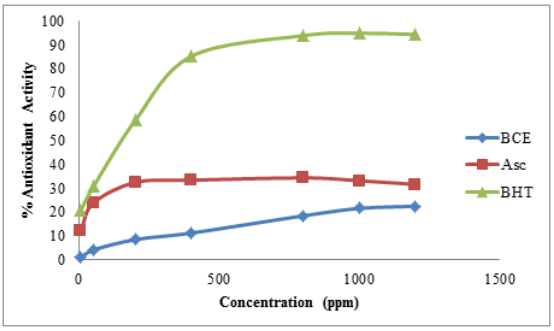

Figure 1 Graphic of antioxidant activity

Antioxidant assay using DPPH method showed IC_{50} value of BCE was 2743.59 whereas IC_{50} of Asc and BHT were 2685 and 213.79, respectively.

Table 3 IC_{50} value each samples

No	Sample	IC_{50}
1	BCE	2743.59
2	Asc	2685.00
3	BHT	213.79

Reaction antioxidant compound and DPPH was displayed in following figure 2.

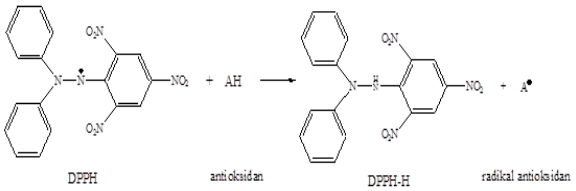

Figure 2. Reaction antioxidant compound and DPPH

Asc and BHT have inhibition mechanism different each other. Reaction BHT and DPPH was displayed in figure 3. And reaction Asc and DPPH was showed int Figure 4.

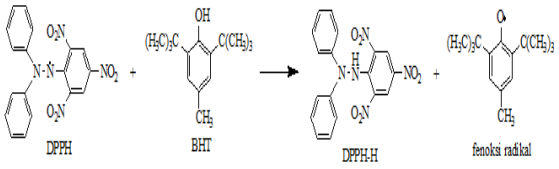

Figure 3 Reaction between BHT and DPPH

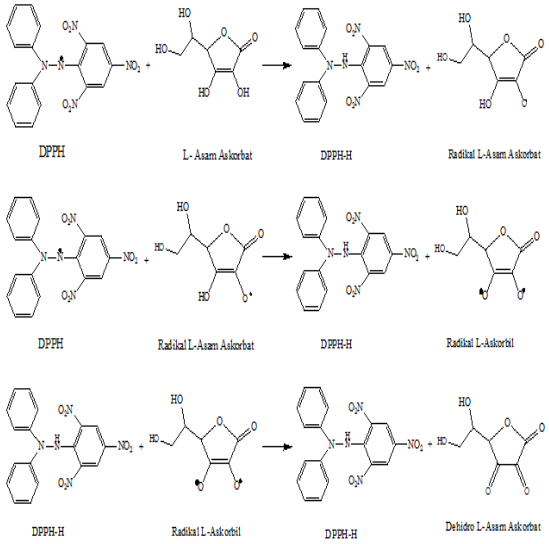

Figure 4 Reaction between Asc and DPPH

Phytochemical Screening

Phytochemical screening is a method to undestand plant base on the compound and a first information to determine secondary metabolite and bioactivity of plant (Teyler, 1988). The screening was applied to terpenoid, flavonoid, saponin, tannin and alkaloid. The screening tests showed in Table 4.

Table 4 The screening result of secondary metabolite of BCE

Compound	Result
Terpenoid	-
Flavonoid	+
Saponin	-
Tannin	+
Alkaloid, including: ▪ Mayer Reagent ▪ Dragendorff Reagent ▪ Wagner Reagent	 - ++ ++

IV. CONCLUSION

- The antioxidant activity of ethanol extract of black cumin was 22.483% and IC_{50}2743.59.
- Phytochemical screening showed presence of flavonoid, tannin and alkaloid.

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