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Study of antioxidants from herbal plant extracts *in vitro*

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Abstract

Background: *Antioxidants are substances that can prevent the formation of free radical reactions, people in Indonesia use a lot of natural ingredients as traditional medicine, one of which as antioxidants for free radical handling. Free radicals are compounds or molecules that contain one or more unpaired electrons in their outer orbitals. The presence of unpaired electrons causes these compounds to be very reactive looking for partners, by attacking and binding electrons around them so that they can trigger disease.*
Destination *The purpose of this study was to discuss the antioxidant activity of wormwood, neem, cinnamon, and brotowali plants in vitro.*
Method: *The method that can be used in antioxidant test research was the DPPH method in vitro through effective concentration (EC_{50}), identification of phytochemical screening of each extract, and total flavonoid test.*
Resuls : *The results of the study obtained phytochemical screening of wormwood, neem, cinnamon, brotowali extracts contained secondary metabolites in the form of flavonoids, tannins, saponins, steroids and triterpenoids which have a role as antioxidant test. Total flavonoid test of wormwood extract had the largest total flavonoid content (110.353 ± 0.802). The antioxidant test results of each cinnamon extract was 75 ppm, neem extract was 81 ppm, wormwood extract was 479 ppm, brotowali extract was 304 ppm. These results indicated that the effective antioxidant extract was cinnamon extract.*

Keywords: *Herbal Plants, Antioxidants, Total Flavonoids, In vitro*

INTRODUCTION

Along with the progress of the times, people have changes in lifestyle. Factors that can affect one of them are food and unhealthy diet that can cause heart disease, cancer and other degenerative diseases (Fajriatus Sakinah 2017). More and more diseases developments in Indonesia. make people use natural ingredients as treatment by utilizing antioxidants from natural ingredients. Antioxidants are substances that can prevent the formation of free radical reactions. (Anggraeny et al. 2021).

Free radicals are compounds or molecules that contain one or more unpaired electrons in their outer orbitals. The presence of unpaired electrons causes these compounds to be very reactive looking for electron paired, by attacking and binding electrons around it so that it can trigger the disease. Some natural ingredients that have antioxidant potential, among others are wormwood, neem, cinnamon, brotowali, while compounds that generally have potential as antioxidants are flavonoid, phenolics and alkaloids compounds (Budiana et al. 2017).

This study discussed the antioxidant activity of wormwood, neem, cinnamon, brotowali plants using the DPPH method *In vitro* through the effective concentration (EC_{50}) of each extract in scavenging radical compounds of DPPH.

METHOD

1. Materials and Equipment

The equipment that was used were oven, grinder, digital scale, rotary evaporator, desiccator, measuring flask, maceration bottle, drop pipette, measuring pipette, test tube, UV-Vis spectrophotometer.

The materials used were neem leaves, wormwood, cinnamon, brotowali stems, aquades, ethanol 96% p.a, 0.5 N HCl, Mayer reagent, Dragendorff reagent, Liebermann Burchard reagent, concentrated HCl, Mg powder, $FeCl_3$(Bahar, K, and Lestari 2021). 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) (Sigma. Co.), methanol (E.Merck) (Tatang Irianti *et al*. 2011).

2. Sampling and preparation

Samples in the form of neem leaves, wormwood, cinnamon, and brotowali stems were obtained from East Semarang City, Central Java Province. Each sample was taken as much as 5 kg where the leaves taken were green and fresh leaves. The sample obtained was tested for determination to determine authenticity of the plant which was carried out at the Pharmacy Biology Laboratory, Pharmacy Science College, Semarang Pharmasi Foundation (Bahar, K, and Lestari 2021).

3. Simplicia Samples Making

Each sample was sorted wet then washed with running water. Samples were chopped using scissors. The chopped samples were dried in an oven at 40 °C. The dried samples were then mashed using a grinder. The fine simplicia powder was stored in a clean, dry and tightly closed container and protected from direct sunlight (Bahar, K, and Lestari 2021).

4. Extract Making

Extracts were made by maceration as long as 3 x 24 hours using 96% ethanol. The results obtained from maceration were then concentrated using a rotary evaporator to obtain a thick extract. The extract obtained was then calculated as the percentage of yield. (Bahar, K, and Lestari 2021).

5. Phytochemical Screening

The test results in the form of alkaloids, flavonoids, tannins, saponins, steroids and triterpenoids compounds. (Sentat and Permatasari 2017).

6. Total Flavonoid Test

6.1 Flavonoids Identification

The research was conducted using alkaline test, Pb test, acetate and ammonia using filter paper.(Bakti, Triyasmono, and Rizki 2017).

6.2 Total flavonoid levels Determination

6.2.1 Maximum wavelength determination

The total flavonoid content was determined spectrophotometrically, to determine the number of samples to be tested. Put extract into a 10 mL volumetric flask, 4 mL of distilled water and 0.3 mL of 10% NaOH solution was added then left for 5 minutes. Then the solution was added with 4 mL of 10% NaOH and distilled water to 10.0 mL. The solution was left for 15 minutes. Absorbance value was measured with a wavelength of 510 nm against the blank. The Quercetin solution was used to make standard calibration curves. The total flavonoid content was used for gram equivalents. (Bakti, Triyasmono, and Rizki 2017).

6.2.2 Determination of Operating Time

Solution of 100 ppm quercetin was taken as much as 1 mL added with 10% $AlCl_3$ and 8 mL of 5% acetic acid. Furthermore, absorbance of the solution was measured at maximum wavelength that had been obtained with a time interval of 2 hours until resulting in stable absorbance data. (Bakti, Triyasmono, and Rizki 2017).

6.2.3 Analysis of Antioxidant Test

Antioxidant activity test was done using 2,2-Diphenyl-1-Pikrihidrazil (DPPH) method. DPPH is an antioxidant test used *In vitro* to obtain the potential biological activity of a sample (Arrohmatus Syafaqoh 2021). The advantages of DPPH method usage were simple, fast, easy, sensitive, and required little amount of samples. In principle, quantitative measurement of antioxidant activity by measuring the scavenging of DPPH radicals by compounds that have antioxidant activity. (Tatang Irianti *et al*. 2011).

7. Phytochemical screening test

Phytochemical screening was done to determine the secondary metabolites contained in the extract. Phytochemical screening was done by observed alkaloids, flavonoids, tannins, saponins, steroids and triterpenoids compounds using each reagent so that results were obtained as in the previous literature. (Anggraeny et al. 2021).

8. Data analysis of each test parameter

8.1 Total Flavonoid Test

Analysis of the test data for the total flavonoid content with spectrophotometric readings at wavelength of 510 nm which resulted in the percentage (%) of total flavonoids content. (Hanin and Pratiwi 2017).

8.2 Antioxidant Test of each sample

Antioxidant test analysis with the DPPH method got the best IC_{50} value. The smaller IC_{50} value, the better antioxidant activity of the sample. Based on research. A sample was said to be a very strong antioxidant if the IC_{50} value was less than 50, strong category if the IC_{50} value was 50-100, moderate category if the IC_{50} value was 100-150, and weak category if the IC_{50} value was 150-200.

RESULTS

1. Phytochemical Screening Test Results

Table 1. Phytochemical screening test results

Chemical Compounds	Result			
	Cinnamon	Neem	Worm wood	Brotowali
Alkaloid				
Mayer	-	-	-	-
Dragendorff	-	-	-	-
Lieberman Burchard	-	-	-	-
Flavonoid	+	+	+	+
Tannin	+	-	-	-
Saponin	+	+	+	+
Steroid and triterpenoid	+	+	+	+

2. Total Flavonoid Test Results

Total flavonoid content test result could be seen in Table 2. Total flavonoid content test based on the quercetin standard.

Table 2. Test Results of Total Flavonoid Equivalent Quercetin.

Sample	Total Flavonoid Content (QE%)
Cinnamon Extract	95.838 ± 0.697
Neem Leaves Extract	105.657 ± 0847
Wormwood Extract	110.353 ± 0.802
Brotowali Extract	74.217 ± 0.540

3. Antioxidant Test Results

Antioxidant activity test in this study used the DPPH method (Antasionasti, 2021). The test results were shown in Table 3, 4, 5 and 6.

Table 3. **Cinnamon Extract Antioxidant Test Results**

Concentration (ppm)	Abs	% Antioxidant Activity	IC_{50} (ppm)
DPPH	0.886	-	-
52.5	0.634	28.44	
62.4	0.440	50.34	
72.8	0.427	51.81	73
83.2	0.377	57.40	
93.6	0.352	60.27	

Table 4. Neem Extract Antioxidant Test Results

Concentration (ppm)	Abs	% Antioxidant Activity	IC_{50} (ppm)
DPPH	0.865	-	-
50.1	0.502	41.97	
60.1	0.497	42.54	
70.1	0.488	43.58	81
80.2	0.429	50.40	
90.8	0.400	53.76	

Table 5. Wormwood Antioxidant Test Results

Concentration (ppm)	Abs	% Antioxidant Activity	IC_{50} (ppm)
DPPH	0.925	-	-
101.4	0.651	29.621	
202.8	0.598	35.329	
304.2	0.537	41.945	479
304.6	0.537	41.945	
405.0	0.448	51.545	

Table 6. Brotowali Antioxidant Test Results

Concentration (ppm)	Abs	% Antioxidant Activity	IC_{50} (ppm)
DPPH	0.928	-	-
102.0	0.608	34.48	
204.0	0.516	44.38	
306.0	0.446	51.94	304
408.0	0.403	56.57	
510.0	0.343	63.04	

DISCUSSION

Results of phytochemical screening tests of cinnamon, neem, wormwood and brotowali extracts could be seen in Table 1. Based on the table it was known that the extracts contained secondary metabolites in the form of flavonoids, tannins, saponins, steroids and triterpenoids. There were several differences in the secondary metabolites contained in each extract. This was due to differences in the samples used. Screening of these secondary metabolites could be used as an illustration of the extract potential of each sample. In inhibiting ultraviolet radiation, it was known to have activity as antioxidants that could prevent free radicals activities. (Farhan 2020).

Cinnamon, neem, wormwood and brotowali leaves extract samples based on the literature had good total flavonoid content. Flavonoids are a common and broad group of

plant phenolic compounds. Determination of total flavonoids using visible spectrophotometry through mechanism of reaction of metallic Al complexes produced a yellow color which then reacted with NaOH to become pink with increasing color intensity. This test was carried out using a wavelength at 510 nm. The total flavonoid content of each extract sample was expressed in quercetin equivalents (EK). Based on previous research, the largest content of each extract sample was a strong result as a total flavonoid test. (Antasionasti, 2021).

Based on the research that had been done, comparing the total flavonoid of each extract sample, wormwood extract had the largest total flavonoid content, namely (110.353 ± 0.802), meanwhile the Brotowali extract had the smallest total flavonoid content, namely 74.217 ± 0.540.

Research on antioxidant activity of herbal plants was carried out using the 2.2-diphenyl-1-picrylhydrazyl (DPPH) method using a 96-well microplate reader (berthold technology) at a wavelength of 520 nm. The result obtained in the form of IC_{50} value compared to other test samples. The IC_{50} value of cinnamon extract samples was in table 3, neem extract was in table 4, wormwood extract was in table 5 and brotowali extract was in table 6.

The first treatment in the antioxidant test of cinnamon, neem, wormwood, brotowali extracts was started by weighing 10 grams of extract, then soaked in 96% ethanol. The sample extraction process was carried out to extract the chemical components contained in each sample. (Sepriyani 2020).

Antioxidant activity using the DPPH method was chosen because it was simple, easy, fast and required a small amount of sample. Measurement of extract of samples with different concentrations in the table showed that the greater the concentration of extract, the greater scavenging of DPPH radicals which was marked by color changing from purple to yellow. The antioxidant activity was marked with DPPH radical inhibition, then made into a curve between the concentration of extract solution and the percent inhibition to obtain linear regression IC_{50} value (Sepriyani 2020).

The value of the concentration of antioxidants that could inhibit free radicals was seen from the IC_{50} value. A compound could be said to be a very strong antioxidant if the IC_{50} value was less than 50 ppm, if it was strong it had value of 50-100 ppm, if it was moderate, it had value of 100-150 ppm, and if it was weak it had value of 150-200 ppm. In this study, the antioxidant value of cinnamon extract was 75 ppm, neem extract was 81 ppm, wormwood extract was 479 ppm, brotowali extract was 304 ppm. These results indicated that the most effective antioxidant among samples was cinnamon extract.

CONCLUSION

Based on the result obtained of this study, we can concluded that:

Phytochemical screening test of extracts of wormwood, neem, cinnamon, brotowali contained secondary metabolites in the form of flavonoids, tannins, saponins, steroids and triterpenoids which had a role as antioxidants. Total flavonoid test of wormwood extract had the largest total flavonoid content (110.353 ± 0.802). In the antioxidant test, the results showed that the most effective concentration was cinnamon extract with IC_{50} value of 75 ppm, IC_{50} value of neem extract was 81 ppm, IC_{50} value of wormwood extract was 479 ppm, IC_{50} value of brotowali extract was 304 ppm. Based on these results, it indicated that the most effective antioxidant among samples was cinnamon extract.

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