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SYNTHESIS AND ANTIOXIDATION OF AVOCADO (Persea Americana) LEAVES EXTRACT BY ABTS METHOD *IN VITRO*

Sintesis dan Antioksidan dari Ekstrak Daun Alpukat (Persea Americana) dengan metode ABTS secara *In Vitro*

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ABSTRACT

Antioxidants play a role in inhibiting the development of metabolic syndrome, which is associated with several diseases. Increased free radical production can cause the body's system to require support from other antioxidants. The use of synthetic antioxidants is still limited due to their potential side effects. Natural antioxidants from natural ingredients, such as Avocado leaves, are needed. The purpose of this study was to determine compounds with antioxidant potential by testing the synthesis and bioequivalence of Avocado leaves extract *in Vitro*. The research methods used were Fourier Transform Infrared (FTIR) spectroscopy, Gas Chromatography-Mass Spectrometry (GC-MS), and ABTS assay. The results of the FTIR analysis of the Avocado leaves extract showed that the functional groups of organic compound molecules were N-H, H-C=O, C=C, C-O-H, and C-N. This result indicated that the Avocado extract contained phenolic compounds. GC-MS analysis result showed that the phytochemical components with the highest abundance in the Avocado leaves extract were the compounds at peak numbers 1 and 4, namely 9-Octadecenoic acid (Z)-, methyl ester (CAS) and 9-Octadecenoic acid (Z)-(CAS). Antioxidant test showed a linear regression equation of y = 0.267x + 35.007, R^2 = 0.9491. This linear regression equation can be interpreted as a very strong and positive correlation. The IC_{50} value of Avocado leaves extract was 56.154, that categorized as a very strong antioxidant property. So it can be said that Avocado leaves extract has potential as an antioxidant.

Keywords: *ABTS, Antioxidants, Avocado, FTIR, GC-MS*

ABSTRAK

Antioksidan berperan dalam menghambat berbagai penyakit seperti penyakit metabolik sindrom. Peningkatan produksi radikal bebas dapat menyebabkan sistem tubuh semakin membutuhkan bantuan antioksidan lain. Penggunaan antioksidan sintetik masih terbatas karena dapat menimbulkan efek samping sehingga diperlukan antioksidan alami dengan memanfaatkan bahan alam seperti daun alpukat. Tujuan penelitian mengetahui senyawa yang berpotensi sebagai antioksidan dengan menguji sintesis, bioekivalen dari ekstrak daun alpukat secara *In Vitro*. Metode penelitian uji infrared spectrophotometer (FTIR), Gas Chromatography-Mass Spectrometry (GC-MS), antioksidan (ABTS). Hasil penelitian Analisa hasil uji FTIR dari ekstrak daun alpukat yang telah diujikan memiliki gugus fungsi suatu molekul senyawa organik yaitu

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gugus N-H, H-C=O, C=C, C-O-H, C-N dari uji tersebut menandakan ekstrak alpukat memiliki senyawa fenol. Analisa GC-MS uji komponen fitokimia menunjukkan bahwa senyawa yang memiliki kelimpahan terbesar dalam ekstrak daun alpukat adalah senyawa pada puncak no. 1 dan 4 yaitu senyawa 9-Octadecenoic acid (Z)-, methyl ester (CAS) dan 9-Octadecenoic acid (Z)-(CAS). Hasil uji antioksidan dengan metode ABTS didapat hasil bahwa ekstrak daun alpukat memiliki nilai =0.267x + 35.007, R^2 = 0.9491 yang dapat diartikan hasil tersebut mengindikasikan korelasi yang sangat kuat dan positif, nilai IC50 56.154 dapat dikategorikan sampel tersebut sangat kuat. Sehingga dapat dikatakan bahwa ekstrak daun alpukat memiliki potensi sebagai antioksidan.

Kata kunci: *ABTS, Antioksidan, Daun Alpukat, FTIR, GC-MS*

INTRODUCTION

The government is trying to improve public health levels by increasing the production and distribution of traditional natural medicine. Pharmaceutical manufacturers are encouraged to develop natural ingredients as treatments. Up to now, researchers have discovered compounds with potential as natural treatments [1]. Free radicals are compounds or molecules containing one or more unpaired electrons that are highly reactive and seek partners by attacking and bonding with surrounding molecules [2].

The mechanism of damage caused by free radicals can result in oxidative stress and lead to cell damage [3]. Oxidative stress is a condition in which there is an increase in free radicals that is unbalanced by the increase in antioxidants in the body. The role of oxidative stress in the body is crucial, especially in human pathophysiology, with several cases of degenerative diseases, one of which is diabetes mellitus [4]. Antioxidants are compounds that can stabilize free radicals and oxidative damage because antioxidants in the body react first with free radicals [5]. Antioxidants are easily oxidized and act as strong reducing agents compared to other molecules. This suggests that the more easily an antioxidant is oxidized, the more effective it is [6]. Furthermore, antioxidants can balance the body's nutritional needs.

Antioxidants play a role in preventing various diseases, such as metabolic syndrome. Increased free radical production can cause the body's system to require the assistance of other antioxidants increasingly. Exogen antioxidants can be obtained through synthesis or naturally. The use of synthetic antioxidants is still limited due to potential side effects, so natural antioxidants are needed. Avocado leaves are a natural antioxidant with high antioxidant properties due to their rich phenolic compound content [7]. Antioxidant activity testing can be used to measure the total antioxidant characteristics, but no single method is considered the most ideal. Different methods of measuring activity can result in different antioxidant mechanisms. One of the antioxidant activity measures is ABTS (2,2-Azinobis (3-Ethyl-Benzothiazolin-6-Sulfonate)) (Gülçin et al., 2010).

ABTS is fundamentally capable of stabilizing free radicals from antioxidants, which is indicated by color fading. The blue-green color of the ABTS radical cation, which will reduce the antioxidant, changes to a non-radical, colorless form [8]. The relative ability of antioxidants to reduce free radicals, reduce redox-active compounds, and apply appropriate standards to measure antioxidant capacity using spectrophotometry at a wavelength of 734 nm [9].

ABTS reduction mechanism with secondary metabolite is Flavonoids react with ABTS cation and establish more stable ABTS radicals. This formation causes the radical oxidation process to reduce color intensity because the ABTS molecule is reduced, resulting in the compound becoming colorless or fading. The ABTS assay has advantages, including enabling the determination of a wide variety of antioxidant compounds (phenols, amino acids, vitamin C, and vitamins). E) components that are simple to operate, and have specific absorbance readings related to visible wavelengths and fast reaction times. Another advantage is that both aqueous and organic solvents

can be used, allowing the detection of lipophilic and hydrophilic compounds [10].

ABTS assay was used to test antioxidant activity, with the ABTS maximum wavelength was 730 nm. The operational time was calculated to determine how long the ABTS radicals react with the sample until a stable compound that reacts with antioxidants is formed. The resulting operational time was used to set the incubation time before measuring the absorbance. The smaller IC_{50} value, the greater antioxidant activity of the sample [9].

MATERIAL AND METHODS

Equipment and Materials

Glassware (Pyrex), rotary evaporator (IKA RV 10), vortex (Gemmy VM300), analytical balance (Ohaus), glass jar stirrer, flannel cloth, waterbath, spectrophotometry (Shimadzu UV-1800), GC-MS. Avocado leaves, ethanol 96% pa, ABTS (2,2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid).

Research Procedures

Preparation of the simple drug

The Avocado leaves were wet-sorted, washed, chopped, and dried in an open area with good air circulation, covered with a black cloth for 6 days. The dried Avocado leaves were dry sorted, then powdered using a blender and sieved using 30- and 40-mesh sieves. This research was conducted in the Biology Laboratory, STIFAR Yayasan Pharmasi Semarang [11].

Extract Preparation

Extraction is the process of separating or withdrawing a substance (compound) from its mixture using a certain solvent based on differences in solubility, polarity, or other chemical properties, this study used the liquid-liquid extraction method using 96% ethanol solvent, 100 grams of avocado leaf powder was put into a glass jar and added 1000 ml of 96% ethanol. The sample: solvent ratio is 1:10. Re-maceration was carried out by soaking for 7 x 24 hours for the first time, followed by the second and fourth soaking, replacing the solvent every 24 hours, stirring for approximately 5-10 minutes, and filtering. The macerate was concentrated using a rotary evaporator at 50°C and evaporated using a water bath to form a thick avocado leaf extract. This study was conducted at the Chemistry Laboratory, STIFAR Yayasan Pharmasi Semarang. [11].

Fourier Transform Infrared (FTIR) Spectroscopy Analysis

FTIR spectrophotometer is a widely used instrument to determine the vibrational spectrum of molecules and its benefits in predicting the structure of chemical compounds. The samples used in this study were 2 mg of water, ethyl acetate, and n-hexane fractions. This research was conducted at the Chemistry Laboratory, STIFAR Yayasan Pharmasi Semarang [12]

GC-MS (Gas Chromatography-Mass Spectrometry) Analysis

This analysis was conducted to determine the compound profile of Avocado leaves extract which can be assumed to have a potential antioxidant effect. The results were obtained in the form of a chromatogram showing a graph and several peaks. Each peak represents a single compound. The sample was injected into a 30 m x 0.25 mm i.d. column with a 0.25 µM thin film. The carrier gas used was helium at 1 ml/min. The injector was purged at 200°C, and the column temperature was programmed at 50-250°C at a rate of 10°C/min. The MS used an ionization voltage of 70 eV, a temperature of 250°C, and a mass range of 50-600. This resulted in a chromatogram and mass spectrum of the unknown compound, which were then compared with the spectra of known compounds. This research was conducted at the Chemistry Laboratory of Ahmad Dahlan University, Yogyakarta [13].

In Vitro Antioxidant Test of Avocado Leaves Extracts

The in vitro antioxidant test used the ABTS (2,2-Azinobis (3-Ethylbenzothiazolin) 6-Sulfonate) method. 7 mg ABTS solution was prepared with 3.5 mg potassium persulfate. The two materials were dissolved in Aqua Pro Injection in 5 mL volumetric flask, then added to 25 mL volumetric flask. The solution was incubated for 12-16 hours and stored in a lightproof container. 1 mL sample was pipetted into 10 mL volumetric flask and

made up to the mark. The maximum wavelength was then measured using a UV-Vis spectrophotometer at 400-800 nm. The sample was then diluted to create a 1000 ppm stock solution in three concentrations (200, 300, and 400 ppm). The sample was pipetted and mixed with ABTS solution. The mix solution was incubated and measured at a wavelength of 600-800 nm and a wavelength of 734 nm until stable absorbance was achieved. This procedure was conducted at Pharmacology Laboratory, STIFAR Yayasan Pharmasi Semarang [10].

RESULTS

Figure 1. FTIR Test Results of Avocado Leaves Extract

The FTIR analysis results of Avocado leaves extract has a functional group of an organic compound molecule, namely the N-H, H-C=O, C=C, C-O-H, C-N groups. This result indicated that the Avocado extract has phenol compounds with the values in table 1.

Table 1. FTIR Test of Avocado Leaves Extract

Type of Vibration	Frequency (cm^{-1})
N-H	3315
H-C=O	2924
C=C	1556
C-O-H	1340
C-N	1053

FTIR spectrophotometric analysis used to determine the functional groups of certain organic compound molecules. In this study, the reference compound used was (Ismu Rohmah Rusmaningtyas ; Endang Dwi Siswani, 2012The results of the infrared spectrum test of quercetin standards which are semi-polar compounds contained N-H groups supported by wavelength absorption of 3315 cm-1; H-C=O groups are characteristics of flavonoids with a wavelength of 2924 cm-1, the presence of sharp aromatic C=C groups is supported by absorption at a wavelength of 1556 cm-1, alcohol C-O=H groups are supported by wavelength absorption of 1340 cm-1, aldehyde C-N groups are supported by sharp band absorption is wavelength absorption of 1053 cm-1.

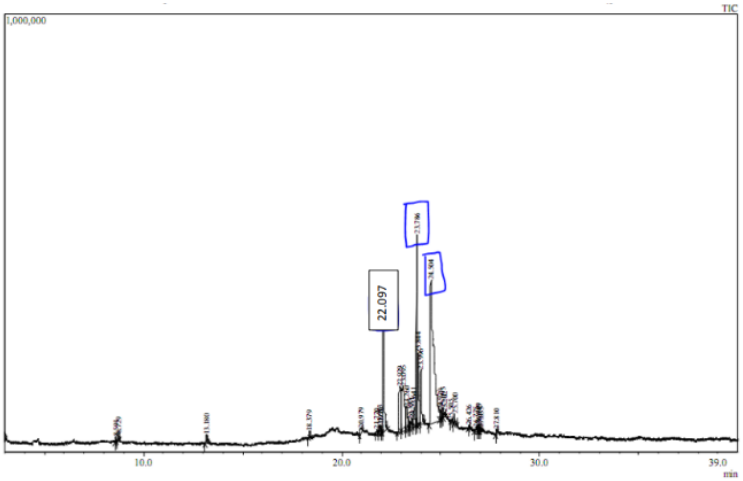

Figure 2. GC-MS Analysis Results of Avocado Leaves Extract

Table 2. Phytochemical Compounds Identified in Avocado Leaves Extract

No	R.Time	Area (%)	Height %	Molecular Weight	Compound
1.	23.786	10.18	22.65	2.54	9-Octadecenoic acid (Z)-, methyl ester (CAS)
2.	23.844	6.07	8.57	4.00	9-Octadecenoic acid (Z)-, methyl ester (CAS)
3.	23.996	5.30	6.47	4.62	Octadecenoic acid (Z)-, methyl ester (CAS)
4.	24.504	36.18	17.03	11.99	9-Octadecenoic acid (Z)-(CAS)

The results in Table 2 showed that the compounds with the greatest abundance in Avocado leaves extract are compounds at peaks no. 1 and 4 (seen from the height's peak), namely 9-Octadecenoic acid (Z)-, methyl ester (CAS) and 9-Octadecenoic acid (Z)-(CAS) compounds, which potentially have antioxidant activity.

Struktur Senyawa Ekstrak Daun Alpukat

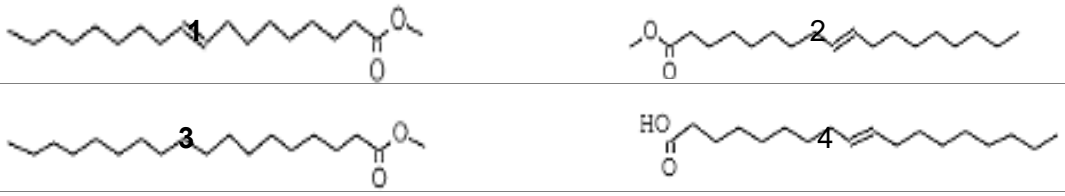

Figure 3. 9-Octadecenoic acid (Z)-, methyl ester (CAS), Octadecenoic acid (Z)-, methyl ester (CAS) 9-Octadecenoic acid (Z)-, (CAS).

The results of GC-MS analysis showed that several compounds from Avocado leaves extract may have the potential to be antioxidants. The compound listed in number 1 was 9-Octadecenoic acid (Z)-, methyl ester (CAS) has an R.Time value of 23,786, an area of 10.18%, a height of 22.65%, and a molecular weight of 2.54. The compound in number 2 was 9-Octadecenoic acid (Z)-, methyl ester (CAS) has an R.Time value of 23,844, an area of 6.07%, a height of 8.57%, and a molecular weight of 4.00. The compound in number 3 was Octadecenoic acid (Z)-, methyl ester (CAS) has an R.Time value of 23,996, an area of 5.30%, and a molecular weight of 4.62. The compound at number 4 was 9-Octadecenoic acid (Z)-(CAS), which has an R.Time value of 24.504, an area of 36.18, a height of 17.03, and a molecular weight of 11.99. Therefore, it can be observed that the potential compounds were at peaks number 1 and 4, which have the potential to act as antioxidants (Suhaili et al.,2020).

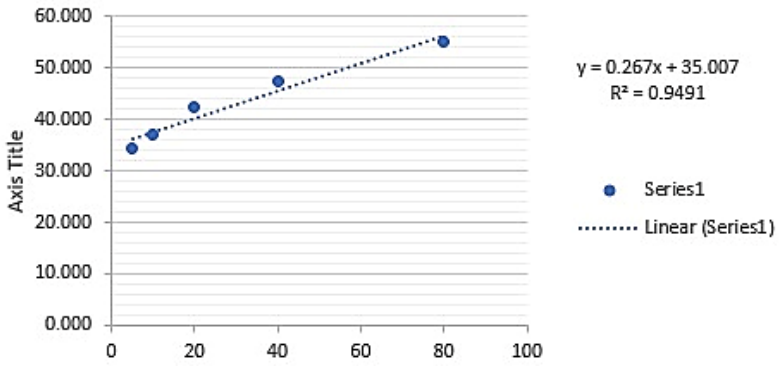

Figure 4. Antioxidant Test Results of Avocado Leaves Extract using the ABTS method

The results of the antioxidant test of Avocado leaves extract obtained a linear regression equation of =0.267x + 35.007, R^2 = 0.9491. This equation indicated a good relationship between variables and antioxidant capacity.

Table 3. IC_{50} Value of Avocado Leaves Extract in Antioxidant Test with ABTS Methol

Concentration (ppm)	Absorbance	Inhibition Value	Linear Regression Equation	IC_{50}
5	0.224	34.311	y= 0.267x + 35.007 R^2 = 0.9491	56.154
10	0.215	36.950		
20	0.196	42.522		
40	0.179	47.507		
80	0.153	55.123		

Incubate and measure the wavelength of 600-800 nm with a wavelength of 734 nm until a stable absorbance is obtained. Antioxidant activity with the ABTS method resulted in Avocado leaves extract having a value of = 0.267x + 35.007, R2 = 0.9491 which can be interpreted as indicating a very strong and positive correlation, the IC_{50} value of 56.154 can be categorized as a very strong antioxidant properties. So it can be concluded that Avocado leaves extract has potential as an antioxidant [15].

CONCLUSION

Based on the analysis, avocado leaf extract showed a strong correlation between lipid (fat-derived) compound content and antioxidant activity. FTIR analysis identified functional groups such as N–H, H–C=O, C=C, C–O–H, and C–N, indicating the presence of phenolic compounds and other organic compounds that act as antioxidants.

GC-MS results showed that the dominant compounds in the extract were 9-octadecenoic acid (Z)- (oleic acid) and its derivatives in the form of methyl esters. These compounds are known to belong to the group of unsaturated fatty acids with biological activities, including acting as antioxidants and protecting against lipid oxidation.

Antioxidant activity testing using the ABTS method produced a linear regression equation with an R^2 value of 0.9491, indicating a very strong correlation between extract concentration and free radical scavenging activity. The IC_{50} value of 56.154 µg/mL indicates that avocado leaf extract has very strong antioxidant activity.

Thus, it can be concluded that the lipid compounds, particularly unsaturated fatty acids such as oleic acid, along with the phenolic compounds identified by FTIR, contribute synergistically to the antioxidant activity of avocado leaf extract. This indicates that avocado leaf extract has the potential to be an effective source of natural antioxidants.

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