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THE EFFECT OF SOLVENT TOWARDS ANTIOXIDANT ACTIVITY OF *Vernonia amygdalina* Delile LEAVES

M. Muhammad[1,✉], E.D. Putra[2], H. Cintya[2] and D. Satria[1]
[1]Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, 20155
[2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia, 20155
✉Corresponding Author: mahatir.muhammad@usu.ac.id

ABSTRACT
Antioxidants guard against reactive oxygen free radicals. Antioxidants neutralize free radicals by adding electrons to them, preventing bodily harm. African leaves (*Vernonia amygdalina* Delile.) contain an antioxidant. African leaves are the leaves of a thousand diseases because they have pharmacological effects on various diseases. African leaves contain steroids/triterpenoids, glycosides, saponins, tannins, and flavonoids. This study examined *Vernonia amygdalina* Delile. leaf combination ethanol extract antioxidant properties. The extracts were prepared with the reflux method using methanol, butanol, isopropanol, ethyl acetate, chloroform, and n-hexane. Antioxidant activities were determined with DPPH, ABTS, FRAP, CUPRAC, O-Phenanthroline, Hydroxyl radical scavenging, total phenolics, and total flavonoids method. The data were analyzed using the Principle Component Analysis (PCA) multivariate exploration technique, which was analyzed using the Minitab software. The highest antioxidant activity was found in African leaf reflux extract with methanol solvent by DPPH method (43.8968 ± 0.021), followed by ABTS (73.6223 ± 0.091), FRAP (89.2924 ± 0.087), CUPRAC (13.4745 ± 0.042), O-Phenantroline (99.0977 ± 0.30), and Hydroxyl radical scavenging (33.5703 ± 0.071). The findings show that *Vernonia amygdalina* Delile. extract exhibit antioxidant activity. According to the findings, *Vernonia amygdalina* Delile leaf extract with methanol solvent provided the maximum antioxidant activity.
Keywords: Antioxidant, Solvent, Extract, *Vernonia amygdalina* Delile. Leaves.
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INTRODUCTION

Free radicals have unpaired electrons in their outer orbitals. Unpaired electrons make the complex reactive, attacking and binding electrons to nearby molecules to find a mate. Free radicals is cause oxidative damage and degenerative diseases like hypertension, diabetes, obesity, and cardiovascular disease.[1] Antioxidants from the body or outside can prevent free radical damage and neutralize free radical activity.[1] Antioxidants consist of artificial and natural antioxidants. However, natural antioxidants are more in demand because they are safer for the body when consumed. Besides being safe, natural antioxidants are widely found in fruits and vegetables that are easily found in the environment; another feature is that natural antioxidants tend to have affordable prices but contain extraordinary antioxidants for the body.[2] Antioxidants are chemicals that can stop or slow down other molecules' oxidation events, which result in the production of free radicals.[3, 4] Endogenous (body-made) and exogenous (food-derived) antioxidants exist. Enzymatic and non-enzymatic antioxidants exist. SOD, GPX, and CAT are important antioxidants.[5,6] In the tropical parts of Africa, particularly in Nigeria, Cameroon, and Zimbabwe, a shrub known as *Vernonia amygdalina* , Delile often known as "bitter leaf," can reach heights of up to three meters. It is thought to have several positive health effects. It has been demonstrated that the plant's organic fraction extracts have lethal effects on human nasopharynx cancer cells.[7] It works well to treat gastrointestinal problems and amoebic dysentery[8,9], and it also has antibacterial and antiparasitic properties.[10,11] Saponins and alkaloids[12], terpenes, steroids, coumarins, flavonoids, phenolic acids, lignans, xanthones, and anthraquinone[13], edotides, and sesquiterpenes are the biologically active components of *Vernonia amygdalina.*[14,15] Antioxidant activity measurement methods have improved greatly in recent decades. Thus, early antioxidant efficiency assessments focused on lipid oxidation. Antioxidant activity has been measured by scavenging multiple forms of free radicals or ROS, reducing power, and metal chelation using various chemical tests and

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susceptible and automated detection systems. Oxidizing substrates have expanded from food model systems to chemical substances, biological materials, cellular pathways, and even living tissues.[8] Numerous tests, including the DPPH, FRAP, CUPRAC, ABTS, Hydroxyl radical scavenging, and phenanthroline techniques, are available for the direct assessment of hydrogen atom transfer or electron transfer from antioxidants to free radicals. with respect to *Vernonia amygdalina* Delile. In order to combat common antioxidants like ascorbic acid, gallic acid, and quercetin that are polar and represent the structure of all antioxidants in general that leaves are utilized.

EXPERIMENTAL

Preparation of Extract

Vernonia amygdalina Delile. air-dried and powdered leaves (100 g) were extracted using the reflux method with methanol, ethanol, ethyl acetate, isopropanol, butanol, chloroform, and n-hexane (Merck). After collecting the filtrate, a viscous fraction was obtained by evaporating it under reduced pressure, and it was then dried to dry.[4]

Testing for Free Radical Scavenging Activity

The 1,1-diphenyl-2-picrylhydrazyl (DPPH•) technique was used to assess the free radical scavenging activity. 100 µl of a 0.2 mM DPPH• solution in methanol was produced and added to various amounts of single and combined extracts. At 516 nm, absorbance was measured after 60 minutes.[17]

Radical Scavenging Assay for ABTS

ABTS radical cation decolorization assay measured plant samples' free radical scavenging. 7 mM ABTS in water and 2.45 mM potassium persulfate (1:1) formed ABTS·+ cation radical. At least three measurements were taken. The formula for percent absorbance inhibition at 734 nm.[18]

Ferric Reducing Antioxidant Power

5 mg of extract was diluted in 5 mL of ethanol, then 1 mL pipette, 1 mL 0.2 M phosphate buffer (pH 6.6), 1 mL $K_3Fe(CN)_6$ 1%, 1 mL TCA, and centrifuged at 3000 rpm for 10 minutes. After centrifugation, 1 mL of the pipette's top layer was transferred to the test tube with 1 mL of distilled water and 0.5 mL of 0.1% $FeCl_3$. After 10 minutes, 700 nm absorbance was determined.[19]

Cupric Reducing Antioxidant Capacity (CUPRAC)

A 250 mL volumetric flask was filled with 0.4262 grams of $CuCl_2.2H_2O$ to make $CuCl_2$ solution using distilled water to dissolve the powder. In a 250 mL volumetric flask, 19.27 g of NH_4Ac was dissolved in distilled water to make ammonium acetate pH 7. 0.039 g of neocuproine (Nc) was dissolved in a 25 mL volumetric flask and diluted with ethanol.[20]

O-phenanthroline Assay

O-phenanthroline, $FeCl_3$, methanol, and extracts made up the reaction mixture. After 20 min at 30°C, the absorbance of an orange-red solution was measured at 510 nm against a reagent blank to compute the % inhibition. Positive standards were quercetin.[21]

OH-Radical Scavenging

1 mL extract, 2 mL salicylic acid, 0.6 mL $FeSO_4$, and 0.5 mL H_2O_2 were combined. The mixes' absorbance was measured at 510 nm after 30 min at 37°C. A positive control is quercetin.[22]

Total Phenol Measurement

Folin reagents measured the sample's total phenol concentration (TPC). Briefly, 100 µL of extract (500 µg/ml) was combined with 7.9 mL of distilled water and 0.5 mL of folin-ciocalteu's reagent (1:10 v/v) and vortexed for 1 minute. After mixing, 1.5 mL of 20% aqueous sodium bicarbonate was added and the mixture stood for 90 min with intermittent shaking. A spectrophotometer measured 775 nm absorbance.[23]

Total Flavonoid Measurement

As previously reported, spectrophotometry evaluated total flavonoids in the extracts. 2 mL of extract in methanol was combined with 0.10 mL of 10% aluminum chloride ($AlCl_3.6H_2O$), 0.10 of sodium acetate (1 M), and 2.80 mL of purified water. A spectrophotometer measured absorbance at 432 nm after 40 min.[24]

Analytical Statistics

A mean and standard deviation (SD) have been used to present the data. Using the Minitab program, the data were analyzed with the Principle Component Analysis (PCA) multivariate exploration technique.

RESULTS AND DISCUSSION

Antioxidant activity of Ethanol Extract *Vernonia amygdalina* Delile. Leaf with DPPH, ABTS, FRAP, CUPRAC, O-Phenanthroline, Hydroxyl Radical Scavenging, Total Phenolic Dan Total Flavonoid

The DPPH technique works by hydrogen atoms from antioxidant chemicals binding to free electrons in radical molecules, converting them into non-radical compounds (*diphenylpicrylhydrazine*). It turns purple to yellow (antioxidants diminish free radical molecules).[25, 26] The reasoning behind using the ABTS method to test antioxidant activity is to take away the cation's color to gauge the antioxidant capacity, which directly interacts with the cation radical. Antioxidants can decrease the colorless-to-colorless ABTS radical, which has a nitrogen center and is often blue-green in hue, into a non-radical state. The ABTS technique is extremely light-sensitive. Even the production of ABTS•-needs 12–16 hours of dark incubation time.[27, 28] The FRAP approach works effectively for antioxidant substances that may decrease ferri-tripy-ridyl triazine (Fe(III)TPTZ) to Fe(II)TPTZ complexes.[29] The CUPRAC method uses bis(neocuproine) copper (II) ($Cu(Nc)_2^{2+}$) as a chromogenic reagent. The blue $Cu(Nc)_2^{2+}$ reagent will be reduced to $Cu(Nc)^{2+}$, which is yellow.[20] The phenanthroline method uses $FeCl_3$ as a source of Fe^{+2} ions and orthophenanthroline as an iron complexing agent. The mechanism that occurs is that Fe^{+3} from $FeCl_3$ will oxidize compounds that are antioxidants; as a result, Fe^{+3} will be reduced to Fe^{+2}, resulting in the formation of a complex red-orange compound caused by the complex cation $[Fe(C_{12}H_8N_2)_2]^{2+}$.[22] Hydroxyl radicals can be formed in biological systems from hydrogen peroxide (H_2O_2), which reacts with ferrous metal ions (Fe^{2+}), called the Fenton reaction. In addition, hydroxyl radicals can also be generated from the reaction between hydrogen peroxide superoxide anion ($O2^{\bullet-}$) with a ferrous metal ion catalyst known as the Haber-Weiss reaction, $H_2O_2 + O2^{\bullet-} \rightarrow OH + {}^{-}OH + O2$.[22] The principle of total flavonoids is that flavonoid compounds can form complexes with Al^{3+} metal, namely on the bordering hydroxy groups (ring A) and ketones (ring C) and on ortho-dihydroxy (ring B), which can produce a yellow color. Flavonoid content in the extract increases with yellow color intensity. Folin–Ciocalteu reagents measure total phenol concentration by decreasing the phenolic hydroxy group, which forms a complex blue molecule.[23, 24]

Table-1: The Results of IC_{50} Values from Various Antioxidant Methods Against the Extract of *Vernonia amygdalina*, Delile

No	Solvent	DPPH	ABTS	FRAP	CUPRAC	O-Phenathroline	Hydroxyl Radical Scavenging	Total Phenolic	Total Flavonoid
1.	Methanol	43.89 ± 0.02	73.62 ± 0.09	89.29 ± 0.08	13.47 ± 0.04	99.09 ± 0.30	33.57 ± 0.07	211.88 ± 0.11	11.65 ± 0.05
2.	Ethanol	54.22 ± 0.09	85.10 ± 0.04	102.99 ± 0.04	16.65 ± 0.02	117.81 ± 0.13	74.35 ± 0.09	55.77 ± 0.14	11.24 ± 0.03
3.	Buthanol	56.40 ± 0.05	126.03 ± 0.07	121.07 ± 0.01	18.67 ± 0.05	132.05 ± 0.11	78.23 ± 0.05	36.88 ± 0.12	10.72 ± 0.09
4.	Isopropanol	62.81 ± 0.01	103.33 ± 0.01	114.07 ± 0.09	27.47 ± 0.03	137.66 ± 0.11	108.80 ± 0.02	23.55 ± 0.08	2.42 ± 0.04
5.	Ethyl Acetate	63.45 ± 0.02	173.62 ± 0.04	204.49 ± 0.03	49.35 ± 0.01	139.97 ± 0.11	113.64 ± 0.04	20.77 ± 0.14	0.26 ± 0.03
6.	Chlorofom	155.82 ± 0.04	132.79 ± 0.03	189.17 ± 0.03	124.34 ± 0.02	161.05 ± 0.098	458.92 ± 0.08	19.11 ± 0.11	0.88 ± 0.02
7.	Hexane	166.89 ± 0.06	161.35 ± 0.02	199.62 ± 0.04	159.62 ± 0.02	458.16 ± 0.19	793.16 ± 0.21	7.44 ± 0.09	0.57 ± 0.019

According to Table-1, African leaf reflux extract with methanol solvent by DPPH method (43.89 ± 0.02), ABTS (73.62 ± 0.09), FRAP (89.29 ± 0.08), CUPRAC (13.47 ± 0.04), O-Phenantroline (99.09 ± 0.30), and Hydroxyl radical scavenging (33.57 ± 0.07) had the highest antioxidant hexane, chloroform, ethyl acetate, and isopropanol. This is so that the bioactive elements present in the methanol-solvent extracted African leaf play a more active antioxidant role in lowering free radicals. O-Phenanthroline, hydroxyl radical

scavenging, total phenolics, and total flavonoids. DPPH, ABTS, FRAP, CUPRAC, and FRAP. Because secondary metabolites like chlorophyll and other polyphenolic chemicals that act as antioxidants are readily soluble in acetone solvents, African leaf extract can be extracted effectively using methanol as a solvent.[30,31] Many assays directly detect antioxidant-to-free radical hydrogen atom or electron transfer. This series of approaches report antioxidant activity by neutralizing specific radical species. However, data on hydrogen atom transfer or electron-donating ability from some of these approaches can reveal their innate antioxidant potential with minimum environmental impact. These assays require no lipid substrate. They use a chemical system with the oxidant (free radicals or other ROS), the oxidizing substrate (other assays do not need it), and the antioxidant under examination. Standard methodologies for the dietary component antioxidant activity must meet certain ideal requirements.[32]

Results of Principal Component Analysis

By decreasing the data, PCA (Principal Component Analysis) evaluates the data. A matrix's number of variables has been lowered while keeping the information that the data contain. Scores of primary components are the new variables that result. There are four separating quadrants that can discriminate between the solvents methanol, ethanol, butanol, isopropanol, ethyl acetate, chloroform, and hexane, according to the PCA analysis results in a score plot shown in Fig.-2. According to the PCA analysis results, each sample is categorized at a different proximity to the others. The difference between samples reveals how similar they are. The similarity between each sample decreases with increasing distance. For instance, it is clear that methanol and ethyl acetate are located in distinct quadrants. The graph demonstrates the ability to discriminate between groups of ethyl acetate and methanol.[33]

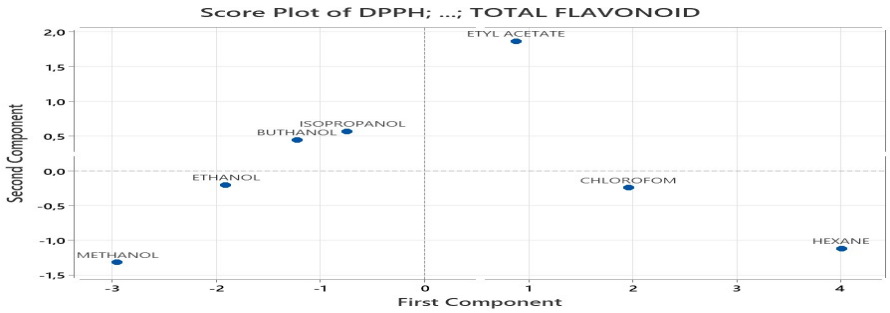

Fig.-2: Solvent Score Plot Graph

PCA analysis begins with calculating the correlation value between variables because PCA analysis can be done if the existing variables correlate. This analysis is done by calculating the correlation of each variable and formed in a correlated matrix. PCA will later analyze the correlated matrix by looking at the eigenvalues that exist in each variable. Finally, a new variable (Principal Component) is formed based on more than one eigenvalue. Fig.-3 describes the principal components and eigenvalues. The requirement for one main component to be formed is that it has an eigenvalue of ≥1.[34] The scree plot displayed is a two- or three-dimensional plot consisting of PC-1 and PC-2. Table 3 shows that the value of the proportion of PC-1 = 73.6% and PC-2 = 88.1%.

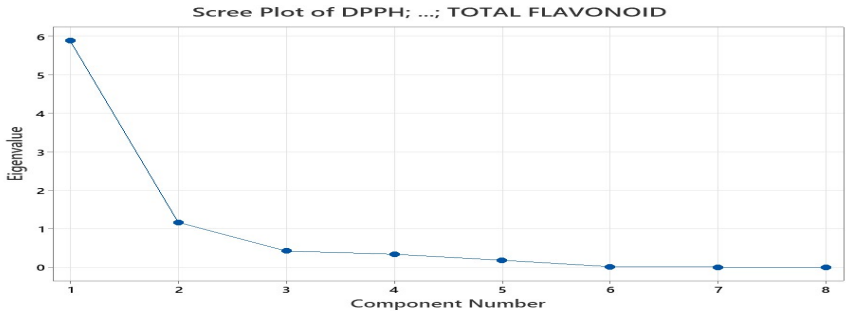

Fig.-3: Scree Plot Graph

Based on Fig.-4, it is found that Principal Component 1 is represented by antioxidant methods DPPH, ABTS, FRAP, CUPRAC, O-Phenantroline, and Hydroxyl Radical Scavenging (with the same correlation, which is around 0.4 for each variable to PC-1). In other words, PC-1 is a component related to the

antioxidant activity of African leaf extract. Meanwhile, PC-2 was represented by total phenol and total flavonoid with a correlation of -0.456 between total phenol to PC-2 and -0.316 between total flavonoid and PC-2, which illustrates the vertical direction, the higher the total phenol and total flavonoid which is related with a smaller antioxidant IC_{50} value.

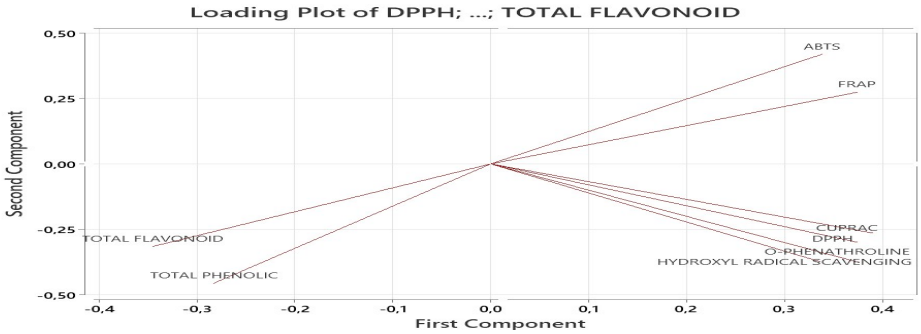

Fig.-4: Antioxidant Method Loading Plot Graph

CONCLUSION

According to the research results, the *Vernonia amygdalina*, Delile leaf extract prepared using methanol solvent contained the greatest concentration of antioxidants.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication. The research profile of the authors can be verified from their ORCID numbers given below:

M. Muhammad https://orcid.org/0000-0002-7895-5144
E.D. Putra https://orcid.org/0000-0003-4726-4102
H. Cintya https://orcid.org/0000-0001-6678-9858
D. Satria https://orcid.org/0000-0003-4724-3256

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