

Free radical scavenger of complex compound derived from bidara plant (*Ziziphus mauritiana*) nanopowder

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Abstract

Natural antioxidants are less likely to cause side effects than synthetic antioxidants. As free radical scavengers, natural complex compounds can be used as antioxidant sources. The bidara plant, for example, has antibacterial, anti-inflammatory, and antioxidant properties and has potential as a free radical scavenger (*Ziziphus mauritiana*). The goal of this study was to identify antioxidants and their roles in complexes formed in bidara plants to scavenge free radicals. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was used in the antioxidant assay, and its physical properties were determined using Field Emission Scanning Electron Microscopy (FESEM)-Energy Dispersive X-ray Spectroscopy (EDS). According to DPPH analysis, the 2.7 M concentration leaf-bark had the highest inhibition activity, accounting for 43.794%, indicating an effective scavenging activity. Bidara contains elements, some of which are metals like sodium (Na), potassium (K), magnesium (Mg), and calcium (Ca), according to FESEM-EDS analysis. The bidara plant is a powerful antioxidant and free radical scavenger. In terms of capturing free radicals, complex compounds differ from single compounds. Several elements found in Bidara plants are required for scavenging and life processes.

Keywords: Scavenger, complex, antioxidants, bidara, elements

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Introduction

Antioxidants are compounds that can remove excess free radicals from the body. Excessive free radicals can cause various diseases. According to Phaniendra et al. (2014) and Kurutas (2016), scavenging free radicals and chelating redox metals at physiologically appropriate levels are ideal antioxidant. Endogenous or exogenous antioxidants are obtained as part of the diet or as dietary supplements. Some dietary compounds are not able to neutralize free radicals. Increased endogenous activity, on the other hand, can be classified as antioxidants.

Nature's complex compounds can be used as free radical scavengers. Complex compounds are used without extracting a component in order to obtain only one compound. All compounds in plants are used to produce complex compounds. According to Jayanti et al. (2018), complex antioxidants have more electrons than single antioxidants, making them more effective. During the cycling redox process, complex antioxidants would reduce the excess of Reactive Oxygen Species. Jayanti et al. (2018) compared single and complex antioxidants in their study. As a result, complex antioxidants are more effective than single antioxidants at reducing DPPH as free radicals.

The Bidara plant, for example, has the potential to pose as a free radical scavenger (*Ziziphus mauritiana*). *Z. mauritiana* have significant anti-inflammatory,

antibacterial, antioxidant, cytoprotective, and antiulcer activities (Gupta et al., 2012; Abdallah et al., 2016; and Yahia et al., 2020). The leaves constitute as immunostimulant and have cardiovascular benefits. According to Butt et al. (2021), *Z. mauritiana* has a high concentration of total protein, reducing sugars, flavonoids, ascorbic acid content, polyphenols, carotene, and tannins. Due to these desirable contents, *Z. mauritiana* has been reported as a good antioxidant and H₂O₂ scavenger.

Bidara's natural antioxidants can aid in the removal of free radicals; Bidara is made into nanoparticles to maximize these functions. Bidara in nano-size will be more convenient in the drug delivery system. According to Misra et al. (2010), the ability of nanoparticles to deliver drugs to the target site crosses several biological barriers, including the blood-brain barrier. Nanoparticles are directed to specific sites and interact biochemically with receptors expressed on target cells.

In addition to antioxidants, plants contain elements such as Ca, Co, Cu, Fe, K, Mg, Mn, Na, Ni, and Zn. Plants, including Bidara plants, contain elements. Plant elements play an important role in life, including their ability to benefit humans. These elements are linked to electron transfer and antioxidant activity in terms of free radical reduction. According to Sumitro and Sukmaningsih (2018), electron transfer protein, substrate binding and activation, atom and group transfer chemistry, and metallic properties in biological chemistry have significant implications in bioinorganic chemistry. According to Sukmaningsih et al. (2019), the structure of bioinorganic compounds is similar to that of

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Superoxide Dismutase (SOD) and hemoglobin. Free radical scavengers SOD and hemoglobin.

The goal of this study was to identify antioxidants and their potential role in the complexes formed to scavenge free radicals in thistles. Many studies have been conducted on antioxidants in bidara, such as *Z. mauritiana* (Javed et al., 2022). However, the effect of complex compounds and nanoparticles on the antioxidant activity has not yet been investigated.

Methods

Plant material

Bidara plants were obtained from the Qur'an garden of the Universitas Islam Malang; the leaves, bark, and a leaf-bark mixture were collected and used as samples. The bidara leaves were taken as young and bright green in color.

Sample preparation for nanopowder

Bidara leaf and bark samples were weighed at 10 g each, washed, and cut into pieces. The samples were fermented in distilled water for 3x24 h before being blended. All plant samples were centrifuged for 15 minutes at 2800 rpm using a centrifuge (PLC Series 03, Taiwan). The supernatant was freeze-dried (Alpha 1-2 LD Plus, Germany) after 48 h. This stage was completed at the Halal Center Laboratory of Universitas Islam Malang in Indonesia.

Antioxidant assay

The dried samples were then tested for antioxidants using 2,2-Diphenyl-1-picrylhydrazyl (DPPH). The test was conducted at the Faculty of Medicine, Universitas Islam Malang. A concentration of 0.1 mM DPPH (Sigma Aldrich) was added to 3 ml of sample solution. After 30 min incubation, spectrophotometric measurement (EPOCH2C Spectrophotometer) was done with a wavelength of 517 nm. Percentage inhibition is calculated as following (Abalaka, et al., 2011):

$$\text{Percentage of inhibition} = [(A_0 - A_1) / A_0] \times 100$$

Where A_0 = Absorbance of the control dan A_1 = Absorbance of the sample

Field Emission Scanning Electron Microscopy (FESEM) - Energy Dispersive X-ray Spectroscopy (EDS) Analysis

The sample is placed on a stub with double-sided carbon tape, Gold Sputter coating. The samples were observed by Scanning Electron Microscopy (FESEM) facility (FEI Quanta FEG 650) at the Central Laboratory of Life Sciences, Brawijaya University (LSIH-UB). The magnifications used in this observation were 500x and 50,000x.

Results

The results showed that the 2.7 M leaf-bark treatment possessed the highest inhibitory activity, at 43.794%, followed by 2.7 M leaf and 3 M bark at 31.946% and 30.818%, respectively (Table 1). In contrast, 3.3 M leaves had the lowest inhibitory activity at -27.856. The

detailed inhibitory values on different plant parts are shown in Table 1.

Table 1. Percentage of Bidara Inhibition

Concentration	Average A	Inhibition %
Blank	0,473	
2,7 M Leaves	0,332	31,946
3 M Leaves	0,471	0,423
3,3 M Leaves	0,604	-27,856
2,7 M Bark	0,404	14,457
3 M Bark	0,327	30,818
3,3 M Bark	0,468	1,058
2,7 M Leaves-Bark	0,266	43,794
3 M Leaves-Bark	0,327	30,748
3,3 M Leaves-Bark	0,380	19,676

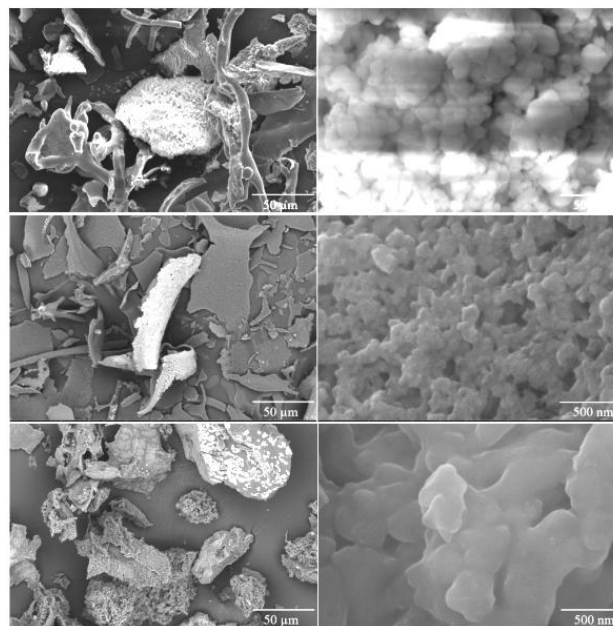


Figure 1. Bidara leaves (top), bark (middle), and leaf-bark (bottom), FE-SEM observed nanopowder at 500x magnification (left) and 50,000x magnification (right)

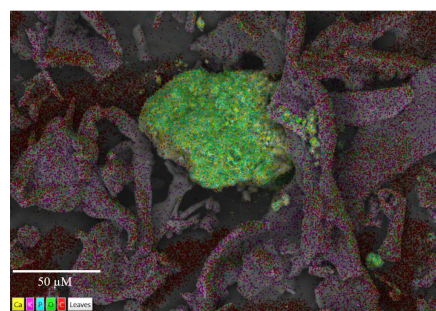


Figure 2. Elements detected in bidara leaves observed by FE-SEM at 500x magnification

In addition, based on observations with FE-SEM at 500x (left) and 50,000x (right) magnification (Figure 1), the physical structure of the bidara nano powder is smaller and more regularly shaped. The distribution of metals found in leaves, stem bark and leaves-stem bark is depicted in Figure 2-10. Different colors indicate different elements in the FE-SEM results (Figure 2-7). The FE-SEM - Energy Dispersive X-Ray Spectroscopy (EDS) profiles of the elements detected in the leaves, bark, and leaf-bark of bidara are shown in Figure 8. The EDS results show that the leaf-bark has the highest peak in all samples, which is comparable to its inhibitory activity value.

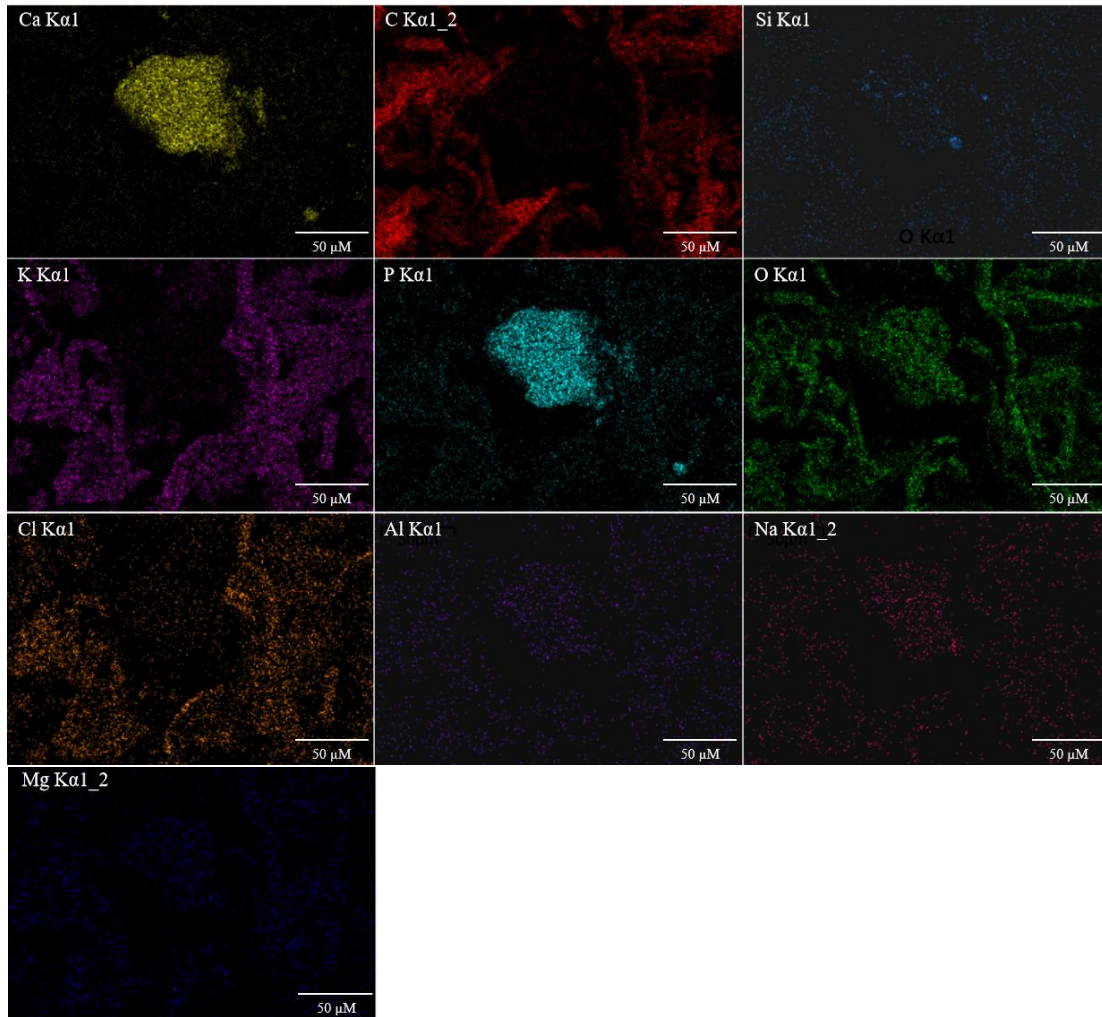


Figure 3. Elements distribution detected in bidara leaves observed by FE-SEM at 500x magnification

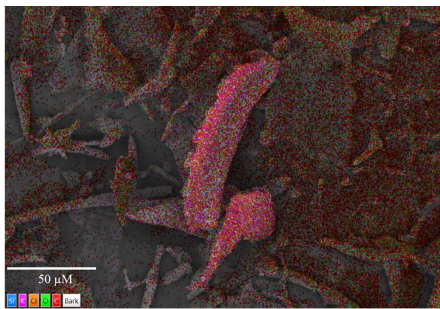


Figure 4. Elements detected in bidara bark observed by FE-SEM at 500x magnification

The highest inhibition (31.946%) in the leaves sample was obtained at 2.7 M concentration, however it yielded negative results at a concentration of 3.3 M. Negative results due to the influence of the sample. In the bark sample, the highest value was obtained at 3 M, accounted about 30.818%. While, the highest inhibition value in the leave-bark was 2.7 M, accounted about 43,794 (Table 1). The high percentage of inhibition indicates the effectiveness of the sample in scavenging free radicals (DPPH) compared to other samples.

According to Dureja and Dhiman (2012), DPPH method was used to test the ability to act as free radical scavengers or hydrogen donors and can be used on any sample for antioxidant capacity, whether solid or liquid, and is not limited to specific antioxidant components. The DPPH free radical is purple and has an odd number of electrons, allowing it to absorb strongly at 517 nm. The color changes from purple to yellow because the DPPH radical's molar absorptivity is reduced when the DPPH radical's odd electron pairs with the hydrogen atom of the free radical scavenging antioxidant to form reduced DPPH-H. (1,1-diphenyl-2-picrylhydrazine).

The different contents of flavonoids, polyphenols, and other ingredients make it possible for different parts of the tree to have different levels of antioxidant activity. Additionally, it's conceivable that various components in each portion of the bidara tree influence the antioxidant activity. This study demonstrates that combining samples of bark and leaves yields results with high values and more complex compounds. The highest antioxidant absorption value among the leaves-bark samples was at a concentration of 2.7 M. Complex compounds have a

greater capacity to scavenge free radicals than simple ones. The complex compounds are the same in all samples (leaves, bark, and leaves-bark). Complex compounds utilize every component without first extracting one of the constituent compounds. Complex compounds have many electrons and donate them to reactive electron-deficient free radicals. Free radicals' electron counts can be balanced to prevent them from

becoming reactive and starting a chain reaction. Free radicals can also be cleaned up. In their paper, Hutasoit, et al. (2021) claimed that molecules strong enough to lessen or prevent the oxidation of other molecules are referred to as antioxidants or free radical scavengers. An oxidizing agent receives electrons from a substance through oxidation

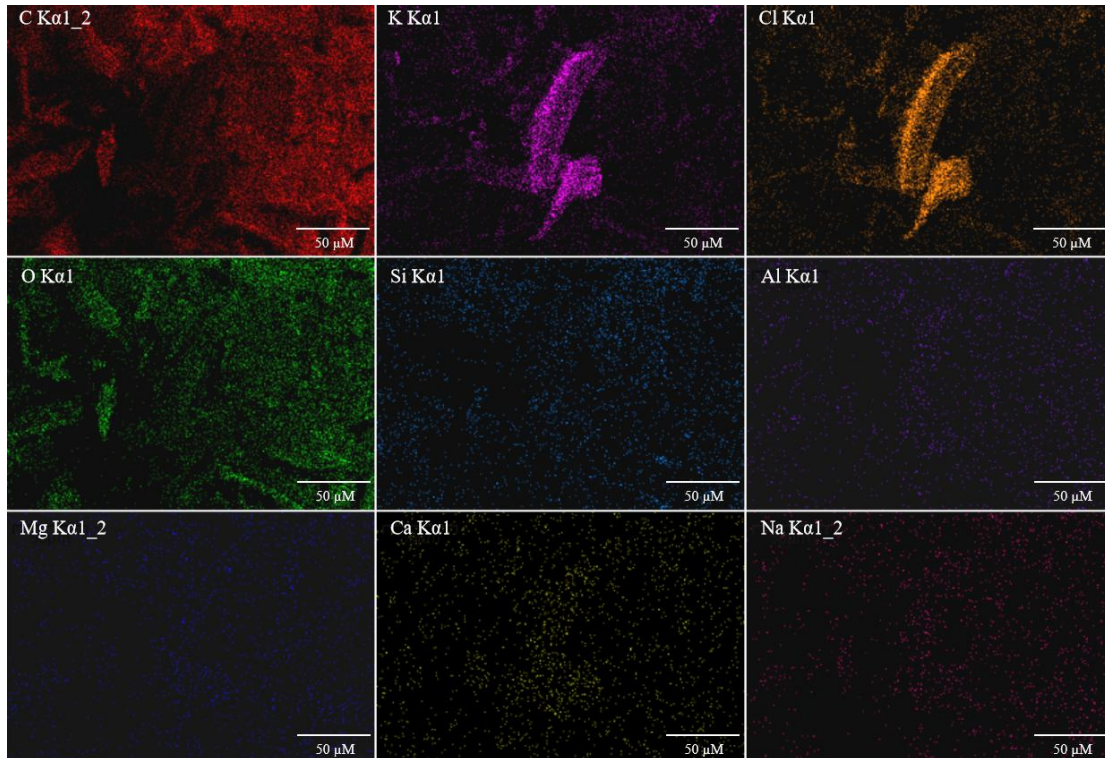


Figure 5. Elements distribution detected in bidara bark observed by FE-SEM at 500x magnification

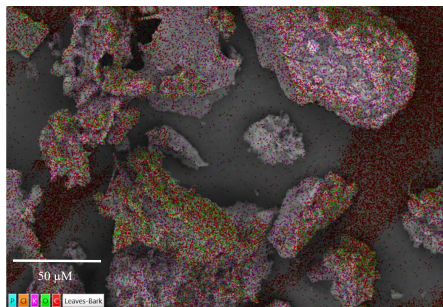


Figure 6. Elements detected in bidara leaves-bark observed by FE-SEM at 500x magnification

According to Sumitro and Sukmaningsih (2018), herbal medicines use both plant and animal materials as their medicinal components. In the study of herbal medicine, complex compounds mixed with extracts are frequently discussed. The majority of them still have their original structure and are composed of intricate compounds. They have complex compounds as their original structure. Herbal medicine made of bioorganic low molecular weight complex compounds can bind toxic metals. Metals are easily removed from the body by these molecules, whether they are intracellular or extracellular.

In addition to complex compounds, nanometer-sized samples are used in this study. Nanotechnology has been extensively applied to many fields, including medicine. For this study, samples were centrifuged and freeze-dried to produce nanometer-sized and uniformly shaped particles. Azizah et al. (2020) reported that the size of the molecules could be formed from the speed of the centrifugation process while using a centrifuge speed of 2800 rpm. Figure 1 depicts the sample's overall crystal structure and reveals a regular shape at a magnification of 500,000x. After freeze-drying, the crystal structure was created. The orderly sample powder is a result of the crystal structure. According to Sukmaningsih et al. (2018), a crystal is a substance with a regular pattern and three-dimensional, organized, and directional atomic bonding. Furthermore, according to Pande and Bhaskarwar (2016), the properties of nanoparticles vary depending on their size compared to bulk materials. because of two main reasons: (1) As size decreases, the surface area to volume ratio rises, and (2) particle size moves into the realm of quantum effects. The number of atoms on the surface rises relative to those inside as the particle size decreases.

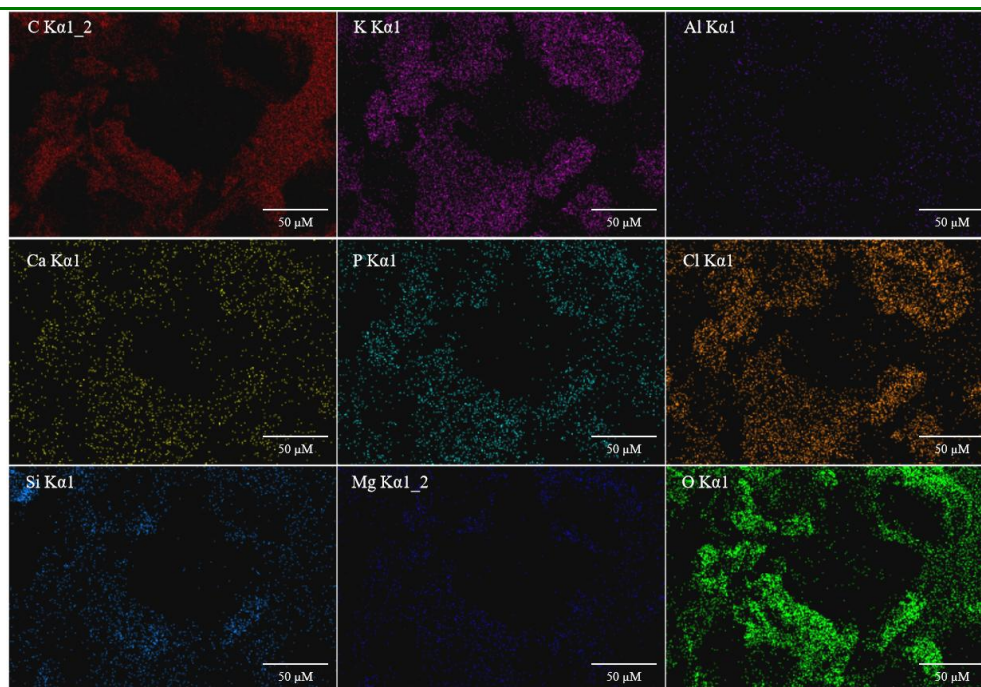


Figure 7. Elements distribution detected in bidara leave-bark observed by FE-SEM at 500x magnification

The element distribution found in bidara leaves is depicted in Figure 2. Ca (yellow), C (red), Si (light blue), K (purple), P (cyan), O (green), Cl (brown), Al (light purple), Na (bright red), and Mg (dark blue) were the elements found in bidara leaves (Figure 3). The elements C, K, Cl, O, Si, Al, Mg, Ca, and Na were found and distributed throughout the bark of the bidara tree (Figures 4 and 5). The elements C, K, Al, Ca, P, Cl, Si, Mg, and O were found and distributed throughout the leaves and bark of the bidara tree (Figures 6 and 7). With the exception of P, all of the elements in the three samples (leaves, bark, and leave-bark) are the same. Metals such as K, Ca, Mg, and Na are among these elements. In Gupta (2018), some metals that play a role in normal biological functions in the human body are sodium (Na), potassium (K), magnesium (Mg), and calcium (Ca) which are included in the main element group, and vanadium (V), chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), molybdenum (Mo), and cadmium (Cd) which are classified as transition metals in the periodic table.

The detected atomic intensity in leaves samples was higher than in bark and leaves-bark samples. Metals, including those related to antioxidants, play critical roles in life. Biochemistry with metals as the center of chemical coordination, according to Sumitro and Sukmaningsih (2018), is research with biologically relevant complexes. Bioinorganic is a multidisciplinary study that is important in understanding the regulation of polymerization, combustion, and chemical energy transfer processes in physiology. As a result, characterization of metal ions in biological systems is required. The bidara plant's EDS profile elements (Figure 8) show peak elements with the C atom as the higher element and the O atom as the lower element. Tables 2

(leaves), 3 (bark), and 4 show the percentages of the elements (weight, weight sigma, and atomic weight) (leaves-bark).

In conclusion, this research shows that the bidara plant acts as an antioxidant complex as a free radical scavenger. Complex compounds have different properties than single compounds in free radical scavenging. The bidara plant contains several essential elements for the scavenging process and life.

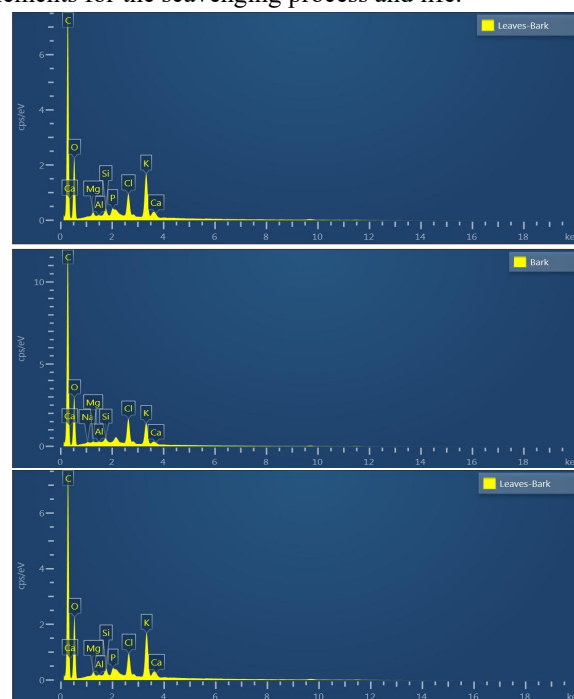


Figure 8. EDS profile elements detected in bidara: (a) leaves, (b) bark, (c) leaves-bark

Table 2. EDS profile elements detected in bidara leaves

Element	Line Type	Weight %	Weight % Sigma	Atomic %
C	K series	54.46	0.38	65.52
O	K series	32.32	0.35	29.19
K	K series	4.45	0.06	1.65
Cl	K series	1.41	0.03	0.57
Ca	K series	4.26	0.06	1.53
P	K series	2.22	0.05	1.04
Mg	K series	0.44	0.03	0.26
Si	K series	0.29	0.02	0.15
Na	K series	0.11	0.03	0.07
Al	K series	0.04	0.02	0.02
Total		100		100

Table 3. EDS profile elements detected in bidara bark

Element	Line Type	Weight %	Weight % Sigma	Atomic %
C	K series	67.62	0.32	75.25
O	K series	27.42	0.31	22.91
Cl	K series	2.08	0.04	0.78
K	K series	2.27	0.04	0.78
Ca	K series	0.19	0.02	0.06
Na	K series	0.1	0.03	0.06
Mg	K series	0.08	0.02	0.04
Al	K series	0.04	0.02	0.02
Si	K series	0.21	0.02	0.1
Total		100		100

Table 4. EDS profile elements detected in bidara leaves-bark

Element	Line Type	Weight %	Weight % Sigma	Atomic %
C	K series	63.42	0.39	72.12
O	K series	29.56	0.38	25.23
K	K series	4.09	0.07	1.43
Si	K series	0.39	0.02	0.19
Cl	K series	1.64	0.04	0.63
Mg	K series	0.27	0.03	0.15
P	K series	0.29	0.03	0.13
Ca	K series	0.31	0.03	0.11
Al	K series	0.03	0.02	0.01
Total		100		100

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Conflict of Interest

The authors declare no conflict of interest in this research.

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