



RESEARCH ARTICLE

Study of Phenolic Compounds of Moringa oleifera Leaf Extracts and Their Potential as Antioxidants

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ARTICLE INFO	ABSTRACT
Received: May 21, 2024 Accepted: Jun 27, 2024	This study aimed to determine the content of Moringa oleifera leaf extracts of phenolic and flavonoid compounds and their antioxidant capacity. The method of quantitative estimation of phenolic and flavonoid compounds was used, and the DPPH test was used, measuring the reducing power, the ability to bind the ferrous ion, and the ability to scavenge the peroxide radical for the extracts. The results of estimating the total content of phenolics showed a significantly exceeded of the alcoholic extract, recording the highest phenolic content of 864.4 mg/100g compared to the aqueous extract, which amounted to 353.2. mg/100g, while the results of estimating the total content of flavonoids showed a significantly exceeded of the alcoholic extract of Moringa oleifera leaves, giving the highest content of 225.1 mg/100g compared to the aqueous extract of 42.7mg/100g. The results of the DPPH test for both the aqueous and alcoholic extracts of Moringa oleifera leaves showed a significantly exceeded of the alcoholic extract, as it gave the highest percentage of inhibition, which amounted to 81.36% at a concentration of 1 mg/ml, while the aqueous extract gave the highest percentage of inhibition at the same concentration, which amounted to 72.46%. The results of examining the reducing power of Moringa oleifera leaf extracts indicated a significantly exceeded of the alcoholic extract, which recorded 85.67% compared to the aqueous extract, which reached 64.00%. The results of the ability to bind ferrous ions of the Moringa oleifera leaf extracts indicated that there were significant differences in the ability to bind between the aqueous and alcoholic extracts, which was 14.90. 21.33%, respectively. The results of measuring the ability to capture hydrogen peroxide of Moringa oleifera leaf extracts showed significant differences in the aqueous and alcoholic levels, which amounted to 47.24 and 66.32%, respectively. It is noted from the results of this study that there are high bioactive compounds (phenols and flavonoids), which have many important functions. The antioxidant capacity results also indicated the high capacity of the extracts and their role as natural antioxidants, and thus the possibility of their use in food applications.
Keywords Phenolic compounds Moringa oleifera Antioxidants Plant extracts	
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INTRODUCTION

The use of plant extracts in many food applications is considered because they contain substances that have effective antioxidants and inhibitors of microorganisms that cause food spoilage, and it has become a promising direction for eliminating and dispensing with industrial additives (Diniz do Nascimento et al., 2020; Jam et al., 2018). Phenolic compounds are one of the most important main

types of antioxidants and are of plant origin. Their presence in food works to provide protection against food oxidation through preparation and storage, in addition to their work as antioxidants within the body, as they prevent the oxidation of unsaturated fatty acids through their interaction with radicals. Free radicals are formed, as flavonoids are considered one of the most important natural antioxidants that play an effective role in this field. They are natural polyphenolic compounds found in almost all plant parts, such as fruits, leaves, roots, and flowers. Attention has been paid to flavonoids, as it has been found from studies and research that they have activity. Physiologically, as well as its effectiveness as antioxidants, many studies have proven its role as antifungal, antibacterial, antiviral, and anticancer (Segwatibe et al., 2023; Rashid et al., 2023). *Moringa oleifera* is one of the perennial and fast-growing trees whose scientific name is (*Moringa oleifera*). It reaches a height of 7-12 m. It is considered one of the most nutritional and medicinal plants in the world and is the most widespread species of the *Moringa* family (Alhassan et al., 2022). It grows in a temperature range between 25-35°C and can withstand high temperatures up to 48°C and extreme cold (Muzammil et al., 2023; Kanval et al., 2024). He explained that the extract of *Moringa oleifera* leaves contains a large group of nutritional antioxidants such as flavonoids, phenols, beta-carotene, ascorbic acid (vitamin C), alphanolophenol (vitamin E), and antioxidant enzymes (SOD), POD, CAT) and others, as the extract showed a powerful scavenging effect. to various free radicals compared to many leaf extracts referred to as antioxidants in many vegetables and fruits. Therefore, this extract can prevent oxidative damage to the main biomolecules and provide great protection for them Jacob and Shenbagaraman (2011). This study aimed to know the content of moringa leaf extracts from Phenols and measuring their ability as antioxidants.

MATERIALS AND METHODS

Plant leaves used in the study and their source:

The leaves of *Moringa oleifera* plant were obtained from some people interested in growing rare and medicinal trees in Babylon Governorate, and the tree was confirmed and diagnosed with the help of Professor Dr. Ibrahim Radhi - Al-Furat Al-Awsat Technical University - Al-Musaib Technical College - Department of Plant Production Technologies - Fasalja plant (a specialist in this field) collected the leaves, washed them well, then dried them naturally, then ground and sieved them to obtain a fine powder, then kept them in the refrigerator until use.

Preparation of plant extracts: preparation of plant extracts

Alcoholic Extract

The alcoholic extract of the plant was prepared according to the method of (Ahmed et al. (1998), where 20 gm of *Moringa oleifera* leaf powder was mixed with 400 ml of 98% ethyl alcohol and the mixture was left for 24 hours on a magnetic stirrer at a temperature of 25°C, then filtered by Filter paper (Whatman No. 1). The filtrate was concentrated using a rotary vacuum evaporator, then the material was incubated at refrigerator temperature until use.

Water Extract

The aqueous extract of *Moringa oleifera* leaves was prepared according to the above method (2-2-1) described by (Ahmed et al (1998), replacing the alcohol with distilled water.

Determination of total phenols

The total phenolic content of *Moringa oleifera* leaf extracts was estimated according to the method used by Ayoola et al., (2008), where 2.5 ml of Folin's reagent was taken and added to 0.5 ml of prepared extracts, equivalent to (1 mg/ml), then the mixture was left for a period 10 minutes at room temperature (25°C), after which 2 ml of 7.5% sodium carbonate was added and the mixture was left to react for 30 minutes, after which the absorbance was measured at a wavelength of 760 nm.

Determination of Total Flavonoids

The amount of flavonoids in aqueous and alcoholic extracts of *Moringa oleifera* leaves was estimated based on the method described by Ayoola et al. (2008), which includes taking 2 ml of aluminum chloride prepared at a concentration of 2% in ethanol, then 2 ml of the extracts were added, after which the mixture was shaken vigorously. Leave it well for an hour at room temperature at 25°C, after which the absorbance was measured at a wavelength of 420 nm.

Antioxidant activity:

DPPH test

The antioxidant activity of *Moringa oleifera* leaf extracts was estimated using the DPPH reagent to estimate the effectiveness of free radical suppression, as stated in the method described before. Okunade (2002). Dissolve 0.04 g of DPPH in 100 ml of methanol to obtain a concentration of 400 µg/ml of DPPH, then mix 1 ml of the sample solution with concentrations (1, 0.50, 0.25, 0.12) mg/ml with 4 ml of DPPH dissolved in methanol, then shake the mixture well with a magnetic stirrer, then leave it at room temperature for 30 minutes. Then, the optical absorption at the wavelength of 517 nm was read using a spectrophotometer, and the percentage of free radical scavenging activity was calculated using the following equation:

$$\text{Free radical suppression \%} = \frac{\text{Optical absorbance of control} - \text{Optical absorbance of the sample}}{\text{Optical absorption to control}} \times 100$$

Measurement of Reducing Power

The reducing power of the studied extracts was estimated based on the method described by Benzie and Strain (1996), in which 1 ml of the extract was taken and mixed with 2.5 ml of 1% Potassium Ferricyanide solution, after which 2.5 ml of phosphate buffer solution with a concentration of 0.2 molar at pH was added. 6.6 Then the mixture was incubated at 50°C for 20 minutes, after which 2.5 ml of 10% Trichloro acetic acid (TCA) was added. Then the centrifugation process was carried out at 1900 x g for 10 minutes, then 2.5 ml of the filtrate was taken and 2.5 One ml of distilled water and 0.5 ml of 0.1% ferric chloride. The control sample was prepared by adding all the materials except the addition of the extract. The mixture was left for 30 minutes, after which the absorbance was measured at a wavelength of 700 nm with a spectrophotometer, and to calculate the reducing power, the following equation was applied:

$$\text{Reducing power \%} = \frac{(\text{absorbance reading of the control sample} - \text{absorbance reading for the sample})}{\text{read the absorbance of the control sample}} \times 100$$

Chelating ability of ferrous ion

The ferrous ion binding capacity of *Moringa oleifera* leaf extracts was estimated based on the method of Gülçin et al., (2003), which includes taking 0.4 ml of the extracts with 0.4 of ferrous chloride 2 mM and then adding 0.4 ml of 8-hydroxyquinoline at a concentration of 5 M (prepared with 98% ethanol). The mixture was incubated for 10 minutes at room temperature in a dark place, then the absorbance was measured at a wavelength of 562 nm. The control sample was prepared with the same previous steps except for the addition of the extract. The ability to bind the ferrous ion was calculated based on the following equation:

$$\text{Ferrous ion binding capacity \%} = \frac{1 - \text{absorbance reading of the sample}}{\text{absorbance reading of Control sample}} \times 100$$

Ability to scavenge hydrogen peroxide radical

The hydrogen peroxide radical scavenging capacity was measured according to the method (Rush et al., (1989), which includes taking 1 ml of the studied extracts and adding 0.6 ml of hydrogen peroxide

with a concentration of 2 mM in a phosphate buffer solution with pH (7.4) and leaving the mixture for 10 minutes, then the absorbance was measured. At a wavelength of 230 nm, the control sample was prepared in the same way except for the addition of the extract. The scavenging capacity of hydrogen peroxide radical was calculated based on the following equation:

$$\text{Hydrogen peroxide radical scavenging \%} = \frac{\text{absorbance reading of control sample} - \text{absorbance reading of model sample}}{\text{absorbance reading of control sample}} \times 100$$

Statistical Analysis and Design

A completely randomized design was used to estimate the total content of phenols and flavonoids of *Moringa oleifera* leaf extracts, in addition to tests for antioxidant activity (hydrogen peroxide capture, ferrous ion binding capacity, reducing power) with the use of the least significant difference (LSD) test to compare means. For the DPPH test, the data were analyzed as a factorial experiment (3×2) according to a completely randomized design, at a probability level of 0.05 (Al-Rawi and Khalfallah, 2000). The results were analyzed using the statistical program (Genstat., 2009) Genstat V.12.

RESULTS AND DISCUSSION

Determination of the total content of phenols and flavonoids in extracts of *Moringa oleifera* leaves.

Figure (1) shows the total content of phenols and flavonoids in *Moringa oleifera* leaf extracts. Statistical analysis showed significant differences in the amount of phenols between the alcoholic and aqueous extracts. The amount of phenols in the alcoholic extract of *Moringa oleifera* leaves reached 864.40 mg/100 g, which is much higher than the amount of phenols in The aqueous extract amounted to 353.20 mg/100 g. Statistical analysis showed that there were significant differences in the amount of flavonoids for the alcoholic and aqueous extract, amounting to 225.10 and 42.70 mg/100 g, respectively. (2022) et al., Ezz El-Din Ibrahim showed that the amount of phenols and flavonoids for leaf extracts *Moringa oleifera* extracted alcoholically amounted to 689.64 and 147.6 mg / 100 g, respectively. Phenols and flavonoids play an important role in reducing fat oxidation and increasing their stability, preventing protein degradation and other biological functions. As found (2022) et al., Olvera- Aguirre stated that the total amount of phenols in the aqueous and alcoholic extracts of *Moringa oleifera* leaves reached (121.60, 130.57) mg/100g, respectively, while the amount of flavonoids was (2.66, 3.68) mg/100g, respectively. (Do Nascimento et al., (2017) found that the amount of phenolics in the alcoholic extract of the flowers, leaves, and seeds of the *Moringa oleifera* plant is (114.49, 170.07, 22.43) mg/100 g. (Singh et al., 2013) found when he studied the phenolic composition of the *Moringa oleifera* seed extract. The amount of phenols was 780.0 mg/100 g, while the amount of flavonoids was 133.3 mg/100 g. It was also shown that *Moringa oleifera* seed extract contains a high amount of phenolic compounds, enabling it to become a natural preservative agent and the possibility of use in many food applications in the future.

(Al-Owaisi et al., 2014) studied the amount of phenols and flavonoids in *Moringa oleifera* leaf extracts using several solvents: coliform, ethyl acetate, methanol, and hexane. The amount of phenols and flavonoids in the methanol extract was higher than the rest of the extracts, while their presence in the hexane extract was not indicated. The difference may be due to The amount of total phenols in the *Moringa oleifera* leaf extracts studied compared with previous studies of the same plant due to the difference in the method of extraction, the type of plant, and the difference in environmental conditions of the plant. Many studies have shown the presence of polyphenols (tannins and flavonoids), steroids, alkaloids, carbohydrate glycosides, cardiac glycosides, and terpenoids in ethanolic extracts of moringa leaves, as they have effective biological properties such as antioxidants,

antidiabetic, hepatoprotective, anti-inflammatory, antibacterial, and anti-cancer. (2013, Kumar and Pandey).

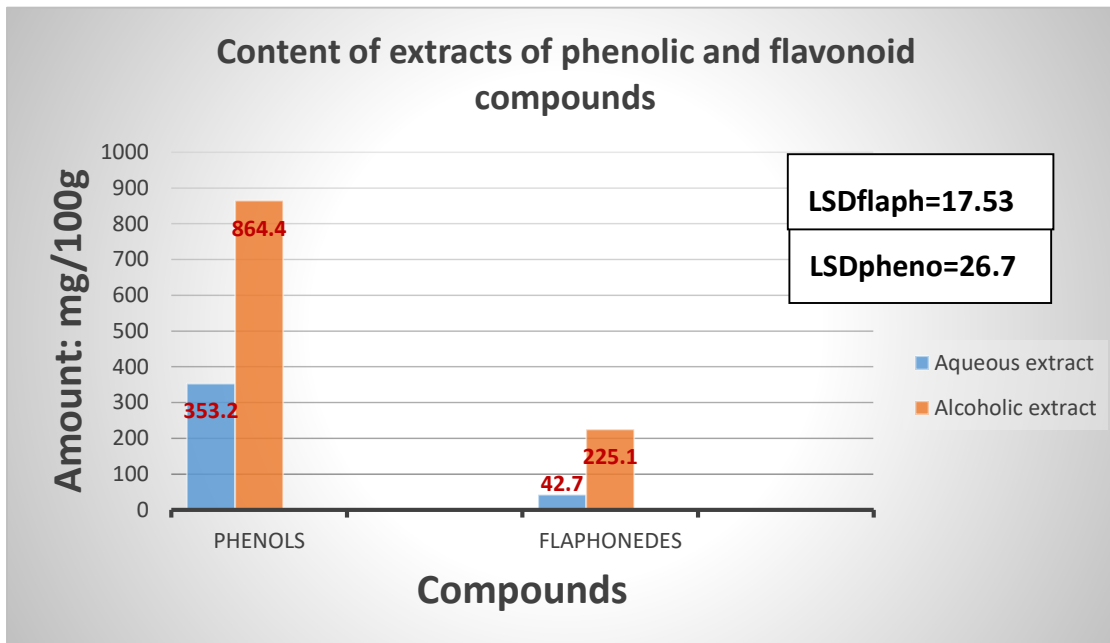


Figure (1): Total content of phenols and flavonoids of the aqueous and alcoholic extract of *Moringa oleifera* leaves.

DPPH test for *Moringa oleifera* leaf extracts

Figure (2) shows the results of the antioxidant activity of DPPH for the aqueous and alcoholic extracts of *Moringa oleifera* leaves. There is a significant increase in the effectiveness of free radical suppression of the alcoholic extract compared to the aqueous extract and for all concentrations, where the concentration of 1 mg/ml gave the highest effectiveness of DPPH, which amounted to 80.36%, while it was 72.64% in the aqueous extract at the same concentration, while it was less effective for the alcoholic extract at 56.40% at a concentration of 0.12 mg/ml, while in the aqueous extract it was 53.06% at the same concentration, while the interaction between the type of extract of *Moringa oleifera* leaves and the concentration of the alcoholic extract at the same concentration appeared. 1 mg/ml was the highest, reaching 80.36%. This is due to the number of hydrogen-donating hydroxyl groups and their location on the rings, which greatly affects the activity of removing free radicals.

These results agreed with (Alhakmani et al., 2013), who confirmed that the effectiveness of inhibiting free radicals of *Moringa oleifera* flower extract increases proportionally with the concentration in the DPPH test as a result of the increased effect of the active compounds (phenols, tocopherols, carotenoids) present, as these compounds work to inhibit Free roots. (2021) et al., Prasajak found that the effectiveness of free radical suppression of aqueous and alcoholic extracts of *Moringa oleifera* leaves and pods reached (63.60, 53.07, 59.07, 54.14) %, respectively. The content of phenols and flavonoids is responsible for the antioxidant activity in the crude extract, and they are secondary plant metabolites that represent common natural antioxidants in the plant. The high amounts of chlorogenic acid and isoquercetin in moringa leaves are responsible for the high antioxidant activity (Adebayo et al., 2018).

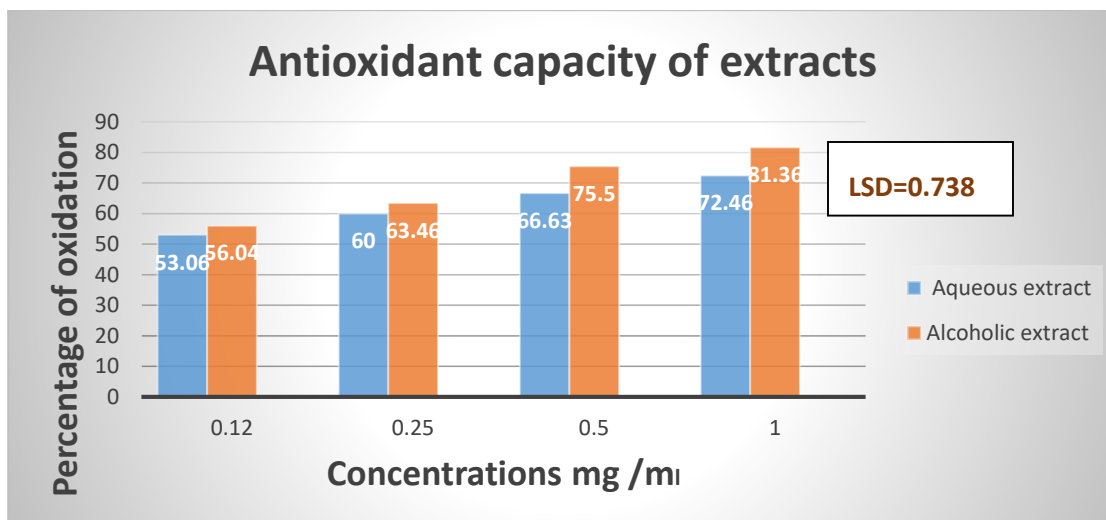


Figure (2) DPPH test for alcoholic and aqueous extracts of *Moringa oleifera* leaves

The ability of *Moringa oleifera* leaf extracts to absorb hydrogen peroxide

Figure (3) shows the ability of *Moringa oleifera* leaf extracts (aqueous and alcoholic) to capture hydrogen peroxide. The results indicated a significant difference between the alcoholic extract and the aqueous extract, which amounted to (66.32, 47.24) %, respectively.

When Kumar et al., (2012) studied the ability to capture hydrogen peroxide of the alcoholic extract of *Moringa oleifera* leaves using different concentrations of 20, 40, 60, 80, and 100 $\mu\text{g/ml}$, it was found to be 15.08, 47.16, 50.55, 58.12, and 64.49%, respectively. He noted (et al., 2014 Al-Owaisi) when studying the ability to capture peroxide radicals in *Moringa oleifera* leaf extracts using different solvents (ethyl acetate, methanol, hexane, and chloroform), the activity of the extract is affected by the type of solvent used and the extract's content of active compounds, especially polyphenols.

Hydrogen peroxide is characterized by its high ability to interact with iron and copper ions to produce hydroperoxide radicals, which when accumulated have toxic effects. Therefore, the amount of hydrogen peroxide must be controlled biologically in the cells, and the active compounds have a clear effect in stopping its formation, as their presence in plant extracts leads to To inhibit the formation of hydrogen peroxide radical (Smirnov and Arnaud (2019).

The reductive power of *Moringa oleifera* leaf extracts

Figure (3) shows the reducing power of *Moringa oleifera* leaf extracts (aqueous and alcoholic). The results of the statistical analysis showed that there were significant differences at a significance level of 0.05, as the alcoholic extract recorded the highest reducing power, amounting to 85.67%, while the aqueous extract recorded the lowest reducing power, which amounted to 64.00%. . These results were consistent with the findings of (2023). Al-Shebli and Al-Anbari, when studying the reductive power of aqueous and alcoholic extracts of *Moringa oleifera* leaves and comparing them with synthetic antioxidants, were 57.71, 90.12, and 84.46%, respectively. (Moyo et al., 2012) studied the reductive power of *Moringa oleifera* leaf extracts and compared them with vitamin C and BHT. They found that the reductive power of the acetone extract is significantly effective compared to the aqueous extract, but it is lower than that of vitamin C and BHT. The reductive power is an indicator that reflects the antifungal activity. For oxidation, the effectiveness of the reductive force is determined by reducing the ferricyanid complex Fe^{+3} to Fe^{+2} . The presence of phenols and flavonoids in these extracts causes the reduction of Fe^{+3} and the production of Fe^{+2} . The formation of Fe^{+2} can be inferred by the appearance of a blue or green dye at a wavelength. 700 nanometers, as the active compounds that have a high reducing power have a greater ability to donate an electron, which in

turn can interact directly with free radicals and transform them from an unstable state to a more stable state, thus preventing their reproduction (Al-Halafi, 2016).

Ability of *Moringa oleifera* leaf extracts to bind ferrous ion

The results showed in Figure (3) the ability of aqueous and alcoholic extracts of *Moringa oleifera* leaves to bind ferrous ions. There were significant differences between the aqueous and alcoholic extracts, as the highest effectiveness of the alcoholic extract was 21.33%, while the aqueous extract recorded the lowest effectiveness, which amounted to 14.90%. Between (2023), Al-Shebli and Al-Anbari, when studying the ferrous ion binding capacity of aqueous and alcoholic extracts of *Moringa oleifera* leaves and comparing them with synthetic antioxidants, were (80.50, 88.20, 76.90) %, respectively. When studying the ability of the alcoholic extract of quinoa seeds to bind the ferrous ion in comparison with BHT and ascorbic acid, they found (Nasser et al., (2019) that the ability to bind the ferrous ion of the alcoholic extract was higher compared to BHT and similar to that of ascorbic acid. The ability of the alcoholic extracts of plants to bind the ferrous ion depends on The ability of phenolic compounds such as phenols, tannins, and flavonoids to bind metal ions such as iron and copper depends on their content of multiple hydrogen groups and their ability to donate hydrogen to hold these ions. Phenolic compounds have a chelating property It binds the ions of metal elements that stimulate oxidation, such as iron and copper, thus preventing the formation of free radicals and Then the oxidation process occurs (Al-Halafi and Al-Moussawi, 2011; Jam et al., 2014).

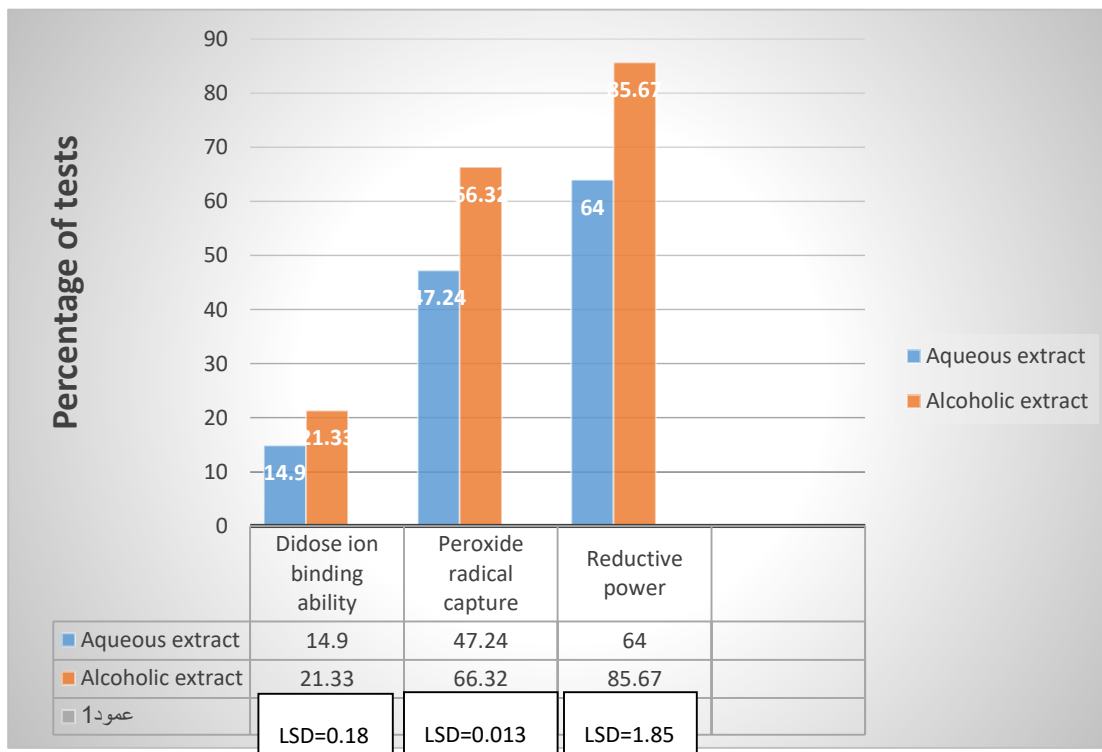


Figure (3) shows the chemical antioxidant activities of *Moringa oleifera* leaf extracts

CONCLUSION AND RECOMMENDATIONS

The alcoholic extracts of *Moringa oleifera* leaves contained a higher amount of phenols than the aqueous extract, and the alcoholic extracts of *Moringa oleifera* leaves contained a much greater amount of flavonoids than the aqueous extract. Alcoholic extracts of *Moringa oleifera* leaves contained the highest antioxidant activity through inhibition of DPPH free radicals, while aqueous

extracts recorded the lowest antioxidant activity. The alcoholic extracts of *Moringa oleifera* leaves recorded the highest value in terms of capturing hydrogen peroxide compared to the aqueous extract, which recorded the lowest ability to capture hydrogen peroxide. The alcoholic extracts of *Moringa oleifera* leaves recorded significant differences in terms of reducing power compared to the aqueous extract, which recorded the lowest reducing power. The alcoholic extracts of *Moringa oleifera* leaves recorded the highest ability to bind the ferrous ion, while the aqueous extract recorded the lowest ability to bind the ferrous ion.

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