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# Antiradical Properties of *Moringa oleifera* (Saharan Plant from Southwestern Algeria)

## Propriétés antiradicalaires de *Moringa oleifera* (plante saharienne du Sud-Ouest algérien)

**A. Seghir · A. Moussaoui · M. Draoui · A. Saad**



**Abstract** The diversity of biological properties of medicinal plants is certainly related to the therapeutic virtues attributed to an arsenal range of bioactive molecules synthesized by these plants. The aim of this study was to evaluate the anti-free radical activity by means of three prepared macerates from *Moringa oleifera* leaves, from Southwestern Algeria: firstly we proceed to a phytochemical screening of *Moringa oleifera* leaves. The quantitative estimation of the total phenols and flavonoids is carried out by colorimetric methods. The antioxidant test revealed the presence of a strong reducing activity of crude extracts. Furthermore, the results of the antioxidant activity showed that the acetone extract had the highest inhibition percentage of the free radical DPPH 76% compared with the hydromethanolic extract 71.71% and with the aqueous extract 63% at the concentration of 1 mg/ml. On the other hand, the ferric reducing antioxidant power assay (FRAP) also illustrated a high reduction power proportionally to the concentration of the extract. Consequently, this plant can serve as a good source of natural antioxidants and could be potentially considered as a functional food or functional food ingredient.

**Keywords** *Moringa oleifera* · Antioxidant capacity · DPPH · Reducing power FRAP · Southwestern Algeria

**Résumé** La diversité des propriétés biologiques des plantes médicinales est certainement reliée aux vertus thérapeutiques attribuées à un large arsenal de molécules bioactives synthétisées par ces plantes. Le but de cette étude est d'évaluer l'activité antiradicalaire libre au moyen de macérats de feuilles de *Moringa oleifera* du Sud-Ouest algérien. Tout d'abord, nous avons procédé au criblage phytochimique des feuilles de *Moringa oleifera*. L'estimation quantitative des phénols et des flavonoïdes totaux est effectuée par des méthodes colorimétriques. Les tests antioxydants ont révélé la présence d'une forte activité réductrice des extraits totaux. Bien plus, les résultats d'activité antioxydante montrent que l'extrait acétonique a le plus grand pourcentage d'inhibition des radicaux libres (DDPH : 76 %) comparé à l'extrait hydrométhanolique (71,7 %) et à l'extrait aqueux (63 %) avec une concentration de 1 mg/ml. Par ailleurs, l'essai du pouvoir antioxydant de réduction ferrique (FRAP) confirme aussi un haut pouvoir de réduction proportionnellement à la concentration de l'extrait. En conséquence, cette plante peut servir comme une bonne source d'antioxydants naturels et pourrait être considérée comme aliment fonctionnel ou ingrédient d'aliment fonctionnel.

**Mots clés** *Moringa oleifera* · Capacité antioxydante · DPPH · Pouvoir antioxydant de réduction FRAP · Sud-Ouest Algérie

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## Introduction

In the recent years, many researches on natural substances with antioxidant properties have been conducted to substitute the commercial synthetic antioxidant molecules such as butylhydroxyanisole (BHA) and butylhydroxytoluene (BHT) because of their potential toxicological risks. Development in scientific research in various specialties has allowed researchers to extract, quantify and identify many compounds from several natural sources, especially medicinal plants and agro-food products [1–3].

This work was interested to study the species of *Moringa oleifera*, also called "miracle tree," and "tree of life" because of its many nutritional, medicinal and industrial potentialities [4,5], whose leaves are recognized worldwide as an excellent dietary supplement [6,7].

*Moringa oleifera* belongs to the family of Moringaceae which comprises of about 13 species [8]. This plant is widely distributed in tropical and subtropical countries and it is very resistant to arid conditions, thanks to its tuberous roots [9]. It is a plant with rapid growth; it reaches 4 m in a year. It grows optimally under direct sunlight and below 500 m altitudes. *Moringa oleifera* prefers neutral or low acid soils (6.3–7.0), but seems to grow well in difficult environments (seaside, poor soils and arid climate) [10].

Chemical investigations of different plant parts of the genius of Moringa have led to the characterization of various secondary metabolites such as essential oils in the leaves (contains 70–73% of oleic acid, 1.4% of palmitoleic acid, 7.7% bohenic acid, 6.2% palmitic acid and 5.7% of stearic acid), riboflavin, β-carotene, nicotinic acid, folic acid, pyridoxine, amino acids and various phenolic flavonoids (quercetin and kaempferol), tanins and phenolic acids (cafeic acid and ferulic acid) [11,12]. Different pharmacological effects of *Moringa oleifera* species extracts have been reported such as antitumor, anti-inflammatory, antiulcer, antispasmodic, antioxidant, antiproliferation [13], antidiabetic, antibacterial, antifungal, antidiarrheic, diuretic and antihypertensive activities basically because of the vicinity of high measures of polyphenols, anti-obesity, antiatherogenic [14] and it also has the hepatoprotective activity as well [15].

Therefore, the aim of the present study was to evaluate the anti-free radical activity in vitro by means of three prepared macerates from *Moringa oleifera* leaves, collected from Tabelbala region, situated in the Southwestern of Algeria, using DPPH and ferric reducing antioxidant power (FRAP) methods.

## Materials and Methods

### Collection of Plant Material

Fresh leaves of *Moringa oleifera* were harvested in October 2016, in Tabelbala region, Southwestern Algeria. The leaves were dried, then ground into fine powder and stored in a separate glass bottles away from light and moisture before analysis.

### Phytochemical Screening

Phytochemical screening plays a crucial role in the characterization of chemical groups in a plant extracts, using commonly employed precipitation and coloration reactions. Different groups of compounds (alkaloids, quinones, flavonoids, saponins, tannins, sterols and reducing sugar) were highlighted by qualitative methods [16]. The used extracts for the phytochemical screening were obtained by using extractions with increasing polarity solvents as follow: diethyl ether to extract lipophilic compounds (sterol and triterpenes), methanol to extract polar compounds (phenolic compounds including tannins and flavonoids) and water to extract very polar compounds (such as polysaccharides) (Table 1).

### Preparation of Extracts

A weight of 10 g powdered plant material was macerated in 100 ml of methanol (70%) with frequent agitation for 72 h. The extract was stored at 4 °C for 24 h and then filtered. The solvent was evaporated to dryness under reduced pressure at

**Table 1** Chemical groups, identification reactions and indicators used in phytochemical screening [17]

| Chemical Groups | | Reagents | Indicator (Positive Reaction) |
|---|---|---|---|
| Sterols and polyterpenes | | Acetic anhydride<br>Concentrated sulfuric acid | Appearance at the interphase of a purple ring, turning blue then green |
| Polyphenols | | Ferric chloride $FeCl_3$ (2%) | Appearance of a darkish blue or dark green color |
| Flavonoids | | Hydrochloric alcohol<br>Magnesium ships<br>Isoamyl alcohol | Heat release then pink–orange or purplish color appearance |
| Tanins | Catechic | Formaldehyde<br>Concentrated hydrochloric acid | Gelatinous precipitate (in large flakes) |
| | Gallic | Sodium acetate<br>Ferric chloride | Intense blue–black color |
| Quinones | | Ammonia | Appearance of a color ranging from red to purple |
| Saponins | | Foam index | Appearance of a persistent foam |
| Alkaloids | | Dragendorff (potassium iodo-bismuthate solution)<br>Mayer | Appearance of a reddish-brown precipitate |

60 °C, using a rotary evaporator (Buchi Rotavapor R-200) [18]. In the same way, the other macerates were prepared by replacing methanol with water and acetone [19].

### Determination of Total Phenols (TP) and Total Flavonoids

The amount of TPs was determined by the Folin–Ciocalteau reagent based on the method of [20]. The content was expressed as milligram of gallic acid equivalents (GAE)/g of extract.

The total flavonoid content was determined using the Dowd method, as adapted by Arvouet-Grand [21]. The content was expressed as milligram of Vanillin equivalents/g of extract.

### Determination of Antioxidant Activity

#### *Qualitative High Performance Thin Layer Chromatography (HPTLC) Test*

In order to confirm the antioxidant power of the extracts, a test on TLC plates was performed.

The plates were exposed to spraying with a methanolic solution of DPPH 0.004% (w/v). After 30 min, the appearance of a dark yellow or green spots indicates a positive test; however, a violet spot color indicates a negative test [22].

#### *DPPH Antiradical Scavenging Test*

1,1-Diphenyl-2-dicrylhydrazyl (DPPH) is a stable free radical, with purple color in solution ($\lambda_{max}$ = 515–517 nm) [23]. It possesses an unpaired electron on nitrogen atom. Due to this delocalization, the molecules of the radical do not form dimers and remain in their monomeric form relatively stable at ambient temperature. In the presence of free radical scavengers, purple DPPH is reduced to yellow (2,2-diphenyl-1-picrylhydrazyne) [24,25].

The intensity of the color is proportional to the ability of antioxidants to give protons. The anti-free radical capacity is determined by arithmetic expression as a function of the concentration of the solution to eliminate 50% of free radicals. The results are expressed in $IC_{50}$. The concentration was calculated from the curves by giving the variation of inhibition percentage as a function of the concentration of the solution. Generally, the smaller $IC_{50}$ value the greater antiradical activity.

The antioxidant test was carried out with the DPPH method [26]. About 1.9 ml of DPPH solution (0.025 g/l) was added to 100 µl of each concentration of the dissolved extracts in methanol (from 0.0156 to 1 mg/ml). After agitation, the flasks ware placed in the dark at room temperature for 30 min. The reading was performed by measuring the absorbance at 517 nm (AA) by a UV-visible spectrophotometer, the DPPH radical solution were prepared and measured daily at the same wavelength (AB), using methanol as white.

The results were expressed as the average of three separate measurements. The positive control was represented by a solution of ascorbic acid, a standard antioxidant; whose absorbance was measured under the same conditions as the samples, and for each concentration the test was repeated three times. The results were expressed as percent of inhibition (%). The $IC_{50}$ values were determined graphically by linear regression.

The radical scavenging activity of *Moringa oleifera* was calculated from a calibration curve.

DPPH Scavenged (%) = ((AB–AA)/AB) × 100

#### *Ferric Reducing/Antioxidants Power Assay (FRAP)*

The using of FRAP assay was essentially as previously described by Oyaizu [27]. The extracts prepared (from 1 to 5 mg/ml) in 1 ml of distilled water were mixed with 2.5 ml of phosphate buffer solution (0.2 M, pH 6.6) and 2.5 ml of potassium ferric-cyanide [$K_3Fe(CN_6)$] (1%). The mixtures were incubated at 50 °C for 20 min. Afterwards, 2.5 ml of trichloroacetic acid (10%) was added. The whole mixer was centrifuged at 3000 g for 10 min. At the end, 2.5 ml of the supernatant of each concentration was mixed with 2.5 ml of distilled water and 0.5 ml of $FeCl_3$-$H_2O$ (0.1%), and the absorbance was measured at 700 nm.

## Results and Discussion

Crude extracts are beginning to be of great interest as a potential source of bioactive natural molecules. They are the subject of several studies for their possible use as an alternative for the treatment of infectious diseases and for the protection of foods against oxidation [28,29].

### Phytochemical Screening

The phytochemical screening was done using color forming and precipitating chemical reagents on the leaves of *Moringa oleifera* to generate preliminary data on the constituents of the plant. The results obtained from the tests were summarized in table 2.

The results of the phytochemical screening (Table 2) revealed the presence of many phytoconstituents, namely tannins, flavonoids, sterols, triterpenes, saponosids, anthracenosids, anthocyanins, alkaloids and reducing compounds. The phytochemical characterization showed also the absence of coumarins. This change may be related to the polarity and capability of the extracted substances to be dissolved in the used solvent [30].

**Table 2** Phytochemical screening of *Moringa oleifera*

| Chemical compounds | Results |
|---|---|
| Alkaloids | ++ |
| Flavonoids | ++ |
| Anthocyanins | + |
| Tannins | ++ |
| Saponins | ++ |
| Sterols and triterpenes | ++ |
| Phenolic compounds | ++ |
| Reducing compound | + |
| Coumarins | – |

+: present; –: absent

These results were in agreement with previous studies confirming data yielded, which indicates the presence of some flavonoid pigments such as kaempherol, rhamnetin, isoquercitin and kaempferitin in *Moringa oleifera* leaves [31,32].

These results were also in agreement with the previously conducted studies on plants of the same genus. The phytochemical screening of *Moringa oleifera* revealed the presence of the active phenolic compound quercetin, a powerful antioxidant in the flowers [33].

Due to their extensively reported pharmacological activities, the flavonoid compounds shown in these studies must be evaluated as precursor for many clinical therapies. A higher content of ascorbic acid, flavonoids, carotenoids, phenolic compounds, vitamins A, B and C have been reported in *Moringa oleifera* leaves [34].

The presence of these principle chemicals in the *Moringa oleifera* leaves could
justify its uses, especially in the prevention of cardiovascular diseases and other pathologies (hepatitis, ulcers, etc.) and the management of many infections [35,36].

## Determination of Total Phenols (TP) and Total Flavonoids

The total phenols (TP) content was estimated using the Folin–Ciocalteu colorimetric method. The phenolic substances were oxidized by the Folin–Ciocalteu reagent resulting in a blue color. The aluminum chloride colorimetric method was based on the formation of the stable complexes by the C-4 keto group of aluminum chloride and either the C-3 or the C-5 hydroxyl group of flavones and flavonols. In addition, aluminum chloride formed acid labile complexes with the orthodihydroxyl groups in the A- or B-ring of flavonoids [37]. The concentrations of flavonoid compounds were calculated according to the following equation that was obtained from the standard vanillin graph [38].

The total phenol and total flavonoid contents of extracts are shown in table 3.

According to table 3, it was shown that acetonic extracts had the highest total phenolic content and antioxidant capacity (2.15 mg of GAE/g ± 0.2310) compared with the other extracts; methanol 70% (1.4 mg of GAE/g ± 0.0998) and aqueous (0.65 mg of GAE/g ± 0.1055).

The total phenolic content obtained in this study was higher than that reported by Saddiq [39], who obtained values of 1.94 ± 0.02 g/100 phenolic content in terms of gallic acid in the cake extract of sesame.

These findings were correlated with previous studies [40] mainly those concerning phenolic compounds and their antioxidant properties.

## Evaluation of the Antioxidant Potential of the Extracts

### *Qualitative HPTLC Essay*

To screen the antioxidant capacity of *Moringa oleifera* leaves, HPTLC method was performed. The samples producing yellow spots on the purple background were considered as antioxidants. As shown in figure 1, all the selected extracts exhibited antioxidant activity. However, the highest activity was found in the hydroacetone and methanol extracts, and the lowest antioxidant activity was found in aqueous extract. These outcomes were not surprising, because the natural compounds mainly phenolics, flavonoids, β-carotene, vitamin C, protein, iron, potassium, sterols, alkaloids and glycosides contributing for radical activity were found in the acetonic and methanol extracts. This activity can be explained by their ability to donate hydrogen radical and neutralize DPPH free radical [41].

These antioxidant substances were a part of the therapeutic treatments against atherosclerosis, chronic polyarthritis, asthma and cancers [28].

### *Quantitative Essays*

To give the complexity of the oxidation process, it was clear that a single method was not sufficient to characterize the antioxidant potential of a sample. It was therefore necessary

**Table 3** Total phenolic and total flavonoids contents of *Moringa oleifera* leaves extracts

| Extracts | Total Phenolics (mg GA.eq/g Extract) | Total Flavonoides (mg V.eq/g Extract) |
|---|---|---|
| Acetonic extracts | 2.15 ± 0.2310 | 7.68 ± 0.35 |
| Methanolic extracts | 1.4 ± 0.0998 | 5.02 ± 0.39 |
| Aqueous extracts | 0.65 ± 0.1055 | 3.39 ± 0.40 |

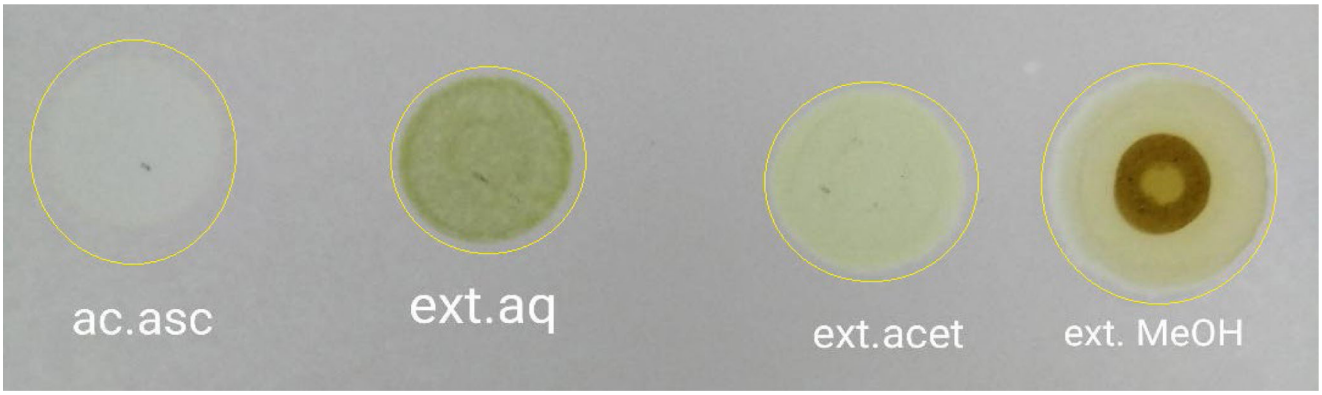


**Fig. 1** Photographic illustration of a DPPH-sprayed TLC plate

to combine results of different and complementary tests. This is why it was chosen two chemical tests based on a color change followed by the reading of the absorbance at specific wavelengths.

**DPPH Free Radical Scavenging Activity**

In this study, ascorbic acid was used as a standard, while methanol was used as a DPPH solvent. The antioxidant activity of methanol, acetone and aqueous extracts of *Moringa oleifera* leaves and the standard antioxidant (ascorbic acid) vis-à-vis the DPPH radical were evaluated using a spectrophotometer followed by the reduction of this radical which was accompanied by its color passage from the violet ($DPPH^{\bullet}$) to the yellow (DPPH-H) measurable at 517 nm. This reduction ability was determined by the decrease in the induced absorbance of the antiradical substances [42].

The hydroacetonic extract of *Moringa oleifera* showed the highest inhibition percentage of the free radical DPPH compared with the other extracts. At a concentration of 1 mg/ml, it reached a percentage of inhibition equal to 80%, while the hydromethanolic and aqueous extracts reached only 75.28% and 65.96%, respectively, at the same concentration.

It can be found from figure 2 that the hydroacetonic and the hydromethanolic macerates had shown the most obvious activity. This antioxidant activity could be explained by the presence of tannins and flavonoids [43].

According to Zhao [44] the extraction of phenolic compounds is a crucial step for the valorization of active ingredients. It depends on both the extraction solvent and the nature of the organ studied. Thus, the selection of the suitable solvent system remains one of the most important steps in the optimization of the polyphenols, flavonoids and other antioxidant compounds extraction.

The shown $IC_{50}$ values in table 4 allowed to evaluate and to compare the activity of different extracts. It is important to remind that the lower $IC_{50}$ value, the more extract is powerful against the free radicals. Table 4 showed that the acetonic and alcoholic extracts had an important antioxidant power. Their respective $IC_{50}$ were 0.52 and 0.58 mg/ml. These two values were very close compared to the ascorbic acid. A compound (ascorbic acid) known for its high antioxidant activity whose value was 0.21 mg/ml. This effect was probably attributed to their richness in phenolic compounds and flavonoids.

Indeed, phenolic compounds particularly flavonoids were recognized as potentially antioxidant substances with the ability of scavenging the radical species and reactive formed of oxygen [45,46]. These results were in accordance with Anwar et al. [34] work. Their work was conducted on aqueous extracts of *Moringa oleifera* leaves. It exhibited high antioxidant activity due to the presence of different varieties of antioxidants such as ascorbic acid, flavonoids, phenolic compounds and carotenoids.

The previous study also reported that methanol extract of *Capparis spinosa* buds showed results of rich in flavonoids including several quercetin and kaempferol glycosides. They demonstrated to possess strong antioxidant/free radical scavenging effectiveness [47].

**Ferric Reducing/Antioxidants Power Assay (FRAP)**

The antioxidant capacity of leaf extracts was evaluated by FRAP assay, because it also showed high reproducibility [48]. The antioxidant activity of the acetonic, methanolic and aqueous extracts of *Moringa oleifera* was evaluated using the FRAP method. It can be applied both in plants, plasmas and in organic and aqueous extracts. The presence of reducing agents in plant extracts causes the reduction of $Fe^{3+}$ in ferric cyanide complex to the ferrous form. Therefore, $Fe^{2+}$ can be evaluated by measuring and monitoring the density augmentation of the blue color in the reaction medium at 700 nm [49].

Phenolic antioxidants possess the ability to act as scavengers by donating a proton, and thus inhibit the autoxidation

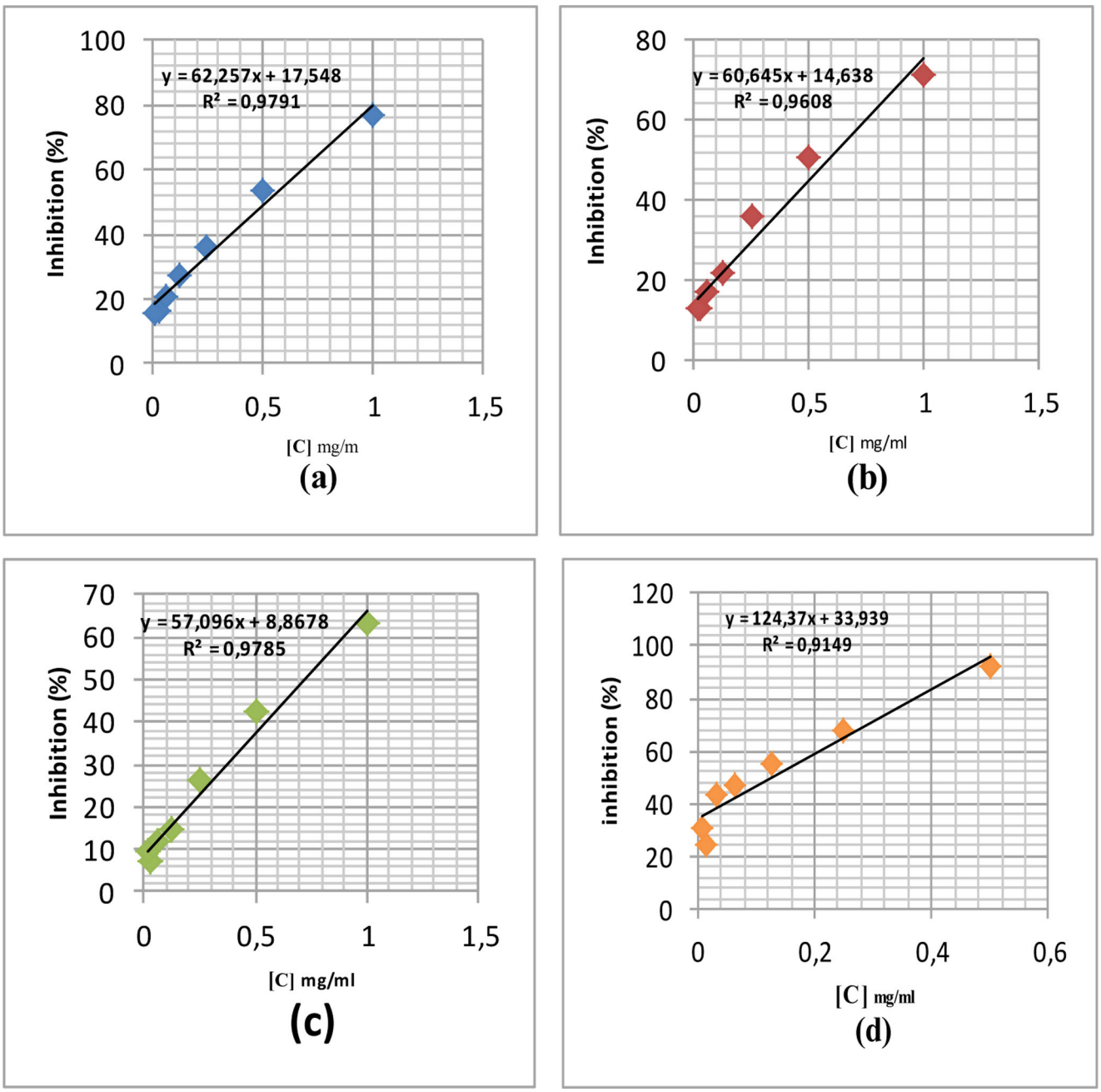


**Fig. 2** Percentage of inhibition of the free radical DPPH as a function of different concentrations of *Moringa oleifera* leaves macerate. a: hydroacetonic extract; b: hydromethanolic extract; c: aqueous extract; d: Ac ascorbic

**Table 4** DPPH radical scavenging activity expressing the effective concentration 50% in mg/ml

| **Extracts Leaves** | **$IC_{50}$%** |
|---|---|
| Acetonic | 0.52 ± 0.029 |
| Methanolic | 0.583 ± 0.33 |
| Aqueous | 0.73 ± 0.007 |
| Ascorbic Acid (Control) | 0.12 |

process. Many higher plants contain useful and effective antioxidants Middleton [50].

The results of figure 3 showed that FRAP values were higher in hydroacetone extract which displayed the highest antioxidant capacity, compared to that of the aqueous and hydroalcoholic extract, reflected by the optical densities obtained at different concentrations (maximum OD = 1.73 at the concentration of 5 mg/ml), even closer than that of reference antioxidant ascorbic acid (OD = 1.88) for the same concentration 5 mg/ml.

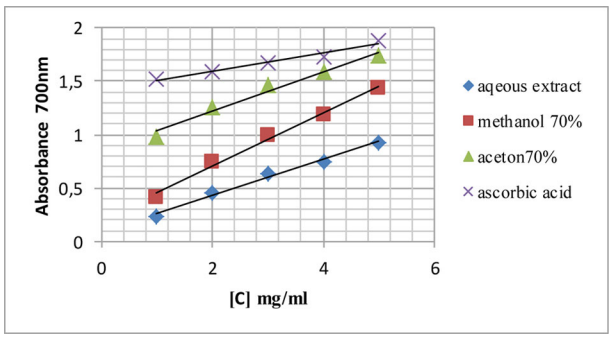


**Fig. 3** Reducing power of *Moringa oleifera* extracts and ascorbic acid

In general, for the three extracts of the studied plant, the iron reduction profile increased with the increasing of the used concentrations. The iron reduction power of the different studied extracts at the concentration of 5 mg/ml can be classified as follows:

Ac. ascorbic acid > Hydroacetone extract > Hydroalcohol extract > Aqueous extract

The grapevine leaves extracts were found to have strong antioxidants results [51]. Those extracts exhibited the presence of condensed tannins, which are strong antioxidants.

Tanaka [52] have observed a direct correlation between the antioxidant activity and the reducing power of certain plant extracts. This reducing power might be due to the hydrogen donating ability, and is generally associated with the presence of reductones [49].

The reducing power of *Moringa oleifera* species was probably due to the presence of hydroxyl groups in the phenolic compounds that can serve as electron donors. Therefore, the antioxidants are considered as reducing and inactivating oxidants [53].

## Conclusion

This study of the antioxidant activity of *Moringa oleifera* species extracts by iron reduction and DPPH free radical scavenging methods had shown that both acetonic and hydroalcolic extracts had an important antioxidant activity. These extracts could therefore be an alternative to certain synthetic additives. However, this antioxidant activity stays much lower than that of ascorbic acid. It was therefore very likely that they contain compounds which, once purified, may exhibit activity comparable to that of ascorbic acid. These results recommend that this plant can serve as a good source of natural antioxidants and could potentially be considered as a functional food or functional food ingredient. Further researches are needed to identify, isolate and purify these constituents.

**Conflicts of interests:** the authors have no conflicts of interests to declare.

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