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A COMPARATIVE STUDY OF THE PHENOLIC AND FLAVONOIDS CONTENTS, AND ANTIOXIDANT ACTIVITY OF *ZIZIPHUS MAURITIANA*'S LEAVES, RIPE AND UNRIPE FRUIT EXTRACTS FROM UAE

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Abstract. This study evaluates the antioxidant properties of leaves, unripe and ripe fruits of Ziziphus mauritiana from the UAE. Phenolic compounds show a strong correlation with antioxidant activity, with the leaves exhibiting the highest efficiency. The results suggest that Ziziphus mauritiana leaves are a promising source of natural antioxidants with potential therapeutic applications.

Keywords: *Ziziphus mauritiana*, jujube, antioxidants, Al Sider, DPPH, UAE.

1. Introduction

Free radicals and other reactive oxygen species (ROS) considerably contribute to the oxidative damage of the human body such as cardiovascular diseases or cancer[1, 2], and could cause immune system depletion[2–4].

Antioxidants can be natural or synthetic. The ability of natural antioxidants produced by the human body is not enough to resist the damage caused by ROS, so supplemented antioxidants are used to enhance the body's capacity to neutralize or decrease these damages. In general, antioxidants work by donating an electron to a free radical to neutralize it[5–7] or binding to metals[8]. It has been reported that synthetic antioxidants might induce negative health effects[1] and, therefore, several studies have been conducted on natural antioxidants to replace the synthetic ones[8, 9] to control the formation of free radicals[10]. Some enzymes such as catalase and vitamin C[11] are known to be natural antioxidants. Other natural antioxidants are found in different spices and herbs or extracted from some plants[12].

Many plants used in traditional medicine are receiving increased interest from researchers because of their nutritional and medical value. These plants are being investigated as significant sources of natural antioxidants and as considerable sources of compounds that have medicinal applications such as atherosclerosis, Alzheimer's, cerebral cardiovascular events, diabetes, hypertension, and cancer[13–16].

In recent years, novel chemopreventive agents have been extracted from fruits and vegetables. Some plants are used as medications that showed promising growth inhibitory effects in preclinical studies using skin and ovarian cancer models[17–19]. *Ziziphus mauritiana,* commonly referred to as jujube, is well known in Arab countries as Al Sider or Nabag. It belongs to the Rhamnaceae family and is commonly found in tropical and subtropical regions[20, 21]. The unripe fruit has a green color and, then when it ripens it turns brown passing through the yellow color. In general, the ripe fruits have a sweet-sour taste, and fruit sizes diameter ranges from 1 to 3 cm. *Ziziphus mauritiana* fruits are common in the diet of several Arab countries, particularly in the Gulf region. Their widespread cultivation in Gulf countries is attributed to their adaptability, ease of cultivation, and ability to thrive in arid conditions. These fruits are highly valued for their early maturation, resilience to harsh environments including drought and salinity stress, minimal input requirements, and significant nutritional and economic benefits[22, 23]. In the United Arab Emirates (UAE), *Z. mauritiana* is an alien species commonly grown for its edible fruits and medical importance. It is often found in gardens, along streets, and in public parks. Its cultivation by the government along main roads and in parks, as well as by individuals on private farms and around homes, highlights its popularity and economic importance in the region.

Different parts of *Ziziphus mauritiana* (Al Sider) have been traditionally used for the treatment of different diseases such as asthma, allergies, depression, ulcers, and inflammations[24]. Reported studies have shown that the

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extracts of *Ziziphus mauritiana* parts (leaves, fruits, seeds, roots, bark, and bulbs) exhibit antioxidant activity and possess cytotoxicity against different cancer cell lines[25–28]. Al Sider trees, their leaves, in particular, are of religious and cultural value according to Arabic traditions and conventions. For the sake of completion, the ripe fruits of Al sider have been commonly used in the Arab regions as a source of sweet fruits while the unripe ones are not. This study aimed at determining the total polyphenol and flavonoid content of ethanolic extract of *Ziziphus mauritiana* leaves, unripe and ripe fruits collected from Fujairah, UAE and comparison of the antioxidant activity of these samples.

2. Experimental

2.1. Chemicals and Materials

All solvents were of analytical grade and were used without further purification. Ascorbic acid, ferric chloride, aluminum chloride, potassium acetate, quercetin, DPPH reagent, Folin-Ciocalteau reagent, gallic acid, sodium carbonate, methanol, and ethanol were obtained from Sigma Chemical Co. (Sigma Aldrich, USA). Millipore deionized water was used throughout. Agilent Cary 60 UV-Vis Spectrophotometer was used in all spectrophotometric measurements. The extracts were dried using a Telstar Cryodos freeze-dryer machine.

2.2. Plant Sample and Extract Preparation

Ziziphus mauritiana leaves, ripe and unripe fruits were collected from a private farm in Fujairah Emirate, United Arab Emirates (UAE). The collected samples were washed with water and then left to dry on filter paper in a well-ventilated place at room temperature. The dried samples were ground using an electric blender. Ten grams of leaves, ripe and unripe powder were separately extracted with 150 mL solution of 70 % ethanol and 30 % water and macerated for 48 hours at room temperature. The extraction was repeated three times, followed by filtration using Whatman filter paper (grade 1). The obtained solvents were combined. Several solvents were used to extract the bioactive materials from *Ziziphus mauritiana* including methanol[29], methanol (80 %)[30], ethanol[31, 32], hexane[31, 32], ethyl acetate[31, 32], and water[31]. In this study, ethanol (70 %) was chosen due to its notable efficacy as shown in our reported studies[14, 15]. Another study found that ethanol extract had the highest total phenolics and the highest antioxidant capacity in all the tested assays except for the superoxide scavenging assay[32]. The solvents were selectively removed under reduced pressure and dried using a TELSTAR CRYODOS freeze dryer machine. Stock solutions of 30 mg/mL of all extracts were prepared separately in a solution of 50 % ethanol and 50 % water. The obtained extracts and solutions were thereafter stored at 4.0 ^{o}C.

2.3. Total Polyphenol Content (TPC)

The total polyphenol content (TPC) of *Ziziphus mauritiana* extracts was measured using the Folin-Ciocalteu assay[33, 34]. Each extract was diluted to a 10 % solution from stock solutions (30 mg/mL) using 50 % ethanol. Subsequently, a mixture of 100 µL of this solution, 200 µL of the Folin-Ciocalteu, and 2 mL of de-ionized water was prepared in a brown glass vial with a screw cap and then incubated for 3 min at room temperature. A 1 mL of 20 % (w/w) aqueous solution of sodium carbonate was added, then the solution was incubated for 1 hour at room temperature. The absorbance of these solutions was measured at 765 nm using Agilent Cary 60 UV-Vis Spectrophotometer. A control sample was prepared using the same procedure, with the extract replaced by 100 µL of 50 % ethanol. Experiments were conducted in triplicates.

The same procedure was repeated with a series of gallic acid concentrations ranging from 0.5 to 26 µg/mL to construct the gallic acid calibration curve. Experiments were conducted in triplicate. The total phenolic content was calculated using the equation obtained from the gallic acid calibration curve and expressed as mg gallic acid equivalents (GAE) per g dry weight of plant material.

2.4. Determination of Total Flavonoids

The total flavonoid content of *Ziziphus mauritiana* extracts was measured using the aluminum chloride colorimetric method as described previously[35]. The methanolic solutions (600 µg/mL) of the extracts were prepared separately. A mixture of each methanolic extract (500 µL), aluminum chloride (0.1 mL, 10 % (w/v)), potassium acetate (0.1 mL, 1 M), methanol (1.5 mL), and distilled water (2.8 mL) was prepared and thoroughly mixed, then it was incubated for 30 min at room temperature. Subsequently, the absorbance of the solutions was measured spectrophotometrically at 415 nm using an Agilent Cary 60 UV-Vis Spectrophotometer. All experiments were conducted in triplicate.

The last procedure was repeated using various concentrations of quercetin (5–25 µg/mL) instead of the extract samples in order to construct its calibration curve. The total flavonoid content was determined from the resulting calibration curve and represented as mg of quercetin equivalents (QE)/g of the dry extract. Experiments were conducted in triplicate.

2.5. Free-Radical Scavenging Activity

Free radical scavenging activity of *Ziziphus mauritiana* leaves, unripe and ripe extracts was determined using 2,2-diphenyl-1-picrylhydrazil (DPPH) assay as previously described[36]. A methanolic solution of DPPH (60 µg/mL) was prepared in non-transparent vials and directly covered with aluminum foil to protect it from the light. *Ziziphus mauritiana* extracts with different concentrations (0.15, 0.30, 0.60, and 1.5 mg/mL) were prepared using methanol. Subsequently, 200 µL of each extract was mixed with 3.8 mL of the prepared methanolic solution of DPPH, vigorously shaken, and then incubated in the dark for 30 min at room temperature. The absorbance of the mixture was measured at 517 nm. A control sample was prepared using the same procedure, with the extract replaced by an equivalent volume of methanol. Experiments were conducted in triplicate. The free radical scavenging activity was expressed as % Inhibition, which was calculated using the following equation[37]:

$$\%Inhibition\ of\ DPPH\ radicals = \frac{A_{control} - A_{sample}}{A_{control}} \times 100.$$

where $A_{control}$ and A_{sample} are the absorbance values for the control and extract sample, respectively.

The antioxidant activity is also expressed as EC_{50} value (half maximal effective concentration), representing the antioxidant concentration needed to achieve a 50 % inhibition of radicals. EC_{50} value (µg/mL) was determined using GraphPad Prism 5.01 software from the calibration curve of ascorbic acid and % Inhibition values[37].

A calibration curve of ascorbic acid was prepared using the same procedure mentioned above, with a series of ascorbic acid concentrations ranging from 5 to 25 µg/mL. The total antioxidant activity was determined from ascorbic acid calibration. Experiments were conducted in triplicate.

2.6. Statistical Analysis

The data of all experiments were presented as means ± standard deviations and were conducted using SPSS IBM Statistics software version 25. The results were considered significant at $P < 0.05$.

3. Results and Discussion

3.1. Plant Extraction

The extracts of *Ziziphus mauritiana* leaves, unripe and ripe fruits were prepared by the complete evaporation of ethanol/water producing an amorphous solid of leaves and a sticky solid in the case of unripe and ripe fruits. The w/w percent yield was calculated to be 15.72 %, 17.20 %, and 28.92 % for leaves, unripe and ripe fruits, respectively, based on the used solvent[38].

3.2. Total Polyphenol Content (TPC)

Polyphenols are defined as aromatic secondary plant metabolites and considered the main bioactive phytochemicals that have been shown efficacy in the prevention of certain chronic diseases such as cancers, because of their free radical-scavenging activities[36, 39, 40]. Total polyphenol content is commonly estimated using the Folin-Ciocalteu method. A blue-colored complex is formed when the Folin-Ciocalteu reagent reacts with phenolic compounds. The quantity of this complex is measured using a UV-spectrophotometer at 765 nm[41].

The obtained calibration curve of gallic acid showed linearity in the range of 0.5–26 µg/mL, with a correlation coefficient (R^2) of 0.974 (Fig. 1). *Ziziphus mauritiana* leaves showed (Fig. 2) the highest polyphenol content (60.8 ± 1.55 mg GAE/g), whereas unripe fruit extract exhibited total phenolic content (51.0 ± 0.33 mg GAE/g, Table 1) slightly more than ripe ones (49.0 ± 0.73 mg GAE/g, Table 1). In addition, the unripe fruit extract showed TPC lower than the leaves by 16.1 % and ripe fruits – lower than the leaves by 19.4 %. In general, the phenolic content decreases during the ripening stages, and the highest concentration was found within the greenest stage[26, 42]. These findings are almost similar to the results obtained by Al-Saeedi *et al*. for the Omani *Ziziphus jujuba L* leaves (68.10 ± 0.09 mg GAE/g) and fruits (64.89 ± 0.44 mg GAE/g)[34]. However, our results are lower than those reported by Gao *et al*. for Chinese jujube fruits[43] and by Wojdyło *et al.* for Spanish jujube fruits[44]. The differences may be attributed to the used extraction solvents, extraction methods, cultivars, harvesting time, and climate conditions[45]. *Ziziphus mauritiana* fruits contain different types of phenolic compounds such as proanthocyanidins, catechin, epicatechin and rutin hydroxycinnamate, flavonols, flavan-3-ols, and especially procyanidins[30, 46]. The most common phenolic acids are caffeic, ferulic, and *p*-coumaric acids, however, these compounds are almost found in negligible quantities[47]. In the early stages of fruit development, the existence of phenolic substances like catechins is directly linked to undesirable tastes such as bitterness and astringency. Xue *et al.*[48] found that the total content of phenolic acids and flavonoids was higher in the leaves of Chinese Ziziphus jujuba than in the fruits. They found that there was a difference in the composition of the leaves and the fruits, quercetin and rutin present in the leaves, and (+)-catechin, epicatechin, and rutin in the fruits. In addition, the quantities of (+)-catechin, rutin, quercetin, spinosin, and luteolin decreased with fruit development stages while the quantities of epicatechin, kaempferol, gallic acid, and chlorogenic acid initially increased and then decreased[48]. However, as the phenolic content decreases during the ripening process, these harsh tastes fade away, leading to a delightful taste of the fruit[42].

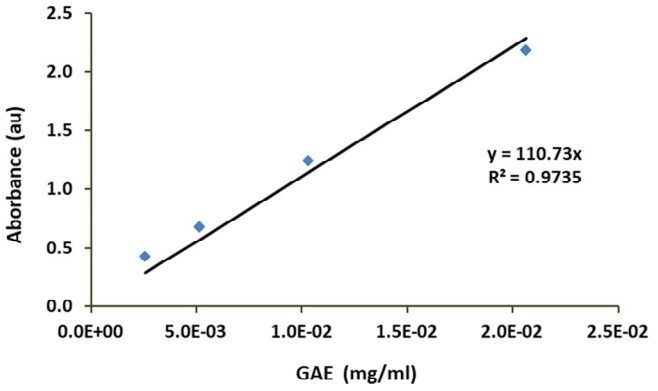

Fig. 1. Calibration curve of gallic acid in the range of 0.5–26 µg/mL

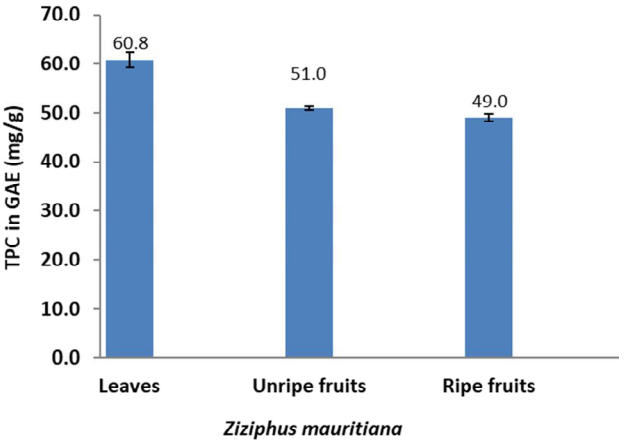

Fig. 2. Total phenolic content of *Ziziphus mauritiana* leaves, unripe and ripe fruits extracts determined by the Folin – Ciocalteu assay and calculated as mg GAE/g extract based on dry weight. Results are the average of triplicates ± SD

Table 1. Total phenolic content (TPC) of *Ziziphus mauritiana* leaves, unripe and ripe fruits extracts

Z. M. Extract	The used solvent	Assay	R^2 Calibration curve of gallic acid	TPC, mg GAE/g
Leaves	Ethanol (70 %)	Folin-Ciocalteu	0.974	60.8 ± 1.55
Unripe fruits	Ethanol (70 %)	Folin-Ciocalteu	0.974	51.0 ± 0.33
Ripe fruits	Ethanol (70 %)	Folin-Ciocalteu	0.974	49.0 ± 0.73

Total phenolic content of *Ziziphus mauritiana* extracts was determined by the Folin-Ciocalteu assay and calculated as mg GAE/g extract based on dry weight. Results are the average of triplicates ± SD.

3.3. Total Flavonoid Contents

Flavonoids are polyphenolic molecules that are widely found in plants and contribute to their antioxidant properties[49, 50]. Flavonoids content was investigated in the extracts using the aluminum chloride assay[51] and calculated from the regression equation of quercetin calibration curve as quercetin equivalents. The obtained calibration curve of quercetin showed linearity in the range of 1–25 µg/mL, with a correlation coefficient (R^2) of 0.98 (Fig. 3). The total flavonoids content of *Ziziphus mauritiana* leaves (Fig. 4, Table 2) was (3.50 ± 0.18) mg QE/g dry extract. Unripe and ripe fruit extracts showed very low flavonoid content (0.24 ± 0.10) and (0.10 ± 0.04) mg QE/g dry extract, respectively, compared to the leaves. In the present study, the contribution of flavonoids to the total polyphenols value of the *Ziziphus mauritiana* extracts varied from 5.76 % in leaves, 0.39 % in unripe fruits, and up to 0.16 % in ripe fruits. These findings are close to the results obtained by Al-Saeedi *et al*. for the Omani *Ziziphus jujuba L* leaves (90.28 ± 0.09 µg GAE/g) and fruits (0.29 ± 0.44 µg GAE/g)[34]. The differences in phenolic and flavonoid contents among various parts of *Ziziphus jujuba L* may be attributed to the chemical characteristics, solubility, and presence of the extracted compounds[52]. A reported study showed that the antioxidant capacity of *Ziziphus mauritiana* is attributed to the presence of total phenolic compounds including flavonoids (in particular, polymeric proanthocyanidins and quercetin derivatives) and ascorbic acid[44].

3.4. DPPH Radical Scavenging Activity

In general, the ability of the plant extract to reduce DPPH radical is used to measure its antioxidant activity[36]. Reduction of the radical can occur by accepting an electron or hydrogen radical from the extract components forming a stable diamagnetic molecule that causes a color change from blue to yellow[53]. Such color change is used to measure the radical scavenging activity which is represented as ascorbic acid equivalent/g dry extract[54].

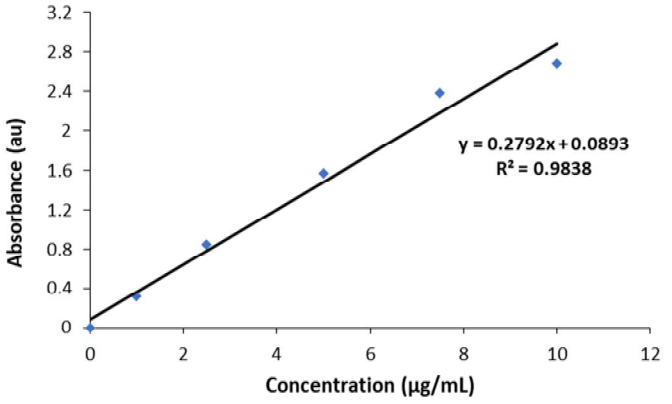

Fig. 3. Calibration curve of quercetin in the range of 0.5–26 µg/mL

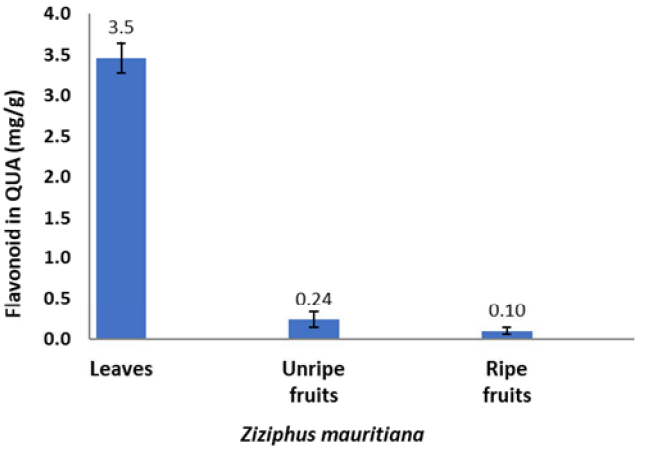

Fig. 4. Flavonoids content of *Ziziphus mauritiana* leaves, unripe and ripe fruits extracts using aluminum chloride colorimetric method and expressed as mg of quercetin equivalents (mg QE)/ g of the dry extract. Results are the average of triplicates ± SD

Table 2. Total flavonoid contents (TFC) of *Ziziphus mauritiana* leaves, unripe and ripe fruit extracts

Z. M. Extract	The used solvent	Assay	R^2 Calibration curve of quercetin (QE)	TFC, mg QE/g
Leaves	Ethanol (70 %)	Aluminum chloride	0.98	3.50 ± 0.18
Unripe fruits	Ethanol (70 %)	Aluminum chloride	0.98	0.24 ± 0.10
Ripe fruits	Ethanol (70 %)	Aluminum chloride	0.98	0.10 ± 0.04

Flavonoids content of *Ziziphus mauritiana* extracts was determined using the aluminum chloride colorimetric method and expressed as mg of quercetin equivalents (QE)/ g of the dry extract. Results are the average of triplicates ± SD.

The obtained calibration curve of ascorbic acid showed linearity in the range of 5–20 µg/mL, with a correlation coefficient (R^2) of 0.994 (Fig. 5). *Ziziphus mauritiana* leaves (Fig. 6) showed the highest DPPH scavenging activity which ranged between (93.5 % ± 0.05) at 1.50 mg/mL and (55.7 % ± 3.68) at 0.15 mg/mL followed by unripe fruits which ranged between (79.0 % ± 2.77) at 1.50 mg/mL and (12.7 % ± 0.85) at 0.15 mg/mL. Ripe fruits showed the lowest DPPH scavenging activity which ranged between (56.0 % ± 4.07) at 1.50 mg/mL and (7.7 % ± 0.56) at 0.15 mg/mL. The decrease in antioxidant activity of *Ziziphus mauritiana* fruits during ripening stages can be attributed to the decline in phenol and flavonoid concentrations indicating that there is a positive correction between antioxidant activity and total polyphenol content.

Furthermore, DPPH radical scavenging ability of Ziziphus mauritiana extracts was assessed as half maximal effective concentration (EC_{50}, µg/mL) value since low EC_{50} values indicate high free radical scavenging activity (high potency of the plant). Ziziphus mauritiana leaves showed the highest free radical scavenging activity with EC_{50} 83.43 µg/mL while ripe fruits showed the lowest with EC_{50} (1310 µg/mL). The EC_{50} value was 733.8 µg/mL for the unripe fruits as shown in Table 3.

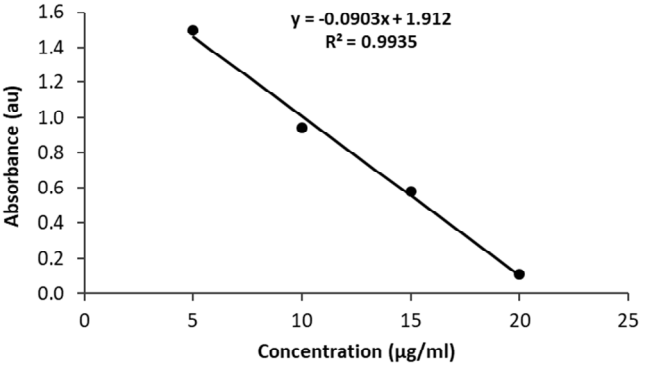

Fig. 5. Calibration curve of ascorbic acid in the range of 5–20 µg/mL

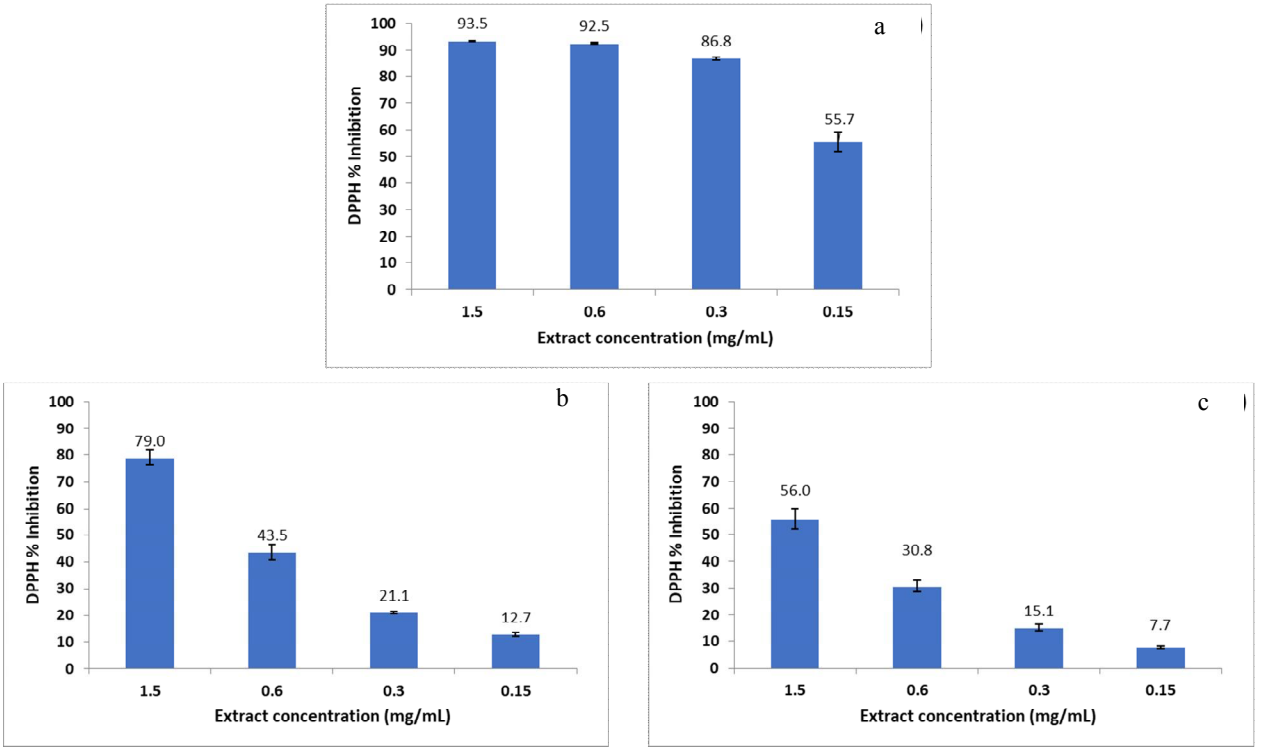

Fig. 6. DPPH % inhibition of *Ziziphus mauritiana* leaves (a), unripe (b), and ripe (c) fruit extracts (1.5, 0.60, 0.30, and 0.15 mg/mL) determined by the DPPH assay and calculated as ascorbic acid equivalent/g dry extract. The results are the mean of triplicates ± SD

Table 3. Antioxidant activity of *Ziziphus mauritiana* leaves, unripe and ripe fruits extracts using DPPH assay.

Z. M. Extract	Concentration, mg/mL	λ_{max}, nm	Inhibition, %	EC_{50}, µg/mL
Leaves	1.50	517	93.5 ± 0.05	83.43
	0.60	517	92.5 ± 0.34	
	0.30	517	86.8 ± 0.50	
	0.15	517	55.7 ± 3.68	
Unripe fruits	1.50	517	79.0 ± 2.77	733.8
	0.60	517	43.5 ± 2.83	
	0.30	517	21.1 ± 0.43	
	0.15	517	12.7 ± 0.85	
Ripe fruits	1.50	517	56.0 ± 4.07	1310
	0.60	517	30.9 ± 2.24	
	0.30	517	15.1 ± 1.32	
	0.15	517	7.7 ± 0.56	

DPPH % inhibition of *Ziziphus mauritiana* extracts was determined by the DPPH assay and calculated as ascorbic acid equivalent/g dry extract. The results are the mean of triplicates ± SD.

3.5. Total phenolic and total flavonoid content correlations with antioxidant activity

In this work, linear regression analysis was used to study the relationship between total phenolic content, total flavonoid content separately, and antioxidant activity based on EC_{50} values. The obtained results indicated a significant positive correlation (R^2 = 0.88, *P*-value <0.05) between antioxidant activity and total phenolics along with a significant negative correlation (R^2 = 0.82, *P*-value <0.05) between EC_{50} (DPPH· scavenging) and total phenolic content. Similarly, there was a positive correlation (R^2 = 0.66, *P*-value <0.05) between antioxidant activity and flavonoid contents while a significant

negative correlation (R^2 = 0.81, *P*-value <0.05) was observed between EC_{50} (DPPH scavenging) and total flavonoid contents. This suggests that the presence of total phenolics and flavonoids contributed significantly to the antioxidant activity. In agreement with our results, a positive correlation between antioxidant activity and total phenolics has been reported using different plants[14, 49, 50, 56].

4. Conclusions

The present study investigated the antioxidant activity, polyphenolic and flavonoid contents of *Ziziphus mauritiana* leaves, unripe and ripe fruits from the UAE. *Ziziphus mauritiana* showed considerable amounts of total phenolic content. Leaves showed higher antioxidant activity than the fruits. A significant correlation was found between total phenolic content and antioxidant activity. The antioxidant activity of leaves, unripe and ripe fruits depends on the total amount of the present phenols and flavonoids. These results highlight the potential of *Ziziphus mauritiana*, particularly its leaves, as a valuable natural source of antioxidants and potential therapeutic applications for the treatment of various diseases. The high phenolic contents obtained in this study highlight the promising role of *Ziziphus mauritiana* as a functional food with health-promoting properties. Exploration of its bioactive components and their mechanisms of action could offer valuable insights into its therapeutic potential and broaden its applications in the healthcare and functional food industries.

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ПОРІВНЯЛЬНЕ ДОСЛІДЖЕННЯ ВМІСТУ ФЕНОЛЬНИХ І ФЛАВОНОЇДНИХ СПОЛУК ТА АНТИОКСИДАНТНОЇ АКТИВНОСТІ ЕКСТРАКТІВ ЛИСТЯ, СТИГЛИХ І НЕЗРІЛИХ ПЛОДІВ *ZIZIPHUS MAURITIANA* З ОАЕ

Анотація. *У цьому дослідженні оцінено антиоксидантні властивості листя, незрілих і стиглих плодів Ziziphus mauritiana з ОАЕ. Для фенольних сполук встановлено сильну кореляцію з антиоксидантною активністю, причому ефективність листя найвища. Отримані результати свідчать про те, що листя Ziziphus mauritiana є перспективним джерелом природних антиоксидантів з потенційним терапевтичним застосуванням.*

Ключові слова: *Ziziphus mauritiana, зизифус мавританський, антиоксиданти, аль-сидер, DPPH, ОАЕ.*