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Detection of the antioxidant activity of natural flavonoid extracts from *Ziziphus mauritiana* Apple buckthorn (Rhamnaceae) fruits In vitro

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Abstract:

This in vitro study investigated the antioxidant activity of flavonoids extracted from the fruit of the (*Ziziphus mauritiana L.*), a traditional medicinal plant. The in vitro antioxidant activity of flavonoids extracted from the fruit of the apple buckthorn (*Z. mauritiana* L.), a tree used in traditional medicine, was also evaluated. The study focused on isolating flavonoids from the fruit, determining their antioxidant activity, and quantifying them using colorimetric and DPPH reducing capacity tests, with vitamin C used as a control. The data indicate that the Mauritanian hawthorn extract has a significant antioxidant effect, which is correlated with its flavonoid concentrations. The study demonstrates that apple buckthorn is an excellent source of antioxidants, due to its high flavonoid content. These compounds effectively scavenge free radicals and combat oxidative stress. The results suggest that apple buckthorn can be considered a promising source of natural antioxidants that can be used to treat chronic diseases.

Keywords: *Z. mauritiana*; Antioxidant activity; flavonoids, Reducing power

الكشف عن النشاط المضاد للأكسدة لمستخلصات الفلافونويد الطبيعية من ثمار *Ziziphus mauritiana* النبق التفاحي (*Rhamnaceae*) في المختبر

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مستخلص:

بحثت هذه الدراسة المختبرية النشاط المضاد للأكسدة للفلافونويدات المستخرجة من ثمرة (*Ziziphus mauritiana L.*)، وهو نبات طبي تقليدي. كما تم تقييم النشاط المضاد للأكسدة في المختبر للفلافونويدات المستخرجة من ثمرة شجرة نبق التفاح (*Z. mauritiana L.*) المستخدمة في الطب التقليدي. ركزت الدراسة على عزل الفلافونويدات من الفاكهة، وتحديد نشاطها المضاد للأكسدة، وتحديد كميتها باستخدام اختبارات قياس اللون واختبارات القدرة الاختزالية لـ *DPPH*، مع استخدام فيتامين C كعنصر تحكم. تشير البيانات إلى أن مستخلص الزعرور الموريتاني له تأثير مضاد للأكسدة ملحوظ، ويرتبط بتركيزات الفلافونويد فيه. توضح الدراسة أن نبق التفاح مصدر ممتاز لمضادات الأكسدة، نظرًا لمحتواه العالي من مركبات الفلافونويد. تعمل هذه المركبات بفعالية على إزالة الجذور الحرة ومكافحة الإجهاد التأكسدي. تشير النتائج إلى أن الكفر النبق التفاحي يمكن اعتباره مصدرًا واعدًا لمضادات الأكسدة الطبيعية التي يمكن استخدامها لعلاج الأمراض المزمنة.

الكلمات المفتاحية: *Ziziphus mauritiana* ؛ النشاط المضاد للأكسدة؛ الفلافونويدات، القدرة المختزلة.

1. Introduction

Ziziphus mauritiana (Apple buckthorn) is a member of the Rhamnaceae family (1). This species is well known in traditional medicine because of its therapeutic activities like the treatment of asthma, anxiety, depression, fever, inflammation, and ulcers. *Z. mauritiana* together with its leaves and fruits has been traditionally used in medicine for the treatment of diarrhea, wounds, abscesses, edema, gonorrhea, liver disease, and even asthma. The nutritional and pharmacological properties of *Z. mauritiana* fruit are well known and appreciated. This plant can be consumed as dried fruits, candied, pickled, and even in juice form (2). *Z. mauritiana* is well known for its antioxidant properties since the extracts of its fruits, leaves, and seeds have shown remarkable antioxidant and anticancer activities. Phytochemical studies on *Z. mauritiana* have identified the presence of cyclopeptide alkaloids, lupanes, and cyanothane terpenes among other compounds (3,4).

This plant is a real treasure trove of helpful compounds, with its fruits, leaves, bark, and even seeds all playing a part. These natural components give it the ability to act as an antioxidant, fight off microbes, reduce swelling, and offer protection to the liver (5). It's loaded with phenolic acids – think of caffeic, ferulic, p-hydroxybenzoic, and p-coumaric acids – which are well-known for their strong antioxidant abilities. A key flavonoid in *Xantha mauritiana*, called naringin, is a major contributor to its antioxidant strength (6, 7). Also present are triterpenoids and saponins, which are interesting because they can perform a range of helpful tasks, including working as antioxidants and antimicrobials, easing inflammation, and even having antidiabetic effects. Studies that looked at the amounts of these compounds showed that the pulp of the *Xantha mauritiana* fruit contained more total phenolic compounds (29.8 mg gallic acid equivalent per gram) than the leaves (25.8 mg gallic acid equivalent per gram). This hints that the fruit's flesh might be a more potent antioxidant (8, 9, 10). The pulp extract also exhibits antimicrobial activity, albeit to a lesser extent. Studies have indicated that *Xantha mauritiana*

leaf extracts can mitigate oxidative liver damage ((11,12,13), likely through the scavenging of free radicals by antioxidant enzymes. This suggests potential benefits in the management of liver-related diseases and inflammatory conditions (14). The study aimed to identify flavonoids as one of the most important active ingredients and to test their antioxidant activity.

2. Materials and Methods

2.1. Plant material and extraction method

10 g of dried fruits were ground and soaked in 75% methanol for 72 h under laboratory conditions (1:10 w/v), then defatted three times using petroleum ether. The extract was collected and concentrated in vacuum (10 ml), extracted with chloroform, acidified with 20% H2SO4 (pH = 5) and extracted with ethyl acetate three times. An interstitial precipitate was observed upon extraction with ethyl acetate. The ethyl acetate fractions were taken as flavonoids for our study (15). Random sampling was used during harvesting. Area sections of *Z. mauritius* were taken from a public garden in Baghdad. They were classified in the Natural History Center/Botany Department.

2.2. Determination of Flavonoids content Briefly, the flavonoid content of the fruits was estimated according to the aluminum chloride solution method [17]. To quantify the flavonoids in our extract, we performed a colorimetric assay. This involved combining one milliliter of our methanol-based extract with one milliliter of a 2% aluminum chloride solution in methanol. The reaction was allowed to proceed for ten minutes, after which the absorbance was measured at 430 nm. By comparing these measurements to a standard curve generated using quercetin, we could express the total flavonoid content of our extract as milligrams of quercetin equivalents per gram of extract (mg/g). This tells us the relative amount of flavonoid-like compounds present**.**

2.3. DPPH assay

We evaluated the extract's ability to neutralize free radicals using the DPPH assay. We took one milliliter of the fruit extract at various concentrations and mixed each with 0.5 ml of a DPPH solution in methanol. These mixtures

were then shaken well and left to react in the dark at room temperature for 30 minutes. We then measured the absorbance of each resulting solution at 517 nm. From this data, we determined the IC50 value, which represents the concentration of the extract needed to inhibit 50% of the DPPH radicals (expressed in micrograms per ml). To get a clear picture of its radical scavenging power, we used a specific calculation:

"DPPH scavenging effect (%)=[(A_0-A_1)/A_0]" Where:

A_0: The absorbance of the control at 30 minutes.

A_1: Is the absorbance of the sample at 30 minutes. BHT was used as standard [18].

2.4. Reducing power

The reducing ability of the fruit extract was evaluated based on a known method [18]. In this assay, 2.5 ml of the extract reacted with 2.5 ml of a sodium phosphate buffer (pH 6.6; 200 mmol/L) and 2.5 ml of potassium ferricyanide (10 mg/ml). This reaction proceeded at 50 °C for 20 minutes. To stop the reaction and prepare for colorimetric analysis, 2.5 ml of trichloroacetic acid (100 mg/ml) was added, and the mixtures were centrifuged at 200 g for 10 minutes to separate components. A 5 ml aliquot of the supernatant was then mixed with 5 ml of deionized water and 1 ml of ferric chloride (1 mg/ml), leading to the formation of a colored complex whose absorbance was read at 700 nm against a blank. The effective concentration (EC50), defined as the concentration of the extract needed to achieve 50% of the maximum reducing effect (expressed as mg extract/ml), was calculated by interpolating from a linear regression curve. Ascorbic acid was used as a positive control to validate the assay [19].

2.4. Statistical analysis

Data are presented as mean ± standard deviation and were statistically analyzed using Student's t-test, followed by Fisher's test ($P < 0.05$ for significance) to compare between the plant extract with standards, using GraphPad Prism 5 Demo.

3. Results & Discussion

3.1. Antioxidant assays

The flavonoid's estimated quantities were, 21 ± 0,52 mg 1,84 mg EQ/GE. The antioxidant power of *Z. mauritiana* flavonoid extract was measured using the DPPH assay, a standard way to compare plant extract activity (Figure 1). DPPH is a stable, methanol-soluble radical with a characteristic 517 nm absorbance. Antioxidants make the DPPH solution lighter as they neutralize the radicals by donating hydrogen [20].

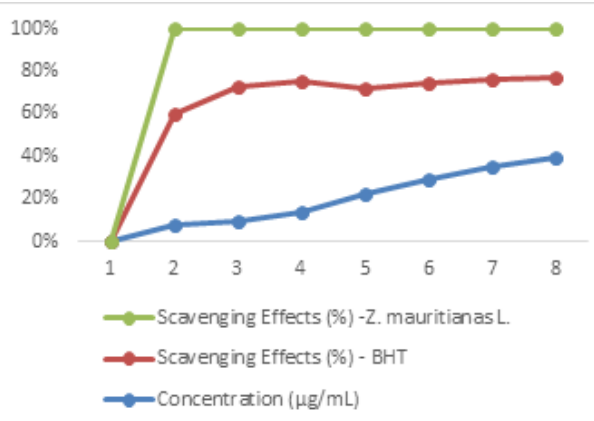

Figure 1: Antioxidant activity of *Z. mauritiana* flavonoids and BHT. (LSD 5%= 2.234)

"Figure 1" compares the in vitro scavenging activity of Apple buckthorn (*Ziziphus mauritiana* L.) extract with that of butylhydroxytoluene (BHT), a synthetic antioxidant standard, across a range of concentrations. The figure shows the percentage scavenging effects of both compounds, providing insights into their relative antioxidant potential. The results reveal that while Mauritanian lime extract exhibits significant scavenging activity, particularly at lower concentrations, BHT exhibits a more sustained and ultimately higher scavenging capacity within the tested range. This comparative analysis confirms the potential of Apple buckthorn as a natural antioxidant source, while also highlighting the efficacy of the synthetic standard.

At the lowest concentration of *Z. mauritiana* fruit extract was found to have a significantly higher scavenging effect (100%) compared to BHT (60%). This indicates that *Z. mauritiana* extract, even at low concentrations, may have greater free radical neutralization capabilities. This may be due to the presence of highly reactive antioxidant compounds in the compound mixture of the plant extracts. With increasing concentration (21), BHT's free radical

scavenging effect shows a gradual and steady increase, reaching nearly 77% at the highest concentration tested. In contrast, *Z. mauritiana* extract appears to immediately (around Concentration 2) reach full scavenging activity at 100% and then plateau, showing no further improvements with increasing concentration in the tested range. This plateau could suggest that the active antioxidant constituents of the extract become saturated in their scavenging capacity at these concentrations or that the assay hits its maximum detection limit (22).

The antioxidant activity noted from *Z. mauritiana* extract is most likely due to the diverse array of its phytochemicals such as phenolic acids, flavonoids (quercetin derivatives, rutin, and kaempferol), tannins, and ascorbic acid (23). With the presence of hydroxyl groups, these compounds are capable of donating hydrogen atoms to free radicals. These compounds possess hydroxyl groups that enable them to donate hydrogen atoms to free radicals, thereby terminating the chain reaction of lipid peroxidation and stabilizing free radical species. Complex interactions, both synergistic and antagonistic, between these various compounds within the extract may contribute to the observed free radical scavenging pattern (24).

Z. mauritiana extract exhibits strong detoxification activity, especially at low concentrations, indicating its potential as a valuable source of natural antioxidants. However, BHT exhibits a more sustained increase in detoxification activity across the tested concentration range.

3.2. Reducing power

“Figure 2” shows the reducing capacity of *Z. mauritiana* extract compared to vitamin C. The extract of fruit C have a high and superior ability to reduce, and a noticeable increase with increasing concentration that may exceed the ability of ascorbic acid at the same concentration. This indicates that there is a direct relationship between the dose and the response to the extract within concentrations from 15mg\ml to 20mg\ml. The extract also shows a decrease in its ability to reduce at a concentration of 15mg\ml.

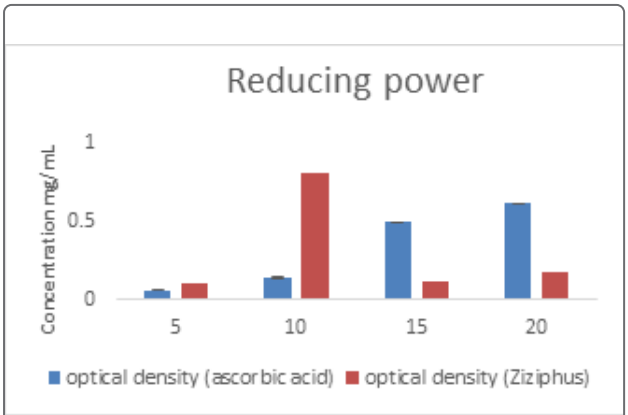

Figure2: Reducing capacity of *Z. mauritiana* flavonoids and vitamin C, LSD 5%= 0.0035)

The high value reducing capacity of ascorbic acid at most tested concentrations is consistent with its proven role as an effective, water-soluble antioxidant. Its molecular structure allows it to be readily electron donor, neutralizing free radicals and reducing ferric ions. This suggests the presence of bioactive compounds within the extract capable of donating electrons and exhibiting antioxidant activity. These compounds may include phenolic acids, flavonoids, tannins, and other phytochemicals known for their antioxidant properties (25). The variation in values with different concentrations may be attributed to the complex mixture of compounds present, as different compounds may exhibit varying activity at different concentrations, or they may interact synergistically or antagonistically.

A lower value of EC50 indicates higher antioxidant activity because less of the substance is required to provide the same level of reducing power. In case these values were obtained using linear regression calculations, comparison of the reported EC50 values will yield a quantitative measure of their relative antioxidant effect. The greater EC50 value for *Z. mauritiana* extracts in relation to ascorbic acid also supports the visual observation that the extract is indeed less effective in reducing power. The reducing power observed in the extracts is mainly due to the varying phytochemicals that these plants possess. Of special importance among these compounds is the phenolic ones that exhibit extraordinary antioxidant properties because of their ability to donate hydrogen atoms or electrons to, and neutralize free radicals through resonance, usually in several different ways by showing. The balance of these compounds with respect to *Z. mauritania* extract determines its total reducing power (26). The part of the plant,

method of extraction, and the place it is grown can all dictate the level of phytochemical content and thus determine the antioxidant ability of the extract, this quantifies its antioxidant potential (27).

Flavonoids work as key antioxidants by either directly giving away a hydrogen atom (HAT) or an electron (SET) to neutralize harmful free radicals. The best antioxidants for stopping these chain reactions usually have a ring structure that can easily donate a hydrogen atom (H•) to the unstable molecules formed when fats break down. The resulting flavonoid radical then becomes stable because its unpaired electron spreads out around the ring, like it's being shared (cutting) (28,29). Phenolic hydroxyl groups have a remarkable ability to scavenge free radicals. On the other hand, flavonoids are biologically important compounds with a wide spectrum of biological activities, such as antioxidant, anticancer, anti-inflammatory, antiallergic, antiangiogenic, and anti-allergic properties (30).

4.CONCLUSION

The antioxidant properties of *Z. mauritiana*'s total flavonoid extract (obtained with organic solvents) were the focus of this study. The DPPH assay showed significant antioxidant activity, nearing that of BHT, which correlates with the high polyphenol and flavonoid content. The fruits and their extracts also exhibited good reducing ability, suggesting they could be a valuable addition for boosting immunity.

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