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Original article

Evaluation of Anti-Oxidant Capacity, Total Phenol, Metal, and Mineral Contents of Ziziphus *lotus* Plant Grown at Some Regions of AlGabal AlKhder, Libya

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ABSTRACT

The Ziziphus plant is widely used in Libyan traditional medical treatment for many infections, it is classified as one of the important medicinal plants. Ziziphus plant growth in Libya in some regions and it is tolerant to the climate in these regions, in semi-desert and mountain regions around Al Gabal Al Khder locations. In this study, the Ziziphus plant was selected and collected from different places, the semi-desert at Marawa station and the Mountain at AlHaeiea station. The contents of total phenols and antioxidant capacity were determined. In addition to the levels of minerals (Sodium, potassium, and Calcium), the quantities of phosphorus and nitrogen were also estimated. The results recorded that the ziziphus plant of the studied areas contained important amounts of antioxidant values, and they ranged between (1.21 - 1.73 mg/g). Also, the total phenol contents ranged between (1.90 -2.87ppm). The concentrations of sodium, potassium, and calcium were fluctuated in the ranges of (10.7 -25.10), (35.4 -49) and (20.3-30 ppm), respectively. While the phosphorous and nitrogen ranged between (1.90– 3.45) and (0.14 – 0.26 ppm), respectively. The results of the study indicated that the ziziphus collected from the selected contains important levels of antioxidants, total phenols, and minerals.

Introduction

Zizyphus Lotus (Z. Lotus), also known as jujube, belongs to the angiosperm *Rhamnaceae* family. This family includes about 135–170 species of Zizyphus [1]. As a tropical and subtropical plant, *Z. Lotus* grows generally in arid and semiarid countries and is widely distributed in China, Iran, Africa, South Korea, and Europe like Cyprus, Spain, Greece, and Sicily [2]. In Africa, *Z. Lotus* is widely distributed in the Mediterranean region, like Algeria, Morocco, Tunisia, and Libya [3]. This plant is employed in nutrition, health, and cosmetics in several forms, for example, honey, tea, jam, juice, oil, loaf, and cake. In addition, in traditional medicine, both in North Africa and the Middle East, several parts of *Z. lotus* are given as anti-urinary troubles agents, anti-diabetes, skin infections, and fever, anti-diarrhea, insomnia agents, sedatives, bronchitis, and hypoglycaemic activities [4]. On the other hand, this plant offers a delicious read fruit (jujube) that is consumed fresh, dried processed as food by local populations in substantial, and amounts [5]

Zizyphus Lotus Grande called “Tahounek” in tamahaq belongs to the botanical family of Anacardiaceae. Sumac is the common name of the genusRhus. *R. tripartita* is an indigenous shrub encountered in the A hagar arid areas especially in shallow soils of the mountains [6]. The presence of abundant foliage year-round, notwithstanding the soil water status characterizes this highly drought-resistant native species [7]. Moreover, the rapid root development is one of its adaptive characteristics to arid conditions. Indeed, the root system of *R. tripartita* is highly developed both in depth and laterally which allows the plant to adapt well to drought conditions. Therefore, shrub can be of great interest for the rehabilitation of degraded habitats in arid and desert areas. However, it is little known and threatened with extinction [8].

Phenolic compounds - flavonoids

Phenolic compounds are widely distributed in plants and abundant in fruits and vegetables. One of the major groups of polyphenolic compounds, the flavonoids, is important in contributing to the flavours and colors of many fruits and vegetables. Flavonoids are widely distributed in leaves, fruits and barks of plants. Over 5000 types of flavonoids out of approximately 8000 types of phenolics have been identified, mainly as flavones, flavanones, flavan-3-ols, flavonols, anthocyanins, flavonones, and isoflavones. Flavonoids are C15, benzo-α-pyrone derivatives consisting of phenolic and pyrane rings, and they are classified according to substitutions. Flavonoids differ in the arrangements of hydroxyl, methoxy, and glycosidic side groups, and

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in the conjugation between the B- and C- rings. During metabolism, hydroxyl groups are added, methylated, and glycosylated typically forming as 3-Oglycosides in foods for anti-inflammatory, antiallergic, and vasodilatory actions. In addition, they also inhibit lipid peroxidation, platelet aggregation as well as the activity of enzyme systems including cyclo-oxygenase and lipoxygenase [9]. Flavonoids exert these effects as antioxidants, free radical scavengers, and chelators of divalent cations and are reported to have unique cardioprotective effects. For example, rats fed a flavonoid-rich diet are reported to exhibit reduced myocardial post-ischemic damage. As flavonoids are believed to be beneficial and occur in food, fruit, and vegetables, the investigation of flavonoids in Malaysian traditional vegetables was one of the objectives of the present study [3]. Oxidative stress is caused by the imbalance between oxidants and antioxidants, potentially leading to damage in plants and humans. Approximately 1-3% of the oxygen consumed in the human body is converted to superoxide and other reactive oxygen species (ROS) called free radicals under physiological conditions. Even though these free radicals perform many important physiological processes such as cell signaling, microbial killing, and gene transcription, they may also damage DNA, protein, or lipids in the human body. These deleterious effects are reported to be responsible for chronic diseases mentioned before [10]. To counteract the threat of free radical-induced damage, the human body has developed an antioxidant defence system, which consists mainly of antioxidant enzymes such as superoxide dismutase (SOD) or catalase and radical scavengers like ascorbic acid or tocopherols. However, during physical stress, such as exercise or certain disorders i.e., fever, this antioxidant system is affected and enhanced, which requires the body to keep the balance between antioxidants and prooxidants. Therefore, to improve the antioxidant defence, one easy way is to increase the dietary intake of antioxidants from food mainly fruits and vegetables, which contain bioactive compounds such as vitamin C, carotenoids, and importantly polyphenolics. Recent studies have emphasized the importance of antioxidants and the mode of action of specific flavonoids as bioactive components of the diet in vivo and in vitro [9]. Thus, it is important to have a clear idea of the major phenolic antioxidant compounds and their levels in fruits and vegetables.

It was reported the antioxidant capacities of extracts from selected fruit and vegetables assessed using the Trolox Equivalent Antioxidant Capacity (TEAC), the Ferric Reducing Antioxidant Potential (FRAP) and Oxygen Radical Absorbance Capacity (ORAC) assays. Fruit such as strawberry, raspberry, and red plum, which are rich in anthocyanins had the highest antioxidant activities, followed by those rich in flavanones, such as orange and grapefruit, and flavanols (e.g. onion, leek, spinach, and green cabbage), while the hydroxy cinnamate containing fruit (e.g. apple, tomato, pear and peach) consistently elicited lower antioxidant activities. In vitro studies have shown the relationships between flavonoid structure and antioxidant activity. Quercetin, the most potent antioxidant among the flavonoids, has three structural properties which contribute to its activity. First, the number and configuration of hydroxyl groups on A and B rings especially the dihydroxycathecol structure of the B ring. Secondly, the planarity of the molecule and third, the double bond about the 4-oxo group of the C ring. Other antioxidant properties of flavonoids were reported to stabilize unpaired electrons, scavenge free radicals from lipid peroxidation, reduce the incidence of DNA damage, and the ability to chelate with transition metal ions, which results in the inhibition of the reactive oxygen species production. There is limited information on the antioxidant activities of Malaysian traditional vegetables although recently, the screening of biological activities such as anticancer, antioxidant, and anti-inflammatory in vitro has been reported (. For example, extracts from Alpinia galanga and Cayratia japonica used traditionally to treat cancer, have been shown to possess cytotoxicity against human lung and breast cancer cell lines (COR L23 and MCF 7) with the IC50 7.8 and 23.9 μM respectively. Extracts from Centella asiatica have also been shown to exhibit high antioxidant activities using linoleic acid and TBARS assays. Therefore, investigation of the biological activities of these plants should be a priority to understand the potential health effects of Malaysian traditional vegetables [3]. This study aims to determine the contents of antioxidants and total phenols. beside the minerals and some metals in leaves and stem samples of Ziziphus plant growth at some Al Gabal Akhder regions.

Methods

Sampling

Due to the importance of many plants used in the AL-Gabal AL-Khder region (Libya), this study was designed to select two different plants (*Ziziphus lotus and Rhus tripartita*). The samples were collected from Al-Gabel Al–Kadar region during the spring season of (2024) year. Stems and leaves of every species of the selected plants were separated and, then dried in the open air (Figure 1).

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Figure 1. Ziziphus lotus

Plant samples preparation

The leaves and steam samples were washed several times with distilled water and then dried in the dark for 2 weeks. The dried samples were ground and stored in pre-cleaned polyethylene bottles until the start of the analysis. (Each sample contained at least three individual plants of the same area that were mixed to compose a single sample).

Determination of antioxidant capacity by Prussian Blue method:

One gram of the sample powder was defatted with petroleum ether. The defatted powder was then extracted sequentially by stirring with 10 ml methanol twice, then with 10 ml 1% hydrochloric acid: methanol(v/v). The three combined extracts were evaporated under vacuum and the residue was dissolvedin 10 ml methanol. Half ml of the solution was diluted with 3 ml of distilled water, 3 ml (0.008 M) of $K_3Fe(CN)_6$ (Potassium Ferricyanide) Was added, 3 ml 0.1M HCl, and 1 ml of 1% $FeCl_3$ (Ferric Chloride). The blue color is allowed to develop for 5 min and the absorbance is measured at 720 nm against the blank [11 -14].

Determination of phenolic compound contents

In a 10-ml flask, aliquots of the extracts were mixed with three ml of distilled water. Next, 0.5 ml of folin ciocalteu reagent (1:1 with water) and 2 ml of Na2CO3 (2%) were added. Following a minute of warming and chilling, the absorbance of the test solutions at 650 nm was measured and compared to the reagent used as a blank. A standard calibration plot was created at 650 nm using known tannic acid values between 4 and 20µg/ml [15-16].

Determination of metals and minerals:

The metals of (Cu, Fe, and Ni) (Copper, Iron, and Nickel), were metals with an Atomic absorption (Perkin Elmer 800) according to the method described by [17-20] Soluble sodium, calcium, and potassium contents measured by a Flame Photometer (JENWAY Flame Photometer) according to the method described by [17-20]Total phosphorus was determined spectrophotometrically using the procedure of [17 -20].

Results and Discussion

Total phenol compounds and anti-oxidant contents

The results of the *Ziziphus lotus* showed a significant increase in the content of phenolic compounds and antioxidants. In the coastal *Ziziphus lotus* plant, the leaves contained 2.87 and 2.02 mg/g, whereas the stems recorded 2.37 and 1.90 mg/g in regions 1 and 2, respectively (Table 1). On the other hand, the antioxidant values were 2.03 and 1.82 in leaves and 1.34 and 1.31 mg/g in stems, there are small increases in total phenols content in leaves compared with stems in the studied samples. Increased activity of RNA and DNA polymerases involved in the physiological response to bio-stimulants causes an increase in protein content The nutritional importance of plant species is determined by their content of carbohydrates, proteins, fats, oils, vitamins, and minerals responsible for the growth of plant and animal species. Fats, proteins, and carbohydrates are essential nutrients. The amount and quality of plant protein are key factors to consider when selecting plant species with nutritional value. The high polyphenol content of *Ziziphus lotus* has antioxidant, antibacterial, and immunomodulatory activities [21].

Phenols are one of the most common and diverse groups of chemicals found in plants that all have an aromatic ring with at least one hydroxyl (phenol) substituent. Phenols have antiviral, antibacterial,

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immunostimulatory, hypotensive, anticoagulant, hypocholesterolaemia, anti hepatocellular, and anticancer properties in mammals [22]. The medicinal properties of phenols are related to their ability to modulate gene expression and interact with cellular signalling pathways. Its ability to adsorb metal ions involved in the free radical generation is also credited. Moreover, phenolic structures possess the ability to strongly interact with proteins due to the hydrophobic benzenoid rings and the hydrogen bonding property of phenolic hydroxyl groups. By decreasing protein production, phenol can decrease oxidative stress induced by the opening of mitochondrial junction pores. Phenols also have a role in the activation of several protein kinases, phase II antioxidant detoxification enzymes, and the control of many cells survival and cell cycle genes [22].
An antioxidant is a substrate that keeps molecules in a cell from oxidizing. A well-known chemical process allows electrons or hydrogen to be removed from a material. During the biological oxidation reaction, free radicals are produced. The chain reaction begins at the same time because the radicals are reactive. A cell can be damaged or even killed as a result of this. Antioxidants can stop a chain reaction by removing free radical intermediates; hence, they can be classified as reducing agents. Ascorbic acid, thiols, and polyphenols are some examples. Antioxidants are dietary supplements that have been studied for their ability to prevent diseases including heart disease and cancer [23].
Plants have evolved many antioxidant systems that protect them from potential cytotoxic effects such as the system that reacts with active forms of oxygen and keeps them at a low level [e.g. Secondary metabolite, superoxide dismutase (SOD), catalase (CAT), peroxidases (POD), polyphenol oxidase (PPO)] and the system that regenerates oxidized antioxidants [e.g. glutathione reductase (GR) and acerbate peroxidase (APX)].The enzymatic antioxidant system is one of the protective mechanisms, including SOD, found in various cell compartments that catalyze the disproportion of two O_2 radicals to form hydrogen peroxide (H_2O_2) and O_2. Various antioxidant enzymes such as CAT and POD, which convert H_2O_2 into water, eliminate hydrogen peroxide [24]. Various cytotoxic reactive oxygen species (ROS) are constantly generated in mitochondria, peroxisomes, and cytoplasm during oxidative stress caused by various climatic conditions, which can disrupt normal metabolism through oxidative damage to proteins and nucleic acids, as well as hinder the growth of the vast majority of plants [25]. The relation between protein metabolism and abiotic stress in plants is well, with stress affecting the metabolism of nitrogenous compounds, protein synthesis, and free amino acid pool formation [26].

Table 1. The contents of total phenol compound, and anti-oxidant in the leaf and stem of the Ziziphus lotus plant grown in coastal (R1) and semi-desert regions (R2).

Samples		**Total phenol compound (µg/g)**		**Anti-oxidant(mg/g)**	
		R1	**R2**	**R1**	**R2**
Ziziphus lotus	**Leaf**	2.87	2.02	1.73	1.70
	Stem	2.37	1.90	1.21	1.17
Average		2.5	1.82	1.44	1.411
SD		0.80	0.16	0.27	0.27
P value		0.629		0.891	

The contents of mineral composition

Tables 2 and 3 showed the mineral content in the leaves and stems of Ziziphus *lotus* plants cultivated in coastal and semi-desert areas. The results showed that plants grown in coastal areas resulted in significant increases in levels of ions, Phosphorus, Nitrogen, Potassium, Calcium, Sodium, Iron, Nickel, and Copper compared to plants grown in semi-desert areas. According to the findings, the coastal plant contained a high mineral content, where it had phosphorus in the leaves, followed by sodium, calcium, and phosphorus 3.67 in the stems and soil.
The contents of phosphorus, nitrogen, sodium, calcium, and potassium in leaves were as follows (3.24 and 2.22), (0.26 and 0.18), (25.1 and 19.2), (30 and 24.20) and (49.0 and 45.22 ppm), respectively. On the side, the same metals in stem samples were as follows: (3.45 and 1.90), (0.19 and 0.14), (10.7 and 20) (25.1 and 20.20), and (35.40 and 39.20 ppm). The accumulation of elements in different plant species is influenced by a variety of factors including soil type, fertilization method, plant species, and environmental circumstances [27]. Many micro and macro elements present in plants are responsible for treating different types of diseases. The reason for the increasing public interest in herbal remedies is the high cost and side effects of most modern medicines [28].
It was suggested that Mineral nutrients, such as calcium ions influence intracellular Na^+ and K^+ balance and enhance the K/Na ratio by selective intracellular accumulation in high-temperature conditions. This

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link between K and Na in food aids in the prevention of high blood pressure. Many enzymes have Cu as a structural component. Mg is required for energy metabolism, bone production, and enzyme activity [29]. Fe is essential for the formation of haemoglobin, the delivery of O_2, and the enhancement of immunity in the body. As a result, it is important to perform pharmacological studies of native medicinal plants to determine their nutritional and therapeutic association [30].

Nitrogen makes up roughly 80% of the total mineral nutrients taken up by plants in one form or another. Climate change has been found in several agricultural experiments to limit nitrogen accumulation [31]. Reduced nitrogen uptake happens in unfavourable conditions, resulting in reduced plant growth and productivity [32]. This is due to a reduction in NO_3 uptake or a reduction in water uptake in dry conditions. Phosphorus is an essential component of nucleic acids, nucleotides, phospholipids, and phosphor proteins, making it the second most critical nutrient for plant growth. Phosphate compounds are used as a source of energy in plants. Calcium plays an essential role in the processes that maintain the structural and functional integrity of plant membranes, stabilization of cell wall structures, regulation of ion transport and selectivity, and control of ion exchange behaviour and cell wall enzymatic activities. Since calcium seems to be easily displaced from membrane binding sites by other cations, these functions can be seriously disrupted by reduced calcium availability due to different climatic conditions [33].

In plants, the potassium ion serves as the main cation that balances the negative charge of anions. The potassium ion helps cells maintain their pH, osmotic potential, and turgor pressure. It also plays a key role in the activation of enzymes involved in protein and carbohydrate metabolism and synthesis, as well as in the binding of tRNA to ribosomes [34].

Table 2. The contents of minerals in the leaf, and stem of the Ziziphus lotus plant grown in coastal (R1) and semi-desert regions (R2).

Samples		**Phosphors**		**Nitrogen**		**Sodium**		**Calcium**		**Potassium**	
		R1	**R2**	**R1**	**R2**	**R1**	**R2**	**R1**	**R2**	R1	R2
Ziziphus lotus	**Leaf**	3.24	2.11	0.26	0.18	25.1	19.2	30	24.2	49.0	45.22
	Stem	3.45	1.90	0.19	0.14	10.7	20.0	25.1	20.2	35.4	39.20
Average		3.3	2.10	0.22	0.16	17.6	19.5	27.5	22.2	42	42
P Value		0.014		0.757		0.15		0.60		0.50	

Table 3. The contents of Fe, Ni, and Cu in the leaf, and stem of the Ziziphus lotus plant grown in coastal (R1) and semi-desert regions (R2).

Samples		**Fe**		**Ni**		**Cu**	
		R1	**R2**	**R1**	**R2**	**R1**	**R2**
Ziziphus lotus	**Leaf**	0.51	0.45	0.38	0.22	0.53	0.23
	Stem	0.75	0.34	0.14	0.18	0.43	0.34
Average		0.6	0.4	0.26	0.20	0.46	0.29
P Value		0.19		0.11		0.20	

Conclusion

The results of this study recorded the presence contents of antioxidants, total phenols, minerals, and small quantities of metals which give importance to this plant for medical uses against different types of infections, also the results no record wide variations of the detected compounds between leaves and stems of Ziziphus plant of the studied locations.

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المستخلص

يعتبر السدر من اهم النباتات التي تستخدم في الطب البديل التقليدي في ليبيا ، وقد تم استخدامه نظرا لفعاليته ضد انواع كثيرة من العدوى المرضية التي تصيب الانسان، ويتواجد السدر في العديد من المناطق بليبيا ، كما انه ينمو في المناطق الجبلية وشبه الصحراوية على حد سواء، في هذه الدراسة تم استخدام اوراق وسيقان نبات السدر النامي في مناطق الجبل الاخضر ، حيث تم جمع عينات من مناطق جبلي بالجبل الاخضر وعينات شبه صحراوية على حدود الجبل الاخضر الجنوبي, زلك لقياس مضادات الأكسدة والمركبات الفينولية الكلية وكذلك محتوى المعادن وبعض العناصر. وقد بينت نتائج الدراسة احتواء نبات السدر على تراكيز مهم من مضادات الاكسدة والمركبات الفينولية الكلي وتراوحت تراكيزها ما بين (1.21-1.73) و (1.90 سلطان 2.87 ملىجرام/لتر) في كلا من الأورق والسيقان ، على التوالي ، كما بينت نتائج الدراسة ان تراكيز المعادن الكبرى وهي الصوديوم والبوتاسيوم والكالسيوم انها تتراوح ما بين (10.7-25.10) و (35.4- 49) و (20- 30 جزء في المليون) ، على التوالي .كما بينت نتائج للدراسة احتواء السدر في المناطق قيد الدراسة على تراكيز مهمه من العناصر الحديد والنحاس والنيكل.

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