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Original article

Estimation of Amino Acid Composition, Total Carbohydrate, and Total Protein Content in Ballota pseudodictamnus Plant Extracts from Al Jabal Al Akhdar Region, Libya

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This comparative study was carried out on plant extracts which collected from different regions (Susa and Aldahar Alhmr, Derna) around Aljabal Alkhder area. The contents of amino acids, total protein, and total carbohydrate. The samples were collected during two different seasons (Winter and summer, 2023). The GC –Mass instrument was used to estimate the contents and types of amino acids, while the spectrophotometric method was used to evaluate the total contents of carbohydrates and proteins in the leaves and stems of the studied samples. The results of this study recorded the presence of different amino acids in the studied samples, including: Their names are Leucine, Serine, Glutamine, Phenylalanine, Methionine, Arginine, Valine, Histidine, Isoleucine, and Asparagine. Their contents were small and varied between the leaves and stems. generally, the average values were as follows (0.223, 0.222, 0.811, 0.183, 0.186, 0.224, 0.880, and 0.648 ppm, respectively. On the other side, the contents of carbohydrate fluctuated in the ranges of (20.94 – 20.99 ppm) and (20.53 -20.84 ppm) in leaves and stems, respectively. Also, the results showed that the contents of total protein ranged between (6.21-6.6.80 ppm) and (6.61-7.62 ppm) for the leaves and stems, respectively. The study concluded that there are small variations in the chemical constituents determined in this study between the two selected regions.

Introduction

Plants are the main source of food and medicine for all living creatures, man has known since the beginning of creation and to this day many plant species are still recognized, and he is trying to classify and divide them intowhat is useful to benefit from it or what is harmful to avoid [1]. Aromatic and medicinal plants are among the largest natural resourcesfor medicines and essential oils that play a vital role in alternative medicine and the preservation of communities, especially rural ones. Medicinal and aromatic plants of the world treat different types of human and animal ailments. These therapeutic and preventive uses aroused the interest of scientists and made them intensify efforts to research its ingredients and all the active compounds in it. It is known that medicinal plants contain basic components that are included in food and also in the treatment of diseases, and act as nutritional supplements such as enzymes, vitamins, carbohydrates, fats, and proteins. These active compounds are important to life and are used in different physiological and metabolic activities [2]. The medicinal and antimicrobial properties of the plant's seeds make its used to treat constipation, malaria, measles, and stomach pain and other diseases, the roots and stems are also effectively used as a remedy for diseasesof the teeth and gums, analgesic activity, antibacterial, antidiabetic, antifungal, anti-hypercholesterolemic, antioxidant, prevention of liver disease and neuritis [3].

Lamiaceae occupies the sixth largest family of angiosperms with about 233 genera and 7,172 species distributed the world in both temperate and tropical regions; its main distribution is in the Mediterranean, and many of these and related species are also importantbee plants, providing the nectar and pollen to support bee colonies and provide honey. The essential oils (secreted from glands on the leaves and stems of the plant) are commercially extracted from many species. Menthol and thymol and many other monoterpenes are used in medicine and the food industry. The main objectives of this study were to comparative study of the medicinal value the of *B. pseudodictamnus* L. plant growing in two different locations (semi-desert and mountainous) around the Al- Jabal Al-Akhdar region by estimate some chemical composition contents of amino acids, carbohydrate and total protein in extracts of *B. pseudodictamnus L* plant.

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Methods

Chemical analyses and physiological measurements for this study were carried out in the laboratories of the Department of Botany and Chemistry, Science Faculty, Omar AL-Mukhtar University during the period from 2022 to 2023.

The Study area

This study was conducted in two different locations (Derna andSusa) in the climate in the Jabal Al-Akhdar region. The city of Derna is located between latitudes 6′ 48́ 32 46 46 degrees north and longitudes 21″ 96́ 22́ 37 degrees east. The region has moderate climatic conditions prevailing. As the region is located on the Mediterranean Sea on the north and north-eastern side on one side and a high topography on the other, while it is open to semi-desert terrain on thesouth side. Susa is a limestone plateau 700 to 870 m above sea level with an undulating surface which tips gently to the south. Stretches between the latitudes 9′ .46 32́ 45°N and longitudes 10̋.70 15 43°E. There are two main escarpments, further apart in the west but drawing gradually closer together eastward. large portion of the two benches, especially the second,is dissected by wadis, giving the Jabal a predominantly hill to mountainous appearance, Figure 1.

Figure 1. Google Earth map showing the study area.

Sampling

The plant samples (leaf and stem) were collected from the two study areas (Derna and Susa) in the summer (June) and winter (December) seasons, 2022- 2023. The plant was identified and authenticated as *Ballota pseudodictamnus* L. by Botanists in (Silphium Herbarium) Department of Botany, Faculty of Science, Omar Al-Mukhtar University, Al Bayda – Libya.

Plant Samples Preparation

The collected leaf and stem 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 theanalysis. (Each sample contained at least three individual plants of the same area that were mixed to compose a single sample) [4-15].

Estimation of total carbohydrates

To estimate total carbohydrates, a known weight of 0.2 g of the dried sample was ground, then 5 ml of sulphuric acid was added. After thesamples dissolved, the samples were cooled at room temperature, then a small quantity of Barium carbonate (Ba_2CO_3) was added, and the mixture was heated again. After cooling, the samples were filtered, 1 mL of solution was taken, then 1 mL of 5% phenol was added. The total carbohydrate was determined by the method carried out by many of the studies [4-15]. The absorbance was measured at a wavelength of 490 nm.

Estimation of total soluble proteins

Soluble protein was determined by using a spectrophotometric reagent according to previous studies [4] assay and protein was expressed as mg protein/g FW.

Estimation of amino acid

A gas chromatography-mass–mass spectrometry (GC–MS) method has been used for the determination of

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amino acid content in the leaves of the plants.

Statistical analysis

The test of least significant difference (LSD) at the level of0.05% significance was used to examine differences among treatment meansand interactions. Data were statistically analyzed using the MSTAT-C softwarepackage according to the method described in a previous study [16].

Results

Amino acid composition investigation

The essential amino acid content of each of the two plants is summarized in Figure 2 and Table 1. Their names are Leucine, Serine, Glutamine, Phenylalanine, Methionine, Arginine, Valine, Histidine, Isoleucine, and Asparagine. The results presented in Table 1 and Figure 2 show a significant increase in the number of ten amino acids found to be present in the plants that were grown in the Susa region compared to the plants grownin the Derna region. The contents of the detected amino acids were ranged as follows: (0.01 -0.162), (0.060-0.501), (0.11-0.701), (0.090 -0.093), (0.052-0.084), (0.102-0.122), (0.221 -0.661), (0.176 -0.43 ppm).

Table 1. Comparison of the amino acid content in the leaves of B. pseudodictamnus plant grown in Darna and Susa regions, which were collected in two seasons (summer and winter).

Amino acid	Plant 1(Derna)	Plant 2(Susa)	Average
Leucine	0.071	0.162	0.233
Arginine	0.060	0.501	0.222
Valine	0.111	0.701	0.811
Histidine	0.093	0.090	0.183
Isoleucine	0.084	0.052	0.136
Aspargine	0.122	0.102	0.224
Serine	0.221	0.661	0.88
Glutamine	0.113	0.176	0.648
Phynelanaline	0.901	0.981	1.78
Methionine	0.206	0.611	0.816
Average	2.043	3.89	5.933

Where: - Means having the same letters in a column were not significantly different at p<0.05.Plant [1]= B. pseudodictamnus plant grown in Derna Plant [2]= B. pseudodictamnus plantgrown in Susa.

Carbohydrate

The results are shown in Table 2. There were no significant differences between the carbohydrate content of the leaf and stem extract, but only between seasons. The contents of Carbohydrate were fluctuated in the ranges of (20.53 – 20.99) and (2.11 -7.66 ppm) in leafs and stems at Susa area during summer and winter, respectively, whereas for the same plant at Derna area the carbohydrate contents were ranged as (20.94 and 20.84 ppm) for leafs and stems during summer, respectively, and (2.33 and 2.60 ppm) for leafs and stems during winter, respectively.

Table 2. Comparison of the contents of carbohydrate of B. pseudodictamnus plants grown in the regions of Darna and Susa was collected from different seasons (summer and winter).

Season	Carbohydrate			
	Summer		Winter	
Sample (Type)	leaf	stem	leaf	stem
Plant 1	20.99a	20.53b	7.66c	2.11d
Plant 2	20.94a	20.84a	2.33d	2.60d
LSD 5%	0.31			

Where: - Means having the same letters in a column were not significantly different at p<0.05; Plant. [1]= B. pseudodictamnus plant grown in Derna Plant; [2]= B. pseudodictamnus plant grown in Susa

Protein

The contents of total protein are shown in Table 3. The results recorded that there was a significant increase in the content of both proteins of the *B. pseudodictamnus* plant in summer compared to samples collected

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in winter. On the other hand, the second plant (Susa) was better in the content of the mentioned measurements than the first plant. The stem part gave significant results compared to the leaf in all studied plants Table 3 and Figure 4 (a, b, and c). Generally, the contents of total protein were ranged as follows: (6.21- 6.32 ppm) and (6.38-6.80 ppm) in leaves during summer and winter, respectively. On the other side of the stems, the contents of carbohydrates ranged between (6.70 -7.62) and (6.61 – 7.20 ppm) during summer and winter, respectively.

Table 3. Comparison of the protein content of B.pseudodictamnus plant grown in Darna and Susa regions, which were collected in two seasons (summer and winter).

Compounds	Total protein			
Season	Summer		winter	
Sample (Type)	leaf	stem	leaf	stem
Plant 1	6.32^{c}	6.70^{b}	6.21^{c}	6.61^{b}
Plant 2	6.38^{c}	7.62^{a}	6.80^{b}	7.20^{a}
LSD 5%	0.43			

Discussion

The accumulation of soluble carbohydrates in plants has been widely reported in response to different climatic conditions such as temperature, humidity, drought, etc., despite the significant decrease in the net carbon dioxide uptake rate [17]. The accumulation of soluble sugar may be due to further conversion of starch to sugars or reduced consumption of carbohydrates by tissues under inappropriate conditions [18]. The positive impact of the summer seasonson the production and metabolism of carbohydrates is quite well known in plants [19]. The results presented in this study indicate an increase in the carbohydrates content in leaf and stems *B. pseudodictamnus* plant grown in Derna under summer seasons, which might be caused by an enhanced photosynthetic capacity of tissues account by elevated photosynthetic pigments contents. They concluded that the higher temperature and longer illumination period increases the carbon dioxide assimilation capacity in the Calvin cycle, which is mainly attributed to the increased initial activity of RuBisCO.

According to modern concepts about the contribution of plant compounds to the pharmacological interaction activity, amino acids, as secondary components, are very important for different types of actions [20]. Most often, the presence/absence of certain amino acids is the main factor affecting the central nervous system, thyroid gland, etc [21]. Amino acids are the main component of proteins [22], which in turn are important for stimulating cell growth. They contain both acidic and basic groups and act as buffers, helping to maintain a favorable pH value within the plant cell. Amino acids are also a well-known bio-stimulant that has positive effects on plant growth and yield and significantly alleviates injuries caused by abiotic stresses. The increase in protein content under the influence of summer and high temperature is a consequence of the enhanced activity of RNA and DNA polymerases that are involved in the physiological response to biostimulants. A marked decrease in the contents of soluble proteins was recorded in the leaves, and the highest levels were recorded in the stems (Figure 4 a). Phenols are plant auxiliary metabolites that constitute one of the foremost common and far-reaching bunches of substances in plants. They constitute a huge store of normal chemicals with differing qualities that envelop a colossal range of compounds and proteins and a wide range of components of quality control and of transport of metabolites and chemicals [23].

Amino acids significantly increased the total nitrogen, phosphorous, and potassium in leaves of plants as well as the total yield, weight, vitamin C and total sugars content of medicinal plants [24]. During oxidative stress caused by different climatic conditions, various cytotoxic reactive oxygen species (ROS) are constantly generated in mitochondria, peroxisomes, and cytoplasm, which can destroy normal metabolism through oxidative damage to proteins and nucleic acids [25], impeding the growth and development of the vast majority of plants. The relationship between protein metabolism and abiotic stress in plants is well documented, with stress affecting the metabolism of nitrogen-containing compounds, protein synthesis and free amino acid pool formation [26]. Quantitative determination of amino acid concentration was conducted by GC- Ms. L-cystine ensures elasticity of keratin, and therefore, it is a part of complexes of vitamins for an advance of appearance of the hair, and skin, different additives, and shampoos. L-cystine is also used in complex therapy for the treatment of bronchitis. Alzheimer's disease, diabetes, as well at joint diseases. The study of chemical constituents in many Libyan medical plants and their applications as antibacterial, anti-cancer activities took place in different studies [27-44]. Most of these studies concluded that the area located at Al Gabal Alkhder region contains high values of natural compounds.

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Conclusion

According to the results recorded in this study, the plant extracts of leaves and stems showed the presence of different types of amino acids, carbohydrates, and total protein. The importance of this plant comes from its classification as one of the plants that give an interesting taste to some honey types, which are given the same name in traditional Honey called (Mailla).

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