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Original article

Applying Different Analytical Methods (GC-Mass, Spectrophotometer, Atomic Absorption, and Flame Photometry for Estimation some Chemical Constituents in *leaves and Stems* of the *Quercus coccifera* plant

Aisha Ali[1], Adel Abdulathim[2], Zuhir Akrim[3], Nevein Abdel-Hady[4], Hamad Hasan*[5]

[1]*Botany Department, Faculty of Science, Omar Al-Muhtar University, Libya*
[2]*Medical Technology Department, Higher Institute of Science and Technology, Cyrene, Libya*
[3]*Pharmacolgy and Toxicology Department, Faculty of Pharmacy, Omar Al-Muhtar University, Libya*
[4]*Pharmacognosy Department, Faculty of Pharmacy, Al-Azhar University, Egypt*
[5]*Chemistry Department, Faculty of Science, Omar Al-Muhtar University, Libya*
Corresponding Email. *hamad.dr@omu.edu.ly*

Abstract
Some of the different analytical methods were used to estimate some of the chemical constituents in leaves and fruits of *the Quercus coccifera* plant, where the GC-MS was used for the determination of Fatty acids and phenolic acids. The atomic absorption spectrometry (AAS) was used for measuring the contents of metals such as Zn, Cr, and Cu. Also, the Flame photometer was used for the determination of minerals Na, K, and Ca. On the side the spectrophotometer was applied to measure the contents of phosphorus and Nitrogen The results of this study recorded that, some of phenolic acids were found in the samples including **:**The concentration of phenolic acids in leafs and fruits of the studied plant as following: Chlorogeneic acid (0.0115 mg/g), 3,4-Dicaffeoly guinic acid (0.129 mg/g), 3,5- Dicaffeoylquinic acid (0.309 mg/g), 4,5- Dicaffeoylquinic acid (0.0161 mg/g), 2,5-dihydroxy Benzoic acid (0.00000589 mg/g), and Galic acid (0.166mg/g). *Quercus coccifera* fruits: Chlorogenic acid (0.0197 mg/g), 3,4-Dicaffeoylquinic acid (0.102 mg/g), 3,5-Dicaffeoylquinic acid (0.132 mg/g), 4,5-Dicaffeoylquinic acid (0.205 mg/g), and cinnamic acid (0.00000698mg/g). Small amounts of Fatty acids were recoded and included Saturated fatty (0.081 and 0.087mg/g) and unsaturated fatty acids containing Monounsaturated fatty acids (0.047and 0.048 mg/g) in leaves and stems, respectively, and Polyunsaturated fatty acids (0.033 and 0.049mg/g), respectively. On the other side, the contents of Sodium, potassium, Calcium, Magnesium, Nitrogen, and Phosphorus in leaves were as follows**:** 0.3, 11.5, 1.50, 1.82, 0.69, and 2.27 ppm, whereas the contents of the same elements in fruits were as follows: 0.08, 13.5, 0.50, 1.597, 0.52, and 2.142 ppm, respectively. This study recorded small amounts of Zinc, Copper, and Chromium, and ranged between (0.147 - 0.154), (0.947 -1.132) and (0.0861 -0.0898 ppm), respectively.
Keywords. Analytical Methods, Chemical, Constituents, Quercus Coccifera Plant.

Introduction

Medicinal and aromatic plants are an important source of drugs in Libya. The country has a vast area and a variety of geographic conditions suitable for the cultivation of aromatic and medicinal plants. There is scope for the establishment of small-scale phytochemical industries. There is a lack of R&D activities in the field of medicinal plants. As a result of over-exploitation, some plants have become rare and endangered. There is also an imminent threat of genetic erosion of medicinal plant species because of an increase in heavy grazing, human use, and drought hazards. The economic constraints of the country mean that international assistance is needed for the collection and conservation of the genetic resources of endangered species of medicinal value [1]. Natural products, especially medicinal plants, produce and contain a variety of secondary metabolites that can perform various functions, and many of them exhibit interesting and useful biological activities**.** It`s well established that natural products have been a source of lead compounds for the development of some of the most effective drugs available for the treatment of different human diseases [2]. Investigations have revealed that daily consumption of antioxidants can reduce the incidence of various ailments [3,4]. Many studies were carried out on different plants to evaluate the chemical constituents in different tissues, such as leaves, stems, and fruits [5-34]. This study aims to estimate the contents of Fatty acids, phenolic acids, minerals, metals, and elements in fruits and leaf samples of the *Quercus coccifera* plant.

Methods

Sampling

Selection of medicinal plants for this study

The selected plant of the *Quercus coccifera* in this study was collected from Al-Gabel Al –Kadar Region. The-Samples including leaves and Stems.

Samples preparation

Leaves and fruits were separated and washed with distilled water several times, then dried in the open air for fifteen days. Then samples were ground and stored until the analysis (Figures 1& 2).

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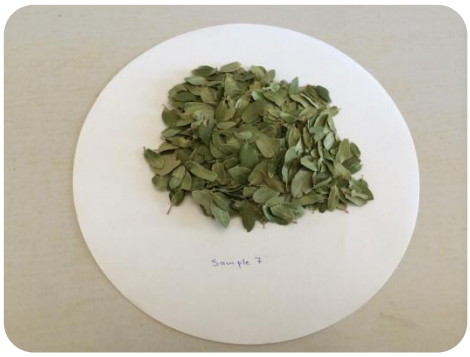

Figure 1. Quercus coccifera leaves

Figure 2. Quercus coccifera fruits

Taxonomical investigation

The samples were kindly identified by the Plant Taxonomy of the Botany Department. The samples were kept in the Seliphium herbarium, Faculty of Science, Omar Al Mukhtar University. (Figure 3), show the plant taxonomy,

Figure 3. Herbarium sample of Quercus coccifera.

Chemical studies

Total phenolic content (TPC)

Total phenolic was estimated using the colorimetric method based on Folin-Ciocalteu reagent "100,200,300,400 and 500µl" of methanol extract of leaves and fruits of the selection plant were diluted with 2ml of distilled water and mixed with "600µl" of Folin-Ciocalteu reagent. The mixture was allowed to stand for 5 min. and then 2ml of 20% Na_2CO_3 was added and kept at a boiling water bath for 1 minute. After cooling, the blue colour formed was measured at 765 nm by a UV-visible spectrophotometer. Quantification was done with respect to stander calibration curve of Pyrogallol, the results were expressed as pyrogallol "µg/ml".

Fatty acids (Gas Liquid Chromatographic Analysis)

5 grams of powdered extract for 30 minutes with 20ml mixture of chloroform and methanol (2:1) and filtered. The marc (remained powdered) was re-extracted three times as mentioned (chloroform/ methanol). Combine the extracts and wash with distilled water. The extracted layer was concentrated to a residue The analysis of fatty acid was carried out by shinadzu-8A GLC, in the Faculty of Science, Alexandria University, Egypt.

Determination of minerals

0.5 gram of each sample was digested by Nitric acid. The samples were put in 250 ml conical flask containing 25 l distilled water and 5 ml Nitric acid on a hot plate. The samples were heated until near dryness. The samples were then removed and left until cooled, then filtered. To the filtrate, the distilled water was added to complete the valium at 100 ml in a measuring flask. The mineral content of the samples, Na, K, and Ca were measured by Flam photometer, while the metals of Mg, Cu, Zn, and Cr were determined by atomic absorption, whereas the P and N were estimated by a Spectrophotometer according to the method described by previous studies. After digestion with HNO_3 acid**,** were determined using the Atomic Absorption Spectrophotometer. These methods were used for estimating minerals and metals in solid and liquid samples [35-40].

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Results

Total phenolic acids content

The concentration of phenolic acids in leaves and fruits *Quercus coccifera* as follows: *Quercus coccifera* leaves: Chlorogeneic acid (0.0115 mg/g), 3,4- Dicaffeoylquinic acid (0.129 mg/g), 3,5-Dicaffeoly guinic acid (0.309 mg/g), 4,5- Dicaffeoylquinic acid (0.0161 mg/g),2,5-dihydroxy Benzoic acid (0.00000589 mg/g), and Galic acid (0.166mg/g). *Quercus coccifera* fruits: Chlorogenic acid (0.0197 mg/g), 3,4-Dicaffeoylquinic acid (0.102 mg/g), 3,5-Dicaffeoylquinic acid (0.132 mg/g), 4,5-Dicaffeoylquinic acid (0.205 mg/g), and cinnamic acid (0.00000698mg/g). Phenolic acid content in the studied plant is given in (Table 2). The concentration of

Table 2. Phenolic acids contents µg/g in Quercus coccifera plant

Plants / **Phenolic acids µg/g**	**leaves**	**fruits**
Chlorogenic acid	0.0115	0.0197
Caffeic acid	_	_
3,4-Dicaffeoly guinic acid	0.129	0.102
3,5-Dicaffeoyl guinic acid	0.309	0.132
4,5-Dicaffeoly guinic acid	0.0161	0.205
2,5-dihydroxy Benzoic acid	0.00000589	_
Cinnamic acid	_	0.00000698
Galic acid	0.166	_

Fatty acids

Total Saturated and Unsaturated fatty acid

The concentration of Saturated fatty acids in *Q. coccifera* leaves and fruits is as follows: (0.081 and 0.087mg/g). concentration of unsaturated fatty acid in *Q. coccifera* leaves, and fruits as follows: Monounsaturated fatty acid (0.047and 0.048 mg/g) respectively. Polyunsaturated fatty acid (0.033 and 0.049mg/g) respectively, (Table 3).

Table 3. Total Saturated (T SFA) and unsaturated (Un SFA) fatty acids

Plant / **T SFA and T UnSFA µg/g**		***Quercus coccifera***	
		leaves	**fruits**
SFA		0.081	0.087
Un SFA	MUFA	0.047	0.048
	PUFA	0.033	0.049

Saturated fatty acids

Theconcentration of saturated fatty acids in leaves and fruits of *Quercus coccifera* is as follows: Hexadecanoic (0.037, 0.023mg/g) and Octadecanoic (0.044, 0.064mg/g) respectively, (Table 4).

Table 4. Saturated fatty acid content in the studied plant (leaves and fruits).

Plants / **Fatty acids µg/g**	***Quercus coccifera***	
	leaves	**fruits**
Hexadecanoic	0.037	0.023
Octadecanoic	0.044	0.064

Unsaturated fatty acids

The concentration of unsaturated fatty acids in leaves and fruits of *Quercus coccifera* is as follows: *Quercus coccifera* leaves: Oleic (0.047mg/g) and ɣ-linoleic (0.033mg/g). *Quercus coccifera* fruits: Oleic (0.048 mg/g) and ɣ-linoleic (0.049mg/g), (Table 5).

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Table 5. Unsaturated fatty acid content in the studied plant (leafs and fruits).

Plants / Fatty acids µg/g		*Quercus coccifera*	
		Leaves	**Fruits**
Mono-unsaturated fatty acid	Oleic	0.047	0.048
poly poly u	ɣ-linoleic	0.033	0.049

Mineral element contents of the Leaves and fruits of the studied Plants

The mineral element constituents of the studied plant are shown in (Table 6). The concentration of macro elements. The high contents of potassium were recorded in fruits and leaves of values 13.5 and 11.5 ppm, respectively. Small amounts of sodium were recorded in leaves and fruits (0.33 and 0.08 ppm), respectively. The contents of calcium, phosphorus, Nitrogen in leaves were as following 1.50 ,2.271, and 0.69 ppm, respectively, whereas the contents of the same elements in fruits were 0.50 ,2.142 and 0.52 ppm, respectively. The contents of Zn, Cu, Cr, and Mg in the leaves were as follows: 0.154, 0.947, 0.0898, and 1.824 ppm, respectively, while their contents in fruits were 0.147,1.132 0.0861, and 1.597 ppm, respectively (Table 6).

Table 6. Mineral contents of Leaves and fruits of studied plants (ppm)

Plant / Elements ppm		Leaves	Fruits
Macro elements	Na	0.33	0.08
	Ca	1.50	0.50
	K	11.5	13.5
	P	2.271	2.142
	N	0.69	0.52
Microele ments	Zn	0.154	0.147
	Cu	0.947	1.132
	Cr	0.0898	0.0861
	Mg	1.824	1.597

Discussion

A lot of researches now taking place everywhere in the world to evaluate the safety, therapeutic use, and the composition of the traditional medicine. The Mediterranean climate in Libya favors the growth of a great number of plant species, some of which have various medicinal and antioxidant properties. There are more than a hundred species used by local people in Libyan folk medicine for medicinal purposes. Recently, free radicals have attracted a great deal of attention from researchers. The free radicals are mainly derived from oxygen and nitrogen, and are produced in our body systems, exposure to physicochemical conditions, or could be related to some diseases. Free radicals can cause adverse effects on lipids, proteins, and oligonucleotides, including DNAs and RNAs, and are also involved in aging and many human diseases [41]. The use of plant extracts and phytochemicals with antioxidant activity can be of great significance in the treatment of many diseases [42].

One of the most important roles played by natural products as therapeutic agents is through their antioxidant activity. Plants can produce a large number of antioxidants to manage the oxidative stress resulting from the sunbeams and oxygen, which makes the plants good sources for the new compounds with antioxidant activity. Antioxidants can be of immense therapeutic importance in the treatment of free radical-linked pathogenesis like cancer, cardiovascular disease, atherosclerosis, and ageing [43]. Estimating the contents of fatty acids and phenolic acids by modern methods as GC-Mass is very important, because these method scan detect very low concentrations of the samples, also to atomic absorption instrument can estimate low levels of heavy metals in many studies on different samples [44 -105].

Conclusion

According to the results recorded in this study, there are different amounts and types of Fatty acids and phenolic acids, beside the presence of important amounts of elements such as potassium, sodium, calcium, phosphorus, and Nitrogen.

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Conflict
No conflict between the results recoded in this study and other studies.

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