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Original article

Using Flame Photometer and Spectrophotometric Methods to Estimate the Minerals, Anti-oxidant Capacity, Total Phenol, and Total Carbohydrate of *Nicotiana Glauca* R.C. Graham. *Plantago Major* L. Sp. Pl and *Phillyrea Latifolia* L. Sp. Pl Plants

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Keywords.
Minerals, Carbohydrate, Antioxidant, Phenols, Plants, Flame, Spectrophotometer.

ABSTRACT

The main aims of this study are to evaluate the concentrations of some minerals, potassium, sodium, and calcium, besides antioxidant, total phenols, and carbohydrate in leaf and stem samples of some plants, including *Nicotiana glauca R.C. Graham, Plantago major L. Sp. Pl* and *Phillyrea latifolia L. Sp. Pl.* selected from some eastern regions of Libya. The methods of the Flame photometer were used to estimate the contents of Minerals, and the Spectrophotometric method was used to estimate the concentration of carbohydrates, total phenols, and antioxidants. The results of this study showed that: all the studied samples containing higher values of potassium comparing with calcium and sodium contents, generally the contents of sodium were ranged from 0.708 to 1.45 ppm in leafs and from 1.68 to 9.88 ppm in stems, whereas the concentrations of potassium fluctuated in the ranges of 3.36 -37.56 ppm in leafs and from 36.96 to 75.76 ppm in stems. On the other side, the concentrations of calcium showed lower values compared with potassium and sodium, where the calcium contents ranged between 0.12 – 0.48 ppm in leaves and from 0.291 to 0.708 ppm in stems**.** The results also recorded that the contents of total phenols ranged between 195.49 – 345.33 ppm; a high content was observed in stems of *Nicotiana glauca R.C. Graham.* On the other side, the amounts of anti-oxidant were ranged from 9.124 ppm to 10.45 ppm in leaves and from 9.54 ppm to 10.008 ppm in *Phillyrea latifolia L. Sp. Pl* plant stems. Whereas the concentrations of total carbohydrate were fluctuated in the ranges from 0.054 to 0151 ppm in leaves and from 0.134 to 0.191 ppm in stems. Also, the phytochemical screening of the aqueous extracts of the selected leaf and stem samples showed the presence of different of natural product compounds.

Introduction

Since ancient times, medicinal plants, also known as medicinal herbs, have been found and utilized in conventional medical procedures. For a variety of purposes, including defense and protection against insects, fungi, illnesses, parasites, and herbivorous mammals, plants produce hundreds of chemical compounds [1-2]. Because they are more accessible and less expensive than contemporary medications, medicinal plants are frequently employed as folk medicine in non-industrialized civilizations. Traditional medicine is not well regulated in many nations, but the World Health Organization organizes a network to promote its safe and sensible use. The market for botanical herbs has come under fire for being ill-regulated and for having pseudoscientific and placebo goods with no scientific backing for their therapeutic claims [3]. In addition to general dangers like habitat destruction and climate change, medicinal plants also suffer the particular hazard of over-collection to satisfy market demand [4]. There are three main types of benefits that medicinal plants can offer: financial benefits to those who harvest, process, and distribute them for sale; health benefits to those who use them as medicines; and societal benefits like employment oppmortunities, tax revenue, and a healthier workforce [5]. However, inadequate funding, shoddy drug development procedures, and scant scientific data all hinder the creation of plants or extracts with potential medical use [5]. The measurements of the chemical constituents of medicinal plants were taken in many studies. In Libya, the medicinal plant studies are one of the most important studies, because Libya has a variety of huge herbal plants [6-25]. The studies of the chemical constituents, metals, and minerals in many plants collected from different locations were established [26-

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60]. This study aims to estimate some of the chemical constituents (Carbohydrates, total phenols, and antioxidants) in some selected plants. Using phytochemicals of leafs and stems. To measure the contents of the (minerals: Na, K, and Ca) in leafs and stems of *Nicotiana glauca R.C. Graham, Plantago major L. Sp. Pl* and *Phillyrea latifolia L. Sp. Pl.*

Methods

Sampling

Three different species of plants were selected in this study, including *Nicotiana glauca R.C. Graham, Plantago major L. Sp. Pl* and *Phillyrea latifolia L. Sp. Pl.* The leafs and stems of each plant were separated. The samples were collected from different locations, including the valley called Wadi Derna, and Karsah in the West, Al-Dhahr Al-Ahmar in the South, and the Mediterranean coast in the North. The study area is located on the second terrace of El-Jabal El-Akhdar mountain that lies in Wadi Derna in the Derna region, north-east Libya, where the Wadi divides the city into two parts, between longitudes (33°00'-32° 30'N and 22°30'- 22°45'E). The elevation of the Wadi ranges between 40m to 300m above sea level. The climate of the study area is comparable to that of El Jabal El Akhdar with a mean temperature of about 20 ^{o}C. The average rainfall ranges between 200- 300 mm.

Sample extraction

10 grams of each dried sample were taken and transferred to a beaker containing 100 ml of distilled water, and the mixture was mixed. Then the extraction was carried out by an evaporator system at 75 ^{0}C. After two hours, the mixture was filtered, and the filtrate was used to determine the phytochemical screening.

Phytochemical Analysis

All the phytochemical screening tests were carried out according to the standard methods in the central lab of the Faculty of Science, Omar Al Mukhtar University. The methods are described by previous studies [7-10].

Test for sterols and/or triterpines: Libermann-Burchard's test

One ml of the alcohol and aqueous extracts of each sample and 0.3 ml of acetic anhydride were added, then a few drops of concentrated sulphuric acid were added along the side of the dry test tube. A reddish-violet color is produced at the junction of the two layers, and the chloroform solution acquires a green color in case of presence of sterols and/or triterpines.

Test flavonoids

The extracts (alcohol and aqueous) of the tested species were further extracted with 1% hydrochloric acid. Each extract was subjected to the following test: 10 ml of each extract was rendered alkaline, where a yellow color is produced in the case of the presence of flavonoids.

Test for alkaloids

The alcohol and aqueous extracts of the tested species were further extracted with 20 ml of dilute hydrochloric acid, cooled, and rendered alkaline with dilute ammonium hydroxide solution, and then extracted with chloroform. The chloroform extract is subjected to the following tests:
Dragendorff, the preparation of the reagent:
Solution (a): About (0.85 g) of basic bismuth nitrate was dissolved in a mixture of 10 ml of acetic acid and 40 ml of distilled aqueous. Solution (b): about (8 g) of potassium iodide was dissolved in (20) ml of aqueous solution. Stock solution: Equal volumes of solutions (a) and (b) are mixed. A few drops of chloroform extract were appmlied to filter paper, allowed to dry, and sprayed with the reagent. Orange color is observed in cases of the presence of alkaloids.

Test for tannins

The extracts (aqueous) of the tested species were further extracted with ethanol 50% then filtered, and the hydro-alcoholic clear solution was subjected to the following test: Ferric chloride test: One ml of the reagent (1% $FeCl_3$) was added to the alcohol and aqueous solution. Blue color develops in cases of the presence of pyrogallol tannins.

Test for anthraquinones

Bornträger's test

One ml of each alcohol and aqueous extracts of the successive aqueous ammonia or caustic soda is added and shaken. Rose-red color in the aqueous layer develops due to the presence of anthraquinones

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glycosides.

Modified-Bornträger's test
One ml of each alcohol and aqueous extracts of the successive extracts of the tested plants is hydrolyzed with alcoholic potassium hydroxide, the acidified and continues as Bornträger's test. Rose-Red develops in the aqueous layer in cases of the presence of anthraquinones.

Test for Saponine
Five ml of tap aqoues is added to (1 ml) of each alcohol and aqueous extracts, then shaken vigorously for five minutes, a froth develops, having (1cm) and persists for (15minutes) indicating the presence of Saponine.

Determination of Phenol Compounds by the Folin-Ciocalteu Method
This experiment was carried out to determine Phenolic compounds, where the amount of total phenolic in the Extracts was determined by (Folin Ciocalteu) reagent according to the method of Slinkard and Singleton (10) using gallic acid as a standard. Samples (two replicates of the sample) were introduced into test cuvets, then 1.0 ml of Folin-Ciocalteu reagent and 0.8 ml of Na_2CO_3 (7.5%) were added. The absorbance of all samples was measured at 765 nm using the Shimadzu UV – Vis spectrophotometer after incubating at 30 °C for 1.5 h. Results were expressed as ppm of fresh weight. of Slinkard and Singleton (10) using gallic acid as a standard. Samples (two replicates of sample) were introduced into test cuvets, then 1.0 ml of Folin-Ciocalteu reagent and 0.8 ml of Na_2CO_3 (7.5%) were added. The absorbance of all samples was measured at 765 nm using the Shimadzu UV – Vis spectrophotometer after incubating at 30 °C for 1.5 h. Results were expressed as ppm of fresh weight.

Determination of antioxidant capacity by the Prussian blue method
One gram of the powdered sample was defatted with petroleum ether. The defatted powder was then extracted sequentially by stirring with 10 ml of methanol twice, then extracted again with 10 ml 1% hydrochloric acid: methanol (v/v). The three combined extracts were evaporated under vacuum, and the residue was dissolved in 10 mL of methanol. Half ml of the solution was diluted with 3 distilled waters, 3 ml (0.008 M) of $K_3Fe(CN)_6$was added,3 ml 0.1M HCl, and 1 ml 1% $FeCl_3$. The blue color is allowed to develop for 5 minutes, and the absorbance is measured at 720 nm at the central lab of the Faculty of Science, Omar Al-Mukhtar University**.**

Determination of 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 completion,the samples were 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. One ml of solution was taken, then one ml of 5% phenol was added. The total carbohydrate was determined by the method carried out in a previous study. Where the absorbance was measured at wavelength of 490 nm.

Determination of Minerals
After digestion of 0.5 gram of each sample by nitric acid (HNO_3). The sodium and potassium, calcium contents were measured by a Flame Photometer (JENWAY Flame Photometer) according to the method described by some studies in the central lab of the Faculty of Science, Omar Al-Mukhtar University.

Results
The results of phytochemical investigation showed the absence of sterols in the *Nicotiana glauca R.C. Graham.* plant and stems of *Plantago major L. Sp. Pl* plant, and presence of high sterols in *Phillyrea latifolia L. Sp. Pl plant.* The flavonoids were observed in leafs of all the studied leaf samples, but they are absent in stems of *Plantago major L. Sp. Pl* plant. Also, the alkaloids are not recorded in the stems of *Plantago major L. Sp. Pl* and *Phillyrea latifolia L. Sp. Pl*. The results also recorded the presence of Tannins in all leafs and stems of samples under investigation, but they are relatively higher contents were detected in stems compared with leafs. The anthraquinones showed higher contents in the leafs of *Plantago major L. Sp. Pl* and *Phillyrea latifolia L. Sp. Ppm*lants comparing with the stems of the same plants, whereas they are completely absent in the leafs and stems of the *Nicotiana glauca R.C. Graham* plant. The saponines were detected in all leaf and stem samples (1&2).

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Table 1. The phytochemical screening of sterols, flavonoids, and Alkaloids for the studied plants

Scientific name	Sterols		Flavonoids		Alkaloids	
Compounds	Leafs	Stems	Leafs	Stems	Leafs	Stems
Nicotiana glauca R.C. Graham.	-	-	+	+	+	+
Plantago major L. Sp. Pl	+	-	+ +	-	+ +	-
Phillyrea latifolia L. Sp. Pl	+ + +	+ + +	+ +	+	+	-

Table 2. The phytochemical screening of of the studied plants

Scientific name	Tannins		Anthraquinones		Saponines	
Compounds	Leafs	Stems	Leafs	Stems	Leafs	Stems
Nicotiana glauca R.C. Graham.	+	+ +	-	-	+ +	+ +
Plantago major L. Sp. Pl	+	+ +	+ + +	+ +	+ +	+ + +
Phillyrea latifolia L. Sp. Pl	+ +	+ +	+ + +	+	+ +	+ +

Total phenols, Anti–Oxidant, and Carbohydrate Contents

The results of this study recorded that the studied plants contained different amounts of total phenols, where the higher values were recorded in stems compared with leafs and ranged from 195.49 ppm in *Plantago major L. Sp. Pl* to 345.33 ppm in stems of *Nicotiana glauca R.C. Graham.* plant, also the results recorded small amounts of anti-oxidant in both stems and leafs, their contents were ranged as follows: from 9.124 ppm to 10.45 ppm in leafs and from 9.54 ppm to 10.008 ppm in *Phillyrea latifolia L. Sp. Pl* plant stems. On the side, the contents of carbohydrate showed lower values in the leafs of *Plantago major L. Sp. Pl* (0.054 ppm), generally no wide variations were observed in carbohydrate contents in the studied plants and fluctuated in the ranges of (0.054 – 0.151 ppm) in leafs and from 0.134 to 0.191 ppm in stem samples (Table 3).

Table 3. The contents (ppm) of Phenols , Anti-oxidant, and Carbohydrate in the studied samples

Scientific name	Total Phenols		Anti-Oxidant		Carbohydrate	
Samples	Leafs	Stems	Leafs	Stems	Leafs	Stems
Nicotiana glauca R.C. Graham.	286.74	345.33	9.41	10.12	0.151	0.134
Plantago major L. Sp. Pl	195.49	305.89	10.45	9.54	0.054	0.191
Phillyrea latifolia L. Sp. Pl	208.59	327.26	9.124	10.008	0.135	0.171

Minerals

This study showed the presence of sodium, potassium, and calcium in leafs and stems of the studied plants. The contents of sodium ranged from 0.708 to 1.45 ppm in leafs and from 1.68 to 9.88 ppm in stems, whereas the concentrations of potassium fluctuated in the ranges of 3.36 -37.56 ppm in leafs and from 36.96 to 75.76 ppm in stems. On the other side, the concentrations of calcium showed lower values compared with potassium and sodium, where the calcium contents ranged between 0.12 – 0.48 ppm in leafs and from 0.291 to 0.708 ppm in stems. The results showed that the potassium recorded higher values in stems compared with leafs and compared the contents of other metals (Na and Ca). On the other side, Calcium contents recorded lower values in all leaf and stem samples. Higher contents of potassium were recorded in stems of *Nicotiana glauca* R.C. Graham plant, followed by leafs of *Plantago major* L. Sp. Pl plant (Table 4).

Table 4. The contents (ppm) of minerals (Na, K, and Ca) in the studied samples

Scientific Name	Leafs			Stems		
	Sodium Na	Potassium K	Calcium Ca	Sodium Na	Potassium K	Calcium Ca
Nicotiana Glauca R.C. Graham.	1.291	12.16	0.48	7.68	75.76	0.541

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Plantago Major L. Sp. Pl	1.45	37.56	0.48	9.88	67.16	0.708
Phillyrea Latifolia L. Sp. Pl	0.708	3.36	0.12	1.68	35.96	0.291

Discussion

This study was carried out on the leafs and stems of three different types of plants collected from AlGabal AlAkhder region, Libya. The phytochemical investigation was carried out according to color tests. The results showed the presence of different natural products of compounds, including flavonoids, alkaloids, anthraquinones, phenols, sterols, and tannins, the tests depend on the color changes after adding specific reagents of each compound. The colors show the amounts of the natural products according to their intensity. The variations of colors between the studied samples are usually coordinated with the presence of different compounds of aromatic chemical compounds. The presence of these compounds gives importance to them for medical and pharmaceutical uses, therefore most of these plants were used for many years in traditional medicine. Every plant produces chemical substances that provide it with an evolutionary advantage, such as salicylic acid, a hormone used in plant defenses, or defense against herbivores [61–62]. If experimentally verified, the pharmacological activity and content of these phytochemicals in medicinal plants provide the scientific foundation for their prospective appmlication as medications in contemporary medicine. For example, nine families of alkaloids, including galantamine, which has been appmroved for use against Alzheimer's disease, are found in daffodils (Narcissus). The toxic and bitter-tasting alkaloids are concentrated in plant portions like the stem that are most likely to be consumed by herbivores; they may also offer protection against parasites [63–64].The Medicinal Plant Transcriptomics Database, which by 2011 had a sequence reference for the transcriptome of over thirty species, is organizing current knowledge about medicinal plants [63]. Examples of plants that contain the major types of plant phytochemicals are given. There are different factors that affect the contents of minerals in plant tissues as the type of soil, the geochemistry of the studied plant locations, and water. Also, the period and duration of sample collection may be affecting the distributions of the mineral contents, many methods as AAS, ICP, XRF, and others, were used to estimate the contents of metals and their composition in different samples [65-75]. Different studies were carried out by many instrumental methods as XRF, atomic absorption, flame photometer [76-95], to estimate the types and contents of minerals and metals in different natural and environmental samples, included soil, sediment, water, plant, vegetable, and others.

Conclusion

The studied samples of *Nicotiana glauca R.C. Graham, Plantago major L. Sp. Pl* and *Phillyrea latifolia L. Sp. Pl.* Plants showed presence of of different amounts of Minerals (Potassium, sodium, and calcium), beside small variations in total carbohydrate concentrations. Also, the contents of anti-oxidants and total phenols not did showed high variations between leafs and stems of the selected plants in this study.

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