

Phytochemical Profiling and Nutritional Assessment of *Artemisia herba-alba*, *Arbutus pavarii*, and *Annona cherimola* from Selected Libyan Regions

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Abstract

The contents of anti-oxidant capacity, total phenol content, mineral concentration, total carbohydrate, and Phytochemical screening of *Artemisia herba alba* Asso, *Arbutus pavarii* Pamp, and *Annona cherimola* Mill were selected from some eastern regions of Libya. The results of this study recorded that the contents of phenols and antioxidant activity were fluctuated in the ranges of (176.01 – 332.046) and (9.42 – 10.64 ppm), respectively. For Carbohydrate, the results showed small amounts in most studied plants in this study, its values ranging between (0.024-0.461) and (0.11-0.142 ppm) for leaves and stems, respectively. For the phytochemical screening, the results of this study showed the presence of different natural product compounds as sterols, flavonoids, alkaloids, tannins, anthraquinones, saponins, and phenols. Also, the results exhibited variations in the contents of these compounds between the selected plants and between leaves and stems. The detection of sterols, flavonoids, alkaloids, tannins, anthraquinones, saponins, and phenols was applied by Color tests according to standard methods. For Potassium, from (1.76 -34.16 ppm) for leaves and from (48.56-84.36 ppm) for stems. For Sodium (0.291-1.375 ppm) for leaves and from (3.48-8.28 ppm) for stems. And for Calcium from (0.08-0.48 ppm) for leaves and from (0.291-0.958 ppm) for stems.

Keywords: Phytochemical Investigation. Chemical Constituents, Plants, Libya

Introduction

Libya is a country in the Maghreb region of North Africa. It has an area of 176,0000 m² consisting mainly of desert (more than 90%) and the Mediterranean coast, Coastal area, and El-Jabal El-Akhdar, known as the green mountain region, which contributes to about 50% of the total plants in the whole country. While the other plants are distributed in regions such as the Al-Jabal Al-Gharbi (Gharian), Ghadames, Fezzan, and Tripolitania regions. The vegetation cover is one of the natural resources of any country in the world, and Libya has a distinct vegetation cover. The interest in studying is increasing day by day in the inventory, definition, classification, and preservation of plants in the herbaria to preserve them from the danger of extinction and disappearance [1]. Al-Jabal Al-Akhdar is an upland region located south of the coastal belt in the north-eastern part of Libya in Cyrenaica. It extends along the coast to about 300 km and rises to some 881 m above sea level, cut by several wades and receiving some 250 - 600 mm of precipitation annually, with red soil or heavy clay. Wadi Derna is one of the most important wades (Valleys) in Al-Jabal Al-Akhdar. The Wadi is located within a Mediterranean climate [2]. Plants have been an important source of medicine since ancient times. Early written reports on the use of plants as medicine appeared around 2600 BC, when plants were used as medicine by [3]. Since then, plants have been used to treat ailments such as headaches, toothaches, stomach aches, diarrhoea, wounds, tumours, and sexually transmitted diseases [4], just to mention a few. However, the potential of several plants as medicinal agents has not been fully characterized and established. A review by Funnell, Lindsey, McGraw, Sparg, and Stafford, states that about 122 drugs were estimated to have been discovered through ethno botanical leads of 94 plant species. In Libya, the medicine plant studies are one of the most important studies, because Libya has a variety of huge herbal plants [5-20]. The studies of the chemical constituents, metals, and minerals in many plants collected from different locations were established [21-42]. This study aims to estimate some of the chemical constituents (Carbohydrates, total phenols, and antioxidants) in some selected plants. Using phytochemicals of leaves and stems. To measure the contents of the (minerals: Na, K, and Ca) in leaves and stems of some selected plants.

Methods

The study area

The study area is a Derna that starts from Al-Fatihah in the East, and the region is mediated by a 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 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 °C. The average rainfall ranges between 200- 300 mm [43] (Figure 1).

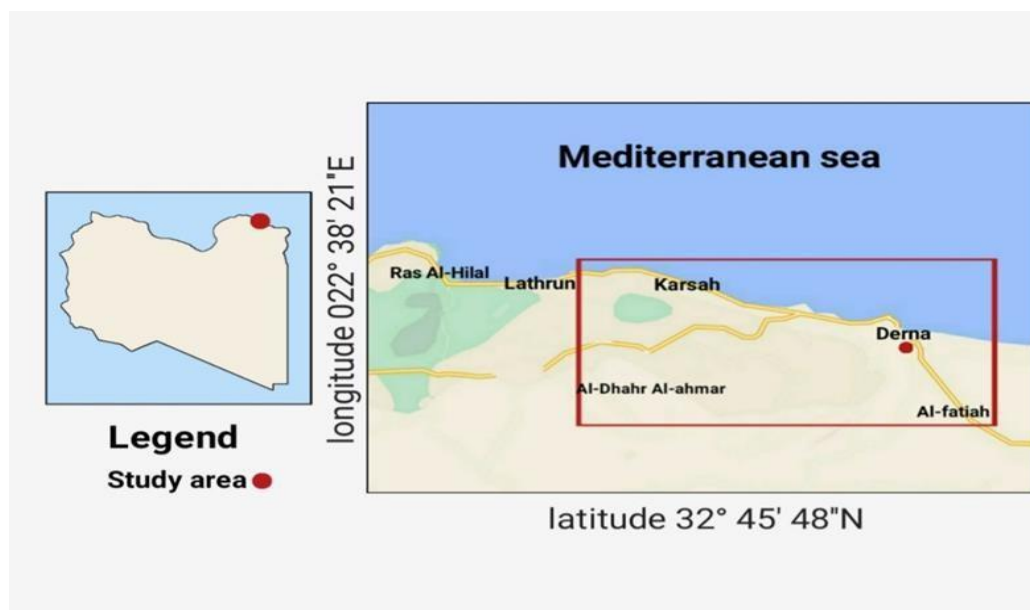


Figure 1. A map showing the study area

Sample collections, and preparation

Three different plant species of *Artemisia herba alba* Asso, *Arbutus pavarii* Pamp and *Annona cherimola* Mill Grow at some Al Gabal AlKhder region were collected. The leaves and stem samples were separated from each plant, then dried in a dry place for one to two weeks. After drying the samples were ground by mortar and stored until analysis.

Sample extraction

10 gram of each derided sample was 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 °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 [20-23].

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 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 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. Stock solution: Equal volumes of solutions (a) and (b) are mixed. A few drops of chloroform extract were applied 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 (alcohol and 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 in the presence of anthraquinone 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 milliliters of aqueous solution 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 (15 minutes), indicating the presence of Saponine.

Determination of Phenol Compounds by Folin-Ciocalteu Method

This experiment was carried out to determine Phenolic compounds, where the amount of total phenolic in the Extracts was determined by the 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 cuvetts, 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 water, 3 mL (0.008 M) $\text{K}_3\text{Fe}(\text{CN})_6$ 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 of the dried sample was ground, then 5 ml of sulphuric acid was added. After, 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 by previous study. Where the absorbance was measured at wavelength of 490 nm. determined by the method was carried out by previous study. Where the absorbance was measured at wavelength of 490 nm.

Determination of Minerals

Soluble sodium and potassium, calcium contents were measured by a Flame Photometer (JENWAY Flame Photometer) according to the method described by some studies [41-45] in the central lab of the Faculty of Science, Omar Al-Mukhtar University.

Table 1. The plant species selected for chemical studies

Scientific Name	Local name	Family
<i>Artemisia herba alba</i> Asso	Shayh	Asteraceae
<i>Arbutus pavarii</i> Pamp.	Shamari	Ericaceae
<i>Annona cherimola</i> Mill.	Qishta	Annonaceae

Results

Tables 2&3 showed the presence of sterols, flavonoids, Alkaloids, Tannins, Anthraquinones, and saponins in the studied plants. The results of this study showed the presence of sterols in all the leaves and stems used in this study; a relative increase in their contents was observed in the stems and leaves of the *Artemisia herba alba* plant. But the sterol compound is completely absent in the *Annona cherimola* plant and stems of the *Arbutus pavarii* plant. The flavonoid compounds were detected in all the studied samples, all of the studied leaf samples exhibit a high content of flavonoids, including stem samples. High contents of alkaloids were recorded in the *Arbutus pavarii* plant. Small contents were observed in *Annona cherimola*. Stems. For the tannin compounds, the results indicated that all the studied plant samples containing tannins, a relative increase of their contents was observed in the stems of *Artemisia herba alba* and *Arbutus pavarii* plants. The Anthraquinones and saponin compounds were detected in all the studied leaf and stem samples; high contents were observed in *Artemisia herba alba*. Plant samples, on the side, lower contents of Anthraquinones and Saponine compounds were present in the *Annona cherimola* plant sample in both leaves and stems.

Table 2. The phytochemical screening of sterols, flavonoids, and Alkaloids of the studied plants

Scientific name	sterols		Flavonoids		Alkaloids	
Compounds	Lea fs	Stem s	Leafs	Stems	Leaf s	Stems
<i>Artemisia herba alba</i> .	++	+++	+++	++	++	++
<i>Arbutus pavarii</i>	++	-	+++	++	+++	+++
<i>Annona cherimola</i> .	-	-	+++	+	++	+

Table 3. The phytochemical screening of the studied plants

Scientific name	Tannins		Anthraquinones		Saponine	
Compounds	Leafs	Stems	Leafs	Stems	Leafs	Stems
<i>Artemisia herba alba</i> .	++	+++	+++	+++	+++	++
<i>Arbutus pavarii</i>	++	+++	+++	++	++	+
<i>Annona cherimola</i> .	++	+	++	+	+	+

Phenols, Anti-Oxidant – Oxidant and Carbohydrate Contents

(Tables 4 & 5) showed that all studied plants contain phenols and antioxidant activity, their values fluctuated in the ranges of (176.01 – 332.046) and (9.42 – 10.64 ppm), respectively. For the phenol compounds, the results recorded increase their values in stems of *Artemisia herba alba* plant compared with other samples, while the lower values of total phenols were observed in *Annona cherimola* plant leaves. On the other side, no wide variations (10.085 – 10.32 ppm) and (9.42-10.65 ppm) were observed for anti-oxidant between stems and leaves. For Carbohydrate, the results showed small amounts in most studied plants in this study, its values ranging between (0.024-0.461) and (0.11-0.142 ppm) for leaves and stems, respectively, in the studied plants in this study.

Table 4. The contents of Phenols (ppm), Anti-oxidant (ppm), and Carbohydrate (ppm) in the studied samples

Scientific name	Total Phenols		Anti-Oxidant		Carbohydrate	
Compounds	Leafs	Stems	Leafs	Stems	Leafs	Stems
<i>Artemisia herba alba</i> .	296.18	332.646	9.42	10.085	0.024	0.111
<i>Arbutus pavarii</i>	327.385	297.867	10.64	10.283	0.461	0.142
<i>Annona cherimola</i> .	176.01	305.658	10.65	10.32	0.099	0.123

Minerals

The results of Minerals showed high contents of potassium in both leaves and stems of the studied samples compared with contents of Sodium and Calcium (Table 5), where the contents of Minerals were ranged as follows: For Potassium, from (1.76 -34.16 ppm) for leaves and from (48.56-84.36 ppm) for stems. For Sodium (0.291-1.375 ppm) for leaves and from (3.48-8.28 ppm) for stems. And for Calcium from (0.08-0.48 ppm) for leaves and from (0.291-0.958 ppm) for stems. In this study, the *Annona cherimola*. Leaves of the plant showed relatively high contents of sodium; on the other hand, the *Artemisia herba alba* leaves of plant showed higher values of potassium, and a relative increase of calcium contents was recorded in *Artemisia herba alba* leaves. The potassium contents recorded higher values in the studied samples compared with the other elements of sodium and potassium

Table 5. The contents of minerals (Na, K, and Ca) in the studied samples

Scientific name	Sodium		potassium		Calcium	
	Leafs	Stems	Leafs	Stems	Leafs	Stems
Artemisia herba alba.	1.125	3.48	34.16	54.76	0.48	0.375
Arbutus pavarii	0.291	6.68	1.76	48.56	0.12	0.291
Annona cherimola.	1.375	8.28	4.76	84.36	0.08	0.958

Discussion

Some of the phytochemical analyses were carried out in this study. Many of the plants were selected. The Chemical investigation included leaves and stems. The deflection of sterols, flavonoids, alkaloids, tannins, anthraquinones, saponins, and phenols was applied by Color tests according to standard methods. For the chemical analysis, most of the selected plants contain very important contents of Natural products, as sterols, phenols, Tannins, anthraquinones, and carbohydrates. Most of the selected plants have antioxidant and phenol compounds, which may be attributed to the presence of different aromatic chemical compounds. Most medicinal plants contain benzene derivatives and different substitutions alkanes, which give special odors to most of them. Plants generally produce several secondary metabolites like phenols, Flavonoids, quinines, tannins, alkaloids, saponins, and sterols, which are important sources of biocides and many other pharmaceutical drugs [44]. Medicinal plants are important in pharmacological research and drug development [45]. About 7.000 medicinal compounds used in the Western pharmacopoeia are derived from plants [46]. The screening of plant extracts led to the discovery of important anticancer compounds such as Camptothecin, Taxol, and Vinblastine, which are used clinically in the treatment of cancer. Camptothecin, Taxol, and Vinblastine were isolated from extracts of Camptotheca acuminata, Taxus brevifolia, and Catharanthus roseus, respectively. Plants are still being used among different indigenous groups to treat different diseases and ailments. Despite this, the compounds responsible for the healing actions in most of the Namibian medicinal plants are not yet investigated due to a lack of scientific studies and exposure [3&4].

Medicinal plants are frequently used as raw materials for the extraction of active ingredients, which are used in the synthesis of different drugs. Like in the case of laxatives, blood thinners, antibiotics, and anti-malarial medications, which contain ingredients from plants. Moreover, the active ingredients of Taxol, vincristine, and morphine are isolated from foxglove, periwinkle, yew, and opium poppy, respectively [4]. The minerals as sodium, potassium and calcium are normally present the natural plants, potassium showed high values in plants due to its importance during some of chemical reactions during photosynthesis process, the contents of minerals in plants also deepened on their contents in the soil surrounding the location of plant samples, the chemical composition of natural soil samples containing high values of calcite, dolomite and different soil ores [48-75] Some studies used different instrumental methods as XRF, atomic absorption, flame photometer [76-89], to estimate the types and contents of minerals and metals in different natural and environmental samples including soil, sediment, water, plant, vegetable, and others.

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Conclusion

The results of this study, which was carried out on the leaves and stems of three plants grown at Alqabal Akhder and around some of Derna city locations, showed the presence of different natural compounds as sterols, flavonoids, phenols, Alkaloids, Tannins, Anthraquinones and saponins in the studied plants. There are some variations of the detected compounds between the leaves and stems; the results also showed high contents of total phenol compounds, besides all the samples exhibited antioxidant activities. Small carbohydrates were recorded in the leaves and stems of the investigated plant, also very important values of potassium were also recorded.

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Conflict of interest. Nil

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