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Comparative study of the chemical composition of leaves and stems of five Lebanese plants

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ABSTRACT

Our study was focused on the quantification and determination of the chemical composition of both aqueous and ethanolic extracts from dried leaves and stems of five Lebanese plants Astragalus coluteiodes, Astragalus angulosus, Euphorbia macroclada, Trigonella berythea and Anacyclus nigllifolius. In order to detect the presence of phenol, saponin, coumarin, tannin, alkaloid, flavonoid and volatile oil, a preliminary phytochemical screening has been done. The quantification of saponin, alkaloids, lipids, ash, elements contents and humidity has been done using the classical methods. The phytochemical screening showed that aqueous and ethanolic extracts from both the leaves and stems of the five studied plants contain polyphenol, flavonoid, tannin, saponin and terpenoid in different concentrations. On the other hand, our plants contain fewer amounts of some heavy metals (Fe, Cd, Mn, Cr and Cu). From our work, it can be concluded that both leaves and stems of the five plants contain good compounds that may be used in the prevention or treatment of some diseases.

Keywords: Lebanese plants, Phytochemical screening, Heavy metals.

INTRODUCTION

Many plants have been used for the different purposes, such as food, drugs and perfumery. Researchers are interested in biologically active compounds isolate from plant species for the elimination of pathogenic microorganisms because of the resistance that microorganisms have built against antibiotics [1].

The protective effects of plant products are due to the presence of several components which have distinct mechanisms of action, some are enzymes and proteins, other are low molecular weight compounds such as vitamins, carotenoids and phenolic compounds [2,3]. The beneficial health-related effects of some phenols or their potential antioxidant properties, especially when these compounds are present in large quantities in foods, are importance to be consumers. The antioxidant compounds present in edible plants have recently promoted as food additives because they display little or no toxic side effects [4].

The ancient use of plants for healing purposes forms the origin of much of modern medicine. Many conventional drugs originate from plant sources: a century ago, most of the few effective drugs were plant based. Examples include aspirin (from willow bark), digoxin (from foxglove), quinine (from cinchona bark), and morphine (from opium poppy). The development of drugs from plants continues, with drug companies engaged in large-scale pharmaceutical screening of herbs [5].

The subject of traditional medicine in Lebanon has received little attention in the literature, as regards the use of medicinal plants. Lebanon is known for the wealth of plant species especially with medicinal properties. In fact 2607 wild species of which 92 are endemics can be found in only 10452 km^2.

This study aimed to compare the chemical composition of two crude extracts, ethanolic and aqueous from dried leaves and stems of five Lebanese plants.

MATERIALS AND METHODS

Chemicals

All the chemicals were used of analytical grade. Absolute ethanol and sodium hydroxide, n-butanol, petroleum ether, ammonium hydroxide, acetic acid, sodium chloride, nitric acid were purchased from BDH England. Hydrogen peroxide, sodium carbonate and were purchased from Unichem, India.

Preparation of crude extracts

10 grams of powdered leaves and stems of five studied plants were putted into a flask with 500 mL of ethanol, and the mixture was then extracted by agitation for 5 h at 25 °C. Then, a maceration of the extracts was done overnight for 24 h. After, the ethanolic layer containing the extract was taken. The extraction was repeated on the remaining amount of the precipitate using 150 mL of ethanol and all extracts were filtered by using a 0.45 millipore filter paper. After that, the two fractions of extracts were mixed together and then concentrated using a rotary evaporator at 40 °C under reduced pressure. Then, the extracts were stored at -20 °C till their usage in the different tests. The extracts resolved in ethanol and distilled water. The aqueous extract has been prepared using the same steps of ethanolic extraction except the temperature of the extraction should be 60 °C [6].

Saponin determination [7]

1 g of powdered plant has been added to 100 mL ethanol (20 %) and kept in a flask on stirrer for half hour and then heated for 4 h at 45 °C with mixing. The mixture was then filtered using a filter paper whatman N 1 and the residue was again extracted with another 100 mL ethanol (25 %). The combined extracts were concentrated by using rotary evaporator at 40 °C to get 40 mL approximately. The concentrate was then transferred into separator funnel and extracted twice with 20 mL diethyl ether. The ether layer was discarded while the aqueous layer was kept and then re-extracted with 30 mL n-butanol. The n-butanol extract was washed twice with 10 mL aqueous sodium chloride (5 %). The remaining solution was evaporated. After that, the samples were dried in the oven at 40 °C to a constant weight. The saponin content was calculated using the following formula:

% Saponin = [final weight of sample / initial weight of extract] × 100

Total alkaloids [7]

100 mL of 10 % acetic acid in ethanol were added to 1 g of dry powdered plant and then the extracts were covered and allowed to stand for 4 h. After that, the extracts have been filtrated and concentrated on a water bath to 25 mL of its original volume. The droplets of concentrated ammonium hydroxide were added to the extract until the precipitation and the whole solution was allowed to settle. Then, the precipitates were washed with dilute ammonium hydroxide and then filtered using filter paper whatman N 1. The residue was dried in the oven at 40 °C and weighed. The alkaloid content was determined using the following formula:

% Alkaloid = [final weight of the sample / initial weight of the extract] × 100.

Determination of total lipids [7]

10 g of powders from leaves and stems of the five plants were added in Soxhlet apparatus with 200 mL of petroleum ether (40-60 °C) and extracted during 8 hours. After that, the solvent was filtered using Büchner funnel under reduced pressure, and then an evaporation using a rotary evaporator at 40 °C has been done. Finally, the weight of lipids has been calculated.

Estimation of the proportion of ash [8]

1 g of powders of leaves and stems of the five studied plants has been tested to estimate the proportion of ash. 1 g was putted in and burned in a furnace burning (muffle furnace) at 550 °C for 5 h till the obtaining of an ovary gray color of the powders. Then, we weighted the residues and the percentage of ash has been determined according to the essential dry weight of plant powder.

Microwave digestion

The method of microwave digestion was used to determine the elements Fe, Mn, Cd Cr, and Cu in dried leaves and stems of the five studied plants. This method provides the acid digestion of the dried plant tissue in a closed vessel device using a temperature control microwave heating (Milstone Ethos DG-AG-02) for the metal determination by spectroscopic methods. Weighed 0.5 g of dried plant samples were placed into microwave digestion inside TFM vessels and 8 mL of a freshly prepared mixture of concentrated HNO_3 (65 %), H_2O_2 (30 %) (7:1, v/v) were added to each vessel and stood for 10 min. The microwave system digestion concentrated on two steps, 10 min for every step on 200 ˚C and the microwave power up to 1000 Watt. The samples left one day and resulting solutions after cooling diluted and the volume was completed to 50 mL with ultra-pure water and then filtrated by 0.25 µ filter units to take the solutions which can be used to determine the metal with atomic absorbance spectrometry.

Determination of humidity content [7]

1 g of fresh leaves and 1 g of fresh stems have been taken and placed in an oven at 105 °C for 1 h. Then, they were putted in a desiccator for half hour. After that, the mass has been noticed. Again, the two samples were returned to the oven for another 1 h. After heating, they were placed again in the desiccator for half hour. These steps have led to dry leaves and stems and their mass has been noticed again in order to calculate the percentage of humidity.

Statistical Analysis

All analyses were carried out in triplicates. The results were performed from the averages of all samples reading Mean ± SD used Excel 2003.

RESULTS AND DISCUSSION

Antioxidants perform multiple functions which includes defending against oxidative damage and cell signaling. One major function of antioxidants in biological system is to prevent the damage of cellular components by reactive oxygen species. The obtained results from the phytochemical screening showed that aqueous extract from both the leaves and stems of five Lebanese plants contains polyphenols, flavonoids, tannins, saponins and terpenoids in different concentrations as reported in Table 1. These results argue those obtained by Farhan et al. [9,10] indicated the presence of various actifs compounds in both leaves and stems of some Lebanese plants. The saponin was present at high amount in all leaves and stems of the five plants. In addition, the aqueous extract from leaves of the plant *A. nigllifolius* contains the higher amount of polyphenol, flavonoid and saponin among the five plants. On the other hand, the aqueous extract from the five plants doesn't contain tannin, resin and alkaloid.

Table 1: Phytochemical screening of aqueous extract from leaves and stems of five plants.

Active compound	Polyphenol	Flavonoid	Saponin	Alkaloid	Resin	Tannin	Terpenoid
Leaves 1	+	+	++	-	+	-	+
Stems 1	+	+	+	-	-	-	+
Leaves 2	+	+	++	-	-	-	++
Stems 2	+	+	++	-	-	-	+
Leaves 3	+	+	++	+	-	-	+
Stems 3	+	+	++	-	-	-	+
Leaves 4	+	+	+	-	-	-	+
Stems 4	+	+	+	-	-	-	+
Leaves 5	++	++	+++	-	-	-	+
Stems 5	+	+	++	-	+	-	+

(1.Astragalus angulosus, 2.Astragalus coluteiods, 3.Trigonella berythea, 4.Euphorbia macroclada, 5.Anacyclus nigllifolius)
+++ = high amount after added of reagent immediately; ++ = moderate amount after 5 minutes of reagent added;+ = low amount after 10 minutes of reagent added and - = absent of active compound after 20 minutes

The obtained results from the phytochemical screening showed that ethanolic extract from both the leaves and stems of five Lebanese plants contains polyphenols, flavonoids, tannins, saponin, alkaloid, resin, tannin and terpenoid in different concentrations as reported in Table 2. The stems of the plants *Astragalus angulosus* and *Euphorbia macroclada* don't contain alkaloid, resin and tannin. Also, the stems of the *Trigonella berythea* don't contain resin and tannin. The phytochemical screening revealed that the ethanolic extract from both leaves and stems contain higher amount of terpenoid in the five plants (Table 2).

Vegetable materials contain various compounds that possess higher antioxidant power. Several plants have been considered as sources of potentially safe natural antioxidants; various compounds have been isolated, many of them being polyphenols. Table 3 shows that the amounts of total lipid, total saponin, total alkaloid, and total ash were higher in the leaves than in stems of all studied plants. However, the humidity was higher in the stems than in leaves. Total saponin was at higher level in the leaves and stems of the plant *A. angulosus.* Also, this total saponin

was higher in the leaves of this same plant than in the stems. On the other hand, total alkaloid was at high level in the leaves of *A. nigllifolius* and in the stems of *T. berythea*. The leaves of *T. berythea* contain high amount of total ash in comparison with the leaves of other plants. Therefore the stems of *A. angulosus* contain the higher level of total ash compared to other stems of other plants. In addition, leaves and stems of *T. berythea* showed a high level of total lipid in comparison with those of the other plants.

Table 2: Phytochemical screening of ethanolic extract from leaves and stems of five plants

Active compound	Polyphenol	Flavonoid	Saponin	Alkaloid	Resin	Tannin	Terpenoid
Leaves 1	+	+	+	+	++	+	++
Stems 1	+	+	+	-	-	-	+
Leaves 2	++	+	+	+	+	+	+++
Stems 2	+	+	+	+	-	+	++
Leaves 3	++	+	+	+	+	+	++
Stems 3	+	+	+	+	-	-	++
Leaves 4	+	+	+	+	-	+	+
Stems 4	+	+	+	-	-	-	+
Leaves 5	++	++	+	+	-	+	+
Stems 5	+	+	+	+	+	+	++

(1.Astragalus angulosus, 2.Astragalus coluteiods, 3.Trigonella berythea, 4.Euphorbia macroclada, 5.Anacyclus nigllifolius)
+++ = high amount after added of reagent immediately; ++ = moderate amount after 5 minutes of reagent added; + = low amount after 10 minutes of reagent added and - = absent of active compound after 20 minutes

The humidity was at high level in both leaves and stems of *A. nigllifolius* and it reaches the 73 % as shown in Table 3.

Table 3: The percentage of active contents in leaves and stems of five Lebanese plants

Plant parts	Total saponin	Total alkaloid	Total ash	Total Lipid	Humidity
Leaves 1	9.2±0.012	3.1±0.014	8.8±0.006	6.9±0.038	65.6±0.009
Stems 1	7.7±0.014	1.8±0.008	8.3±0.003	2.2±0.014	68.05±0.046
Leaves 2	8.9±0.011	2.3±0.011	6.7±0.015	5.3±0.013	62.53±0.005
Stems 2	6.4±0.014	2.6±0.009	4.1±0008	4.5±0.03	67.45±0.05
Leaves 3	5.6±0.019	2.9±0.014	9.2±0.009	11.5±0.056	64.4±0.048
Stems 3	4.8±0.016	2.7±0.010	4.3±0.011	7.22±0.01	72.4±0.072
Leaves 4	3.8±0.009	2.5±0.004	9 ±0.006	7.3±0.012	68.93±0.009
Stems 4	3.4±0.014	1.6±0.007	5.4±0.005	5.6±0.002	72.75±0.044
Leaves 5	5.1±0.015	3.6±0.006	6.5 ±0.017	7.5±0.011	70.61± 0.09
Stems 5	4.5± 0.037	2.5±0.007	5.2±0.005	4.6±0.028	73.12±0.009

Values are the average of triplicate experiments and values expressed as mean ±SD
(1.Astragalus angulosus, 2.Astragalus coluteiods, 3.Trigonella berythea, 4.Euphorbia macroclada., 5.Anacyclus nigllifolius)

Table 4: The amounts of five elements in leaves and stems of five Lebanese plants (mg per g of dry weight)

Plant parts	Fe	Cd	Mn	Cr	Cu
Leaves 1	0.016±0.0001	0.005±0.0004	0.03±0.0001	0.03±0.0003	0.006±0.0002
Stems 1	0.008±0.0007	0.003±0.0008	0.05±0.0002	0.02±0.0009	0.001±0.0006
Leaves 2	0.83±0.0005	0.003±0.0001	0.04±0.0002	0.02±0.0006	0.008±0.001
Stems 2	0.9±0.0001	0.003±0.0007	0.04±0.0004	0.02±0.0003	0.005±0.0001
Leaves 3	0.34±0.0002	0.004±0.0001	0.03±0.0003	0.01±0.0001	0.008±0.0006
Stems 3	0.13±0.0007	0.002±0.0001	0.02±0.0004	0.02±0.0008	0.007±0.0001
Leaves 4	0.09±0.0006	0.002±0.0004	0.03±0.0005	0.02±0.0003	0.004±0.0001
Stems 4	0.05±0.0004	0.003±0.0004	0.01±0.0004	0.01±0.0004	0.002±0.0004
Leaves 5	0.1±0.0003	0.002±0.0003	0.03±0.0002	0.03±0.0002	0.005±0.0001
Stem 5	1.1±0.0007	0.002±0.0001	0.03±0.0001	0.02±0.0002	0.008±0.0001

Values are the average of duplicated experiments and values expressed as mean ±SD
1.Astragalus angulosus, 2.Astragalus coluteiods, 3.Trigonella berythea, 4.Euphorbia macroclada, 5.Anacyclus nigllifolius

Heavy metal contents in spices and medicinal plants depend on climatic factors, plant species, air pollution, and other environmental factors [11]. Monitoring levels of toxic metals in medicinal plants is very interesting as the contamination of the general environment with them has increased [12]. The sources of this environmental pollution are quite varied, ranging from industrial and traffic emissions to the use of purification mud and agricultural expedients, such as cadmium-containing dung, organic mercury fungicides, and the insecticide lead arsenate [13-15]. The results obtained in Table 4 showed that the highest mean levels of cadmium and chromium were detected in *A. angulosus* which were recorded as 0.005, 0.03 mg/g in leaves and 0.003 and 0.02 mg/g in stems respectively. However, the leaves of *A. coluteiod*s contained the highest mean levels of Fe (0.83 mg/g), Mn (0.04 mg/g) and Cu (0.008 mg/g). On the other hand, the highest mean levels of Fe (1.1 mg/g) and Cu (0.008 mg/g) were detected in the

stems of *A. nigllifolius*. Moreover, the highest mean levels of Mn (0.05 mg/g) were detected in the stems of *A. angulosus*.

From the monitoring data revealed that the heavy metal contents in the samples under investigation were recorded at different levels. If we compare the obtained results with the data of [16], who investigated the contents of heavy metals in 291 samples of medicinal plants, grown in unpolluted sites, we can note that our plants contain fewer amounts of all heavy metals under investigation. They reported that the contents of Cd, Cu and Cr, in the plants were 0.39, 6.64 and 1.45 µg/g, respectively.

It can be concluded that some types of metal such as Cu and Mn are the natural essential components of coenzymes and they are important for growth, photosynthesis, and respiration. Other metals such as Cr and Cd had no biochemical or physiological importance, so they are considered as very toxic pollutants

CONCLUSION

Heavy metals are present in the five studied medicinal plants at different concentrations, which, in some cases, exceeded the permissible levels. This could be attributed to the use of contaminated irrigation water, the addition of some fertilizers and herbicides, and also contamination from traffic. Sewage sludge, industrial activities, fuel, and automobile tires can also be significant metal sources. Heavy metals can accumulate in plants through both foliage and root systems. On the other hand, heat treatments of medicinal plants by both hot and boiling water can extract different concentrations of the plant metal content into the used water.

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