



MICROSCOPIC, MICROBIAL AND MOISTURE CONTENT EVALUATION OF THE HERBAL PRODUCTS AFFECTING THE URINARY SYSTEM MARKETED IN SYRIA

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*The use of herbal products has increased in recent times. However, there are insufficient studies on their quality. Finished herbal products and crude plants affecting the urinary system in Syria were evaluated in this study. Most of the loss on drying values were above the constitutional limits. 37% of the product samples exceeded the safety limits ($CFU/g \leq 10^3$) of bacterial growth, and 70.3% of the samples exceeded the safety limit for fungal growth ($CFU/g \leq 10^2$). All samples were free of bacterial pathogens; however, the dominant detected fungal species were *Aspergillus flavus* and *Rhizopus*. The Microscopic evaluation demonstrated the presence of the labeled plants on the product. Nevertheless, substantial quantities of starch grains were detected in samples B2 and B3 of product B, and *Ammi visnaga L.* powder was missing from one of the samples. It is critical to monitor the quality of herbal products before and after marketing.*

Keywords: *herbal drugs, microbiological tests, purity, microscopic evaluation, contamination, moisture content.*

INTRODUCTION

Herbal drugs have a long history of traditional use across the world, with knowledge passed down from multiple generations and are still used in modern times. Their use has evolved from the simple use of crude plants and extracts to the use of the finished herbal products that are available at the pharmaceutical market¹. Moreover, many herbal drugs proved their efficacy in treating urinary system disorders, especially urinary stones^{2,3}. Numerous reasons contribute to the wide use of herbal products like the failure of conventional medicines to control symptoms, a better safety profile, lower cost, and a desire to use a more natural approach for overall health⁴. However, many herbal drugs remain untested, and their use is also unmonitored⁵. It is equally established knowledge that the safety of most

herbal products is further compromised by the lack of suitable quality controls⁶.

International health authorities led by the World Health Organization have established some standards for the quality control of herbal drugs. According to the World Health Organization, the quality of medicinal plant materials should be monitored through a series of tests. They include determination of foreign matter, macroscopic and microscopic examination, thin-layer chromatography, and the determination of the following: ash, extractable matter, water, and volatile matter, volatile oils, bitterness value, hemolytic activity, tannins, swelling index, foaming index, pesticide residues, arsenic, and heavy metals, microorganisms, aflatoxins and radioactive contamination⁷.

Microscopic examinations come at the forefront of the identity tests of plant materials. They represent the first step in identifying any

plant, proving its authenticity, and detecting the fraud. The diagnostic elements of the plant are observed and differentiated from the foreign substances and the elements of other plants. As the therapeutic efficacy of medicinal plants depends on the quality and quantity of the chemical components. Confusion may occur between one plant and another because one of the common names is given to two or more different plants, which is one of the most frequent mistakes⁸. Few studies evaluated the microscopic quality of herbal drugs. *Trigonella foenum graecum* L. seeds were studied microscopically and the characteristic diagnostic elements of the powder were documented⁹. The powder of *Equisetum arvense* L. was also studied microscopically¹⁰. In addition, the powder of *Tribulus terrestris* L. was studied for macroscopic, microscopic and physicochemical parameters¹¹. However, there are no studies available to assess the quality of the finished herbal products from a microscopic point of view, especially in case of the polyherbal products.

In terms of purity, several cases of infections caused by the administration of non-sterile preparation contaminated with microorganisms have been documented^{12,13,14}. The presence of microbial contamination in non-sterile pharmaceutical products can reduce or even inactivate the therapeutic activity of the products and has the potential to adversely affect the health of patients taking the medicines. Microbial contamination of medicinal herbal can be influenced by environmental factors such as temperature, humidity, and extent of rainfall during pre-harvesting and post-harvesting periods, handling practices, and the storage conditions of crude and processed medicinal-plant materials. In order to improve the purity and safety of herbal products, observation of basic hygiene during preparation, standardization of some physical characteristics such as moisture content, pH, and microbiological contamination levels are desirable^{12,15,16}. According to USP, the limits of non-sterile oral products are 10³ CFU/g for bacteria and 10² CFU/g for fungi in addition to the absence

of *Salmonella*, *Escherichia coli* and *Staphylococcus aureus*¹⁷.

Moisture content is an inevitable part of the crude drug that needs to be eliminated. An excess of water in herbal materials will encourage microbial growth, the presence of fungi or insects, and deterioration following hydrolysis. The moisture content is determined by several methods. Among them, the WHO has typically prescribed the determination of the loss on drying (LOD). This method is employed to determine the loss of water and volatile matter through heating of the sample¹². In Syria, there are no studies present to prove the quality of finished herbal drugs, especially after 201¹⁸. The Syrian pharmaceutical market provides several herbal products that affect the urinary system. The hard capsule products contain dry powders of single plants such as *Ammi Visnaga* L., *Trigonella foenum-graecum* L., and *Tribulus terrestris* L. The polyherbal product contains the four powders of *Ammi visnaga* L., *Arctostaphylos uva-ursi* L., *Equisetum arvense* L., and *Juniperus communis* L. This study aimed to assess the microscopic characteristics, microbiological contamination, and moisture content of the finished herbal products and crude plants affecting the urinary system locally marketed in Syria.

MATERIALS AND METHODS

Experiments of this study were carried out in the pharmacognosy and microbiology laboratories of the faculty of pharmacy at Al-Baath University in Homs, Syria.

Herbal Samples

Twenty-seven samples (Table 1) of herbal products were purchased from the Syrian pharmacies (three batches of each nine products). Also, crude plants of *Ammi visnaga* L. fruits, *Juniperus communis* L. cone berry, *Equisetum arvense* L. arial parts, *Solidago virgaurea* L. arial parts, *Trigonella foenum-graecum* L. seeds, *Arctostaphylos uva-ursi* L. leaves and *Tribulus terrestris* L. fruits were sourced from various traditional herbal shops in Syria (Table 2). Crude plants were used as comparative materials to the finished products.

Table 1: Samples of the finished herbal products

Product code	Samples N.	Product's contents	Dosage forms
A	3	<i>Ammi visnaga L.</i>	Hard capsules
B	3	<i>Ammi visnaga L.</i> , <i>Juniperus communis L.</i> , <i>Equisetum arvense L.</i> , <i>Arctostaphylos uva-ursi L.</i>	Hard capsules
C	3	<i>Trigonella foenum-graecum L.</i>	Hard capsules
D	3	<i>Tribulus terrestris L.</i>	Hard capsules
E	3	<i>Arctostaphylos uva-ursi L.</i>	Hard capsules
F	3	<i>Solidago virgaurea L.</i>	Hard capsules
G	3	Pinene ($\alpha + \beta$), Camphene, Cineol, Fenchone, Borneol and Anethol.	Soft capsules
H	3	Cucurbita (Pumpkin) seeds oil	Soft capsules
I	3	Pinene ($\alpha + \beta$), Camphene, Cineol, Fenchone, Borneol and Anethol.	Oral drops

Table 2: Crude plants samples

Crude plants	Used parts	Family
Khella (<i>Ammi visnaga L.</i>)	Fruits	Apiaceae
Bearberry (<i>Arctostaphylos uva-ursi L.</i>)	Leaves	Ericaceae
Juniper (<i>Juniperus communis L.</i>)	Fruits	Cupressaceae
Horsetail (<i>Equisetum arvense L.</i>)	Air parts	Equisetaceae
Goldenrod (<i>Solidago virgaurea</i>)	Flowers	Asteraceae
Fenugreek (<i>Trigonella foenum-graecum L.</i>)	Seeds	Fabaceae
Tribulus (<i>Tribulus Terrestris L.</i>)	Fruits	Zygophyllaceae

Chemical and Equipment

The following materials were used: Nutrient agar and MacConkey agar were obtained from (HiMedia-India), Sabauraud dextrose agar was obtained from (Accumix-India), Gram staining, H₂O₂ 20%, sterilized water, incubator from (UK-Carbolite), alcohol 70% from (Sigma-Aldrich, Germany), sterilized Loup, Petri dishes, sensitive electronic scales, Precisa-XB220, Switzerland, moisture scale, Precisa HA60, Switzerland, optical microscope (Micros-Austria 1500x magnification), Sony Xperia L camera with 13mp magnification, automatic milling machine, chloral hydrate solution 60%, iodine solution 1%. All chemicals were of analytical grade.

Microscopic Evaluation

Microscopic evaluation was conducted on products A, B, C, D which are hard capsules containing raw plant powder. Crude plants of *Ammi visnaga L.*, *Juniperus communis L.*, *Equisetum arvense L.*, *Trigonella foenum-graecum L.*, *Arctostaphylos uva-ursi L.* and *Tribulus terrestris L.* were studied microscopically and used as standards for comparison with the products.

Samples preparation for microscopic tests

Grinding the standard plants was done using an automatic grinding device until reaching a fineness level that is macroscopically appropriate and compatible with the fineness of the studied capsules' powder. Samples were studied using a light microscope after fixation with chloral hydrate solution 60% with heating. Microscopic evaluation was done using eye lenses 10 and 40. Photographs of the samples were taken using a Sony Camera 13 Megapixel.

Detection of starch grains

By adding the potassium iodine solution to the samples slide; the starch grains are colored in purple under the microscope in the presence of starch.

Determination of LOD

Moisture content was evaluated for the products A, C, D, E, F.

Each of them consists of only a single plant powder (Table 1). The crude plants (Table 2) were evaluated too and used for comparison. The constitutional limits of LOD are shown in Table 3¹⁹⁻²¹. For moisture content,

single herb product and crude plants were dried at 105° C for 2 hrs or until their weights remain constant and the difference in weight was calculated from the equation:

Percentage of LOD = material before drying - weight of material after drying / weight of material after drying × 100.¹⁷

Evaluation of microbiological contamination

The microbiological quality of the products was evaluated by following the indications of the American Pharmacopoeia (USP 41, 2018) for microbiological examination of oral non-sterile products with some modifications in accordance with WHO guidance references⁷. These criteria establish a limit of 10³ CFU (Colony-forming unit) of bacteria and 10² CFU of fungi per gram of the product and the absence of *Salmonella*, *E. coli* and *S. aureus*. The specific monograph of *Trigonella foenum-graecum* L powder in USP allows a bacterial growth of 10⁴ CFU/g and fungal growth of 10⁵ CFU/g¹⁷.

Total viable aerobic count

The culture tubes were prepared using distilled water, then diluted in a sufficient ratio to obtain an aqueous suspension that could be implanted and counted (10/100). The preservative efficacy was canceled by dilution to 100 ml. The spread plate method was used

and 0.1 ml of each tube was transferred to Nutrient Agar plates for bacteria and to Sabouraud dextrose Agar plates for fungi and to MacConkey Agar plates for detection of Gram-negative and enteric bacteria. Two plates for each level of dilution. The plates were incubated for 24 hrs at 37°C for bacterial and for 7 days at 25°C for fungal grown. After the incubation, colonies grown on the medium were counted. The number of colony-forming units (CFUs) per gram of sample was calculated, taking the dilution factor into account. A Gram stain was performed on each culture.

Isolation and identification of fungal species

Identification of fungi was done on the basis of morphological and cultural characteristics as described by references²².

RESULTS AND DISCUSSION

Loss on drying

The moisture content of plant material is important from a stability perspective. The moisture content affects the microbial growth, enzymatic activity, the bulk of the material which helps in the storage of the plant material, and after drying, further comminution and size reduction becomes easier for the drug¹².

Table 3: The results of LOD of the four crude plants' powders and the finished herbal products

Sample No.	Samples	Average LOD (%)	limits of LOD
1	<i>Ammi visnaga</i> L. powder	10.5%	10%
2	A1	8%	10%
3	A2	8.9%	10%
4	A3	11%	10%
5	<i>Trigonella foenum-graecum</i> L. powder	16.2%	11%
6	C1	14%	11%
7	C2	16.5%	11%
8	C3	17%	11%
9	<i>Tribulus terrestris</i> L. powder	13.9%	11%
10	D1	15.5%	11%
11	D2	14.8%	11%
12	D3	12.6%	11%
13	<i>Arctostaphylos uva-ursi</i> L. powder	11.4%	10%
14	E1	12%	10%
15	E2	11%	10%
16	E3	9.4%	10%
17	<i>Equisetum arvense</i> L. powder	13.5%	10%
18	<i>Juniperus communis</i> L. powder	12%	12%

Table (3) shows the results of loss on drying of the four crude plants' powders and the finished herbal products which consist of single plant powder, namely products A, C, D, and E. The mean LOD of the three selected samples of each product was compared to the mean LOD of each crude plant. Among product A samples, A3 was the highest in LOD values while the other samples were within the permissible LOD limits for *Ammi visnaga* L. powder. However, the crude powder of *Ammi visnaga* L. was above the permissible limits. For *Trigonella foenum-graecum* L. and *Tribulus terrestris* L. powders, LOD values were the highest in both crude plants and finished herbal products. Similarly, *Arctostaphylos uva-ursi* L., *Juniperus communis* L., and *Equisetum arvense* L. powders had also exceeded the permissible limits for LOD²³. The resulting LOD values may indicate a high moisture content, which can be attributed to various influences, however, storage conditions and drying methods represent the most prominent factors²⁴.

Bacterial and Fungal Contamination

The mean CFU for bacterial and fungal load of all samples of crude plants (Table 4) ranged from 2×10^4 CFU/g (*Ammi visnaga* L. and *Solidago virgaurea* L.) to 7.5×10^6 CFU/g (*Trigonella foenum-graecum* L.). Samples of *Trigonella foenum-graecum* L. had the highest contamination levels (7.3×10^6 CFU/g) for bacterial growth and (7.5×10^6 CFU/g) for fungal growth. In contrast to that, the samples of *Solidago virgaurea* L. were the least contaminated (2×10^4 CFU/g) for bacterial growth and (2.2×10^4 CFU/g) for fungal growth. The United States Pharmacopeia¹⁷ draws the following specifications for non-sterile

products for oral use: 10^3 aerobic bacteria/g, 10^2 fungi/g and absence of *Salmonella* spp, *E. coli* and *S. aureus*. However, the specific monograph of *Trigonella foenum-graecum* L powder in USP allows a bacterial growth of 10^4 CFU/g and fungal growth of 10^5 CFU/g¹⁷. Results showed that all crude plant samples exceeded the constitutional limits (Table 4).

As for the bacterial species in the crude plants (Table 5), *Staphylococcus aureus* was detected in *Equisetum arvense* L. powder. *Klebsiella* was detected in *Juniperus communis* L. powder. *Aspergillus* species were also the main contaminant (Table 5). Sources of contamination vary here as crude plant powders are not subjected to sterilization before selling; they are rather sold to the public as mixes for instant consumption. Stored in poor conditions, and repeatedly opened when quantities are deducted for sale, which makes them exposed to air and contaminants. Elevated temperatures during storage, especially in the summer, also result in high humidity. Thus, the water activity increases leading to increased bacterial and fungal growth. Another possible source of contamination is irrigation water and the harvest procedures. Contaminated water leads to contaminated plants. Inappropriate harvest procedures may also contribute to contamination levels. Plants can be mixed unintentionally with sand, other plant parts, and foreign materials. Also, the source of these plants is an important factor as they may be contaminated during transportation from the country of origin^{12&25}. The resulting high contamination values of the crude plants are in line with previous studies that reported the contamination of plants sold as herbal treatments^{14&26}.

Table 4: Microbial load of crude plants

Crude plant powder	Aerobic organisms (CFU/g) ⁽¹⁾	Fungi (CFU/g) ⁽²⁾
Ammi visnaga L.	5×10^5	2×10^4
Arctostaphylos uva-ursi L.	2.7×10^4	2.5×10^4
Juniperus communis us cominuis L.	3.2×10^5	10^5
Equisetum arvense L.	10^6	2×10^5
Solidago virgaurea L.	2.2×10^4	2×10^4
Trigonella foenum-graecum L.	7.3×10^6	7.5×10^6
Tribulus Terrestris L.	2×10^6	6×10^6

Key: Cfu/g = Colony forming unit per gram, (1) USP limits is no more than 10^3 cfu/g, (2) USP is no more than 10^2 cfu/g

Table 5: Identification of the bacterial and fungal species in the crude plant samples

Crude plant powder	Bacterial species	Fungal species
Ammi visnaga L.	<i>bacillus</i>	<i>Penicillium + Aspergillus niger</i>
Arctostaphylos uva-ursi L.	<i>bacillus</i>	<i>Aspergillus niger+ Rhizopus</i>
Juniperus communis L.	<i>Klebsiella</i>	<i>Aspergillus flavus +A. niger</i>
Equisetum arvense L.	<i>Staphylococcus Aureus</i>	<i>Aspergillus niger+ Rhizopus</i>
Solidago virgaurea L.	<i>bacillus</i>	<i>Aspergillus niger</i>
Trigonella foenum-graecum L.	<i>bacillus</i>	<i>Aspergillus flavus +A. niger+ candida spp</i>
Tribulus Terrestris L.	<i>bacillus</i>	<i>Aspergillus flavus+ candida spp</i>

As for the finished herbal products (Table 6), product A contains *Ammi visnaga* L. powder, all samples were contaminated with fungi above the constitutional limits. Surprisingly, all samples of product B were within the permissible limits of bacterial growth. Product B is a polyherbal capsule that contains four plant powders: *Ammi visnaga* L., *Juniperus communis* L., *Arctostaphylos uva-ursi* L., and *Equisetum arvense* L. This observation is in line with previous studies which found that the bacterial growth of polyherbal drugs was within the permissible limits²⁷. On the contrary, all samples of products C and product D had exceeded the permissible limits of bacterial and fungal growth. Product C contains *Trigonella foenum-graecum* L. seeds powder. Product D contains *Tribulus terrestris* L. fruits powder. Previous studies had reported major poisoning incidents due to contaminated *Trigonella foenum-graecum* L. seeds with *Escherichia coli*²⁸. The drying method also plays a major role in the moisture content of the *Trigonella foenum-graecum* L. seeds. Insufficient drying increases the moisture content resulting subsequently in bacterial and fungal contamination²⁹. The average LOD for *Trigonella foenum-graecum* L. and *Tribulus terrestris* L. was the highest either in crude form or in finished products (Table 4). In addition to that, *Trigonella foenum-graecum* L. seeds were reported to be contaminated with different types of fungi in Sudan³⁰.

As for *Tribulus terrestris* L., it grows as a harmful weed in crops, fields, roadside edges and cultivated fields, which make *Tribulus*

subjected to various contamination sources³¹. On the other hand, all samples of product F were within the bacterial permissible limits. It can be explained by the fact that product F is a dry extract of *Solidago virgaurea* L. aerial part. The extraction process reduces the bacterial load, especially if it includes heating to high temperatures²⁷. Moreover, *Solidago virgaurea* L. may reach to 1-meter length so the used part is more distant from potentials sources of contamination such as soil and water. Similarly, all samples of products G and H were within the permissible limits for bacterial and fungal growth. Both products consist of oily capsules, which may not constitute a suitable medium for the growth of bacteria and fungi. On the contrary, all the samples of the product I had exceeded the permissible constitutional limits of bacterial and fungal growth. Product I is an oral drop containing the same essential oils as Product G. Oral drops are water-based and suitable for the growth of bacteria and fungi. Previous studies had also reported the contamination of oral herbal products^{32&33}.

In light of the microbiological results of this study, the bacterial and fungal load of the crude plants may explain the high contamination values of the finished products. This observation is in agreement with previous studies and reports that investigated the contamination of raw plants and finished products^{24&34}. The resulting bacterial colonies in all products were of *Bacillus*. No pathogens were detected in any of the samples of the finished products. Furthermore, the predominant fungal species in the products

were *Rhizopus*, *Aspergillus niger*, and *Aspergillus flavus*. *Candida* genus and *Penicillium* were also detected in some samples. These observations are in agreement with reports by other researchers who investigated fungal contamination of spices and herbal products^{35&36}. *Aspergillus* species were also the main contaminant in different herbal products and spices samples in previous reports³⁷. Most of the identified molds have been reported to have ability to produce mycotoxins that are of great hazard if consumed by both humans and animals³⁸. Mismanagement of medicinal herbs during processing, storage, and transportation and their intrinsic factors and external environmental conditions all contribute to the

mycotoxigenic fungi contamination³⁹. Traditionally, fungi in medicinal herbs have been divided into two distinct classes: field fungi (e.g., *Fusarium*, *Alternaria*), which invade and produce their toxins before harvest; and storage fungi (e.g., *Aspergillus*, *Penicillium*), which become a problem after harvest⁴⁰. Furthermore, some fungi (e.g., *Aspergillus*) might belong to both classes before and after harvest. As noticed, the predominant microflora isolated in this study were storage fungi which highlight the necessity of better storage conditions. Some of the environmental factors can be controlled by implementing Good Manufacturing Practice (GMP) for producing these medicinal products from herbal or natural sources.

Table 6: Bacterial and fungal load of finished herbal products

Product code	Bacterial count	<i>E. Coli</i>	<i>Staph. Aureus</i>	<i>Salmonella</i>	Fungi	Fungal species
A1	-	-	-	-	2×10 ³	<i>Penicillium + Rhizopus</i>
A2	1.2×10 ²	-	-	-	3×10 ³	<i>Penicillium + Rhizopus+ candida</i>
A3	28×10 ³	-	-	-	10 ³	<i>Rhizopus</i>
B1	4.8×10	-	-	-	10 ²	<i>Rhizopus</i>
B2	-	-	-	-	10 ⁵	<i>Aspergillus flavus+ A.niger</i>
B3	-	-	-	-	2×10 ²	<i>Rhizopus</i>
C1	5×10 ⁶	-	-	-	10 ⁶	<i>Candida</i>
C2	6.2×10 ⁶	-	-	-	1.1×10 ⁶	<i>Candida</i>
C3	5.5×10 ⁶	-	-	-	1.7×10 ⁶	<i>Rhizopus</i>
D1	7×10 ⁶	-	-	-	10 ⁶	<i>Aspergillus flavus+ Candida</i>
D2	6.3×10 ⁶	-	-	-	2×10 ⁶	<i>Aspergillus niger+ Candida</i>
D3	8×10 ⁶	-	-	-	10 ⁶	<i>Aspergillus flavus+ Candida</i>
E1	2×10 ²	-	-	-	10 ³	<i>Aspergillus flavus+ A.niger</i>
E2	10 ³	-	-	-	3.1×10 ³	<i>Aspergillus flavus+ Rhizopus</i>
E3	-	-	-	-	2×10 ⁴	<i>Rhizopus</i>
F1	-	-	-	-	10 ³	<i>Aspergillus niger+ Rhizopus</i>
F2	3×10 ²	-	-	-	3.3×10 ⁴	<i>Aspergillus niger+ Rhizopus</i>
F3	-	-	-	-	2×10 ²	<i>Aspergillus niger</i>
G1	-	-	-	-	10 ²	<i>Aspergillus flavos</i>
G2	1×10 ³	-	-	-	10 ²	-
G3	-	-	-	-	-	<i>Aspergillus flavos+A.niger+ Rhizopus</i>
H1	-	-	-	-	-	-
H2	-	-	-	-	2×10	<i>Aspergillus niger</i>
H3	2×10 ²	-	-	-	-	-
I1	2×10 ⁴	-	-	-	1.5×10 ²	<i>Aspergillus niger</i>
I2	10 ⁵	-	-	-	1.7×10 ²	<i>Aspergillus niger</i>
I3	3.2×10 ³	-	-	-	1.1×10 ²	<i>Aspergillus niger</i>

Key: - = No growth, Cfu/g = Colony forming unit per gram, (1) USP limits is no more than 10³cfu/g, (2) USP is no more than 10²cfu/g

The Microscopic Evaluation

Product A

The microscopic test was conducted on product (A) and the crude powder of *Ammi visnaga* L. which used as comparative material. The powder of product A was dark brownish-black, gritty in texture, with a faint aromatic odor and a bitter taste which corresponds with the organoleptic characters of *Ammi visnaga* L. powder. The microscopic evaluation showed characteristic microscopic features of *Ammi visnaga* L. fruits powder (fig 1), which are: the large-celled parenchyma of the mesocarp, traversed by the schizogenous vittae; the cells are irregularly rectangular and have moderately thickened walls with dark brown pigment. Also, the testa was observed in all samples, which is composed of one or two layers of brown-pigmented, thin-walled cells, associated with the endosperm. The endosperm consists of polygonal, thick-walled, cellulosic parenchyma containing fixed oil, aleurone grains and microsette crystals of calcium oxalate. Moreover, one of the most important distinguishing elements of *Ammi visnaga* L. was the innermost layer of mesocarp which consists of large, polygonal, brown-walled cells, with thick, porous inner walls, it appeared in all samples. Furthermore, starch grains and hairs were absent in all samples. Groups of lignified vessels appeared in the crude powder and fragments of the endocarp cells. No foreign substances appeared in any of

the samples, however, all samples showed oil droplets which are from the fixed oil in the endosperm. These findings correspond to *Ammi visnaga* L. comparative crude plant powder and the references^{19&41}.

Product B

Product B consists of four plant powders which are: *Ammi visnaga* L. fruits powder, *Arctostaphylos uva-ursi* L. leaves powder, sterile aerial parts powder of *Equisetum arvense* L. and ripe cone berry powder of *Juniperus communis* L. Before the microscopic evaluation of the samples of product B, each of the standard four plants powder was studied individually and used as a comparative powder. *Ammi visnaga* L. fruits powder was shown in figure 1 and the observed pharmacognostic elements of each of the three plant powders are included in fig 2. *Arctostaphylos uva-ursi* L. leaves powder was green with bitter taste and aromatic odor. Fragments of the epidermis were observed via 40x lenses; the upper epidermis was free of stomata, composed of large polygonal cells with straight walls. The lower epidermis, however, showed anomocytic stomata, the cells were similar to those of upper epidermis but were smaller. The powder also contains prisms of calcium oxalate, scattered in the parenchymatous cells, groups of thick fibers and groups of vessels. The trichomes were unicellular^{21&41}

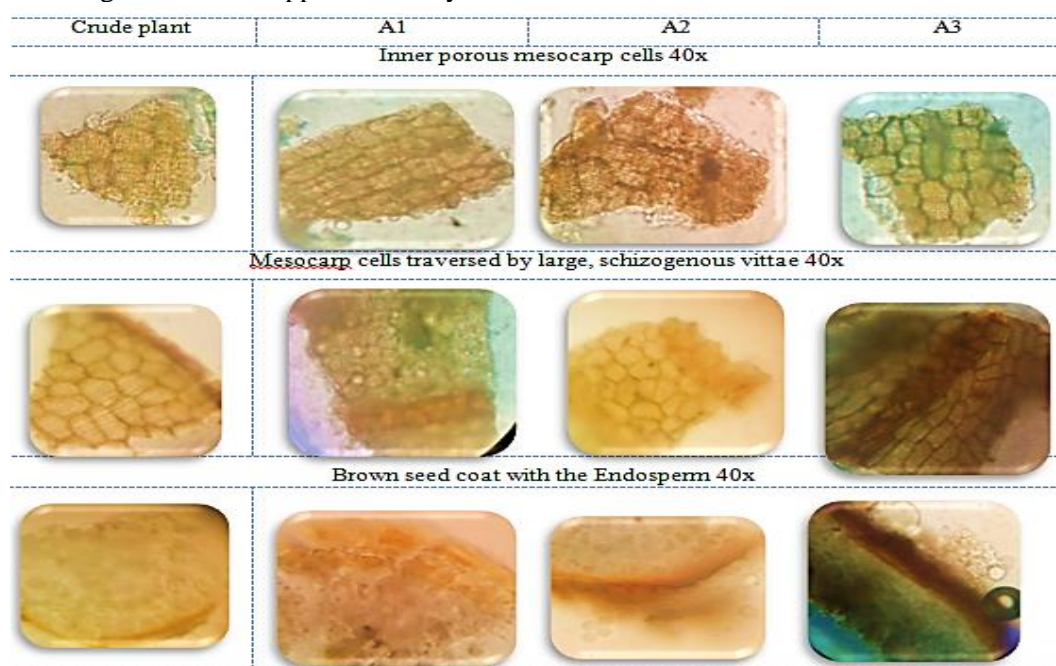


Fig. 1: The Pharmacognostical elements of the product A powder observed using a light microscope 40x

As for *Equisetum arvense* L. powder, it was a greenish grey odorless powder with no taste. The characteristic elements were; fragments of the epidermis in surface view which consists of elongated, wavy-walled cells, silicified, and thickened and the paracytic stomata with the two subsidiary cells cover the guard cells. Fragments of the epidermis in sectional view were also observed with the protuberances formed from the contiguous mass of 2 U-shaped cells. The powder was rich in small silica pilulae which were scattered on the surface of the subsidiary cells. Moreover, fragments of large-celled parenchyma appeared and the spirally thickened vessels²¹

Juniperus communis L. cone berry powder was brown with strong aromatic odor. The diagnostic characters were; fragments of

epidermis of the cone berry wall containing cells with thick, pitted, colourless walls and brown glandular content, with anomocytic stomata. It was also observed the fragments of the hypodermis with collenchymatous thickened cells and fragments of the mesocarp consisting of large thin-walled parenchymatous cells, rounded, with large intercellular spaces and irregular, large, scarcely pitted yellow idioblasts (barrel cells) which were the most dominant elements of the powder. fragments of the testa were observed with thick-walled, pitted, colourless scleroids containing one crystal of calcium oxalate, and fragments of the endosperm and embryonic tissue with thin-walled cells containing fatty oil and aleurone grains appeared too²¹.

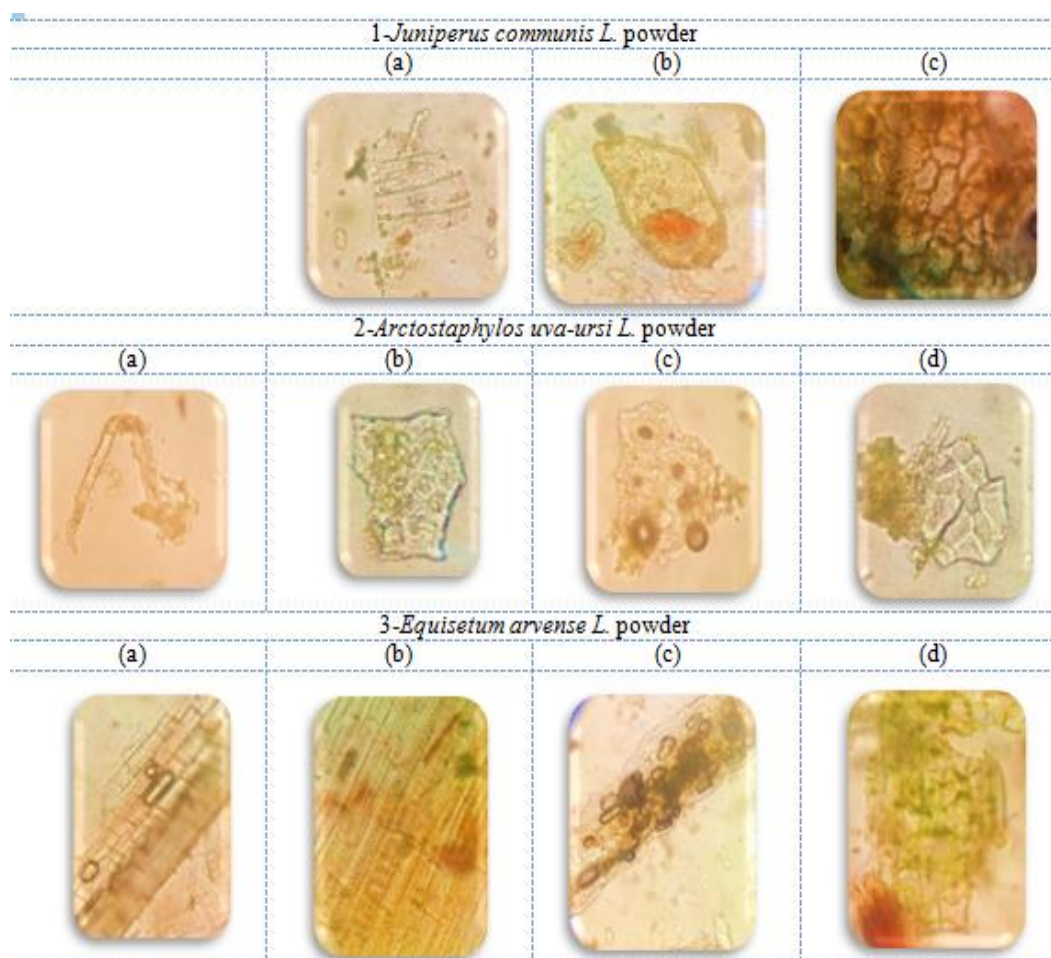


Fig. 2: The Pharmacognostical elements of *Juniperus communis* L. powder, *Equisetum arvense* L. powder and *Arctostaphylos uva-ursi* L. powder. *Juniperus communis* L. powder: (1-a) fragments of the testa with the scleroids containing 1 crystal of calcium oxalate (1-b) idioblast cells of the mesocarp (1-c) epidermis of the cone berry wall. *Arctostaphylos uva-ursi* L. powder: (2-a) trichomes (2-b) spirally thickened vessels (2-c) fragments of lower epidermis with anomocytic stomata (2-d) fragments of upper epidermis. *Equisetum arvense* powder: (3-a) the elongated cells (3-b) vessels with spiral thickening (3-c) Fragments of the epidermis in sectional view (3-d) Fragments of the epidermis in surface view.

Samples of product B

Figure 3 shows the microscopic results of the samples of product B. Fragments of epidermis of the cone berry wall of *Juniperus communis* L. powder were observed in all samples of the product B as well as fragments of the mesocarp cells (barrel cells). As for *Arctostaphylos uva-ursi* L. leaves powder, fragments of upper epidermis were observed in all samples. The epidermis cells were straight walled with no stomata. All samples of product B indicated the presence of *Equisetum arvense* L., as the epidermis of the main stems in surface view which consists of elongated cells were observed in all the samples. However,

Ammi visnaga L. pharmacognostical elements were observed in only the first and the second samples of product B. The absence of *Ammi visnaga* L. powder may be by mistake or in purpose. Starch grains were observed in two samples of product B. In fact, some references reported that starch grains may be present in *Equisetum arvense* L. powder in small amounts, but they do not present in these large quantities as it was observed in samples B2, B3. So, the presence of this amounts of starch grains suggests mixing product B powders with starch powder^{19,21,41}

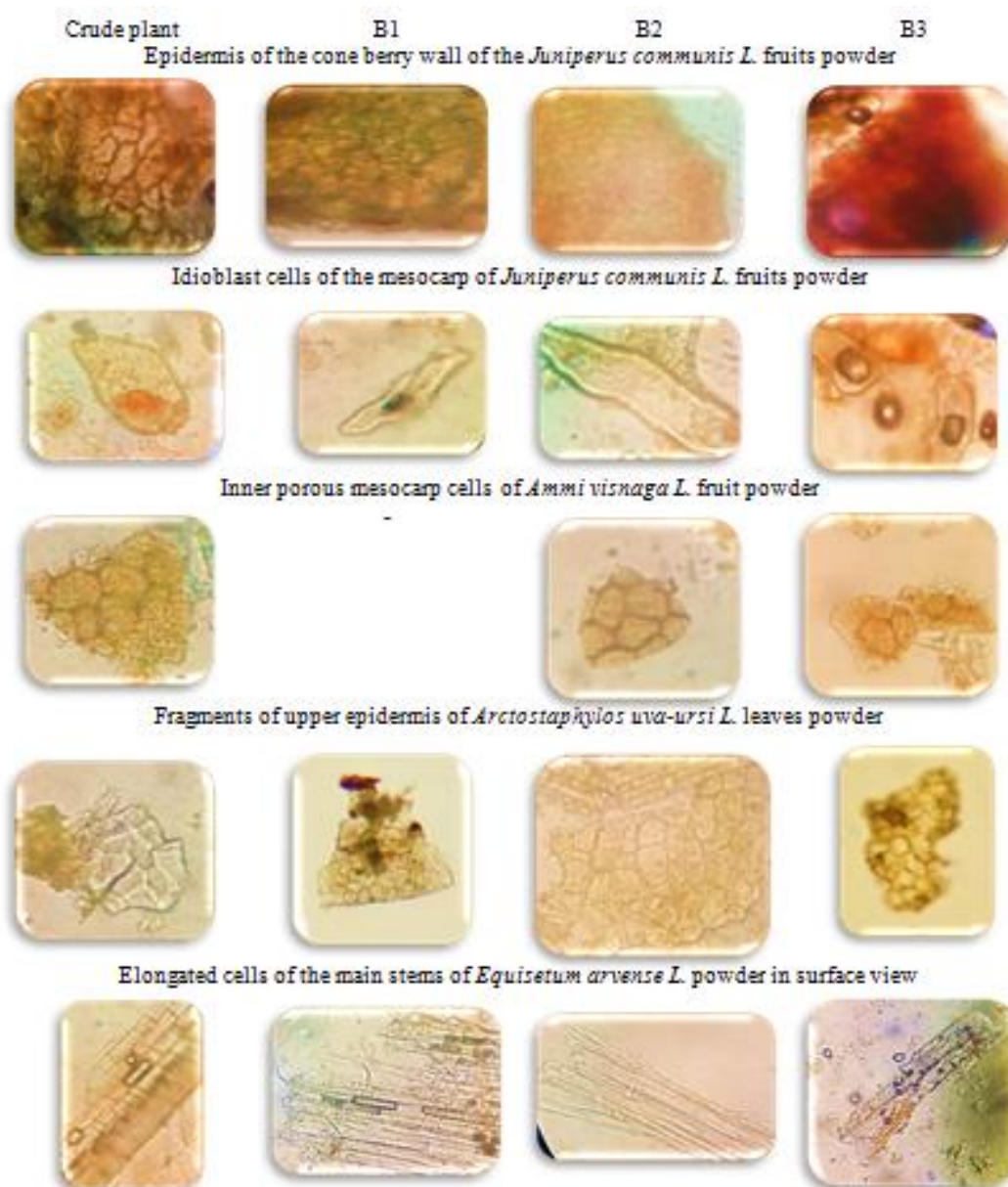


Fig. 3: The Pharmacognostical elements of the samples of the product B via 40X

Product C

Consists of the dried ripe seeds of *Trigonella foenum-graecum* L. (Fabaceae). The crude plant powder was used as a comparative material. The powder of product C is yellowish-brown with a characteristic, spicy odor, and a strong, characteristic taste which is mucilaginous at first and then slightly bitter and unpleasant. This corresponds with the organoleptic characters of *Trigonella foenum-graecum* L. powder.¹⁵ The observed diagnostic characters of the powder (Fig 4) were; fragments of the epidermis in surface view, composed of small polygonal cells with

thickened and pitted walls which appeared in all samples of the product. Fragments of the hypodermis viewed from below, were also observed, they were composed of polygonal cells with bar-like thickenings extending to the upper and lower walls. Also, fragments of endosperm containing mucilage appeared in C2, C3 samples and fragments of the epidermis of the testa in surface view, from below were observed too. Starch grains and foreign substances were absent in all samples and these findings correspond with references and previous studies^{19,21,42}

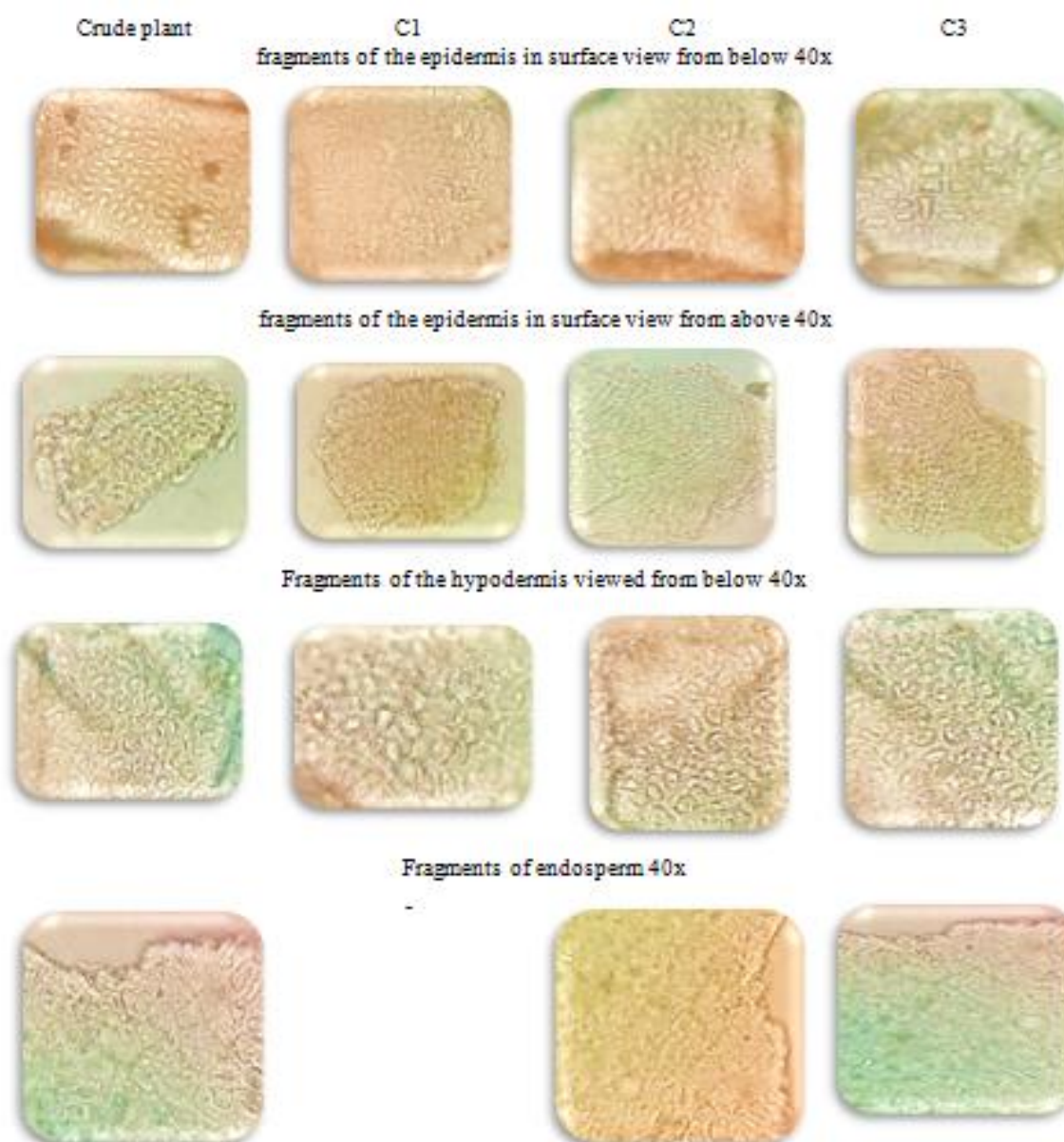


Fig. 4: Pharmacognostical elements of product C samples and *Trigonella foenum-graecum* L. powder using light microscope 40X

Product D

Consists of the dried fruits powder of *Tribulus terrestris* L. (Zygophyllaceae). The powder was fine yellow-green, odorless, with bitter and pungent taste, which corresponds with the organoleptic characters of the *Tribulus terrestris* L. powder. The microscopic elements of *Tribulus terrestris* L. powder appeared in all samples of the product (Fig. 5) which are: epidermis cells of testa showing striated

cuticle, group of thin-walled fibers, groups of stone cells of mesocarp, elongated-elliptical or subrounded, occurring in groups oil globules, prisms of calcium oxalate and spiral vessels. Starch grains were observed in the crude plant powder and in all samples of product D, however, starch is one of the pharmacognostic elements of the *Tribulus terrestris* L. powder. It exists alone or within the endosperm cells^{11,20,43,44}

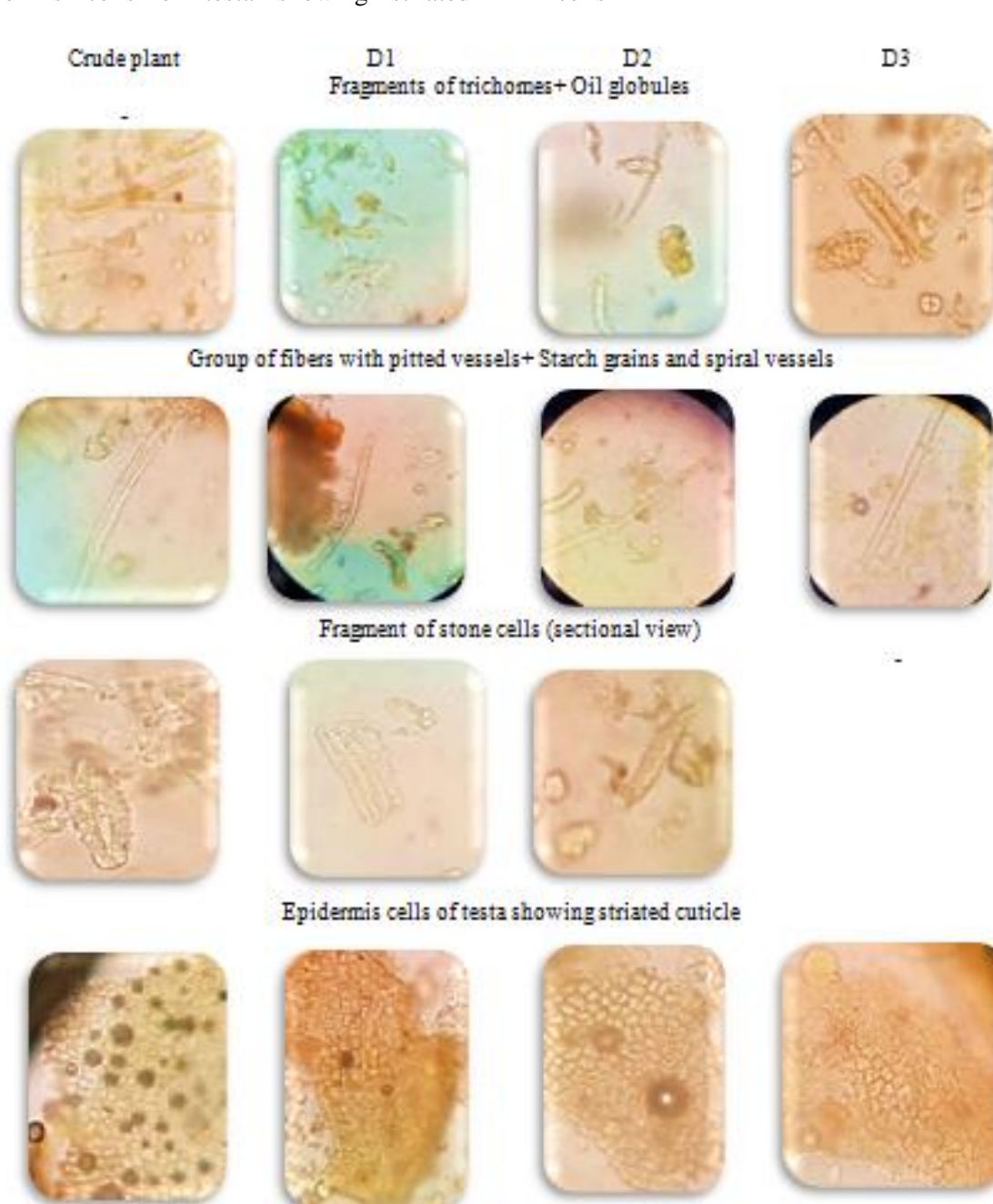


Fig. 5: Pharmacognostical elements of product D powder and *Tribulus terrestris* L. crude powder using light microscope 40X

Conclusion

This study evaluated the herbal products marketed in Syria that affect the urinary system in comparison with the crude plants. Although the microscopic results confirmed the presence of the plants described on the product label, significant amounts of starch grains were observed in two samples and *Ammi visnaga* L. powder was missing from one of the studied samples. Thus, microscopic evaluation of all batches of a powdered herbal product must be performed prior to marketing. In terms of purity and water content, most of the studied samples had a high water content and showed bacterial and fungal contamination above the constitutional limits. Moreover, *Aspergillus* species were detected in most of the samples which carry a high risk of forming toxic spores, and threatens the health of patients. Furthermore, the bacterial species identified are *Bacillus* that may not cause immediate harm but high contamination levels endanger the health of consumers, especially those who are immunocompromised. Finally, it is important to evaluate the quality of herbal medicines before and after marketing, from microscopic evaluation of the powdered plants to the evaluation of purity, as well as monitoring of water content, storage conditions, and harvesting procedures in order to obtain a safe, pure and effective herbal product. The quality of the herbal products can only be ascertained by imposing regulatory standards on these products using the Good Manufacturing Practice (GMP).

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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نشرة العلوم الصيدلانية جامعة أسيوط



لتقييم المجهري والميكروبي والمحتوى الرطب للمنتجات العشبية التي تؤثر على الجهاز البولي المسوقة في سوريا

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زاد استخدام المنتجات العشبية في الآونة الأخيرة ومع ذلك لا توجد دراسات كافية حول جودتها. تم في هذه الدراسة تقييم المنتجات العشبية الجاهزة والنباتات الخام التي تؤثر على الجهاز البولي في سوريا. كانت معظم قيم الفقد بالتجفيف أعلى من الحدود الدستورية وتجاوزت 37% من عينات المنتج حدود السلامة ($CFU / g 10^3$) للنمو البكتيري وتجاوزت 3,70% من العينات حد الأمان للنمو الفطري ($CFU / g 10^2$). كانت جميع العينات خالية من مسببات الأمراض البكتيرية. أما الأنواع الفطرية السائدة الناتجة فهي *Aspergillus flavus* و *Rhizopus*. أظهر التقييم المجهري وجود النباتات المبينة على المنتج. ومع ذلك تم الكشف عن كميات كبيرة من حبات النشاء في العينات B2 و B3 من المنتج B وكان مسحوق الخلة البلدية *Ammi visnaga L* مفقوداً من إحدى العينات. إنه من الضروري مراقبة جودة المنتجات العشبية قبل التسويق وبعده.