COMPARATIVE BOTANICAL STUDY, DNA FINGERPRINTING, CERTAIN PHARMACOPŒIAL CONSTANTS AND MINERAL CONTENT OF *ORIGANUM SYRIACUM* L. SUBSP. *SINAICUM* GREUTER AND BURDET AND *O. MAJORANA* L.

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Received:16-4-2007
Accepted:18-6-2007

Abstract

Comparative macro- and micro-morphological characters of the roots, stems and leaves of two Egyptian Origanum species: O. syriacum L. subsp. sinaicum Greuter and Burdet and O. majorana L. (Family Lamiaceae) were presented with the aim of identification and differentiation of these two species both in entire and powdered forms. Study of DNA fingerprinting, certain pharmacopœial constants and mineral content of both species were also carried out.

INTRODUCTION

Two *Origanum* species are indigenous to Egypt: *Origanum syriacum* L. subsp. *sinaicum* Greuter and Burdet and *O. isthmicum* Danin [1]. Other *Origanum* species have been introduced and cultivated for medicinal purposes, among which is *O. majorana* L. [2] *O. syriacum* L. subsp. *sinaicum* Greuter and Burdet (syn. *O. maru* L. var. *sinaicum* Boiss) is endemic to Egypt [1]. The plant has also been cultivated in several areas for medicinal and culinary purposes [3]. *O. majorana* L. (syn. *Majorana hortensis* Moench.) known as Sweet Marjoram is cultivated in Egypt and widely used as a spice, a warming digestive and a constituent in herbal teas. *O. syriacum* L. subsp. *sinaicum* and *O. majorana* L. are closely related and belong to the Majorana section of *Origanum* [4, 5]. Both are considered as sources of the essential oil of Marjoram [6]. Together with other *Origanum* plants, they have been known for their beneficial effects on upset stomach, headache, colics and nervous complaints as well as on cough and other respiratory ailments [7, 8]. Seasonal variations in the essential oil composition of cultivated *O. syriacum* L. subsp. *sinaicum* were presented in a previous communication [9].

Due to the widespread use of *O. syriacum* L. subsp. *sinaicum* and *O. majorana* L. in the Egyptian market, it was necessary to distinguish between both species. However, little could be traced in the available literature [2,10&11] concerning their botanical characters. It was, thus, necessary to study the macro- and micro-morphological features of the roots, stems and leaves of both *Origanum* species**,** to help in their identification and differentiation in the entire and powdered forms. Further identification of each plant was achieved by a DNA profiling of both species. Certain pharmacopœial constants and the mineral content of the two plants were also studied.

EXPERIMENTAL

Plant material for botanical study:

Samples of roots, stems and leaves of *O. syriacum* L. subsp. *sinaicum* and *O. majorana* L. were obtained in July 2006 from plants grown in the Experimental Station of Medicinal Plants, Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Giza. Identification of *O. syriacum* L. subsp. *sinaicum* has been reported earlier [9]. Identification of *O. majorana* L. was kindly carried out by Dr. Nahed El-Husseiny, Assistant Professor of Plant Taxonomy, Department of Botany, Faculty of Science, Cairo University. Voucher samples of both species are deposited at the Museum of the Pharmacognosy Department, Faculty of Pharmacy, Cairo University. Fresh samples of the different organs, as well as, samples reserved in ethanol 70 percent containing 5 percent glycerin were examined. Air-dried roots, stems and leaves of both species were finely powdered and packed in dark-colored, tightly closed containers for examination of powdered organs. The root, stem and leaf of *O. syriacum* L. subsp. *sinaicum* and *O. majorana* L. were examined macro- and micro morphologically (Figs.1-11). Dimensions in microns of the different elements of the studied organs are recorded (Table 1). In addition, numerical values [12] of the leaves of both species are recorded (Table 2).

Plant material for determination of certain pharmacopœial constants and the mineral content of the two plants:

The air-dried aerial parts of both species were coarsely powdered and packed in dark-colored, tightly closed containers for determination of certain pharmacopœial constants [13] and the mineral content of the two plants [14].

Material for molecular investigations:
Plant material:

Samples of fresh leaves of both species were separately stored at -70°C, freeze-dried and ground to a fine powder using a coffee grinder prior to DNA isolation.

Solutions:
Buffers:

Extraction buffer: 0.7 M NaCl, M Tris (pH 7.5), 0.01 M EDTA, 1% (W/V) N-cetyl-N, N, N- trimethylammonium bromide (CTAB), 1% (v/v) β-mercaptoethanol (added immediately before use), washing buffer: 1:76% ethanol, 0.2 M Na-acetate, washing buffer 2:76% ethanol, 10 mM NH_4O-acetate,TE-buffer: 10 mM tris (pH 8.0), 1mM EDTA,10x reaction-buffer: 100 mM Tris (pH8.3), 500 mM KCl, 0.01% (w/v) gelatin, Chloroform/ isoamylalcohol 24:1 (v/v), isopropanol, dNTP (Pharmacia, Sweden), Taq DNA polymerase (Perkin-Elmer / Cetus, USA, Advanced Biotechnologies, UK)

Primers:

Five primers were used for randomly amplified polymorphic DNA (RAPD) analysis obtained from Operon Technologies Inc., Almeda, California, USA with the following sequence: B-05; TGCGCCCTTC, B-04; GGACTGGAGT, B-03; CATCCCCCTG, A-04; AATCGGGCTG, A-01; CAGGCCCTTC.

Agarose gel: 1.4 % with running buffer TAE.
Molecular weight marker: 100 bp ladder, Promega Corporation, Madisson, USA.

Apparatus:

DNA thermocycler (Hybaid PCR Express) used for amplification of DNA, agarose gel electrophoresis tool (Biorad Wide Mini Sub Cell) used for separation of RAPD fragments according to size and UV Polaroid camera used for visualization of RAPD fragments.

Methods for molecular investigations:
DNA extraction and quantification:

DNA was extracted using CTAB method[15]. Fifty mg of frozen leaf were powdered in liquid nitrogen, extracted with 0.8 ml CTAB, precipitated with isopropanol. The precipitate was washed in 70% ethanol and dissolved in deionized water.

Amplification of RAPD markers:

The polymerase chain reactions were carried out with 100 ηg of genomic DNA template following a thermal cyclic program. Amplified products were analyzed by electrophoresis on 1.8% agarose gel and finally stained with ethidium bromide. A molecular size marker was used as standard marker.

Analysis of RAPD data:

RAPD bands were treated as presence or absence, without considering their percentage. For estimating genetic distance among the tested samples; each of DNA bands was treated as a unit character. Genetic similarity (GS) was analyzed [16].

$$GS = 2N_{1x2} / (N_1 + N_2)$$

Where N_{1x2} is the number of shared fragments between plants 1 and 2; N_1 is the number of scored fragments of plant 1 and N_2 is the number of scored fragments of plant 2. Results are recorded (Tables 3 and 4 and Fig.12).

Determination of certain pharmacopœial constants of the two *Origanum* species:

The moisture content, ash, acid insoluble ash, water soluble ash and crude fiber of the two species were determined by the methods recorded in the Egyptian Pharmacopœia [13]. Results are recorded (Table 5).

Determination of the mineral content of the two *Origanum* species:

The percentage of Na, P, K, Ca and Mg were determined in the aerial parts of both species [14]. Results are compiled (Table 6).

RESULTS AND DISCUSSION

Macro-morphology:
A) *Origanum syriacum* L. subsp. *sinaicum*
General Description of the Plant (Fig.1A)

It is a suffruticose perennial shrub, attaining a height of 40-90 cm. It has an erect, pubescent, monopodially branched stem, bearing broadly grayish green ovate hairy leaves in opposite decussate arrangement and showing verticillasters in terminal panicles of numerous dense spikes. The plant starts flowering in June and carries fruits in September.

The root: (Fig. 2D)

The plant possesses a distinct tap root system with a main tap root and numerous filiform lateral rootlets. The root is conical, dark brown in color with a somewhat rough surface showing faint longitudinal wrinkles; it measures 8-11 cm L and up to 0.7 cm D in the upper part. It breaks with a short, fibrous fracture exposing a yellowish-white solid interior. It is odorless and has a slight characteristic taste.

The stem: (Fig. 2A)

The young stem branches are quadrangular, pubescent and measure 0.2-0.4 cm D. They are solid and break with a flexible fracture. They are grayish green, having a characteristic aromatic, pungent odor and taste.

The older branches are cylindrical, darker in color and more tough, measuring up to 0.4 cm D.

The leaf: (Fig. 2A, B & C)

Leaves are simple, grayish green, cauline, opposite decussate, shortly petiolate, exstipulate and pubescent. Lamina is papery, oval to cordate in shape with an entire margin, recurved acute apex, and symmetric base. Both surfaces are grayish green, the lower is lighter. Venation is palmate reticulate with the big veins slightly projecting on the lower surface. The leaf measures 0.6 to 2.7 cm. L and 0.5 to 2.5 cm W.; petiole measures 1-5 mm L and 1-2 mm D. The leaves have characteristic aromatic pungent odor and taste.

B) *Origanum majorana* L. (Fig.1B)

Sweet marjoram is a bushy perennial sub-shrub, 20-60 cm in height with multi-branched stems.

Macroscopical examination of *O. majorana L.* revealed that the examined organs are more or less similar to those of *O. syriacum* L. subsp. *sinaicum* except in a few aspects. The differences are listed as follows:

The root: (Fig. 2H)

The root shows distinct longitudinal wrinkles.

The leaf: **(Fig. 2E, F&G)**

1. It is faint grayish green in color.
2. Lamina is ovate to oblong with an obtuse apex.
3. It is somewhat narrower, measuring 2.1-3.5 cm L and 0.9–1.1 cm W and the petiole is somewhat longer measuring up to 1 cm L.
4. Venation is pinnate reticulate.

Micro-morphology:

A) *Origanum syriacum* L. subsp. *sinaicum*

The root: (Fig. 3 A & C)

A transverse section in the root (Fig. 3A) is more or less circular with a slightly wavy outline. It shows a relatively narrow bark, which surrounds a central wide cylinder of secondary wood. The phloem and xylem are traversed by uniseriate medullary rays that enclose a tetrarch primary xylem. The bark is formed of a somewhat wide band of a brownish cork that surrounds a parenchymatous phelloderm and a narrow band of phloem.

The cork (Figs. 3C & 4 A) is formed of 3-5 rows of polygonal, tangentially elongated, radially arranged cells, with thick brown, suberized, and lignified walls.

The phelloderm (Figs. 3C) consists of 3-4 rows of polygonal parenchyma cells with thin cellulosic walls and narrow intercellular spaces.

The vascular system (Figs. 3A, C & 4 A):

The phloem (Figs. 3A & C) consists of thin-walled soft phloem elements and is devoid of bast fibers.

The xylem (Figs. 3A, C and 4 A) consists of a continuous ring of lignified xylem elements. The vessels are solitary or arranged in small groups and show mostly bordered-pitted thickening and few spiral vessels. The wood fibers are fusiform with acute or rounded apices, somewhat thick pitted walls, narrow and/ or wide lumina. The tracheids are elongated with lignified pitted walls. The medullary rays consist of rectangular cells with thin cellulosic walls in the phloem region, lignified in the xylem region.

The powdered root *(*Fig. 4A):

The powder is brown in color, with a slight characteristic odor and taste. Microscopically it is characterized by the presence of the following:

1. Fragments of cork cells, which are polygonal, slightly elongated, with somewhat thick, brown, suberized, and lignified walls.
2. Fragments of bordered- pitted and few spirally thickened xylem vessels.
3. Fragments of wood fibers, which are fusiform with rounded or acute apices, somewhat thick lignified walls, narrow or wide lumina.
4. Fragments of elongated tracheids with lignified pitted walls.

The stem (Fig. 5)

A transverse section in the stem (Fig. 5A) is more or less quadrangular in outline. It consists of an epidermis followed by a comparatively wide cortex, which is differentiated into outer collenchymatous zone followed by an inner parenchymatous zone. It is lined internally by a distinct endodermis. The pericycle is parenchymatous. The vascular system consists of a continuous collateral vascular bundle, which is traversed by uniseriate medullary rays. The pith is wide and parenchymatous. A transverse section in an old branch (Fig. 5C) is more or less rounded in outline. It shows a narrow cortical zone. The vascular system is more developed and most of the parenchymatous cells of the pith are lignified and pitted.

The epidermis (Figs. 5E, 6A & 7A) consists of polygonal axially elongated, more or less isodiametric cells with straight or slightly curved, sometimes pitted anticlinal walls and covered with thin striated cuticle. Stomata are of the diacytic type. Trichomes of non-glandular and glandular types are abundant. Non-glandular trichomes are frequent; each arises from one or more epidermal cells[10]. They are tri-to tetra-cellular, uniseriate; the apical cell is sometimes curved or bent at right angles, with an acute, sometimes obtuse apex and covered with a warty cuticle. Glandular trichomes are with uni- to

tricellular uniseriate stalks and unicellular or multicellular disk shaped heads, formed of 8-10 radiating cells [10]. Epidermal cells surrounding the glandular trichomes are sometimes radiating.

The cortex (Figs. 5A & 6A) is differentiated into an outer layer of 1 – 8 rows of collenchyma with thick cellulosic walls. The rest of the cortex consists of 3-4 rows of large polygonal, slightly rounded thin-walled parenchyma cells. The cortex is lined internally by distinct endodermis that consists of large rectangular tangentially elongated cells.

The pericycle (Figs. 5A & 6A) consists of 1-2 rows of thin-walled parenchyma cells.

The vascular system (Fig. 5A, 6A & 7C):

The phloem (Fig. 6A) consists of soft phloem elements. Bast fibers are absent.

The xylem (Figs. 6A & 7C) consists of lignified vessels, tracheids, wood parenchyma and medullary rays. The vessels show reticulate and annular thickening. The tracheids are elongated with lignified pitted walls. Wood parenhyma cells are elongated with thick pitted walls. The medullary rays are uniseriate, with thin cellulosic walls in the phloem region and lignified in the xylem region.

The pith (Fig. 6A) consists of large rounded or polygonal cells with thin cellulosic, sometimes pitted lignified walls and narrow intercellular spaces.

The powdered stem: (Figs.5E and 7A & C)

The powder is grayish green in color with aromatic odor and taste. It is characterized microscopically by the presence of:

1. Fragments of the epidermis, polygonal, isodiametric, somewhat axially elongated cells with straight, slightly curved, sometimes pitted anticlinal walls, covered with striated cuticle, showing few diacytic stomata and numerous non-glandular and glandular trichomes.
2. Numerous multicellular uniseriate non-glandular trichomes having apical cell, curved sometimes bent at right angles, with acute sometimes obtuse apices and covered with warty cuticle; occasional glandular trichomes with uni- to tricellular uniseriate stalks and uni- or multicellular disk shaped heads.
3. Fragments of annular and reticulate xylem vessels.
4. Fragments of elongated tracheids with thick pitted lignified walls.
5. Fragments of elongated wood parenchyma cells with thick pitted lignified walls.

The leaf: (Fig. 8):

A transverse section in the leaf (Fig 8A) shows narrow lamina and midrib depressed on the upper side and convex on the lower side. Upper and lower epidermises enclose a dorsiventral mesophyll. The palisade is discontinuous. The midrib shows 2 rows of sub epidermal collenchyma abutting lower epidermis and a central vascular tissue composed of a collateral vascular bundle.

The epidermis: (Figs. 8C, E & G) The upper epidermal cells are polygonal, elongated with straight, sometimes pitted anticlinal walls. Lower epidermal cells are somewhat isodiametric, sinuous, mostly with pitted anticlinal walls and covered with a finely striated cuticle. Diacytic stomata are more numerous on lower surface. Non-glandular trichomes are similar to those of the stem. Glandular trichomes are with bi-to tetracellular stalks and one to eight celled heads. Trichomes are more frequent on lower surface.

The mesophyll: (Figs. 8A & 9A) is heterogeneous showing a layer of palisade abutting the upper epidermis and discontinuous in the midrib region. The palisade cells are arranged in two rows of columnar, cylindrical, and thin walled cells, the second row of cells is shorter [11]. The spongy tissue is composed of 2-3 rows of irregularly shaped parenchymatous cells.

The midrib: (Fig 8A, G & 9C) Neural epidermal cells (Fig 8A & G) are somewhat axially elongated with straight anticlinal walls. Stomata are lacking. Non-glandular trichomes similar to those of the stem are frequent on neural epidermis. The parenchymatous cells are thin walled with narrow intercellular spaces. The pericycle (Fig.9C) consists of small thin-walled parenchyma cells.

The vascular tissue: (Fig 8A & 9C) Each vascular bundle consists of phloem and xylem, and traversed by narrow medullary rays. The xylem is formed of vessels, tracheids and wood parenchyma and is traversed by uni- or biseriate medullary rays. The xylem vessels are arranged in radial rows that show spiral, annular and pitted thickening. The tracheids are elongated, somewhat fusiform, with lignified spirally thickened walls. Wood parenchyma is similar to those of the stem. The phloem is composed of soft phloem elements and traversed by uni- or biseriate medullary rays which is consisting of thin walled, somewhat radially elongated cells.

The petiole: (Figs. 10A, C)

A transverse section in the petiole is more or less semicircular in outline and is somewhat similar in structure to the midrib of the leaf.

The epidermis: (Fig. 8 I) is formed of polygonal, somewhat isodiametric cells with straight, sometimes pitted, anticlinal walls and covered with a striated cuticle. Non- glandular and glandular trichomes similar to those of the stem are frequent.

The cortex: (Fig. 10C) is somewhat wide, formed of five rows of thin walled parenchymatous cells.

The pericycle: (Fig. 10C) is parenchymatous. The vascular bundle is collateral, composed of phloem and xylem, and traversed by narrow medullary rays. The xylem consists of lignified vessels, and wood parenchyma, similar to those of the leaf. The phloem consists of soft phloem elements.

The powdered leaf (Figs. 8 C, E, G, I,11A & C):

The powder is green in color, having an aromatic pungent odor and taste. The following features characterize it:

1. Fragments of polygonal, sometimes isodiametric or elongated cells with straight or sinuous, sometimes pitted, anticlinal walls, covered with finely striated cuticle, showing diacytic stomata and numerous non-glandular and glandular trichomes from the epidermises of petiole and leaf.
2. Numerous non-glandular and glandular trichomes similar to those of the stem.
3. Fragments of palisade cells.
4. Fragments of broken spiral, annular and pitted lignified vessels.
5. Fragments of wood parenchyma.
6. Fragments of elongated lignified tracheids.

B) *Origanum majorana* L.

Microscopical examination of *O. majorana L.* revealed that the examined organs are more or less similar to that of *O. syriacum* L. subsp. *sinaicum* except in a few aspects. The differences in dimensions and numerical values of the leaf are recorded (Tables 1 & 2). The qualitative differences are listed as follows:

The root: (Figs. 3B, D & 4B)

1. The cork cells are more axially elongated.
2. The phloem region is wider and nearly twice that of *O. syriacum* L. subsp. *sinaicum*.

The stem: (Fig 5F, 7B & D)

1. The epidermal cells sometimes appear beaded in surface view. They are more axially elongated.
2. The glandular trichomes are less frequent, possessing shorter stalks which are uni- or bicellular; few unicellular heads with unicellular stalks (capitate trichomes) are recorded.
3. The cortical region (Figs. 5 B & 6 B) is narrower, showing 4-6 rows. However, it appears wider in the corner.

The leaf: (Figs. 8 B, D & F)

1. Upper epidermal cells are slightly sinuous while lower epidermal cells appear beaded in surface view.
2. The midrib region (Figs. 8 B & 9 D) is characterized by the presence of upper sub epidermal collenchyma (1 or 2 rows) besides the lower sub epidermal collenchyma.
3. The cortical region of the petiole is differentiated into 1 or 2 rows of collenchyma and 3-4 rows of thin walled parenchyma cells.
4. Determination of the numerical values (Table 2) showed slight differences.

The differences between the two species appear to be minor, yet still present. Molecular investigations revealed further discrimination. RAPD-PCR reactions performed with the two *Origanum* species using five random primers indicated distinct differences. The number of fragment patterns generated by each primer was recorded for the detection of genetic diversity (Table 3).

A total of 16 polymorphic bands out of 41 were generated by the five primers (Table 4). Primers OPB-5 and OPB-3 were found to be the most effective in generating polymorphic bands and having the least similarity coefficient (66.67%). Those bands were scored as unique fragments (Table 4). Each species has one or more particular novel sequences. Consequently, these results indicate that each species has a different DNA fingerprint. Comparable results have been previously reported [17] when three *Ruprechtia* species were differentiated.

However, the number of fragments amplified using OPA-4 and OPA-1 was not identical but they almost share analogous bands. These results have been verified by the occurrence of similarity coefficient 90.91% as dictated in Table 4. Correspondent results have been previously reported [18] in the discrimination between eight *Triticum* cultivars.

The presence of only one different base pair in the target sequence of the genome may result in a completely different RAPD profile as previously reported [19]. Accordingly, each primer (10 bp) only covers a limited part of the genome; thus, important non amplified differences could be missed. This fact has to be taken into consideration in the analysis of genetic diversity especially in the closely related species as previously concluded [20].

From table (5), it was concluded that the yield of total ash, acid insoluble ash and water soluble ash was higher in *O. majorana* L.,

16.33%, 4.82% and 3.97% respectively. Crude fibers are higher in *O. majorana* L. (16.92%). However, the moisture content showed a slightly higher value in *O. syriacum* L. subsp. *sinaicum.*

Determination of minerals in the two studied species had important relation to their use in medicine, culinary purposes and as spices. Calcium, magnesium and phosphorus contribute to prevention of osteoporosis. Sodium and potassium, together with calcium and magnesium are crucial for muscle contractions. Besides, the two former minerals help in maintenance of the normal heart rhythm and body water balance [21]. From table (6), it was clear that *O. majorana* L. contains higher percentages of phosphorous, potassium, calcium and magnesium. *O. syriacum* L. subsp. *sinaicum* contains higher percentage of sodium. Detection of these minerals in both plants adds to their medicinal importance.

Table (1): Dimensions of different elements of the organs under investigation in microns of *O. syriacum* L. subsp. *sinaicum* and *O. majorana* L.

Species / Item	*O. syriacum* L. subsp. *sinaicum*			*O. majorana* L.		
	L	W or D	H	L	W or D	H
Root						
Cork	32,60,80	27, 34,45	8, 15,25	45,76,105	15, 27,35	10, 20,30
Vessels		20, 64,105			10,36,72	
Tracheids	50,72,118	10,17,25		74, 90,120	10,18,22	
Wood fibers	204, 724,1020	16, 21,29		174, 326,1325	16,26,34	
Stem						
Epidermis	30,40,66	16, 34,38	10,14,16	14,26,30	12,16,20	6,10,12
Stomata	16,18,20	10,12,14		12,14,15	6,8,10	
Non - glandular trichomes	140, 288 ,380	10,22,45		120, 192 ,280	10,12,16	
Glandular-trichomes with multicellular stalk						
head	12,14,16	9,10,12		-	5,9,10	
stalk	22,69,94	10,12,13		12,13,16	8,10,12	
Labiaceous trichomes						
head	12,16,20	66,69,71		6,9,10	42,44,47	
stalk	8,10,12			5,9,11	23,26,28	
Capitate trichomes						
head	-	-		14,20,24	13,16,27	
stalk	-	-		8,9,11	7, 16,21	
Vessels		13,25,30			10,15,24	
Tracheids	124, 130 ,156	10,20,26		113, 116 ,120	20,30,33	
Leaf						
Upper epidermis	27,35,42	14,17,22	11,14,16	24,30,48	14,23,50	15,17,24
Lower epidermis	14, 25,42	12,13,19	12,14,18	23,38,50	11,36,40	8,13,14
Neural epidermis	56,60,66	14,20,28	9,10,11	14,26,30	9,10,15	13,15,16
stomata	10,11,12	7,8,9		17,18,20	10,12,13	
Palisade	18,28,38	11,15,17		29,38,50	16,18.5,20	
Non-glandular trichomes	280, 350 ,510	10,12,18		66,87,186	7,8,11	

Glandular-trichomes with multicellular stalk						
head	5,11,22	5,8,14		8,9,12	8,10,12	
stalk	16, 23, 118	4,8,10		24, 66,104	8,10,12	
Labiaceous trichomes						
head	9,12,18	44,39,32		14,15,20	64,66,76	
stalk	8,11,12	24,20,22		13,16,22	28,38,48	
Capitate trichomes						
head	-	-		13,17,20	10,12,17	
stalk	-	-		6,9,11	10, 12,13	
Vessels		13,16,19			11,12,13	
Tracheids	71,86,125	10,16,22		-	-	
Petiole						
Epidermis	25,32,38	9,16,42	12,19,22.5	23,41,64	23, 35,41	8,14,19
Stomata						
Non-glandular trichomes				65, 102 , 130	11,13,19	
Glandular-trichomes with bicellular stalk						
head	11,12,13	7,13,15				
stalk	11,15,21	8,9,10				
Labiaceous trichomes						
head		59,60,66			55,60,63	
stalk	7,13,14	21,24,25		20,24,25	20,22,24	
Capitate trichomes						
head	-	-		13,17,20	10,12,17	
stalk	-	-		6,9,10	10,12,13	
Vessels		7,10,13			6,7,11	

Table (2): Numerical values of the leaves of *O. syriacum* L. subsp. *sinaicum* and *O. majorana* L.

Species / Numerical Values	*O. syriacum* L.	*O. majorana* L.
Stomatal index:		
-Upper epidermis	26.6	13.6
-Lower epidermis	25	22
Palisade ratio	4-8	3-4
Vein islet number	4-8	4
Veinlet termination number	24	20

Table (3): Molecular size in base pairs of amplified DNA fragments produced by five decamer primers in two *Origanum* species.

Molecular size (bp)	B-5		B-4		B-3		A-4		A-1	
	2	1	2	1	2	1	2	1	2	1
11595	-	-	-	+	-	-	-	-	-	-
8683	-	-	+	+	-	-	-	-	-	-
6918	-	-	+	+	-	-	-	-	-	-
4672	+	-	+	+	-	-	-	-	+	+
2413	+	-	+	+	+	-	-	-	-	-
1532	+	+	+	+	+	+	+	+	+	+
1353	+	+	-	-	+	+	+	+	+	+
1220	+	-	-	-	-	+	+	+	+	+
1123	-	-	+	+	+	+	-	-	-	+
893	-	+	+	+	-	+	-	-	-	-
757	+	-	-	-	+	-	+	-	-	-
419	+	+	-	-	-	-	+	+	-	-
294	+	+	-	-	-	-	-	-	-	-
273	+	+	+	-	+	+	+	+	-	+
253	-	-	+	-	+	-	-	-	-	-

1: *O. majorana* L.; 2: *O. syriacum* L. subsp. *sinaicum*; (+) and (-): presence and absence of band.

Table (4): The total number of RAPD-PCR fragments, distribution of monomorphic (common) and polymorphic bands and similarity coefficients generated by five decamer arbitrary primers in two *Origanum* species.

Primers	Number of fragments	Monomorphic fragments	Polymorphic fragments	% of polymorphism	Similarity coefficient
B-5	10	5	5	50.00	66.67
B-4	10	7	3	30.00	82.35
B-3	9	4	5	55.56	66.67
A-4	6	5	1	16.67	90.91
A-1	6	4	2	33.33	80.00
Total	41	25	16	Mean 31.11	Mean 77.32

Table(5): Results of certain pharmacopœial constants of the aerial parts of *O. syriacum* L. subsp. *sinaicum* and *O. majorana* L.

Item \ Species	Percentage in	
	O. syriacum L.	*O. majorana L.*
Moisture	54.96	53.54
Total ash	13.12	16.33
Acid insoluble ash	3.33	4.82
Water soluble ash	3.07	3.97
Crude fiber	14.2	16.92

Table (6): Percentage of mineral content of aerial parts of *O. syriacum* L. subsp. *sinaicum* and *O. majorana* L.

Species / Item	Percentage in	
	O. syriacum L.	***O. majorana L.***
Na	0.977	0.723
P	1.042	1.076
K	3.31	4.68
Ca	1.12	2.46
Mg	1.097	1.391

Fig. (1): Macromorphology of two *Origanum* species.
A : A photo of *O. syriacum* L. subsp. *sinaicum*. (X: 0.1)
B: A photo of *O. majorana* L. (X: 0.1)

Fig. (2): Macromorphology of two *Origanum* species.
A :Sketch of a leafy branch of *O. syriacum* L. subsp. *sinaicum*
B: Leaf from upper surface of *O. syriacum* L. subsp. *sinaicum*
C: Leaf from lower surface of *O. syriacum* L. subsp. *sinaicum*
D: Sketch of the root of *O. syriacum* L. subsp. *sinaicum*
E: Sketch of a leafy branch of *O. majorana* L.
F: Leaf from upper surface of *O. majorana* L.
G: Leaf from lower surface of *O. majorana* L.
H: Sketch of the root of *O. majorana* L. All (X: 1)
l., leaf; p., petiole; r., rootlet; s., stem.

Fig. (3): Micromorphology of the root
A: Diagrammatic T.S. of the root of *O. syriacum* L. subsp. *sinaicum* (X: 50)
B: Diagrammatic T.S. of the root of *O. majorana* L. (X: 50)
C: Detailed T.S. of the root of *O. syriacum* L. subsp. *sinaicum* (X: 300)
D: Detailed T.S. of the root of *O. majorana* L. (X:325)
ca., cambium; ck., cork; m.r., medullary ray; ph., phloem; phell., phelloderm; x., xylem; x. v., xylem vessel.

Fig. (4): Micromorphology of the root. The Powder. (X: 367)
A: Powdered root of *O. syriacum* L. subsp. *sinaicum*
B: Powdered root of O. *majorana* L.
ck., cork; f.m., wood fiber of *O. majorana* L.(X: 188); f.s., wood fiber of *O. syriacum* L.(X: 250); tr., tracheids;, w.f., wood fiber; x.v., xylem vessel.

Fig. (5): Micromorphology of the stem.
A: Diagrammatic T.S. of a young stem of *O. syriacum* L. subsp. *sinaicum* (X: 70)
B: Diagrammatic T.S. of a young stem of *O. majorana* L. (X: 45)
C:Diagrammatic T.S. of an old stem of *O. syriacum* L. subsp. *sinaicum* (X: 20)
D: Diagrammatic T.S. of an old stem of *O. majorana* L. (X: 50)
E: Epidermal cells of *O. syriacum* L. (X: 375)
F: Epidermal cells of *O. majorana* L. (X: 375)
ca., cambium; cic., cicatrix; co. cortex; col., collenchyma; end., endodermis; ep., epidermis; g.t., glandular trichome; m.r., medullary ray; n.g.t., non-glandular trichome; per., pericycle; ph., phloem; pi., pith; st., stomata; x., xylem.

Fig. (6): Micromorphology of the stem. "Cont."
A: Detailed T.S. of the young stem of *O. syriacum* L. subsp. *sinaicum* (X: 350)
B: Detailed T.S. of the young stem of *O. majorana* L. at the corner (X: 350)

ca., cambium; col., collenchyma; cu., cuticle; end., endodermis; ep., epidermis; g.t., glandular trichome; m.r., medullary ray; n.g.t., non glandular trichome; par., parenchyma; per., pericycle; ph., phloem; pi., pith; w.p., wood parenchyma; x., xylem; x.v., xylem vessel.

Fig. (7): Micromorphology of the stem. Trichomes and lignified elements
A: Trichomes of *O. syriacum* L. subsp. *sinaicum*
B: Trichomes of *O. majorana* L
C: Lignified elements of *O. syriacum* L. subsp. *sinaicum*
D: Lignified elements of *O. majorana* L. (All X:375)
g.t., glandular trichome; n.g.t., non-glandular trichome; tr., tracheids; w.p., wood parenchyma; x.v., xylem vessel.

Fig. (8): Micromorphology of the leaf.
A: Diagrammatic T.S. of the leaf of *O. syriacum* L. subsp. *sinaicum* (X: 225)
B: Diagrammatic T.S. of the leaf of *O. majorana* L. (X: 80)
C: Upper surface of lamina of *O. syriacum* L. subsp. *sinaicum* (X: 267)
D: Upper surface of lamina of *O. majorana* L. (X: 272)
E: Lower surface of lamina of *O. syriacum* L. subsp. *sinaicum* (X: 300)
F: Lower surface of lamina of *O. majorana* L. (X: 300)
G: Neural epidermis of *O. syriacum* L. subsp. *sinaicum* (X: 200)
H: Neural epidermis of *O. majorana* L. (X: 200)
I: Epidermal cells of petiole of *O. syriacum* L. subsp. *sinaicum* (X: 200)
J: Epidermal cells of petiole of *O. majorana* L. (X: 200)
cic., cicatrix; col., collenchyma; g.t., glandular trichome; l.ep., lower epidermis; n.g.t., non-glandular trichome; pal., palisade; ph., phloem; st., stomata; u.ep., upper epidermis; x., xylem;

Fig. (9): Micromorphology of the leaf."Cont."
A: Detailed T.S. of the lamina of *O. syriacum* L. subsp. *sinaicum* (X: 450)
B: Detailed T.S. of the lamina of *O. majorana* L. (X: 400)
C: Detailed T.S. of a midrib of *O. syriacum* L. subsp. *sinaicum* (X: 400)
D: Detailed T.S. of a midrib of *O. majorana* L. (X: 450)
col., collenchyma; cu., cuticle; g.t., glandular trichome; l.ep., lower epidermis; m.r., medullary ray; n.g.t., non-glandular trichome; pal., palisade; per., pericycle; ph., phloem; sp.ti., spongy tissue; st., stomata; u.ep., upper epidermis; x., xylem.

Fig. (10): Micromorphology of the leaf. "Cont."
A: Diagrammatic T.S. of a petiole of *O. syriacum* L. subsp. *sinaicum* (X: 83)
B: Diagrammatic T.S. of a petiole of *O. majorana* L. (X: 100)
C: Detailed T.S. of a petiole of *O. syriacum* L. subsp. *sinaicum* (X: 425)
D: Detailed T.S. of a petiole of *O. majorana* L. (X: 675)
cu., cuticle; col., collenchyma; ep., epidermis; g.t., glandular trichome; l.ep., lower epidermis; m.r., medullary ray; n.g.t., non-glandular trichome; par., parenchyma; ph., phloem; u.ep., upper epidermis; x., xylem.

Fig. (11): Micromorphology of the leaf. Trichomes and lignified elements
A: Trichomes of *O. syriacum* L. subsp. *sinaicum*
B: Trichomes of *O. majorana* L.
C: Lignified elements of *O. syriacum* L. subsp. *sinaicum*.
D: Lignified elements of *O. majorana* L. (All X: 375)
g.t., glandular trichome; n.g.t., non-glandular trichome; pal., palisade; tr., tracheids; w.p., wood parenchyma; x.v., xylem vessel.

A

B

Fig. (1)

Fig. (2)

Fig. (3)

Fig. (4)

Fig. (5)

Fig. (6)

Fig. (7)

Fig. (8)

Fig. (9)

Fig. (10)

Fig. (11)

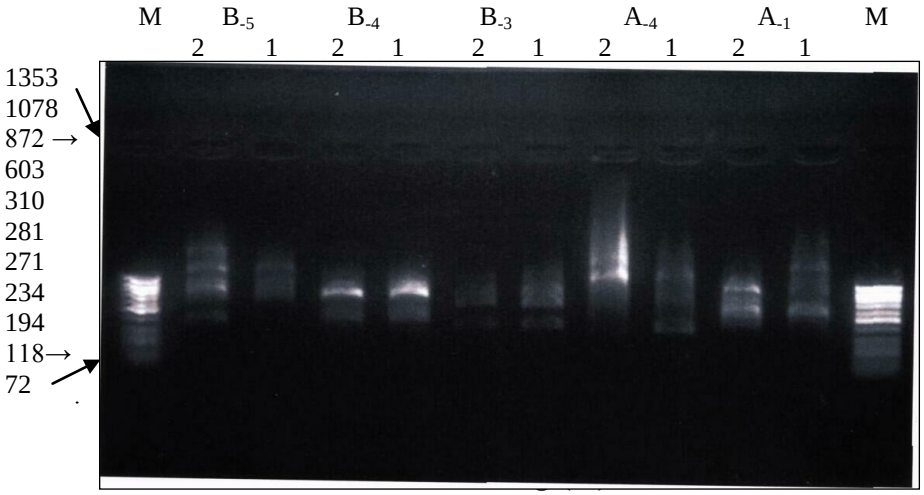

Fig. (12)

RAPD fingerprints detected in two *Origanum* species using primers (OPB-05, 04, 03), lanes: 1-6, and primers (OPA-04, 01) lanes: 7-10. Lane M: standard DNA molecular size marker

ACKNOWLEDGEMENT

The authors are grateful to Dr. Sahar Abd El Tawab, Assistant Professor of Botany and Genetics, Faculty of Girls, Ain Shams University for revising the interpretation of the molecular investigations.

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دراسة تشريحية مقارنة، البصمة الوراثية للحمض النووى، وبعض الثوابت الدستورية، و محتوى المعادن لنبات أوريجانم سيرياكم ل.تحت نوع سينايكم جروتر و بردت و أوريجانم ماچورانا ل.

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يعتبر نباتى أوريجانم سيرياكم ل. تحت نوع سينايكم جروتر و بردت ويعرف باسم زعتر التوابل و أوريجانم ماچورانا ل. ويعرف باسم البردقوش المنتميان للعائلة الشفوية من الأصناف شديدة التقارب لذلك فقد كان من الضرورى اجراء دراسة مقارنة للتفريق بينهما.

وقد اشتملت هذه الدراسة على مقارنة للصفات العيانية والمجهرية لجذور وسيقان و أوراق كلا" من النباتين و كذلك القيم الرقمية بغرض التعرف على أى منهما سواء فى الحالة الصحيحة أو على هيئة مسحوق.

كذلك تم مقارنة البصمة الوراثية (الحمض النووى) للنباتين موضع الدراسة لبيان مدى التشابه بينهما. كما تم تعيين بعض الثوابت الدستورية، و محتوى المعادن لكل منهما.

وقد أظهرت الدراسة وجود فروق دقيقة بينهما مما أتاح امكانية التفرقة بين هذين الصنفين رغم التشابه الشديد