

## ORIGINAL ARTICLES

### Morphological and Anatomical Studies of *Santolina chamaecyparissus* L. (Asteraceae) Ii. Anatomical Characteristics and Volatile Oil

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#### ABSTRACT

A study of two parts about various botanical aspects of lavender cotton (*Santolina chamaecyparissus* L.) was conducted. This is the second part of the study which is dealing with the plant structure and the volatile oil composition. Results are summarized as follows: Adventitious roots develop on the herbaceous stem cuttings. The epidermis consists of a single parenchymatous layer, followed by 8-9 layers of the cortex which is limited internally by a uniseriate endodermis. A hexaprotostele develops and secondary growth takes place in the usual manner. The pith is absent. Apical internode of the main stem represents the primary structure. The cortex consists of 6-7 layers. The stele includes 20 collateral bundles, each having a fibrous cap abutting the primary phloem. Secondary growth develops in internodes of median and basal portions of the main stem. A periderm replaces the epidermis. Secretory canals in large numbers are found in the cortex. Secondary xylem constitutes 70% of secondary vascular tissues. Secondary xylem which forms through the winter season comprises a broad cylinder of xylem with small vessels arranged in radial rows surrounding the pith. Next to this distinguished cylinder of xylem, towards the outside, larger vessels of the secondary xylem differentiating at summer season arrange in bands fluctuate between tangential and diagonal in orientation. Paratracheal lignified parenchyma accompany the vessels also form tangential and diagonal bands; *i.e.*, confluent parenchyma develops. Leaf blade is wavy in outline, bounded by a uniseriate epidermis. Trichomes of woolly hairs type are present. Five collateral bundles are embedded in the ground tissue. The main large one is in the center. The petiole is somewhat flattened in shape. Trichomes are similar to those found on the leaf blade (woolly hairs). In the center there is a main vascular bundle (the largest one), to the right and to the left of this bundle another small bundle develops. Volatile oil of lavender cotton is a mobile liquid of pale yellow colour having an aromatic harsh smell. At flower bud stage, the volatile oil compose 39 components decrease to 36 components at opened flowers stage. Only 22 of these components are identified since the remainder are present as traces. *Santolina* alcohol, 1.8 – cineole and artemisia ketone are the main constituents of the volatile oil.

**Key words:** anatomy, Asteraceae, lavender cotton, *Santolina chamaecyparissus* L., volatile oil.

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#### Introduction

This is the second paper of a two-part study dealing with various botanical attributes of lavender cotton (*Santolina chamaecyparissus* L.) of Asteraceae (Compositae). Flann (2010) compiled the Global Compositae Checklist (GCC) from many contributed datasets. GCC is an integrated data base of nomenclature and taxonomic information for the second largest vascular plant family in the world. Plantae-Magnoliophyta – Magnoliopsida – Asterales – Asteraceae / Compositae (Aster or sunflower family) – 29672 species. One of these, the herbal medicinal species *Santolina chamaecyparissus* L., its morphology was given in the first part of the present study (El-Sahhar *et al.*, 2011). In this part, plant structure and volatile oil composition are considered. The composition of volatile oils from various species of *Santolina* has been investigated, all species produced monoterpene-rich oils and exhibited quite diverse compositions (Villar *et al.*, 1986; Pérez-Alonso and Velasco-Neguerela, 1992; Garg *et al.*, 2001; Teixeira da Silva, 2004; Liu *et al.*, 2007 and Grosso *et al.*, 2009).

Giner *et al.* (1993) separated 36 components from the volatile oil of *Santolina chamaecyparissus* L. The major components were  $\rho$  - cymene + 1.8 – cineole (5.8%),  $\alpha$ - phellendrene +  $\delta$ -3-carene (5%) and borneol (4.78%). In addition to three unknown sesqui-alcohol (18.92%).

Haggag *et al.* (2000) mentioned that the volatile oil of *Santolina chamaecyparissus* L. fresh arial parts (0.33% v/w) was found to contain 74 identified components artemisia ketone (35.49%) is the major one.

*Santolina chamaecyparissus* is widely used in Mediterranean folk medicine. The flowers are used for their anaesthesia, anti-inflammatory, antiseptic, antispasmodic, bactericidal, fungicidal, digestive and vulnerary properties, and is used in phytotherapy for different kinds of dermatitis. Several products (acetylenes, essential oils,

flavonoids and sesquiterpenes) obtained from *Santolina* spp. have been investigated for their biological activities (Teixeira da Silva, 2004).

Akerreta *et al.* (2007) discussed the use of 18 medicinal plants. One of them was *Santolina chamaecyparissus* L. an example of a species used for digestive disorders in Navarra region (Spain). They concluded that edaphological and climatological factors, on the one hand, and culture, on the other, could help in understanding why a plant was replaced by another one for the same purposes, either in the same or in a different area. In many cases, the cultural factor meant that the use of a species was more widespread than its ecological distribution. This might also explain the presence of synonyms and polysemies which are useful for discussing ethnopharmacological data.

At the extent of authors knowledge, information about the anatomical structure of *Santolina chamaecyparissus* L. is scarce. However, previous anatomical studies dealt mainly with the family Asteraceae in general, *e.g.* Metcalfe and Chalk (1950).

## Materials and Methods

### Field work:

The experimental of the field work was described in part I of this research (El-Sahhar *et al.* 2011).

### Anatomical studies:

Microscopical analysis included various vegetative plant organs. Microtechnique procedures given by Nassar and El-Sahhar (1998) were followed. Specimens were killed and fixed for at least 48 hrs in F.A.A. (10 ml formalin, 5 ml glacial acetic acid, 25 ml distilled water, 60 ml ethyl alcohol 95%). After fixation, materials were washed in 50% ethyl alcohol, dehydrated through a normal butyl alcohol series and embedded in paraffin wax (m.p. 56-58°C). Twenty microns tissue sections were cut with a rotary microtome and placed on slides containing Haupt's adhesive and formalin. Sections were stained with safranin-light green or crystal violet-erythrosin before mounting in Canada balsam and cover slips attached. Slides were analysed microscopically and photomicrographs of the permanent sections were taken.

### Analysis of the volatile oil:

Lavender cotton is distinctive by its production of volatile oil. Samples of the aerial part (herb) including leaves and flowering stems were collected in May to obtain the volatile oil at flower bud stage and opened flowers stage. Water distillation of the volatile oil was conducted in duplicate according to the following procedure (Anon., 1980). A 100 g of each investigated fresh herb sample were mixed with ambient amount of water in 2-L spherical flask. The receiver capacity was 5 ml., with graduation of 1/20 ml accuracy. The time of distillation was 3 hrs.

Percentage of the volatile oil was calculated on fresh weight basis according to the following formula:

$$\text{Volatile oil \%} = \frac{\text{Volume of volatile oil in the receiver}}{\text{Sample weight}} \times 100$$

Yield of the volatile oil, ml., of studied plant species was calculated according to the following formula:

$$\text{Volatile oil yield} = \text{Total herb weight of plant, g} \times \text{volatile oil \%}$$

The volatile oil was removed from the receiver using ether to aid its collection, and then placed in a sealed small specimen tube, which contained anhydrous sodium sulphate for 18 hrs. to be dried. The volatile oil obtained was then kept in the dark at a temperature of 0°C. until being required for analysis.

GLC technique was used to separate and detect the volatile oil constituents. Analysis was carried out at the Research Park, Faculty of Agriculture (FARP), Cairo University, Giza, Egypt.

Analysis conditions of the volatile oil were as follows:

Instrument, GLC Trace GC Ultra, Thermo TR-5MS, 5% Phenyl polysil phenylen siloxane). Column, 30 m × 0.25 cm ID × 0.25 µm film. Sample volume, 0.5 ml. Initial temp.: 50°C. Initial time: 3 min. Rate: 5°C / min. Final temp.: 180°C. Hold time: 40 min. Injector temp.: 235°C. Detector temp. : 250°C. FID. Gases flow rate, N<sub>2</sub>: 35 ml/min. H<sub>2</sub>: 35 ml/min. Air: 350 ml/min.

Identification of different constituents were carried out by comparing the relative retention time of each peak with those of authentic samples. The percentage of individual constituents was computed according to their proportional peak area in the chromatogram.

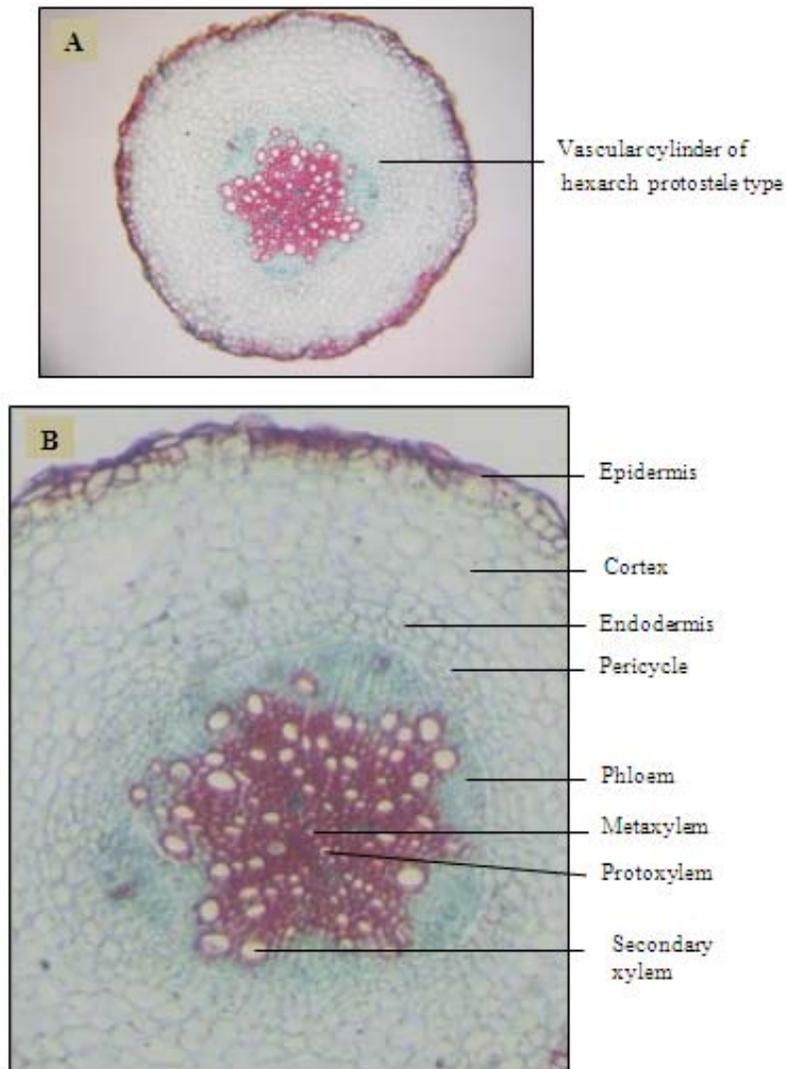
## Results and Discussion

### *I-Anatomical information:*

#### *I-The adventitious roots:*

The anatomical structure of lavender cotton adventitious roots developed on the herbaceous stem cutting (Fig. 1) shows that the root has already completed its primary state of growth and starts its secondary growth. The epidermis is still intact and composed of a single layer of thin-walled parenchymatous cells.

The cortex, underlying the epidermis, consisting of about 8-9 layers of thin-walled parenchymatous cells that are limited internally by a uniseriate endodermis which is regarded as a part of the cortex. The presence of triangular intercellular spaces is characteristic of the cortical layers. The casparian strips are difficult to be distinguished in the endodermis.



**Fig. 1:** Transverse sections of adventitious root of lavender cotton transplant.

A. Whole section (X62).

B. Magnified portion of A (X150).

The pericycle follows the endodermis to the inside and constitutes the outermost part of the stele. The pericyclic cells undergo periclinal and anticlinal divisions. The periclinal divisions cause an increase in the number of pericyclic layers in the radial extent. Secondary growth starts and takes place in the usual manner.

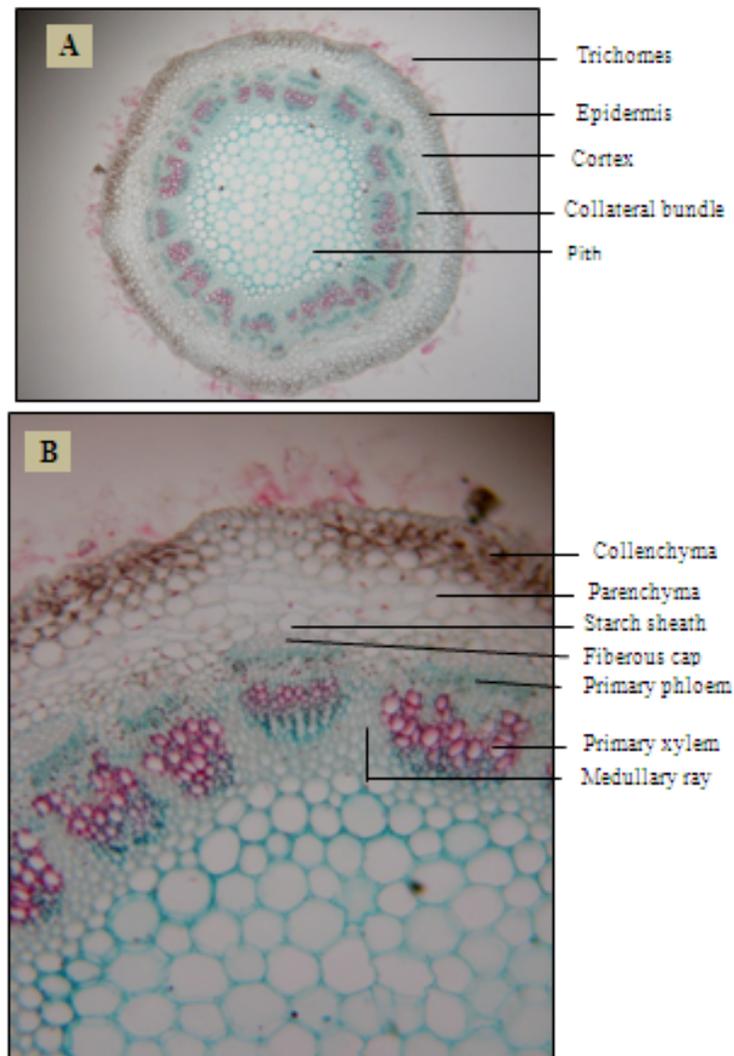
The cambial strips located on the inner face of the phloem begin their activity, producing a few number of xylem vessels towards the inside and small amount of secondary phloem towards the outside. The vascular cylinder is more compact and the stele is composed of six phloem strands, accompanied by a large amount of parenchyma cells, alternating with a similar number of exarch xylem ridges. The metaxylem vessels of xylem ridges occupy the root center forming a solid core. The pith is absent, hence the stele is regarded as a protosteles. The root is of hexarch protosteles type.

## 2. The shoot system:

### 2.1. The main stem:

#### 2.1.1. The apical internode:

The internode directly below the shoot apex of lavender cotton transplant represents the primary structure of the main stem. The transverse sections, (Fig. 2) prove that the stem surface of lavender cotton plant, at its apical portion, is cylindrical in outline. The epidermis consists of a uniseriate layer of barrel shaped cells covered with a thin cuticle layer. Stomata and trichomes, mainly of woolly hairs type are present in the epidermis.



**Fig. 2:** Transverse sections of apical internode of lavender cotton transplant showing its primary structure.

A. Whole section (X62).

B. Magnified portion of A (X150).

The cortex, followed the epidermis, consists of 6-7 layers of which the outer layer is collenchyma underlying the epidermis, followed by a layer of chlorenchyma cells. The remaining layers are parenchyma cells varying in size and associated with obvious intercellular spaces. The inner most layer of the cortex forms the starch sheath.

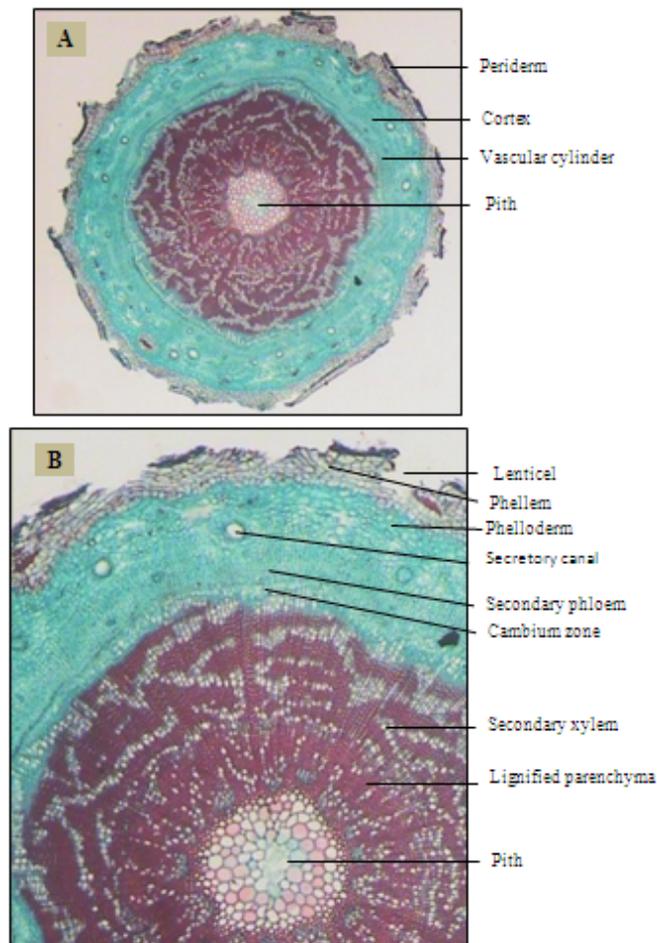
The stele consists of some 20 collateral bundles arranged in a ring forming a dictyostele type. The vascular bundles are separated from one another by the ground tissue. The bundle included a well defined fibrous cap abutting the primary phloem which is followed towards the inside by procambium and xylem. The vessels of primary xylem, in most of the bundles are arranged in 4-5 parallel rows.

The pith occupies a large portion in the center of the section and consists of relatively large polygonal parenchyma cells with relatively small triangular intercellular spaces. Worthy to mention that the pith is connected with the cortex through medullary rays of 3 to 4 rows wide.

The primary structure of the main stem previously mentioned is in accordance with that given by Metcalfe and Chalk (1950) for family Compositae.

### 2.1.2. The median internode:

The transverse sections through the median portion of the main stem of lavender cotton plant (Fig. 3) prove that it is in a secondary state of growth. The stem is almost cylindrical in outline. The secondary growth added to the amount of vascular tissue and includes the formation of a periderm. The phellogen differentiates to a phellem consists of about four layers of cork cells arranged compactly and characterized by suberization of their walls. The phellogen comprised two layers of compactly arranged cells. Lenticels are found through the periderm.



**Fig. 3:** Transverse sections through the median portion of the main stem of lavender cotton plant, 6 months after transplantation showing its secondary structure.

A. Whole section (X62).

B. Magnified portion of A (X150).

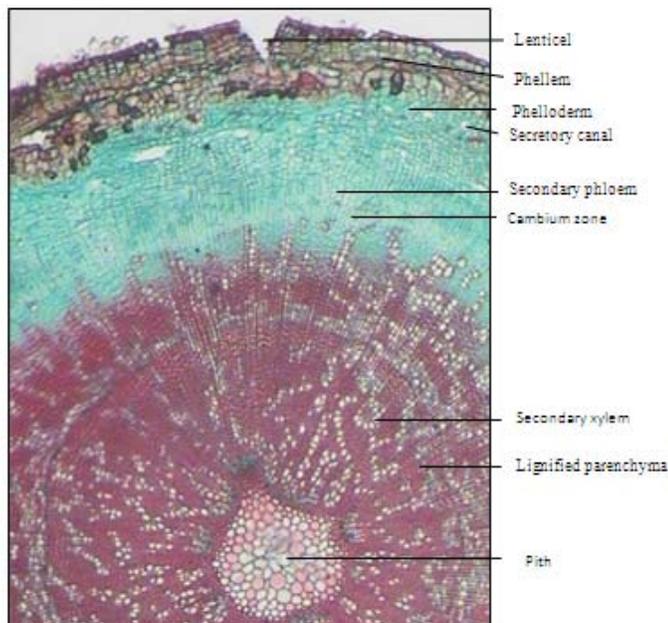
The cortex consists of about nine layers of parenchymatous cells. Schizogenous secretory canals are found in large number through the cortex.

The vascular cambium produces a continuous cylinder of secondary vascular tissues. Phloem and xylem occur in a collateral arrangement with the phloem located outside the xylem in the form of a continuous ring. Secondary xylem constitutes about 70% of the whole thickness of the vascular cylinder. Early differentiated secondary xylem which formed through the winter season, comprised a broad cylinder of xylem with small vessels arranged in radial rows surrounding the pith. Rays of 3-4 cells wide are occasional. Next to this distinguished cylinder of xylem, towards the outside, larger vessels of the secondary xylem differentiating later on (at summer season) arranged in bands fluctuated between tangential and diagonal in orientation. However, the tangential bands predominate and the aggregations form an intersecting network. Consequently, paratracheal lignified parenchyma accompanied the vessels also form tangential and diagonal bands; *i.e.*, confluent parenchyma developed. The pith is composed of thin-walled large polygonal parenchyma cells with relatively small intercellular spaces.

The above mentioned type of secondary growth agrees with that stated by Metcalfe and Chalk (1950) and Fahn (1985).

### 2.1.3. The basal intrnode:

The transverse section through the basal portion of the main stem of lavender cotton (Fig. 4). is generally indifferent qualitatively with that at its median portion. However, an increase in tissues quantity was recorded. The phellem consists of about seven layers of cork cells in compact arrangement. Lenticels are present. The phelloderm which developed inwardly comprised of four layers of paranchymatous cells.



**Fig. 4:** Transverse section through the basal portion of the main stem of lavender cotton plant, 6 months after transplantation showing its secondary structure (X 150).

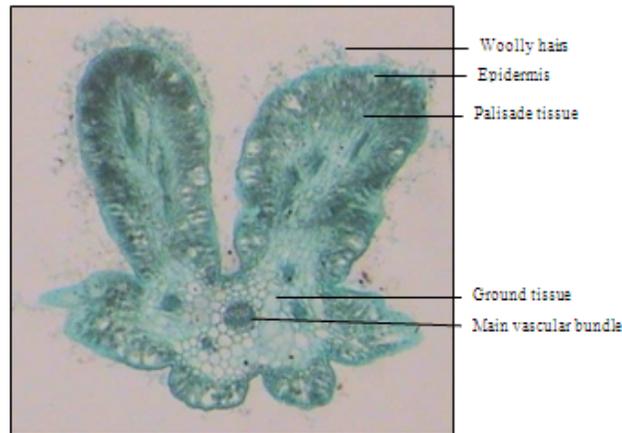
The cortex followed the periderm composed of about 11 layers of parenchymatous cells. Schizogenous secretory canals are present in the outer layers of the cortex.

Vascular cylinder is similar to that mentioned for the median portion of the stem, but wider in diameter. The pith occupied the center and composed of heterogeneous parenchyma cells.

## 2.2. The leaf:

### 2.2.1. The leaf blade:

The transverse section of lavender cotton leaf blade (Fig. 5) is wavy in outline, having distinct ridges and furrows. It seems like amoeba in shape and sometimes the section seems to be palmately lobed. The blade is bounded by a uniseriate epidermis of nearly square-shaped cells. The outer wall of the epidermis is somewhat thickened and covered with a thin layer of cuticle. Trichomes of woolly hairs type are present.



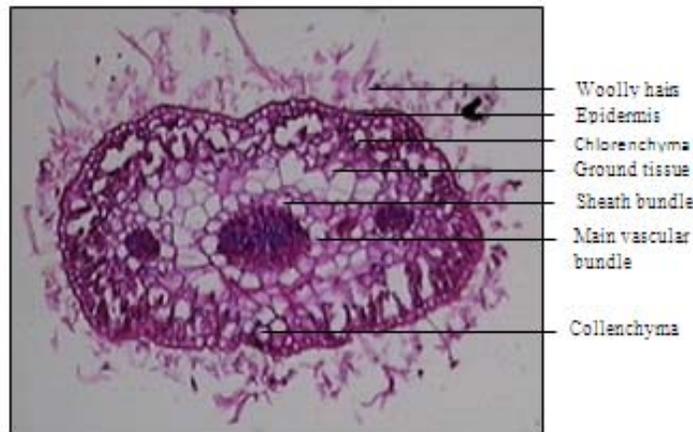
**Fig. 5:** Transverse section through the blade of lavender cotton leaf (X62).

The ground tissue consists of several layers of parenchyma cells of which the outer layer is of palisade type cells bounded by the epidermis.

There are five collateral bundles embedded in the ground tissue. The large one is the main vascular bundle found in the center surrounded by four small ones. The bundle is closed and surrounded by a sheath of one layer of parenchymatous cells.

#### 2.2.2. The leaf petiole:

The petiole of lavender cotton leaf as seen in the transverse section (Fig. 6) is somewhat flattened in shape, bounded by a uniseriate epidermis somewhat thickened and covered with a thin layer of cuticle. Stomata and trichomes are present. Trichomes are similar to those found on the leaf blade (woolly hairs).



**Fig. 6:** Transverse section through the leaf petiole of lavender cotton (X62).

The ground tissue is composed mainly of 6-7 layers of which the outer layer is collenchyma, whereas the remainder layers are chlorenchymatous cells. They are loosely arranged cells with many wide intercellular spaces. In the center there is a main vascular bundle (the largest one), to the right and to the left of this bundle another small bundle develops. Bundles are collateral and closed, being surrounded by a sheath of one layer of parenchymatous cells.

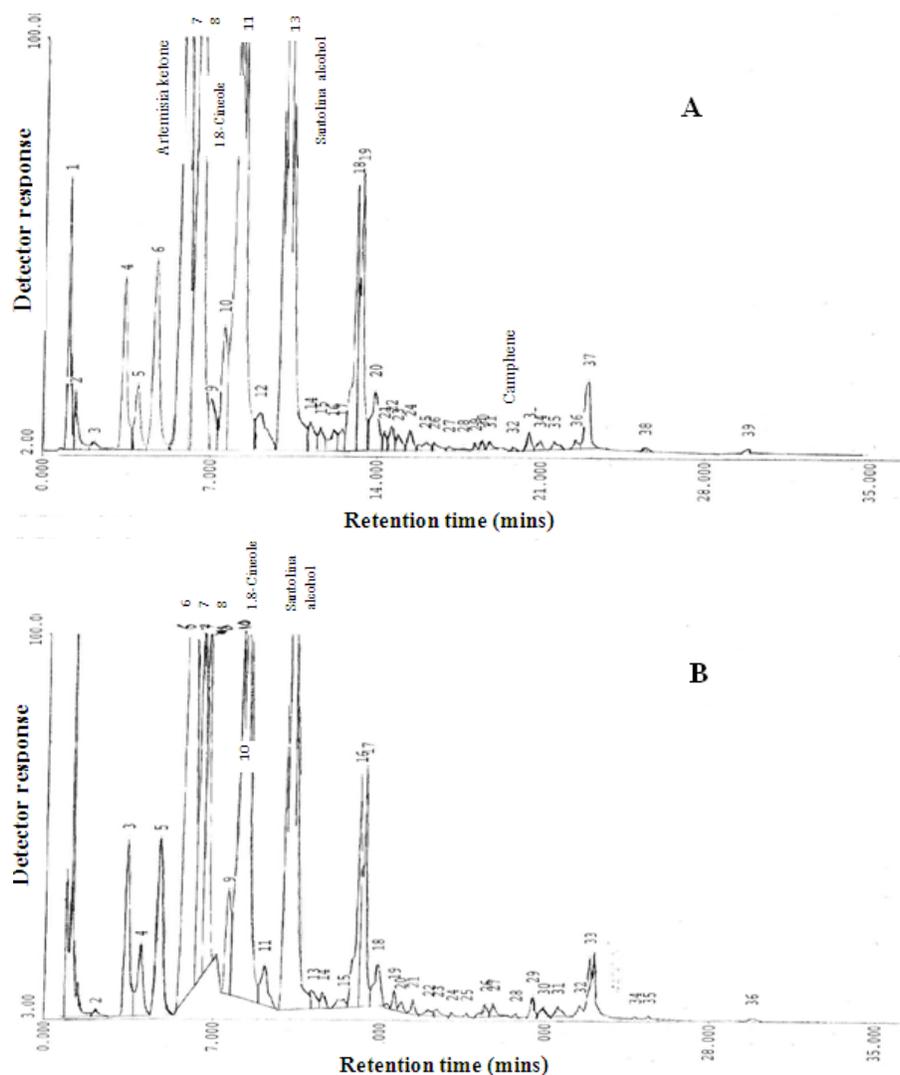
Metcalfe and Chalk (1950) described the type of petiole vascular bundles of *Santolina chamaecyparissus* L. being in conformity with that previously given.

## II. Analysis of the volatile oil:

Samples of lavender cotton herb were collected twice for volatile oil analysis; at flower buds stage and when flowers opened. Distillation procedure at both growth stages was replicated to define the volatile oil quantity in the herb precisely. The average amount of volatile oil at both stages of growth was similar, being 1% v/w, whereas the yield of volatile oil scored 1.63 ml. per plant. These findings are in conformity with those mentioned by Garg *et al.* (2001) for *Santolina chamaecyparissus* L. (1.1% v/w). The volatile oil is a mobile liquid of pale yellow color having an aromatic harsh smell.

From the GLC analysis at flower buds stage, the volatile oil was known to be a complex mixture contains 39 components (Fig. 7 A). The components were designated 1-39 according to their retention times for further reference. Only 22 of these could be identified according to the detector response. The remainder unidentified components, however, were only present as traces.

Concerning the opened flowers stage, GLC analysis of the volatile oil (Fig. 7 B) revealed that it composed of 36 components. For ease of reference, they were designated 1-36 according to their retention times. Twenty two of these components were already known.



**Fig. 7:** Gas chromatogram of *Santolina chamaecyparissus* L. volatile oil obtained using water distillation. Column : 5% phenyl polysil phenylene siloxane, at initial temp. 50°C for 3 min; rate 5°C/min.; final temp. 180°C and hold time 40 min.

A . At flower buds stage. B. At opened flowers stage.

Various characteristics of isolated components of the volatile oil at both stages of growth are listed in Table 1. Worthy to note that investigations indicated that the identified components were 22 in both studied stages being the same qualitatively but differed quantitatively. At flower bud stage, santolina alcohol (18.043%) was the main component of the volatile oil; 1.8-cineole (17.798%) came second and artemisia ketone (15.661%) was the third. In contrast, camphene (0.026%) recorded the least quantity among the identified components. Regarding the second growth stage; 1.8-cineole (19.474%) which was the second at flower buds stage became on top and santolina alcohol (18.413%) replaced it; *i.e.*, santolina alcohol became the second at opened flowers stage. Artemisia ketone (16.641%), however, kept its place as the third component, quantitatively. On the other hand,  $\alpha$ -pinene (0.148%) recorded the least concentration. Camphene which was the least quantitatively at flower bud stage ranked number 20 with concentration of (0.210%) at opened flowers stage followed by terpinolene (0.209%) at position number 21.

**Table1:** Characteristics of the components of *Santolina chamaecyparissus* L. volatile oil at flower buds and opened flowers stages found in the peaks of the corresponding chromatograms (R.T.: Retention time).

Peaks	Flower buds stage			Opened flowers stage		
	Components	R.T. (mins)	Conc. (%)	Components	R.T. (mins)	Conc. (%)
1	Unidentified	1.067	2.771	Unidentified	1.167	5.803
2	Traces	1.383	0.760	$\alpha$ -Pinene	2.200	0.148
3	$\alpha$ -Pinene	2.200	0.187	Sabinene	3.467	3.483
4	Sabinene	3.450	3.089	Terpenen 4-o1	4.067	1.830
5	Terpenen 4-o1	4.033	1.322	$\beta$ -Pinene	4.783	4.477
6	$\beta$ -Pinene	4.750	4.302	Artemesia ketone	6.100	16.641
7	Artemesia ketone	6.033	15.661	Borneol	6.450	7.362
8	Borneol	6.583	14.310	Limonene	6.667	6.395
9	Limonene	7.033	1.115	Camphor	7.567	2.132
10	Camphor	7.533	2.540	1.8-Cineole	8.283	19.574
11	1.8-Cineole	8.300	17.798	$\beta$ -Phellandrene	9.117	1.226
12	$\beta$ -Phellandrene	9.067	1.505	Santolina alcohol	10.267	18.413
13	Santolina alcohol	10.233	18.043	Myrcene	11.217	0.368
14	Myrcene	11.183	0.636	$\alpha$ -Terpinene	11.617	0.248
15	$\alpha$ -Terpinene	11.617	0.445	Traces	12.467	0.252
16	Traces	12.150	0.523	Dimethyl styrene	13.017	3.627
17	Traces	12.483	0.379	Phellandrene	13.250	4.095
18	Dimethyl styrene	13.017	3.822	Myrtenol	13.883	1.160
19	Phellandrene	13.250	4.510	Terpinolene	14.600	0.209
20	Myrtenol	13.900	1.700	Traces	14.867	0.121
21	Traces	14.317	2.273	Thujone	15.400	0.197
22	Terpinolene	14.617	0.395	Traces	16.083	0.182
23	Traces	14.883	0.324	Traces	16.467	0.137
24	Thujone	15.417	0.386	Traces	17.117	0.028
25	Traces	16.100	0.301	Traces	17.717	0.028
26	Traces	16.483	0.185	$\alpha$ -Terpineol	18.550	0.279
27	Traces	17.133	0.064	Camphene	18.850	0.210
28	Traces	17.733	0.083	Traces	19.783	0.036
29	Traces	18.267	0.068	Caryophyllene	20.467	0.353
30	$\alpha$ -Terpineol	18.567	0.126	Traces	20.933	0.222
31	Traces	18.867	0.100	Curcumene	21.517	0.255
32	Camphene	19.817	0.026	Traces	22.467	0.099
33	Traces	20.500	0.249	Traces	22.850	0.299
34	Caryophyllene	20.950	0.159	Traces	24.883	0.013
35	Traces	21.550	0.157	Traces	25.467	0.032
36	Curcumene	22.500	0.151	Traces	29.783	0.066
37	Traces	23.000	1.468	-	-	-
38	Traces	25.500	0.014	-	-	-
39	Traces	29.833	0.053	-	-	-

The aforementioned results are in agreement with those of Villar *et al.* (1986) who found that the volatile oil of *Santolina chamaecyparissus* subsp. *squarrosa* contained a higher proportion of oxygenated components (*e.g.* 1.8-cineole) than hydrocarbons. Pérez-Alonso and Velasco-Negueruela (1992) stated that monoterpenes (*e.g.* 1.8-cineole) predominated in volatile oil of peninsular populations corresponding to *Santolina chamaecyparissus* subsp. *incaca* and *squarrosa*. Giner *et al.* (1993) separated 36 components from the volatile oil of lavender cotton, with 1.8-cineole as a major component. Moreover, several authors reached to similar results to those given in the present study; *e.g.*, Haggag *et al.* (2000), Garg *et al.* (2001) and Grosso *et al.* (2009).

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