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II. PERICARP ANATOMICAL STUDY OF SOME LAMIACEAE NUTLETS IN SAUDI ARABIA AND ITS TAXONOMIC SIGNIFICANCE

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Abstract

The nutlets transverse sections of 23 selected taxa belonging to 12 genera of Saudi Arabia Lamiaceae were examined using light microscope and detailed description of the pericarp anatomical characteristics were provided. Nutlets have shown obvious differences in the presence or absence of hairs, papillae, mucilage, endosperm and crystals, also, in thickness of pericarp layers, parenchymatous and sclerenchymataus layers. The relationships between the studied taxa were presented as phenogram. This study confirms the usefulness of the nutlet anatomical features as additional characters in taxonomical studies of the Lamiaceae especially mucilage production (myxocarpy) and the sclerenchymatous layer (shape, pigment, and cavity) of the endocarp. They are diagnostic at the generic and subfamily levels. The anatomical studies indicate the family is polymorphic in pericarp characters.

Key words: Lamiaceae, Anatomy, Pericarp, Myxocarpy, KSA.

Introduction

Lamiaceae or Mint family is a large cosmopolitan family, mostly abundant in the Mediterranean region to central Asia. It comprises about 252 genera, 6700 species (Mabberley, 1997; Simpson, 2006).

In Saudi Arabia flora, Lamiaceae is represented by 13 genera including 33 species (Migahid, 1996). Collenette (1999) recognized 75 species belonging to 25 genera while Chaudhary (2001) showed 26 native genera of 76 species. Al-Nafie (2004) recognized 26 genera and 70 species. Nutlet anatomy in the Lamiaceae has proved useful at various degrees to different levels of the taxonomic hierarchy. Bentham (1848) who laid the foundations of the classification of the family, used some nutlet characters in the diagnoses of some tribes.

Studies on nutlets in the Lamiaceae have been conducted on the pericarp structure (Ryding, 2001, 2009; Hye-Kyoung & Hong, 2006 and Dinc & Dogu, 2012). Also, anatomical studies of some species of *Salvia* has been examined by some researchers (Wagner, 1914; Wojciechowska, 1961; Hedge, 1970 and Habibvash *et al*., 2007a).

Economically, most members of this family are of great importance, some species are edible, combat pests parasites and of fungicide, bactericide properties. Essential oil is used for perfume others of medicinal value used as culinary herb (International Symposium of the Labiatae, 2006). So, the members of this family need to be reviewed in terms of their systematic positions.

The aim of this study is to provide detailed description of the pericarp anatomical characteristics in some species of Saudi Arabia of Lamiaceae and to establish their interrelationships.

Materials and Methods

In this study 23 taxa belonging to 12 genera, three subfamilies; Nepetoideae, Lamioideae and Teucrioideae of Lamiaceae (21 wild species & two cultivated in Saudi Arabia) were collected from different localities (Table 1) and identified according to Collenette (1999), Migahid, (1996), Chaudhary (2001) and Boulos (2002, 2009).

For pericarp anatomical study: Dry nutlets were placed for 10 days in a mixture of distilled water, 96% ethanol and glycerol taken in equal proportions. The paraffin method was used for preparing a cross sections of nutlets at middle part 8 Mm thickness by using a rotatory microtome. The sections were stained with ersorin 1% (Johansen, 1940). Slides examined by Leica microscope and photos were taken. The thickness of the layers in the nutlets were measured with micromet.

For Myxocarpy (Mucilage) investigation: At least five nutlets were treated with distilled water, examined after 8 hours and measured under the light microscope. The presence of mucilage on the nutlet surface was determined by the method of Ryding (1992b) based on the extent of swelling of mucilaginous cells when nutlet become wet. There were four types of reactions:

1. Strong mucilaginous reaction (mucilaginous cells 0.8-1.5 mm long).
2. Moderate reaction (0.1-0.5 mm long).
3. Weak reaction (> 0.1 mm long).
4. Very weak reaction (No appreciable elongation of mucilaginous cells after swolling in water.

For numerical analysis: In order to prepare a data matrix for numerical analysis the presence or absence of the recorded characters was coded as 1 and 0 respectively .The obtained characters was analysed by NTSYS – PC-program using the UPGMA clustering method (Rohlf, 1993). The relationships between the studied taxa, were demonstrated as phenogram.

Table 1. Shows the collection data and synonyms of the specimens.

S. No.		
1.	(1.1.) *Lavandula citriodora* A. G. Mill	Al-Deraia, Al-Rriyadh
	(1.2.) *L. coronopifolia* Poir. = (*L. stricta* Delie, Descr.)	Al-Seyal, Al-Taif – Makha Road
	(1.3.) *L. dentata* L.	Al-Shafa, Al-Taif
	(1.4.) *L. pubescens* Decne.	Al-What and Al-Wahit, Al-Taif
2.	(2.1.) *Marrubium vulgare* L.	Bany Saad, Al-Taif
3.	(3.1.) *Mentha piperita* L.	Al-Deraia, Al-Rriyadh
4.	(4.1.) *Micromeria biflora* Benth.	Al-Shafa, Al-Taif
	(4.2.) *Micromeria imbricata* (Forssk.) C. Chr. = (*Thymus imbricatus* Forssk.) = (*Thymus biflorus* Buch- Ham. ex D. Don, prodr.) = (*Satureja imbricata* (Forssk.) Briq.) = (*Satureja biflora* (Buch-Ham. ex D. Don) Briq.)	Al-Shafa, Al-Taif
5.	(5.1.) *Nepeta deflersiana* Schweinf. ex Hedge	Al-Shafa, Al-Taif
6.	(6.1.) *Ocimum americanum* L.	Herbarium of Biology Department, Al Taif Univ. –Garwa
	(6.2.) *O. basilicum* L.	Al-Seyal, Al-Taif – Makha Road
	(6.3.) *O. canum* Sims	Herbarium of Biology Department Al-Taif Univ. –Garwa
	(6.4.) *Ocimum filamentosum* Forssk. = (*Becium filamentosum* (Forssk.) Chiov.)	Al-Kalidih, Al-Taif
	(6.5.) *Ocimum forsskalii* Benth. = (*O. menthifolium* Hochst. ex Benth.) = (*O. hadiense* Sensu Boulos) = (*Plectranthus hadiensis* Sensu Boulos)	Al-Deraia, Al-Rriyadh
	(6.6.) *Ocimum tenuiflorum* L. * = *O. sanctum*	Shehar, Al-Taif
7.	(7.1.) *Origanum syriacum* L.* = (*O. maru* L. var. *sinaicum* Boiss.)	Shehar, Al-Taif
8.	(8.1.) *Otostegia fruticosa* ssp., *schimperi* (Benth.) Sebald, Stuttgarter Beitr. = (*Ballota schimperi* Benth.) = (*Otostegig. schimperi* (Benth.) Boiss.) = (*O. Kaiseri* T ckh.)	Al-What and Al-Wahit, Al-Taif
9.	(9.1.) *Plectranthus comosus* Sims = (*P. barbatus* Andr.)	South road, Al-Taif - Al-Baha
10.	(10.1.) *Salvia aegyptiaca* L.= (*S. pumila* Benth	Al-Deraia, Al-Rriyadh
	(10.2.) *S. officinalis* L.	Herbarium of Biology Department Al-Taif Univ. –Garwa
11.	(11.1.) *Stachys* sp. *aff. Schimperi* Vatke	Al-Shafa, Al-Taif
12.	(12.1.) *Teucrium oliverianum* Ging. ex Benth.	Al-Saffa, Jiddah

* Cultivated taxa

Results and Discussion

Nutlet in Lamiaceae referred as indehiscent one seeded fruits, so the pericarp resembles a seed coat in structure (Esau, 1977 & Pandy, 2004). The results obtained from the pericarp anatomical study are presented in (Table 2 & Figs. 1-23) and discussed as the following:

Exocarp: In all the studied taxa, the pericarp is differentiated into three Regions; exo, meso and endocarp. The family is also characterized by the presence of myxocarpy (formation of mucilage when nutlets become wet). It has been reported in many taxa of the family (Ryding, 1992a, b; 1993a, b; 1995 and Hussein, 1995). This character could be used in the systematic of this family, Vaughan *et al*., (1963) and Vaughan (1968) reported that, presence of mucilage in the epidermal cells of Cruciferae seeds of great taxonomic significance at inter or intra-specific level.

The results indicate that myxocarpy is found in 14 species while absent in the remainder 9 taxa. There were clear differences in thickness of mucilaginous layer; it has a strong reaction (1-1.49 mm) in 7 taxa, moderate (0.1–0.5 mm) in another 7 taxa while very weak (0.02–0.04 mm) in the remainder 9 taxa (Table 2).

The mucilaginous cells lies above epidermal cells either pentagonal radially and tangentially elongated, cuboid, crushed or of irregular shape in some taxa (Table 2 & Figs. 1, 5, 12, 4). The results revealed that, in species whose nutlets produce mucilage the exocarp consists of two different types of cells; mucilaginous and non mucilaginous. The latter are much larger, radially elongated, thick walled, the cavity filled with pigment in the centre, while the non mucilaginous cells are often narrow as in *Lavandula citriodora* (Fig. 1).

Table 2. The anatomical aspects of the nutlets of the taxa studied of the Lamiaceae.

No.	Character taxa	Pericarp											Endosperm	
		Exocarp				Mesocarp				Endocarp				
		Myxocarpy	Mucilaginous dimensions (mm)	Mucilaginous reaction	Mucilaginous cells shape	No. of rows	Wall thickness	Cells shape	Pigment colour	Sclerenchymatous cells shape	Sclereid cavity	Crystals	Presence	Shape of parenchymatous cells
1.	*Lavandula citriodora*	+	0.48	Moderate	Pentagonal radially & tangentially elongated	1	Thick	Compressed	Brown	Radially elongated	Fusiform	+	+	Rounded
2.	*Lavandula coronopifolia*	+	0.49	Moderate	Pentagonal radially & tangentially elongated	2	Thick	Compressed	Brown	Radially elongated	Fusiform	+	+	Rounded
3.	*Lavandula dentata*	+	0.16	Moderate	Irregular	2-3	Thick	Compressed	Brown	Radially elongated	Elongated	+	+	Rounded
4.	*Lavandula pubescens*	+	0.60	Strong	Pentagonal radially & tangentially elongated	2	Thick	Compressed	Brown	Radially elongated	Elongated	+	+	Rounded
5.	*Marrubium vulgare*	+	0.12	Moderate	Cuboid	3-4	Thick	Compressed	Dark brown	Non sclerenchymatous	Rounded	–	+	Pentagonal with storage materials
6.	*Mentha piperita*	–	0.02	Very weak	Not easly detected (absent)	2-3	Thick	Compressed	Dark brown	Elongated	Rounded	+	+	Rounded
7.	*Micromeria biflora*	–	0.04	Very weak	–	3-4	Thick	Compressed	Dark brown	Tangentially elongated	–	–	–	–
8.	*Micromeria imbricata*	+	0.59	Moderate	Irregular	2	Thick	Compressed	Brown	Compressed	-	-	+	Rounded
9.	*Nepeta deflersiana*	–	0.04	Very weak	–	1	Thin	Tangentially elongated	Pale brown	Elongated	Ovate	+	+	Rounded
10.	*Nepeta sheliae*	–	0.04	Very weak	–	1	Thin	Compressed	Pale brown	Elongated	Rounded	+	+	Rounded
11.	*Ocimum americanum*	+	0.71	Strong	Irregular	3	Thick	Wavy & Compressed	Dark brown	Small , radially elongated	Rounded	–	+	Rounded

Table 2. (Cont'd.).

No.	Character taxa	Pericarp											Endosperm	
		Exocarp				Mesocarp				Endocarp				
		Myxocarpy	Mucilaginous dimensions (mm)	Mucilaginous reaction	Mucilaginous cells shape	No. of rows	Wall thickness	Cells shape	Pigment colour	Sclerenchymatous cells shape	Sclereid cavity	Crystals	Presence	Shape of parenchymatous cells
12.	*Ocimum basilicum*	+	1.08	Strong	Crushed cells	2	Thick	Wavy & Compressed	Dark brown	Small , radially elongated	Rounded	–	+	Rounded
13.	*Ocimum canum*	–	0.04	Very weak	–	3-4	Thick	Disorganized	Dark brown	Non Sclerenchymatous	–	–	+	Rounded
14.	*Ocimum filamentosum*	+	1	Strong	Crushed cells	3	Thick	Wavy & Compressed	Dark brown	Small, radially elongated	Rounded	–	+	Rounded
15.	*Ocimum forsskalii*	+	1.05	Strong	Large irregular	3	Thick	Wavy & Compressed	Dark brown	Small, radially elongated	Rounded	–	+	Rounded
16.	*Ocimum tenuiflorun*	+	1.49	Strong	Large irregular	3	Thick	Wavy & Compressed	Dark brown	Small, radially elongated	Rounded	–	+	Rounded
17.	*Origanum syriacum*	–	0.03	Very weak	–	2-3	Thick	Disorganized	Brown	Disorganized Sclerenchymatous	Rounded	+	+	Rounded & thick wall
18.	*Otostegia fruticosa* spp. *schimperi*	–	0.03	Very weak	–	3	Thick	Cubic closely spaced	Brown	Compressed	–	–	+	Rectangular with stoarge materials
19.	*Plectranthus comosus*	+	0.25	Moderate	Irregular alternated with clavat hairs	2	Thick	Compressed	Brown	Radially elongated	Star – shape	+	+	Rounded
20.	*Salvia aegyptiaca*	+	0.76	Strong	Irregular	5	4 rows thick Irner one thin	Tangentally elongated	Blackish brown	Radially elongated	Elliptic	+	+	Rounded
21.	*Salvia officinalis*	+	0.13	Moderate	Cuboid	5	Thick	Compressed	Brown	Vertically arranged bone cells	Elongated	–	–	–
22.	*Stachys* sp.aff. *schimperi*	–	0.03	Very weak	–	2	Thick	Disorganized	Brown	Sclerenchymatous layer with clavate – shape cells	Fusiform	–	–	–
23.	*Teucrium oliverianum*	–	0.02	Very weak	–	5	Thick	Disorganized	Brown	Compressed	–	–	+	Pentagonal with storage materials

+ Present - Absent

Mesocarp: The results showed that the number of mesocarp rows ranged from 1-5 rows (Table 2 and Figs. 1-23). The wall are very thick in all the studied taxa except in *Nepeta deflersiana* and *N. sheliae*, which have very thinwall.

The cell shape in most of the studied taxa are compressed cells while compressed and wavy in genes *Ocimum*, cubic in *Otostegia*, tangentially elongated in *Nepeta deflersiana* and *Salvia egyptiaca* while disorganized in the remainder taxa (Figs. 11, 18, 9, 20, 7).

The pigment colour in the majority of the studied taxa are brown – dark brown except in *Nepeta deflersiana*, pale brown and in *Salvia aegyptica* is blackish brown.

Endocarp: The sclerenchymatous cells have been found to be rather consistent within genera and provide very important evidence, useful in the classification at subfamily, tribe and subtribe levels (Ryding, 1993c).

The obtained observations cleared out that sclerenchymatous cells in the studied taxa are radially elongated in two genera *Ocimum* and *Lavandula*, also found in *Plectranthus*, *Salvia*, *Mentha*, *Nepeta sheliae* and *Nepeta deflersiana* (Fig. 9) while tangentially elongated in *Micromeria biflora* (Fig. 7). In *Salvia officinalis* it tackes Bone shape (Fig. 21) while in *Micromeria imbricata*, *Otostegia* and *Teucrium* found as compressed cells (Figs. 8, 18, 23). In *Stachys* it takes clavate shape (Fig. 22), non sclerenchymatous in *Ocimum canum* (Fig. 13) and disorganized in *Marrubium vulgare* and *Origanum syriacum* (Figs. 5, 17). With respect to sclereid cavity, it takes different shapes; fusiform in *Lavandula citriodora*, *L. coronopifolia* and *Stachys*, elongated in *Lavandula dentata*, *Lavandula pubescens* and *Salvia officinalis* star shape in *Plectranthus*, *elliptic* in *Salvia aegyptiaca*, *ovate* in *Nepeta defleresiana*, rounded in *Marrubium vulgare*, *Mentha piperita*, *Nepeta sheliae*, *Ocimum americanum*, *O. basilicum*, *O. filamentosum*, *O. forsskalii*, *O. tenuiflorum* and *Origanum syriacum* while absent in the remainder taxa (Table 2 & Figs. 2, 3, 5, 9, 19, 20).

With regard to crystals depositions, it was recorded in ten taxa and Lacked in 13 taxa as in (Table 2).

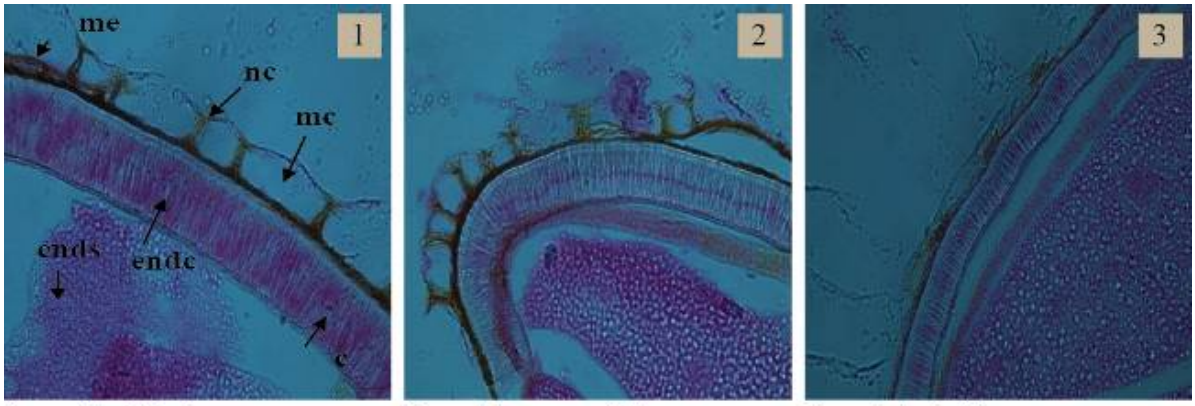
Endosperm: In all taxa studied the endosperm is compared of as multilayers of parenchymatous cells except in *Micromeria biflora*, *Salvia officinalis* and *Stachys*. The parenchmatous cells are either pentagonal in shape as in *Marrubium vulgare* and *Teucrium* (Figs. 5, 23), rectangular in *Otostegia* (Fig. 18) rounded in the remainder 17 taxa (Fig. 2).

To some extent some of the studies made earlier support the findings of the present work as Tobe *et al*., (1987) and in contrast with the other observations.

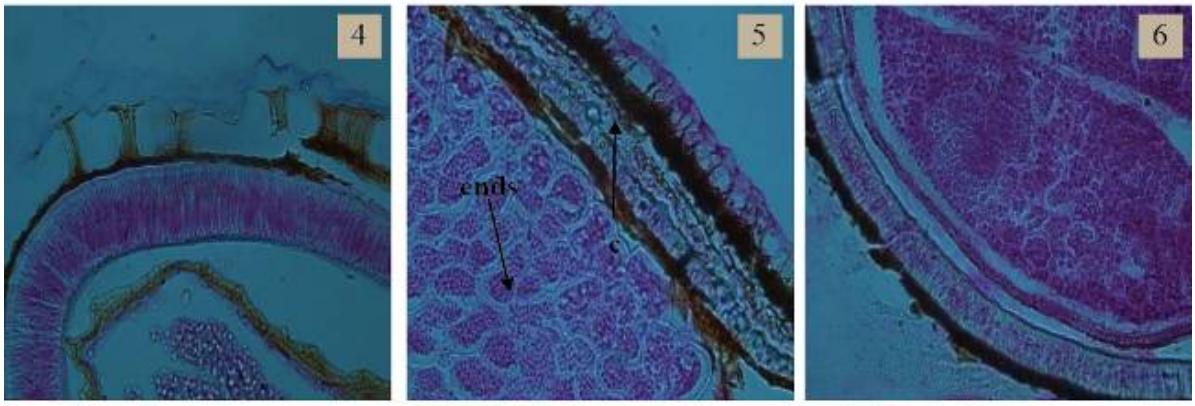
II- Numerical analysis: The phenogram was constructed based on anatomical nutlet characters of 23 taxa of Lamiaceae which were categorized in 14 groups included under six clusters, two subseries and one series (Table 3 & Fig. 24). Some of the investigated taxa were scattered across the phenogram. With regards to the four *Lavandula* taxa and *Plectranthus* were clustered together in (C_1) due to high similarity in the anatomical nutlet features. This result agree with Bentham (1848) who put them in the same tribe: Ocimoideae and with EL-Gazzar and Watson (1970) who put them in the same group.

Table 3. The proposed treatment based on numerical analysis of anatomical nutlet characters of the 23 studied taxa of Lamiaceae [extracted from the phenogram (Fig. 24)].

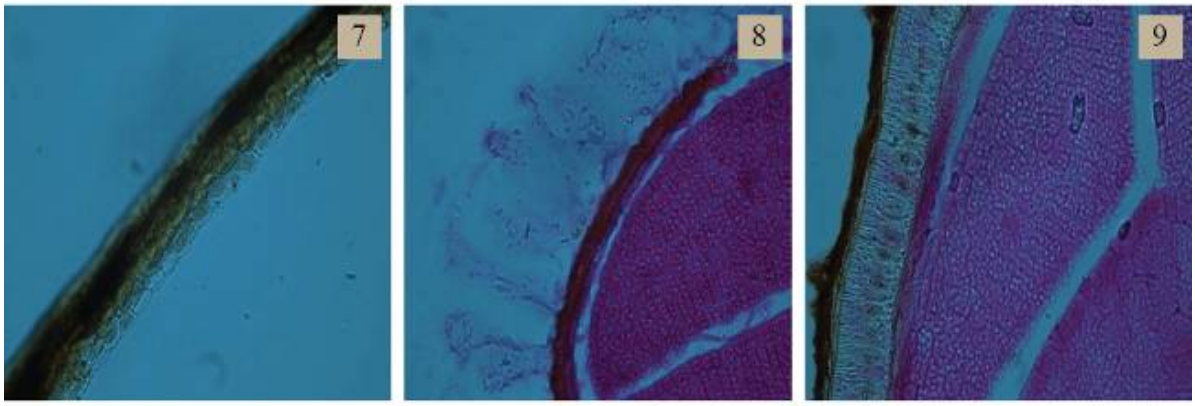
GR_s	Taxa	Clusters (C_{1-6})	Subseries SS_{1-2}	Series S_1
GR_1	• *Lavandula citriodora* • *L. coronopifolia*	C_1	SS_1	S_1
GR_2	• *Plectranthus comosus*			
GR_3	• *Lavandula dentate*			
GR_4	• *Lavandula pubescens*			
GR_5	• *Micromeria imbricata*			
GR_6	• *Salvia officinalis*			
GR_7	• *Marrubium vulgare*	C_2		
GR_8	• *Ocimum americanum* • *O. forsskalii* • *O. tenuiflorum* • *O. basilicum* • *O. filamentosum*			
GR_9	• *Salvia aegyptiaca*	C_3		
GR_{10}	• *Mentha piperita* • *Origanum syriacum*	C_4	SS_2	
GR_{11}	• *Nepeta deflersiana* • *N. sheliae*			
GR_{12}	• *Micromeria biflora* • *Ocimum canum*	C_5		
GR_{13}	• *Otostegia fruticosa* • *Teucrium oliverianum*			
GR_{14}	• *Stachys schimperi*	C_6		



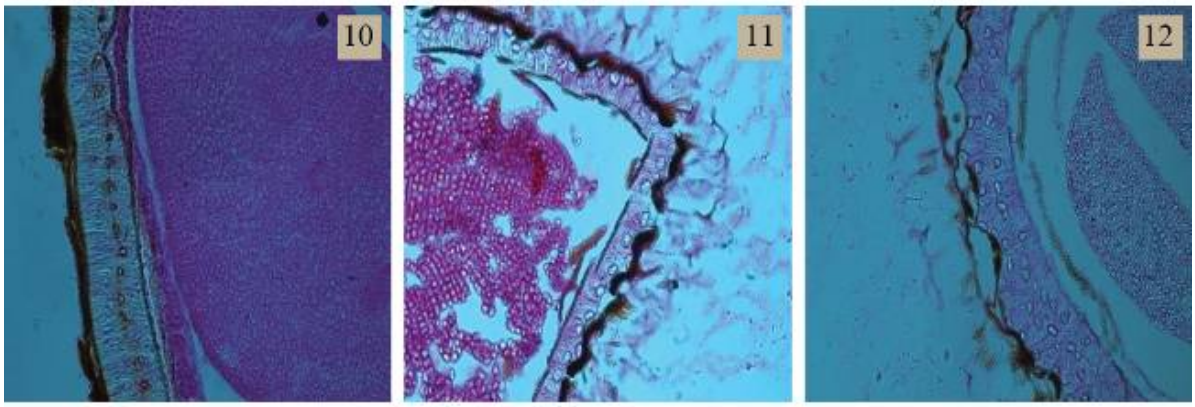
Lavandula citriodora *Lavandula coronopifolia* *Lavandula dentata*



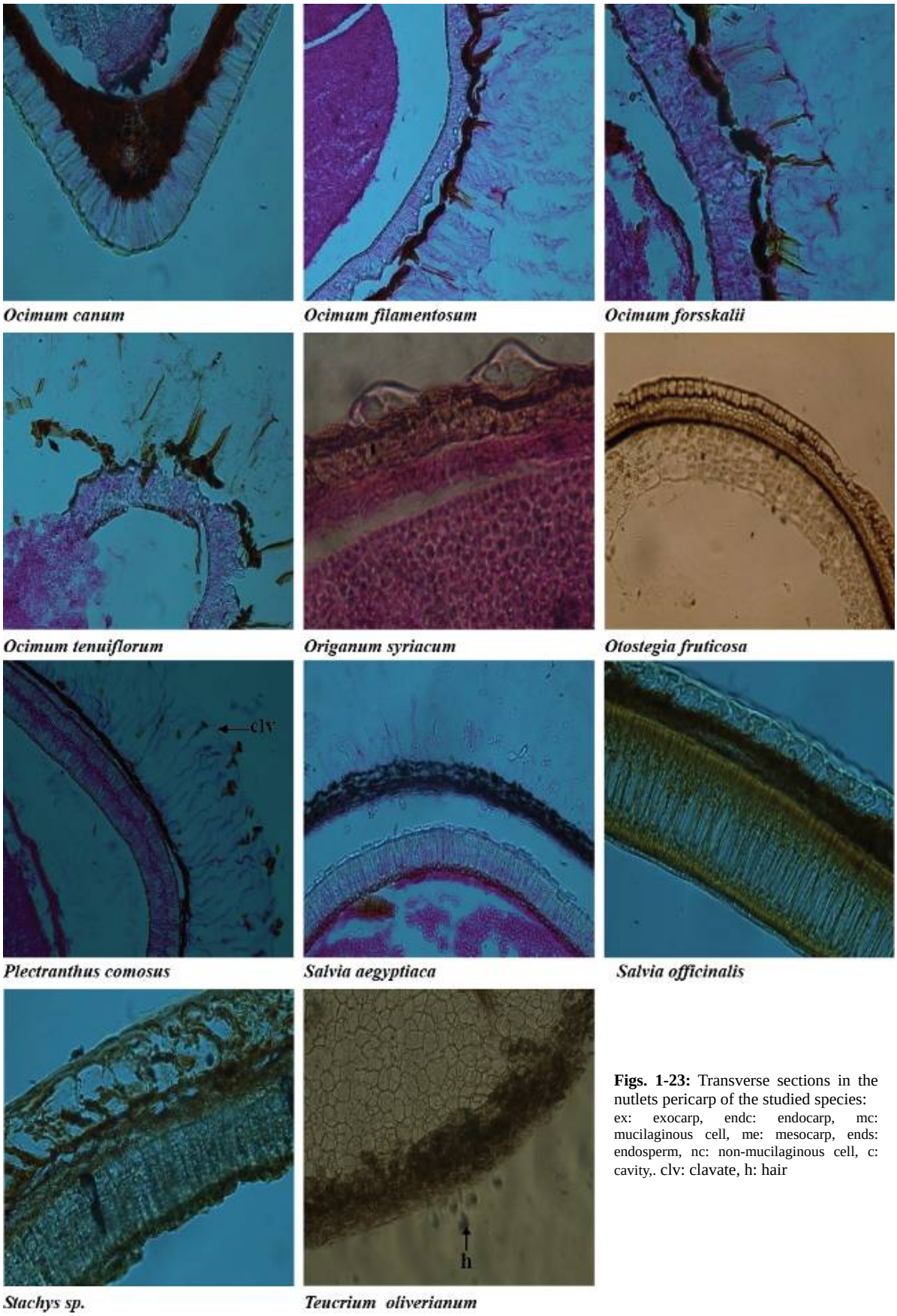
Lavandula pubescens *Marrubium vulgare* *Mentha piperita*



Micromeria biflora *Micromeria imbricata* *Nepeta deflersiana*



Nepeta sheilae *Ocimum americanum* *Ocimum basilicum*



Figs. 1-23: Transverse sections in the nutlets pericarp of the studied species: ex: exocarp, endc: endocarp, mc: mucilaginous cell, me: mesocarp, ends: endosperm, nc: non-mucilaginous cell, c: cavity,. clv: clavate, h: hair

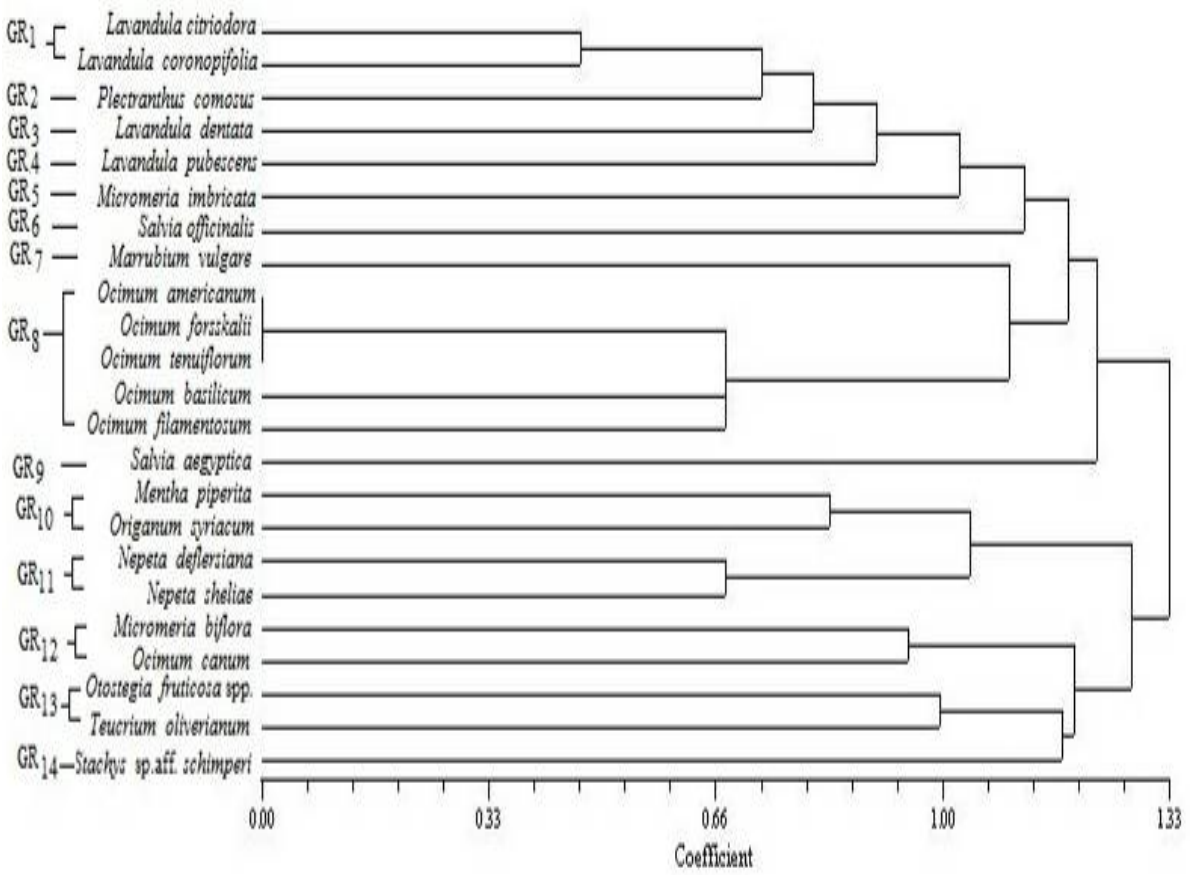


Fig. 24: Phenogram based on Anatomical nutlet characteristics of the 23 studied taxa of Lamiaceae in Saudi Arabia.

Ocimum are clustered in one group (GR_8) except. *O. canum* this agrees with Bentham (1848) who put them in the same tribe: Ocimoideae and with Cantino *et al*., (1992c) who put them in the same subfamily: Nepetoideae and the same tribe: Ocimeae. With respect to *Marrubium vulgare* was splitted in separate group (GR_7), this result agrees to some extent with Bentham (1848). Also, with regard to *Salvia aegyptiaca* separated in this work in one group (GR_9) while *Salvia officinalis* is separated in another group (GR_6) but the two species are clustered together in one cluster (C_1). This finding may support Walker *et al*., (2004) opnion, that *Salvia* is not monophyletic.

Clustering of the two studied taxa *Origanum* and *Nepeta* in one cluster (C_4) according to anatomical features agrees with EL-Gazzar & Watson (1970) who put them in one group according to morphological features while Bentham (1848) put *Mentha* and *Origanum* in the same tribe: Satureieae and the same subtribe: Menthoideae. Our anatomical results agree with the later classification, since *Mentha* and *Origanum* grouped together in (GR_{10}) and the same cluster (C_4).

Grouping of the two studied taxa of *Nepeta* in one group (GR_{11}) agree with Cantino *et al*., (1992c) who put them in the same tribe: Nepeteae and with EL-Gazzar & Watson (1970) who put them in one group . Also, agree with Bentham (1848) who put them in separate tribe: Nepeteae than the other studied taxa.

The two studied taxa of *Otostegia* and *Teucrium* were grouped together (GR_{13}) due to high similarity in anatomical features. This result disagrees with EL-Gazzar & Watson (1970) who put them in two distinct groups. With respect to *Stachys* our results was splitted it in separate group (GR_{14}) than the other studied taxa. This result in accordance with EL-Gazzar and Watson (1970).

Finally this study support the usefulness of using the nutlet anatomical characteristics as additional taxonomic criteria in taxonomical studies on family Lamiaceae, but more work is still needed to the reclassification of this family.

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