

## I. MORPHOLOGICAL NUTLET CHARACTERISTICS OF SOME LAMIACEAE TAXA IN SAUDI ARABIA AND THEIR TAXONOMIC SIGNIFICANCE

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### Abstract

The nutlet characters in the Lamiaceae are of great taxonomic significance. Macro and micro morphology nutlets of 23 taxa belonging to 12 genera of Lamiaceae from Al-Taif, Saudi Arabia have been studied for the first time by both light and scanning electron microscope. Observations revealed that nutlet color, shape, size, presence of areole are of limited taxonomic value. However the pericarp sculpturing are the most important diagnostic characters for differentiating the species through a constructed key. Three main distinct types of nutlets sculpture can be distinguished; smooth, partly sculptured and sculptured, within these types 12 subtypes are also recognized (reticulate, reticulate-foveate alveolate, granulate, rugose-scalariform, favulariate, protuberance, colliculate, ruminant, ocellate, verrucate and tuberculate). The relationships between the studied taxa were demonstrated as a phenogram.

**Key words:** Morphology, Lamiaceae, Nutlet, Saudi Arabia, Sculpture, SEM.

### Introduction

The Lamiaceae (Labiatae) is a large family 252 genera and 6700 species (Mabberley, 1997, Simpson, 2006). In the flora of Saudi Arabia, Lamiaceae is represented by 26 genera including 70 species (AL-Nafiy, 2004).

Various studies on fruit morphology (Nutlet) presented the importance of the pericarp of several belonging to taxa Lamiaceae at different taxonomic levels (Wagner, 1914; Wojciechowska, 1966 & 1972; Hedge, 1970, Ryding, 1992a, b; 1993b and 1995; Husain *et al.*, 1990; Hussein, 1995 and Pandey, 2004). Morphological studies on nutlets were also conducted in same specific genera of Lamiaceae (Mosquero *et al.*, 2005, 2006, Kaya & Dirmencl, 2008 and Canan *et al.*, 2012). Moreover, the use of scanning electron microscope (SEM) which revealed fine details on the fruit surface, was quite helpful in solving taxonomic problems at the family, subfamily, generic, specific or even infraspecific levels (Paton, 1990; Rejdali, 1990; Ryding, 1992a, b; Budantsev, 1993c, d and Hussein, 2002).

Due to the insufficient data about the Lamiaceae nutlet in Saudi Arabia, the present study aims to describe in details the nutlet characters (macro and micro morphology) of 23 Saudi Arabian taxa by the aid of both light and scanning electron microscope. Also to evaluate the usefulness of nutlet characters in taxa delimitations and to study the relationships between them.

### Materials and Methods

This study has been restricted to 23 selected taxa belonging to 12 genera of Lamiaceae; 21 wild and two cultivated species in Saudi Arabia.

These taxa represent three subfamilies viz., Lamioideae, Nepetoideae and Teucrioideae (Cantino *et al.*, 1992c). The species were collected from different localities (Table 1) and identified according to Migahid (1996), Collenette (1999), Chaudhary (2001), Boulos (2002). Synonyms adopted after Boulos (2009).

### Nutlets morphology

**Dimensions:** Five healthy nutlets of each specie were measured under optical microscope using the ocular micrometer. Nutlet colors and areole were examined under a stereomicroscope.

**Wall sculpturing:** Variations in the sculpturing patterns of the nutlet surfaces were examined, using SEM. For this purpose, the dry nutlets were mounted on copper stubs and coated with a thin layer of gold in SPI-MODULE sputter coater. The 23 specimens were then examined by a JEOL JSM-5200 scanning microscope at the central lab. of the Faculty of Agriculture, Cairo University. The magnification power was expressed by (x) for each SEM micrograph. The terminology used in this study for nutlet surface sculpture was followed on the basis of Murely (1951) and Stearn (1992).

**Numerical analysis:** The obtained characters were analyzed by the NTSYS-pc program, using the UPGMA clustering method (Rohlf, 1993). The relationships between the studied taxa are demonstrated as phenogram.

### Results and Discussion

The nutlet micro- and macro morphological characters of the 23 species of Lamiaceae were summarized in (Table 2 and Figs. 1-23).

### Morphological nutlet characters

**Shape:** Three main shapes are recorded; ovoid, elliptic and rounded, with further subtypes. The results show that nutlet shape is variable. Such variability in seed shapes exists within a given species (Mayer & Poljakoff-Mayber, 1975) and referred to as "seed polymorphism".

**Color:** It is ranged from pale dark brown, grey to black. Chung & Heckard (1983); Husain *et al.* (1990) and Hussein (2000) considered the seed color as having a very limited taxonomic value in taxa delimitations, because it is fairly inconsistent and varies within same taxon.

Table 1. Collection data and synonyms.

No. Genera	Taxa	Locality of collection	
1	1.1. <i>Lavandula citriodora</i> A.G. Mill.	Al-Deraia, Al-Riyadh	
	1.2. <i>L. coronopifolia</i> Poir. = <i>L. stricta</i> Delie, Descr.	Al-Seal, Al-Taif – Makah road	
	1.3. <i>L. dentata</i> L.	Al-Shafa, Al-Taif	
	1.4. <i>L. pubescens</i> Decne.	Al-What and Al-Wahit, Al-Taif	
2	2.1. <i>Marrubium vulgare</i> L.	Bany Saad, Al-Taif	
3	3.1. <i>Mentha piperita</i> L.	Al-Deraia, Al- Riyadh	
4	4.1. <i>Micromeria biflora</i> Benth.	Al-Shafa, Al-Taif	
	4.2. <i>M. imbricata</i> (Forssk.) C. Chr. = <i>Thymus imbricatus</i> Forssk. = <i>T. biflorus</i> Buch- Ham. ex D. Don, Prodr. = <i>Satureja imbricata</i> (Forssk.) Briq., = <i>S. biflora</i> (Buch-Ham. ex D. Don) Briq.	Al-Shafa, Al-Taif	
	5	5.1. <i>Nepeta deflersiana</i> Schweinf. ex Hedge 5.2. <i>N. sheliae</i> Hedge & King	Al-Shafa, Al-Taif Al-Shafa, Al-Taif
	6	6.1. <i>Ocimum americanum</i> L.	Herbarium of biology department, Al-Taif Univ. – Garwa
6.2. <i>O. basilicum</i> L.		Al-Seal, Al-Taif – Makah road	
6.3. <i>O. canum</i> Sims		Herbarium of biology department, Al-Taif Univ. – Garwa	
6.4. <i>O. filamentosum</i> Forssk. = <i>Becium filamentosum</i> (Forssk.) Chiov.		Al-Kalidih, Al-Taif	
6.5. <i>O. forsskalii</i> Benth. = <i>O. menthifolium</i> Hoehst. ex Benth. = <i>O. hadiense</i> Sensu Boulos = <i>Plectranthus hadiensis</i> Sensu Boulos		Al-Deraia, Al- Riyadh	
6.6. <i>O. tenuiflorum</i> L.* = <i>O. Sanctum</i>		Shehar, Al-Taif	
7		7.1. <i>Origanum syriacum</i> L.* = <i>O. maru</i> L. var. <i>sinaicum</i> Boiss.	Shehar, Al-Taif
8	8.1. <i>Otostegia fruticosa</i> ssp. <i>Schimperi</i> (Benth.) Sebald, Stuttgarter Beitr. = <i>Ballota schimperi</i> Benth. = <i>O. schimperi</i> (Benth.) Boiss. = <i>O. Kaiseri</i> Täckh.	Al-What and Al-Wahit, Al-Taif	
	9	9.1. <i>Plectranthus comosus</i> Sims = <i>P. barbatus</i> Andr.	South road, Al-Taif - Al-Baha
	10	10.1. <i>Salvia aegyptiaca</i> L. = <i>S. pumila</i> Benth.	Al-Deraia, Al-Riyadh
10.2. <i>S. officinalis</i> L.		Herbarium of biology department, Al-Taif Univ. –Garwa	
11	11.1. <i>Stachys</i> sp. aff. <i>Schimperi</i> Vatke	Al-Shafa, Al-Taif	
12	12.1. <i>Teucrium oliverianum</i> Ging. ex Benth.	Al-Saffa, Jeddah	

\* Cultivated taxa

**Dimensions:** The results showed wide range of variations in nutlets size; The length and the width of the nutlet varies from species to species Thompson, (1981) stated that such attribute is subjected to ecological and physiological variations, i.e. the seed size is often highly adaptive. So, Mourad (1988) stated that seed size is of little use in taxonomic studies.

**Areole:** the areole is an outgrowth developed on the seed or fruit surface of certain plants (Fahn, 1989). In this study, different areole shapes and sizes were recorded among the nutlets of the taxa under investigation. large–small bilobed (9 taxa), straight, triangular while it was absent in some taxa.

**Nutlet coat ornamentation as seen by SEM:** The SEM studies showed that, three main types of coat ornamentation could be distinguished: smooth (type I),

partly sculptured (type II) and sculptured (type III). Within these types, 12 subtypes could be recognized.

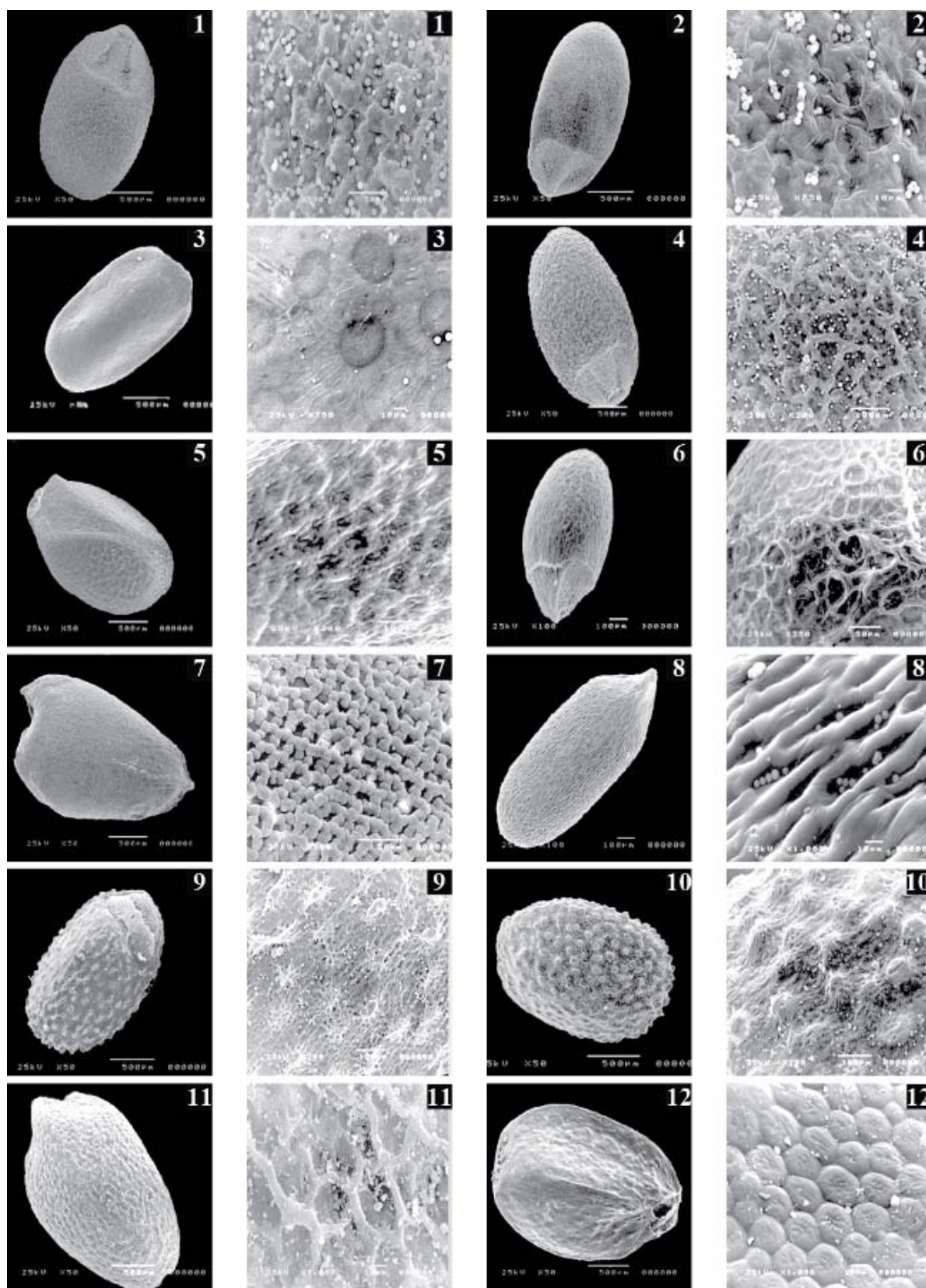
**Type I:** The smooth nutlet may be divided into six subtypes; reticulate, reticulate foveate, alveolate, granulate, rugose– scalariform and favulariate.

**Type II:** The partly sculptured surface is characterized by protuberance or papillae-like cells. Four subtypes are recognized as follows; protuberance, colliculate, ruminant and oscillate.

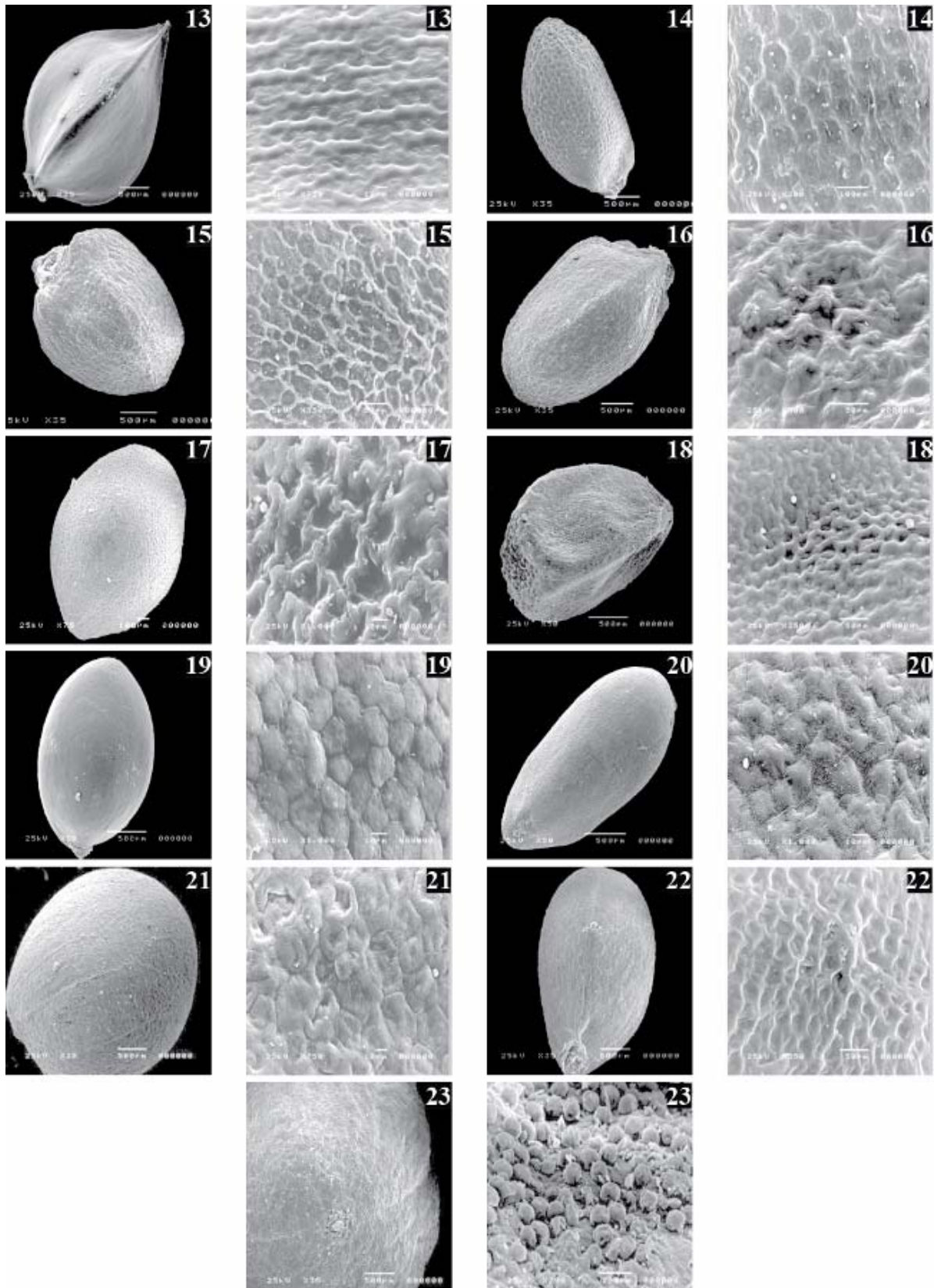
**Type III:** Nutlet surface is sculptured either tuberculate or verrucate. Many authors (Duletie-Lauševiae & Marin, 1999; Budantseva and Lobova, 1997; Hedge, 1992; Ryding, 1992a and 1993a and Turner, 1972) have demonstrated the usefulness of pericarp structure in Lamiaceae classification.

Table 2. Morphological nutlet characters of 23 taxa of the family Lamiaceae.

No.	Character Taxa	Shape	Color	Nutlet Dimensions (mm)			Areole			Overall Nutlet Coat Pattern (Sculpture)	Periclinal Walls			Anticlinal Walls			
				Length	Width	Thickness	Presence	Shape	Size		Level	Texture	Density	Shape	Thickness	Level	
1.	<i>Lavandula citriodora</i> L.	Oblong ovoid	Brown	1.2	0.78	0.78	+	Bilobed	Large	Type III Verrucate	Deep concave	Wrinkled	Many	Rounded	Rugose variegated	Thick	Raised
2.	<i>L. coronopifolia</i>	Oblong ovoid	Brown	1.15	0.62	0.62	+	Bilobed	Large	Type III Verrucate	Shallow concave	Wrinkled	Many	Rounded	Striated	Thick	Raised
3.	<i>L. dentata</i>	Elliptic with convex side	Pale brown	1.21	0.70	0.70	+	Bilobed	Small	Ruminant surrounded by striation	Shallow concave	Smooth	Few	Irregular	Striated	III - defined	III - defined
4.	<i>L. pubescens</i>	Oblong ovoid	Brown	1.29	0.75	0.75	+	Bilobed	Large	Type III Tuberculate	Deep concave	Rough	Many	Rounded	Rugose	Thin	Raised
5.	<i>Marrubium vulgare</i>	Ovoid trigonous	Grey	1.61	0.95	0.95	+	Straight	Small	Reticulate - foveate	Deep concave	Rough	-	-	Rugose striated	Thin	Raised
6.	<i>Mentha piperita</i>	Oblong ovoid	Black	0.52	0.37	0.37	+	Bilobed	Large	Type I Alveolate	Deep concave	Rough	Few	Irregular	Irregular with small striations	Slightly thick	Raised
7.	<i>Micromeritobiflora</i>	Oblong ovoid	Black	1.71	1.22	1.22	+	Bilobed wavy	Large	Type I Granulate aggregated in circular form	Shallow concave	Rough	-	-	Granulate	Thick	Raised
8.	<i>M. imbricata</i>	Long elliptic with acute tip, triangle tip	Pale brown	0.67	0.29	0.29	+	Triang.	Large	Type I Rugose - scalariform	Deep concave	Rough	Few	Rounded	Striated	Thick	Raised
9.	<i>Nepeta deflexiana</i>	Broadly elliptic	Black	1.17	0.68	0.68	+	Straight	Large	Type II Protuberance with irregular mounds	Shallow concave	Rough	Many	Rounded	Rugose	Thick	Raised
10.	<i>N. shetae</i>	Broadly elliptic	Black	1.17	0.75	0.75	+	Straight	Large	Type III Tuberculate	Deep concave	Rough	Many	Mainly Rounded	Rugose	Thick	Raised
11.	<i>Ocimum canariense</i>	Oblong ovoid with acute, triangle tip	Blackish brown	1.66	0.8	0.8	+	Bilobed	Large	Type I Reticulate	Deep concave	Rough	Much	Irregular	Rugose	Slightly thick	Raised
12.	<i>O. basilicum</i>	Ovoid trigonous	Black	1.44	0.81	0.81	+	Bilobed	Small	Type II Colliculate	Shallow concave	Wrinkled, clavate hairs at posterior part	Few	Irregular	Straight	Very thin	Channeled
13.	<i>O. canum</i>	Ovoid trigonous Tapered end	Brown	2.02	1.33	1.33	+	Straight	Small	Type I Favariate	Shallow concave	Smooth	Few	Irregular	Variegated	III - defined	Irregular
14.	<i>O. filamentosum</i>	Elliptic	Grey	1.64	0.98	0.98	+	Straight	Large	Type I Reticulate - foveate	Deep concave	Rough	Few	Irregular	Striated	Thin	Raised
15.	<i>O. forsskalii</i>	Circular - ovoid trigonous	Black	1.71	1.11	1.11	+	Triangular	Large	Type I Reticulate	Deep concave	Rough	Few	Irregular	Straight	Slightly thick	Raised
16.	<i>O. tenuiflorum</i>	Broadly ovoid	Black	1.99	1.15	1.15	+	Triangular with arifoloid	Large	Type III Tuberculate	Shallow concave	Rough	-	-	Wavy	Thick	Raised
17.	<i>Origanum acuminatum</i>	Ovoid	Pale brown	0.89	0.64	0.64	+	Bilobed wavy	Small	Type II Ruminant	Shallow concave	Rough	Few	Irregular	Rugose, Wavy	Thick	Raised
18.	<i>Orostegia fruticosus</i> spp. Schimper	Ovoid trigonous	Grey	1.74	0.92	0.92	-	-	-	Reticulate - foveate with capitate hairs	Shallow concave	Rough with clavate hairs at the posterior part	Few	Rounded	Wavy	Thick	Raised
19.	<i>Plectranthus canosus</i>	Ovoid	Dark brown	1.29	1.03	1.03	+	Straight	Small	Type II Colliculate	Straight	Smooth pitted	-	-	Straight	Thin	Channeled
20.	<i>Sabia aegyptiaca</i>	Narrowly elliptic	Grey	1.67	0.77	0.77	-	-	-	Type III Verrucate	Shallow concave	Rough	Much	Needle like	Irregular	Thick	Raised
21.	<i>S. officinalis</i>	Rounded	Dark brown	2.59	1.99	1.99	-	-	-	Type II Ocellate with central depression	Shallow concave	Wrinkled	Few	Irregular	Rugose	Thick	Raised & channeled
22.	<i>Stachys aff. schimperii</i>	Oblong ovoid	Grey	1.79	1.16	1.16	-	-	-	Type I Reticulate - foveate	Shallow concave	Rough	-	-	Rugose	Thick	Raised & channeled
23.	<i>Teucrium olivertianum</i>	Rounded with constriction	Black	3.27	3.19	3.19	-	-	-	Type I Reticulate with capitate hairs	Shallow concave	Rough and hairy	Few	Irregular	Rugose	Slightly thick	Raised



Figs. 1-23. Scanning electron micrographs of the nutlet as a whole and their coat surfaces in the studied taxa: (1) *Lavandula citriodora*, (2) *Lavandula coronopifolia*, (3) *Lavandula dentate*, (4) *Lavandula pubescens*, (5) *Marrubium vulgare*, (6) *Mentha piperita*, (7) *Micromeria biflora*, (8) *Micromeria imbricate*, (9) *Nepeta deflersiana* (10) *Nepeta sheilae*, (11) *Ocimum americanum*, (12) *Ocimum basilicum*,



Figs. 1-23. Scanning electron micrographs of the nutlet as a whole and their coat surfaces in the studied taxa: (13) *Ocimum canum*, (14) *Ocimum filamentosum*, (15) *Ocimum forsskali*, (16) *Ocimum tenuiflorum*, (17) *Origanum syriacum* (18) *Otostegia fruticosa*, (19) *Plectranthus comosus*, (20) *Salvia aegyptiaca*, (21) *Salvia officinalis*, (22) *Stachys* sp., (23) *Teucrium oliverianum*

**Periclinal cell walls:** Either straight or deep to shallow concave, smooth- wrinkled hairy. Trichomes or hairs are among the most useful taxonomic characters in some genera of family Lamiaceae (Dinc & Öztürk, 2008). Their absence or presence have been used as taxonomic markers in the infrageneric classification of the genus *Teucrium* L. (Navarro & El-Oualidi, 2000). In this study the absence or presence of trichomes is an important taxonomic character for delimitation of *Ocimum basilicum*, *Otostegia fruticosa* and *Teucrium oliverianum*. Hedge (1970) and Ryding (1992b) also suggested that the absence of mucilaginous substance is correlated with certain characteristics. For instance, hairy or glandular nutlets in this study were lacking mucilage production such as in *Micromeria sp.*, *Teucrium oliverianum* and *Otostegia fruticosa* except *Ocimum basilicum* which has hairy nutlets with mucilage.

**Epicuticular wax:** Present with variable density in 18 taxa and absent in the remainder taxa. Its shape either needle like, rounded or irregular.

**Anticlinal cell wall boundaries:** Thick, thin or ill defined and take different aspects; straight, rugose variegated, striated, wavy, granulated or irregular. The boundaries between cells either leveled, raised, channeled, ill-defined or irregular.

The results obtained from the nutlet morphological characters showed that the surface sculpture is of great considerable taxonomic significance at both generic and specific levels, while the nutlet color, shape, size and areole can be of little taxonomic important. Similar conclusions have also been given by Karakish (1993), Hamed & Mourad (1994), Hsieh & Huang (1995), Al- Nowaihi & Mourad (1999), Zou *et al.* (2001); Shaheen (2002) and Kaya & Dirmencl (2008) and Biznet & Teke (2014).

#### Key to the studied taxa of Lamiaceae based on nutlet macro- and micromorphological characters

A1	Nutlet coat sculpture type I (Smooth)	
B1	Reticulate foveate	
C1	Areole present	
d1	Anticlinal wall rugose–striated .....	<i>Marrubium vulgare</i>
d2	Anticlinal wall striated .....	<i>Ocimum filamentosum</i>
C2	Areole absent	
e1	Anticlinal wall wavy .....	<i>Otostegia fruticosa</i>
e2	Anticlinal wall rugose .....	<i>Stachys shimperi</i>
B2	Reticulate	
f1	Areole bilobed .....	<i>Ocimum americanum</i>
f2	Areole triangular .....	<i>O. forsskalii</i>
f3	Areole absent .....	<i>Teucrium oliverianum</i>
B3	Alveolate .....	<i>Mentha piperita</i>
B4	Granulate .....	<i>Micromeria biflora</i>
B5	Rugose–scalariform .....	<i>M. imbricata</i>
B6	Favulariate .....	<i>Ocimum canum</i>
A2	Nutlet coat sculpture type II (partly sculptured) g 1 Ruminant	
h 1	Anticlinal wall striated .....	<i>Lavandula dentata</i>
h 2	Anticlinal wall rugose wavy .....	<i>Origanum syriacum</i>
g 2	Protuberance with irregular mounds .....	<i>Nepeta</i>
g 3	Colliculate	
i 1	Nutlet color black .....	<i>Ocimum</i>
i 2	Nutlet color dark brown .....	<i>Plectranthus comosus</i>
g 4	Oscillate .....	<i>Salvia officinalis</i>
A3	Nutlet coat sculpture type III (Sculptured) J1 Verrucate	
K1	Nutlet color brown	

- L1 Anticlinal wall rugose variegated ..... *Lavandula citriodora*
- L2 Anticlinal wall striated ..... *L. coronobifolia*
- K2 Nutlet color grey ..... *Salvia aegyptica*
- J2 Tuberculate
- m1 Nutlet color brown ..... *Lavandula*
- m2 Nutlet color black
- n1 Areole shape straight ..... *Nepeta sheliae*
- n2 Areole triangular with arioloid ..... *Ocimum tenuiflorum*

**Table 3. The proposed treatment based on numerical analysis of nutlet morphological characters of**

GR <sub>s</sub>	Taxa	Clusters (C <sub>s</sub> )	Subseries (SS <sub>s</sub> )	Series (S <sub>s</sub> )
GR <sub>1</sub>	• <i>Lavandula citriodora</i>			
GR <sub>2</sub>	• <i>Nepeta deflersiana</i>			
GR <sub>3</sub>	• <i>Mentha piperita</i>	C <sub>1</sub>		
GR <sub>4</sub>	• <i>Ocimum americanum</i>		SS <sub>I</sub>	
GR <sub>5</sub>	• <i>Micromeria biflora</i>			
GR <sub>6</sub>	• <i>Micromeria imbricata</i>	C <sub>2</sub>		S <sub>1</sub>
GR <sub>7</sub>	• <i>Marrubium vulgare</i>			
GR <sub>8</sub>	• <i>Otostegia fruticosa</i>	C <sub>3</sub>		
GR <sub>9</sub>	• <i>Lavandula dentate</i>			
GR <sub>10</sub>	• <i>Ocimum canum</i>	C <sub>4</sub>	SS <sub>II</sub>	
GR <sub>11</sub>	• <i>Plectanthis comosus</i>			
GR <sub>12</sub>	• <i>Salvia officinalis</i>	C <sub>5</sub>		

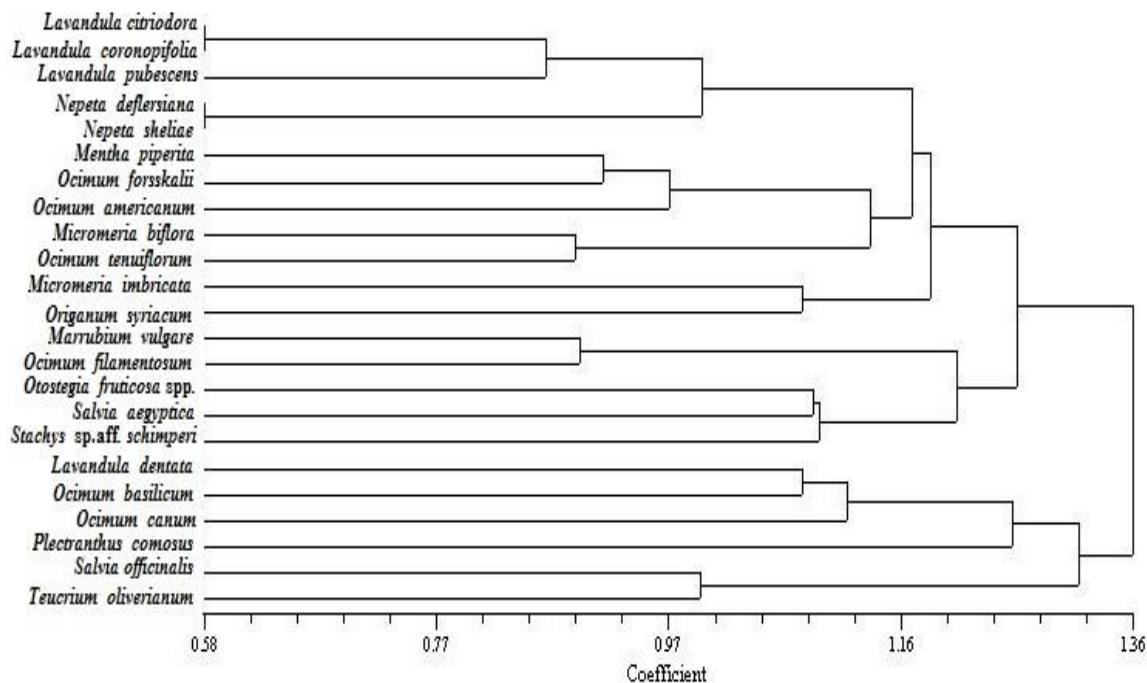


Fig. 24. Phenogram based on exomorphological nutlet characters of 23 studied taxa of Lamiaceae in Saudi Arabia.

**Numerical analysis:** In this study, the constructed phenogram from numerical analysis of the morphological nutlet characteristics of the 23 taxa of Lamiaceae from Saudi Arabia showed their categorization into 12 groups included in five clusters, two subseries and one series (Fig. 24 & Table 3). The results revealed that, generally the investigated taxa of the Lamioideae, Nepetoideae and Teucroideae were scattered across the phenogram. *Lavandula citriodora*, *L. coronifolia* and *L. pubescens* were grouped together in (GR<sub>1</sub>) and the same cluster (C<sub>1</sub>) due to high similarity in the nutlet morphology, while *L. dentata* was widely separated and grouped with *Ocimum basilicum* in group (GR<sub>9</sub>) and the same cluster (C<sub>4</sub>). This relation gives additional support to some views that *Lavandula* is closely related to the Ocimeae (Wagstaff *et al.*, 1995). The clustering of *Lavandula dentata*, *Ocimum basilicum*, *O. canum* and *Plectranthus comosus* in the same cluster (C<sub>4</sub>) agrees with EL-Gazzar & Watson (1970) and Bentham (1876) who classified them in the same tribe Ocimoideae.

Concerning *Micromeria imbricata* and *Origanum syriacum* which were gathered in the same group (GR<sub>6</sub>), this result was, to some extent, in accordance with those of EL-Gazzar & Watson (1970) and Bentham (1876) who suggested their positioning in the same tribe: Satureieae and subtribe: Menthioideae with *Mentha*.

Grouping of *Otostegia fruticosa* ssp. *Schimperi*, *Stachys* sp. and *Salvia aegyptiaca* together show the high similarity between them in the nutlet morphological characters. This result agrees with the conclusions of EL-Gazzar & Watson (1970) and Bentham (1876) with respect to *O. fruticosa* ssp. *Schimperi*, and *Stachys* sp. who suggested their grouping in one tribe Stachydeae, subtribe Lamieae. On other hand, *Salvia officinalis* is separated from the other *Salvia* species and was grouped with *Teucrium oliverianum*. This finding may support the opinion of Walker *et al.* (2004) that *Salvia* is not monophyletic.

Grouping of *Nepeta* in separate (GR<sub>2</sub>) is in agreement with those of EL-Gazzar & Watson (1970) and Bentham (1876) who put it in a separate tribe: Nepeteae. Also, *Plectranthus comosus* is placed in a separate group (GR<sub>11</sub>) and away from the other studied taxa.

## Conclusion

The present study includes valuable information about Lamiaceae nutlet in Saudi Arabia which is given here for the first time.

It was confirmed the usefulness of some nutlet characters i.e. coat ornamentation as a diagnostic feature for taxa delimitation in family Lamiaceae.

Three main coat ornamentations could be distinguished as smooth, partly sculptured and sculptured within these types 12 subtypes recognized. This character used for key construction for separation between the studied taxa.

Numerical analysis of the morphological nutlet characters in accordance to some extent with Bentham (1876) and EL-Gazzar & Watson (1970) classification of the family.

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