Morphological and Anatomical Studies on Selected Lamiaceae Medicinal Plants in Bani Matar District, Sana'a (Yemen)

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Abstract

Morphological and anatomical characters of stem, petiole and leaves of 16 selected medicinal taxa of family Lamiaceae collected from Bani Matar district, Sana'a (Yemen) were examined using both stereo-and light microscopes. The obtained macro- and micromorphological characters (298) were analysed using the software jmp version 13. The resulted dendrogram was discussed on the light of the current taxonomic treatments of Lamiaceae. The measurement of the quantitative characters of macro- and micromorphological characters for the studied taxa was established by multivariate analysis of variance (ANOVA). Evaluation of obtained results revealed that, many characters viz. leaf composition, stomatal types and distribution, number of stamens, stem and petiole vasculature, types of trichomes, nutlet features, number of palisade and spongy layers and types of crystals are of diagnostic value in species identification and delimitation and are potentially valuable at different taxonomic levels. All the quantitative macro- and micromorphological characters except nutlet length are highly significant in species delimitation.

Key words: Anatomy, ANOVA, cluster analysis, Lamiaceae, medicinal plants, morphology, Yemen.

Introduction

Lamiaceae is the sixth largest angiosperm family with about 236 genera and 7173 species distributed throughout the world in both temperate and tropical regions; its main distribution is in the Mediterranean (Harley et al., 2004 & Singh, 2010).

In Yemen, Lamiaceae is one of the most important families due to use in folk medicine and for the commercial production of essential oils. It includes 23 endemic species, (AL-Khulaidi, 2013).

The members of Lamiaceae are generally aromatic, including a number of widely used culinary herbs, such as basil, lavender, marjoram, mint, oregano, rosemary, sage, savory and thyme (Wink, 2003; Celiktas et al., 2007, Hussain et al., 2008).

Traditionally, plant taxonomy has depended mainly upon comparative morphological features because the help in taxa delimitation and identification (Pandey & Misra, 2014). Many publications pointed out on the importance of morphological characters in delimitation and identification in some Lamiaceae species (Satil et al., 2007; Baran et al., 2008; Kahraman et al., 2009; Celep et al., 2011 & Kiliç, 2014).

The anatomical characters of vegetative organs are important for characterization of Lamiaceae taxa (Metcalfe & Chalk, 1950; Abu-Asab & Cantino, 1987; Kahraman et al., 2009, 2010). The glandular hairs and their distribution (Werker, 2006), stomatal distribution and other anatomical features provide significant taxonomic information (Dinç & Öztürk., 2008; Celep et al., 2011 and Venkateshappa & Sreenath, 2013). In addition, these features play an important role in elucidating phylogenetic relationships in many taxa (Pandey & Misra, 2014).

The aim of the present work is to investigate the different morphological and anatomical features of the taxa under study in Bani Matar district Sana'a (Yemen) and evaluate them in taxa identification and delimitation.

Material and Methods:

1. Plant Materials

Sixteen selected medicinal Lamiaceae taxa belonging to 13 genera were collected...
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from their natural habitats except two species from cultivated fields in Bani Mater district, Sana’a Governorate, Yemen; during their flowering and fruiting seasons 2014-2016.

Table (1) represents the data of collection. The identification of the specimens was achieved by utilizing the available taxonomic and floristic literature (Wood 1997; Chaudhary 2001; Boulos 2002; Ibrahim 2013). Nomenclature of taxa followed IPNI. The specimens are kept in Herbarium of Botany Department, Faculty of Science, Ain Shams University and Biology Department, Faculty of Science, Sana’a University.

2. Macromorphological Investigation

The macromorphological characters of the studied taxa; stem, leaf, inflorescence, flower and fruit were described from the living specimens and photographed by using digital Sony camera (HRD- CX 700 -12.3 Mega pixels).

3. Micromorphological Investigation

Cross sections of the stem, petiole and lamina were made using the embedding paraffin wax technique (Johanson, 1940). The sections were examined using Bel. BIO3 T-PL light microscope and photographs were taken by digital camera power =shot G12, 10 Mega pixels.

4. Lamina Epidermal Characters

For leaf epidermal study, at least five leaves were cut at the median portion and soaked in concentrated Nitric acid for two - ten hrs. The appearance of air bubbles indicated the readiness of epidermises to be separated. The samples were transferred to Petri dishes containing water and with the use of fine forceps and dissecting needle the adaxial and the abaxial epidermises were separated (Ibrahim & Ayodele, 2013). The two epidermal layers (Adaxial & Abaxial) were stripped and stained with Saffranin and mounted in glycerin (Sreelakshmi et al., 2014). Photomicrographs were taken using light microscope and camera (Canon powershot G12, 10 mega pixels), the magnification power was expressed by (X). Terminology of epidermal characteristics based on Dilcher (1974).

5. Macro - and Micromorphological Measurements

Measurements of ten quantitative macromorphological characters for the studied taxa viz. stem length, leaf length & width, calyx, corolla, stamen, bract and seed were taken. Also, the micromorphological measurements (viz. thickness of cuticle, epidermal cells size (length x width), cortex, secondary phloem and xylem, pith width, palisade and spongy cells were determined by the ocular micrometer.

6. Statistical Analysis

The obtained macro- and micromorphological data were used to construct a data matrix for numerical analysis. The presence or absence of the obtained characters was coded as 1 and 0 respectively to determine the relationship between the different studied taxa using the software jmp version 13 for Sas Institute Inc (2017) through hierarchical cluster method average linkage modules (Sokal & Michener, 1958). For each of the quantitative measurements, the significant differences between the taxa studied was established by multivariate analyses of variances (ANOVA).

Results and Discussion

1. Qualitative macromorphological characters

Macromorphological characters and their states of all studied taxa were summarized in (Table 2 & Plate 1-3). The most diagnostic characters are: the habit; shrub in Meriandra dianthera, undershrub in the three Lavandula spp. and Otostegia fruticosus or herb in the remaining examined taxa. All taxa studied are aromatic except Lavandula coronopifolia and Marrubium vulgar. This result agreed with that of Harley et al., (2004); Chaudhary, (2001); Wood, (1997).

All studied taxa are erect except Thymus serpyllum is procumbent. The stem is semicircular in Leucas inflata and Thymus serpyllum, circular in Meriandra dianthera, Otostegia fruticosus, Teucrium yemense and squared in all other taxa. The leaves are simple in all studied taxa except, Lavandula spp. are pinnatisect. Leaves petiolate in seven taxa
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while sessile-subsessile in the remaining nine taxa. Other leaf morphological features viz., arrangement, shape, margin, apex, base, texture presented variations between taxa and useful at the interspecific relationships.

In most of the studied taxa, the position and the number of flowers per inflorescence varied. In addition, the corolla color shows great variations among studied taxa. The corolla is four lobed in Mentha longifolia, bilobed in 13 taxa while bilobed densely villous in Leucas inflata and Otostegia fruticosa. Bract was present in all studied taxa except Leucas inflata and Mentha longifolia while bracteoles are absent in all studied taxa except five. Calyx had different shapes and with teeth in all studied taxa expect Otostegia fruticosa and Origanum majorana. The basic number of stamens is four, but in Salvia schimperi and Meriandra dianthera being two.

It was clear that some macromorphological characters viz. lamina shape, flower position and number, corolla color and stamen number are taxonomically significant for Lamiaceae taxa identification and delimitation. This result is in accordance with that of Wood (1997); Chaudhary (2001); Kahraman et al., (2009) and Ibrahim, (2013).

The areole is present, in Lavandula coronopifolia, L. pubescens and Nepeta deflersiana and absent in the remaining taxa. The nutlets showed different shapes, color and size between the studied taxa. The taxonomic value of nutlet characteristics of Lamiaceae as diagnostic character had been discussed by many authors (Husain et al., 1990; Hussein, 1995; Marin et al., 1998; Moon et al., 2009; Salimpour et al., 2014; Hassan & AL-Thobaiti, 2015 and Badamtsetseg, 2016).

Plate 1 (Figs. 1-6): Whole plants of some studied taxa.
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Table (1). Vernacular name, localities and collection data of the studied taxa

<table>
<thead>
<tr>
<th>No</th>
<th>Medicinal taxa</th>
<th>Vernacular name</th>
<th>Village</th>
<th>Elevation (m)</th>
<th>Longitude E</th>
<th>Latitude N</th>
<th>Date of collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Lavandula citriodora</em> A. G. Mill.</td>
<td>Sh'at ran</td>
<td>Nimran</td>
<td>2700</td>
<td>44°10'24.24</td>
<td>15°11'0.14</td>
<td>1/10/2015</td>
</tr>
<tr>
<td>2</td>
<td><em>Lavandula coronopifolia</em> Poir. = <em>L. stricta</em> Delile.</td>
<td>Ha'naz</td>
<td>Hamil</td>
<td>2402</td>
<td>44° 11 11.9</td>
<td>15° 13 44 .5</td>
<td>10/8/2014</td>
</tr>
<tr>
<td>3</td>
<td><em>Lavandula pubescens</em> Decne.</td>
<td>Va'heia</td>
<td>Kusher</td>
<td>2681</td>
<td>44°66 32 9</td>
<td>15° 15 37 .8</td>
<td>14/9/2015</td>
</tr>
<tr>
<td>4</td>
<td><em>Leucas inflata</em> Benth. = <em>physoleucas acrodonta</em> Jaub. &amp; Spach</td>
<td>Ba'udh</td>
<td>Artil</td>
<td>2402</td>
<td>44° 12 06 .3</td>
<td>15°14 18 .6</td>
<td>20/5/2016</td>
</tr>
<tr>
<td>5</td>
<td><em>Marrubium vulgare</em> L.</td>
<td>Hei' wjazp</td>
<td>AL saih</td>
<td>3353</td>
<td>435741.8</td>
<td>15 15 5 8.1</td>
<td>20/5/2016</td>
</tr>
<tr>
<td>6</td>
<td><em>Mentha longifolia</em> L. = <em>M. sylvestris</em> L.</td>
<td>Nā'ud</td>
<td>Subahah</td>
<td>2567</td>
<td>44° 99.75</td>
<td>15°16 25.89</td>
<td>19/9/2015</td>
</tr>
<tr>
<td>7</td>
<td><em>Meriandra dianthera</em> (Roem. &amp; Schult.) Briq. = <em>Meriandra bengalensis</em> (Roxb.) Benth.</td>
<td>darū</td>
<td>Kusher</td>
<td>2681</td>
<td>44° 66 32.9</td>
<td>15° 15 37 .8</td>
<td>10/9/2015</td>
</tr>
<tr>
<td>8</td>
<td><em>Nepeta deflersiana</em> Schweinf. ex Hedge</td>
<td>Ad'hat alnoub</td>
<td>Al- saih</td>
<td>3355</td>
<td>43° 57 41.8</td>
<td>15° 15 5 8.1</td>
<td>9/8/2015</td>
</tr>
<tr>
<td>9</td>
<td><strong>Ocimum basilicum</strong> L. var. basilicum</td>
<td>Ra'ehan abmer</td>
<td>Kusher</td>
<td>2681</td>
<td>44° 66 32 9</td>
<td>15° 15 37 .8</td>
<td>20/8/2015</td>
</tr>
<tr>
<td>10</td>
<td><strong>Ocimum basilicum</strong> var. purpurascens Benth.</td>
<td>Rahan abudh</td>
<td>Kusher</td>
<td>2681</td>
<td>44° 66 32 9</td>
<td>15° 15 37 .8</td>
<td>20/8/2015</td>
</tr>
<tr>
<td>11</td>
<td><strong>Origanum majorana</strong> L.</td>
<td>ba'rduqsh</td>
<td>Kusher</td>
<td>2681</td>
<td>44° 66 32 9</td>
<td>15° 15 37 .8</td>
<td>20/5/2016</td>
</tr>
<tr>
<td>12</td>
<td><em>Otostegia fruticosa</em> (Forssk.) Schwienf. ex Penzing subsp. <em>schimperi</em> (Benth.) Sebald</td>
<td>Sh'iqub</td>
<td>Baty naamah</td>
<td>2686</td>
<td>44°99.75</td>
<td>15°16 25.89</td>
<td>28/8/2015</td>
</tr>
<tr>
<td>13</td>
<td><em>Salvia schimperi</em> Benth.</td>
<td>Ma'smes</td>
<td>Kusher</td>
<td>2958</td>
<td>44°10'32.90</td>
<td>15°15 37.80</td>
<td>20/5/2016</td>
</tr>
<tr>
<td>14</td>
<td><em>Stachys yemenensis</em> Hedge</td>
<td>Ha'lal</td>
<td>Baty al-ahmer</td>
<td>2958</td>
<td>43° 5 81 6.5</td>
<td>15° 14 53.6</td>
<td>12/8/2015</td>
</tr>
<tr>
<td>15</td>
<td><em>Teucrium yemens</em> Deflers</td>
<td>As'abai merem</td>
<td>Baty al-ahmer</td>
<td>2958</td>
<td>43° 5 81 6.5</td>
<td>15° 14 53.6</td>
<td>22/8/2015</td>
</tr>
<tr>
<td>16</td>
<td><em>Thymus serpyllum</em> L. = <em>Thymus laevigatus</em> Vahl</td>
<td>Za'tar, Sa'tar</td>
<td>Al- Bald</td>
<td>3353</td>
<td>43° 5657.2</td>
<td>15° 16 09.3</td>
<td>15/8/2015</td>
</tr>
</tbody>
</table>

**cultivated taxa,** Endemic taxa.
### Table 2. Qualitative macromorphological characters of the studied taxa

<table>
<thead>
<tr>
<th>Character &amp; Character state</th>
<th>Taxa</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Duration</strong></td>
<td></td>
</tr>
<tr>
<td>Perennial</td>
<td>+</td>
</tr>
<tr>
<td>Annual</td>
<td>-</td>
</tr>
<tr>
<td><strong>Habit</strong></td>
<td></td>
</tr>
<tr>
<td>Under shrub</td>
<td>+</td>
</tr>
<tr>
<td>Herb</td>
<td>-</td>
</tr>
<tr>
<td>Shrub</td>
<td>-</td>
</tr>
<tr>
<td><strong>Aromatic</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>+</td>
</tr>
<tr>
<td><strong>External Appearance</strong></td>
<td></td>
</tr>
<tr>
<td>Square</td>
<td>+</td>
</tr>
<tr>
<td>Opposite</td>
<td>+</td>
</tr>
<tr>
<td>Semicircular</td>
<td>+</td>
</tr>
<tr>
<td>Circular</td>
<td>+</td>
</tr>
<tr>
<td><strong>Internal appearance</strong></td>
<td></td>
</tr>
<tr>
<td>Solid</td>
<td>+</td>
</tr>
<tr>
<td>Hollow</td>
<td>-</td>
</tr>
<tr>
<td><strong>Texture</strong></td>
<td></td>
</tr>
<tr>
<td>Sub-glabrous</td>
<td>+</td>
</tr>
<tr>
<td>Glabrous</td>
<td>+</td>
</tr>
<tr>
<td>Tomentose</td>
<td>+</td>
</tr>
<tr>
<td>Woody</td>
<td>+</td>
</tr>
<tr>
<td>Pubescent</td>
<td>+</td>
</tr>
<tr>
<td>Densely pubescent</td>
<td>-</td>
</tr>
<tr>
<td>Opposite deccusate</td>
<td>+</td>
</tr>
<tr>
<td>Clustered</td>
<td>+</td>
</tr>
<tr>
<td>Opposite-whorled</td>
<td>+</td>
</tr>
<tr>
<td>Petioles</td>
<td>+</td>
</tr>
<tr>
<td><strong>Composition</strong></td>
<td></td>
</tr>
<tr>
<td>Pinnatisect</td>
<td>+</td>
</tr>
<tr>
<td>Simple</td>
<td>+</td>
</tr>
<tr>
<td><strong>Shape</strong></td>
<td></td>
</tr>
<tr>
<td>Oblong-linear</td>
<td>+</td>
</tr>
<tr>
<td>Orbicular</td>
<td>-</td>
</tr>
<tr>
<td>Lanceolate</td>
<td>+</td>
</tr>
<tr>
<td>Oblanceolate</td>
<td>-</td>
</tr>
<tr>
<td>Oblate</td>
<td>-</td>
</tr>
<tr>
<td><strong>Margin</strong></td>
<td></td>
</tr>
<tr>
<td>Entire</td>
<td>+</td>
</tr>
<tr>
<td>Crenate</td>
<td>+</td>
</tr>
<tr>
<td>Sharply serrate</td>
<td>+</td>
</tr>
<tr>
<td>Dentate</td>
<td>+</td>
</tr>
<tr>
<td>Irregularly serrated</td>
<td>+</td>
</tr>
<tr>
<td>Serrate</td>
<td>+</td>
</tr>
<tr>
<td><strong>Apex</strong></td>
<td></td>
</tr>
<tr>
<td>Obtuse</td>
<td>+</td>
</tr>
<tr>
<td>Cleft &amp; crenate</td>
<td>+</td>
</tr>
<tr>
<td>Obtuse - acute</td>
<td>+</td>
</tr>
<tr>
<td>Acute</td>
<td>+</td>
</tr>
<tr>
<td>Rounded</td>
<td>+</td>
</tr>
<tr>
<td><strong>Base</strong></td>
<td></td>
</tr>
<tr>
<td>Subacute</td>
<td>+</td>
</tr>
<tr>
<td>Truncate</td>
<td>+</td>
</tr>
<tr>
<td>Subcordate</td>
<td>+</td>
</tr>
<tr>
<td>Cuneate</td>
<td>+</td>
</tr>
<tr>
<td>Cordate</td>
<td>+</td>
</tr>
<tr>
<td>Cuneate - truncate</td>
<td>+</td>
</tr>
<tr>
<td>Acute</td>
<td>+</td>
</tr>
<tr>
<td><strong>Adaxial surface</strong></td>
<td></td>
</tr>
<tr>
<td>Attenuate</td>
<td>+</td>
</tr>
<tr>
<td>Oblique</td>
<td>-</td>
</tr>
<tr>
<td><strong>Color</strong></td>
<td></td>
</tr>
<tr>
<td>Green</td>
<td>+</td>
</tr>
<tr>
<td>Dark green</td>
<td>-</td>
</tr>
<tr>
<td>Green - white</td>
<td>+</td>
</tr>
<tr>
<td>Gray</td>
<td>+</td>
</tr>
<tr>
<td>White</td>
<td>+</td>
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<td>Green</td>
<td>+</td>
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<td>Green - white</td>
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<td>+</td>
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<tr>
<td>Green</td>
<td>+</td>
</tr>
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<td>Dark green</td>
<td>+</td>
</tr>
<tr>
<td>Green - white</td>
<td>+</td>
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### Table 2. Cont’d.

<table>
<thead>
<tr>
<th>Character &amp; Character state</th>
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<tr>
<td><strong>Leaf</strong></td>
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<tr>
<td>Texture</td>
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</tr>
<tr>
<td>Thinly tomentose</td>
<td>+ + + + + + + + + + + + + + + + + + + +</td>
</tr>
<tr>
<td>Thinly pubescent</td>
<td>+ + + + + + + + + + + + + + + + + + + +</td>
</tr>
<tr>
<td>Densely tomentose</td>
<td>+ + + + + + + + + + + + + + + + + + + +</td>
</tr>
<tr>
<td>Tomentose</td>
<td>+ + + + + + + + + + + + + + + + + + + +</td>
</tr>
<tr>
<td>Tomentose &amp; wrinkled</td>
<td>+ + + + + + + + + + + + + + + + + + + +</td>
</tr>
<tr>
<td>Slightly pubescent</td>
<td>+ + + + + + + + + + + + + + + + + + + +</td>
</tr>
<tr>
<td>Densely pubescent</td>
<td>+ + + + + + + + + + + + + + + + + + + +</td>
</tr>
<tr>
<td>Ciliated &amp; Punctuate</td>
<td>+ + + + + + + + + + + + + + + + + + + +</td>
</tr>
<tr>
<td>Terminal panicle</td>
<td>+ + + + + + + + + + + + + + + + + + + +</td>
</tr>
<tr>
<td>Whorl</td>
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</tr>
<tr>
<td>Axial spicates</td>
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</tr>
<tr>
<td>Dense terminal heads</td>
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</tr>
<tr>
<td>Number</td>
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<td>6-10</td>
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<tr>
<td>5-8</td>
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</tr>
<tr>
<td>Present</td>
<td></td>
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<td>Absent</td>
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</tr>
<tr>
<td>Shape</td>
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<tr>
<td>Ovate Acute</td>
<td>+ + + + + + + + + + + + + + + + + + + +</td>
</tr>
<tr>
<td>Orhicular</td>
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</tr>
<tr>
<td>Ovate</td>
<td>+ + + + + + + + + + + + + + + + + + + +</td>
</tr>
<tr>
<td>Linear-lobate</td>
<td>+ + + + + + + + + + + + + + + + + + + +</td>
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<tr>
<td>Breit</td>
<td></td>
</tr>
<tr>
<td>Subulate - hook</td>
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</tr>
<tr>
<td>Linear-lobate</td>
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</tr>
<tr>
<td>Spinoso pubescent subulate</td>
<td>+ + + + + + + + + + + + + + + + + + + +</td>
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<tr>
<td>3- unequal spinose</td>
<td>+ + + + + + + + + + + + + + + + + + + +</td>
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<td>+ + + + + + + + + + + + + + + + + + + +</td>
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<td>+ + + + + + + + + + + + + + + + + + + +</td>
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<tr>
<td>Tubular prickly</td>
<td>+ + + + + + + + + + + + + + + + + + + +</td>
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<tr>
<td>Campanulate with 2 lippes</td>
<td>+ + + + + + + + + + + + + + + + + + + +</td>
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<tr>
<td>Oboconal &amp; bilabiate</td>
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<tr>
<td>Tubular</td>
<td>+ + + + + + + + + + + + + + + + + + + +</td>
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<tr>
<td>Flower</td>
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<td>Sea-shell shaped with flat limb</td>
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<td>Oblong- ovoid</td>
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<td>Obovoid</td>
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<td>Trigonous</td>
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2. Qualitative micromorphological characters

The qualitative anatomical characters of the studied taxa are presented here for the first time and summarized in Table (3)

a. Stem (Table 3; Plate 4)

The epidermal cells are covered with a thick cuticle in eight taxa and thin cuticle in the other eight taxa. Stomata are observed in the three Lavandula spp. only while absent in the remainder taxa. Cortex including patches of sclerenchyma cells in three studied Lavandula spp., Meriandra dianthera and Stachys yemenensis. In addition, the endodermis well defined in all studied taxa except Meriandra dianthera, Otostegia fruticosa and Salvia schimperi. Vascular system is dissected or in the form of continuous siphonostele. Pith is solid in all taxa except Thymus serpyllum and including polyhedral parenchyma in 12 taxa or pentagonal and hexagonal cells in three Lavandula spp. and Leucas inflata. The crystals are needles, solitary, tuft like or duress were observed in the cortex of stem. In the obtained results, the pith is solid in all studied taxa except Thymus serpyllum while, Dinç et al. (2008) reported that some species of genus Stachys are with hollow pith and others with solid pith. Metcalfe & Chalk, (1979) stated that stems of many genera and species of the family Lamiaceae are quadrangular with well-defined collenchyma in four angles. In addition, they reported that sclerenchymatous tissue surround the phloem groups of vascular bundles. This report in accordance with the present results. Also, presence of sclerenchyma cells in a few studied species agree with Salmaki et al.(2011) who reported that the bundle sheath can be sclerenchymatous in a few species of Stachys. Solereder (1908) and Ryding (2010), mentioned that calcium oxalate crystals occur in leaves, stems, flowers and fruits of Lamiaceae in forms of needle-shaped or polyhedral crystals , druses and prismatic .The present results agree with these findings and shows that crystals of great importance in identification and taxa delimitation.

b. Petiole (Table 4; Plate 5)

Present in seven taxa while absent in the remaining taxa. In cross sections five types can be categorized; kidney shape (Marrubium vulgare), trigonometrica (Nepeta deflersiana), arc shape (two Ocimum varieties), half circle with wings (Meriandra dianthera), half circle (Otostegia fruticosa and Salvia schimperi). The epidermal cells are radially arranged in seven taxa while tangentially elongated in Nepeta deflersiana and Meriandra dianthera. The epidermal cells covered with a thick cuticle in five taxa except Otostegia fruticosa and Salvia schimperi. Ground system including one type of tissue in Otostegia fruticosa while the other taxa have two types. The angular collenchyma cells found in Nepeta deflersiana and two Ocimum varieties, Lamellar in Marrubium vulgare, Meriandra dianthera and Salvia schimperi. The shape of the petiole central vascular bundle has diagnostic value in species characterization in Lamiaceae (Shahri et al.,2016; Akçin et al.,2011; Shaheen, 2007; Eric et al., 2007; Metcalfe & Chalk, 1979). In this study, the anatomical petiole characters of the examined seven taxa showed high variation which helped in species delimitation.

c. Lamina (Table 5; Plate 6)

Characterized by isobilateral or dorsiventral, and there are variation in aspects of midrib region, only Thymus serpyllum and Meriandra dianthera characterized by presence of sclerenchyma cells while lamellar and angular collenchyma found in Lavandula pubescens and Stachys yemenensis and the other taxa have lamellar or angular. Midrib region cross sections can be categorized into five types: V shape, circular, flat (ribbon) shape, half circle and U shape. There are differences in vascular system aspects; rounded in Origanum majorana, Lavandula coronopifolia and L. pubescens, crescent in L. citriodora, Leucas inflata, Marrubium vulgare, Salvia schimperi and Teucrium yemense or arc shape in the remaining nine taxa. The number of vascular bundles in midrib region are, two in Lavandula pubescens, three
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in *Salvia schimperi* and one in the remaining 14 taxa. Crystals found only in eight taxa as tuft-like in *Nepeta deflersiana, Mentha longifolia* and *Teucrium yemenense*, needle and solitary in *Marrubium vulgare*, duress in the three *Lavandula* spp. and *Leucas inflata.

The results of the present work showed great differences in the studied taxa regarding lamina structure in dermal system, mesophyll tissue, midrib region and crystals. These parameters were used in studying the relationship among species of Lamiaceae and can be considered for different taxonomic purposes at the genus or species level (Metcalf & chalk, 1950; Inyama et al., 2016). Lamina epidermal surfaces are generally amphistomatic but hypostomatic in *Stachys yemenensis* only. The stomatal types are anisocytic in *Nepeta deflersiana* and *Otostegia fruticosa*, pericytic in *Mentha longifolia* and *Thymus serpyllum*, but more commonly diacytic in most taxa studied. Anticlinal wall is rounded in *Thymus serpyllum*, wavy in *Marrubium vulgare*, *Mentha longifolia*, two *Ocimum* varieties, *Origanum majorana*, *Salvia schimperi* and *Teucrium yemenense* while straight in the eight remaining taxa. Inamdar & Bhatt (1972) found that in majority of species, the stomata occurred exclusively on the lower leaf surfaces. However, Cantino (1990) mentioned that both amphistomatic and hypostomatic lamina are found in the members of the family. The present results in accordance with Cantino (1990) finding. The results showed also, three types of stomatal development within the studied species; diacytic, anisocytic and pericytic and this result agree with EL-Gazzar & Watson (1970), Inamdar & Bhatt, (1972) and Grozeva et al. (2016). While Metcalfe & Chalk (1979), stated that, diacytic stoma type was the most common in Lamiaceae.

**d. Trichomes morphology (Table 6 and Plates 8 & 9)**

In the present results, the types of trichomes observed among studied taxa are summarized in table (6) and selected micrographs of all types of trichomes are presented in plates (8 & 9). The types of trichomes showed considerable variation among the studied taxa and therefore are potentially valuable for different levels of classification. This result agree with Ascensão et al., (1995); Bokhari & Hedge, (1976); Xiang et al. (2010) and Hu et al. (2012).

All taxa studied have eglandular and/or glandular trichomes. Peltate, capitate trichomes are the most diagnostic, and the stellate type is unique in *Marrubium vulgare* while capitate biramous in *Meriandra dianthera*. In some genera of Lamiaceae, trichrome morphology is helpful in infrageneric classification (Marin et al., 1994; Navarro & El-Oualidi, 2000; Yousefi et al., 2015), as well as at specific levels (Bruni et al., 1987; Giuliani et al., 2008).

**2. Quantitative macromorphological measurements (Table 7)**

The mean measurements of ten quantitative macromorphological characters of the stem length, leaf, nutlet length and width, calyx, corolla, stamen, bract length of both the studied taxa and the analysis of variance (ANOVA) are summarized in Table (7).

The results showed highly significant differences at $P \geq 0.05$ between studied taxa in all characters except nutlet length. On the other hand, the stem length and leaf size of *Teucrium yemensis, Thymus serpyllum* have the smallest values (12,17 cm), (1 X 0.5 & 0.9 X 0.4 mm) respectively while *Meriandra dianthera* and *Otostegia fruticosa* have the largest values of stem (200 &150 cm ), also *Meriandra dianthera* and *Salvia schimperi* have the largest values of leaf size (10 X 3.5 & 7.5 X 2.8 cm) respectively. The calyx, corolla, stamen, bract length of both *Otostegia fruticosa* and *Salvia schimperi* have the largest values: (12 and 19 mm), (22 and 35 mm), (15 and 25 mm) and (7 and 18 mm) respectively among 16 taxa while the remainder taxa show smallest and moderate length.

The nutlet length showed no significant differences $P = 0.6$ among the studied taxa while nutlet width showed significant differences with $P= 0.0001$.The *Marrubium vulgare* and *Mentha longifolia* showed the smallest nutlet width.
Table (3): Stem micromorphological characters of the studied taxa

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<tr>
<td>Semicircular</td>
<td>- - - - - - + - - + - - - +</td>
</tr>
<tr>
<td>Circular</td>
<td>- - + - - - - - - - - - - +</td>
</tr>
<tr>
<td>Cuticle</td>
<td></td>
</tr>
<tr>
<td>Thick</td>
<td>+ + + + + + + + + + + + - +</td>
</tr>
<tr>
<td>Thin</td>
<td>- - - - - + + + + + + + + +</td>
</tr>
<tr>
<td>Epidermal cells</td>
<td></td>
</tr>
<tr>
<td>Tangentially &amp; radially</td>
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</tr>
<tr>
<td>Tangentially</td>
<td>- + + - + + + + + + + + + +</td>
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<tr>
<td>Radially</td>
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<td>Absent</td>
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</tr>
<tr>
<td>No. of Tissues</td>
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</tr>
<tr>
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<td>+ + + - - - - - - - - - - -</td>
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<td>2</td>
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Table (4): Petiole micromorphological characters of seven studied taxa (nine taxa sessile).

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<td>Outline in T.S.</td>
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<td>Half circle with wings</td>
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<td>Trigonometric</td>
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<tr>
<td>Arc</td>
<td>-</td>
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<tr>
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<td>-</td>
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<td>Cuticle</td>
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<td>Thin</td>
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**Plate 5 (Figs. 1-5).** Petiole microphotographs showing different outline aspects of the petiole, in the studied taxa of Lamiaceae (LM), (X=20). 1: Trigonometric, 2: Kidney, 3: Half circle, 4: Half circle with wings, 5: Arc. 1: Nepeta deflersiana, 2: Marrubium vulgare, 3: Otostegia fruticosa, 4: Meriandra dianthera, 5: Ocimum basilicum var. basilicum.
<table>
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<td>+</td>
</tr>
<tr>
<td>Thin</td>
<td>-</td>
</tr>
<tr>
<td>Tangentially &amp; bulliform</td>
<td>-</td>
</tr>
<tr>
<td>Tangentially</td>
<td>+</td>
</tr>
<tr>
<td>Radially</td>
<td>-</td>
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Plate 6 (Figs. 1-6). Lamina microphotographs showing different mesophyll characteristics in the studied taxa of Lamiaceae (LM), (X=20-40).  

Plate 7 (Figs. 1-3). Lamina microphotographs showing different types of stomata and anticlinal wall in Teucrium yemense, Mentha longifolia, Leucas inflata.
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T: Taxa; C: Characters; (+):Present; (-):Absent. Type I: unicellular head and uni-bicellular stalk, Type II: bicellular head and uni-bicellular stalk, Type III: unicellular head and multicellular stalk, Type V: multicellular head and unicellular stalk, Type V: biramous, U: unicellular, B: bicellular.

4. Quantitative micromorphological measurements (Table 8)

The stem, petiole and lamina micromorphological characters showed that there are significant differences with P= 0.0001, among 16 taxa (Table 8). The results showed that Lavandula citriodora, L. coronopifolia, have very thick stem cuticle (1, 2.3 μm) respectively while Lavandula citriodora, Leucas inflata and Meriandra dianthera have very thick lamina cuticle (1.5, 1, 1.2μm) respectively. On other hand, the cortex, secondary phloem thickness and pith width of Salvia schimperi has the largest values (38.8, 13, 26.6 & 225 μm) respectively. Leucas inflata, Meriandra dianthera, and two Ocimum varieties, Otostegia fruticosa has the largest cortex thickness (34, 47, 28.57, 28.57 & 28 μm) respectively while Thymus serpyllum has the smallest 11.28 μm. Meriandra dianthera has the largest value of secondary phloem (22.8μm) while Thymus serpyllum has the smallest (3.6 μm). While, Lavandula pubescens, Marrubium vulgare, Mentha longifolia, Meriandra dianthera and Nepeta deflersiana have the largest values of secondary xylem thickness (40, 48.8, 45, 56 & 40 μm). Thymus serpyllum has the smallest pith width (19.2 μm) while Lavandula pubescens, Otostegia fruticosa have the largest values (208 & 240 μm) respectively. On other hand, the adaxial epidermal cell lengths and width are larger than abaxial among all studied taxa.

5. Cluster analysis of macro – and micromorphological characters

In the present study, 16 medicinal taxa of family Lamiaceae belonging to 13 genera were used as the operational taxonomic unites (OUT’S). A total of 298 attributes are extracted from the macro & micromorphological characters of the studied taxa. The presence or absence of each character was treated as binary character in a data matrix using the software jmp version 13 for Sas Institute Inc (2017) through Hierarchical cluster method average linkage modules, (Sokal & Michener, 1958) and resulted in a dendrogram (Fig.1) depending on the degree of dissimilarity of cluster. It was compared with the current taxonomic treatments of the Lamiaceae provided by Bentham & Hooker, (1876); Briquet, (1895 – 1897); Thonner, (1962); Cantino et al. (1992c) and Takhtajan, (2009). The resulted data from the dendrogram are categorized in ten groups included in two clusters, two subseries and two series at relatively high-rescaled dissimilarity distance (Fig.1).

The obtained data from the dendrogram revealed that:

1. Thymus serpyllum was separated in (G1) under Series I, due to high dissimilarity distance 26.03 with other taxa in most morphological and anatomical characteristics. This result agrees with that of Bentham and Hooker (1876), Briquet (1895 – 1897) and Thonner, 1962 classification, who put Thymus under subtribe Thymineae and disagree with Cantino et al. (1992) who put Thymus under tribe Mentheae and Takhtajan (2009) who put it under tribe Satureiae.

2. Salvia schimperi was separated in (G2) under Series II, this result agrees with Thonner (1962) and Takhtajan (2009) classification who put the Salvia under tribe Salviæae and disagreed with Cantino et al., (1992) who put Salvia under tribe Mentheae.

3. The two studied taxa Meriandra dianthera and Otostegia fruticosa were separated in one group together (G3) at dissimilarity distance (24.32). This result agrees with Thonner (1962) who put Meriandra and Otostegia under subfamily Stachyioideae. However, the results disagreed with Cantino et al. (1992) and Takhtajan (2009) classification who put Meriandra under subfamily Nepetoideae and Otostegia under subfamily Lamioideae. In addition, this result support the earlier views of Thonner (1962) who put the two genera Meriandra and Otostegia under the same subfamily of Stachyioideae and two separated tribes Meriandreae and Stachyeae respectively.

4. Leucas inflata was separated in (G4), this result agrees with Takhtajan (2009), Thonner, (1962) classification, who put it under subtribe lamieae and Cantino et al., (1992c) who put the genus under subfamily lamioideae.
Morphological and Anatomical Studies

5. *Stachys yemenensis* was separated in (G5), this result agrees with Bentham and Hooker (1876), Briquet (1895 – 1897) classification, who put it under tribe Stachyaeae or Stachydeae alternatively, and Takhtajan (2009) who put it under lamiaeae tribe.

6. *Teucrium yemense* was separated in (G6), this result agrees with Cantino et al., (1992) classification, who put it under subfamily Teucrioideae and Takhtajan (2009) who put Teucrum under tribe Teucrieae.

7. *Marrubium vulgare* was separated in (G7) this result agrees with Bentham and Hooker (1876) classification, who put it under subtribe Marrubiareae and Briquet (1895 – 1897), Thonner (1962), Takhtajan (2009), classification, who put it under tribe Marrubiareae while Cantino et al. (1992) put it under subfamily lamioidae.

8. *Nepeta deflersiana* was separated in (G8), this result agrees with Bentham and Hooker (1876), Thonner (1962), Takhtajan (2009) classification who put it under tribe Nepeteae or Nepetaceae and disagreed with Cantino et al., (1992) who put under tribe Mentheae.

9. The two *Ocimum* varieties, *Origanum majorana* and *Mentha longifolia* were grouped together in (G9), this result agrees with Cantino et al.(1992) and Takhtajan (2009) classification who put these taxa under subfamily Nepetoideae. Among (G9), we found that the dissimilarity distance was 4.4 between the two *Ocimum* varieties due to the different flower colors.

10. *Lavandula citriodora*, *L. coronopifolia* & *L. pubescens* were separated in one group together (G10), this result agrees with Cantino et al.(1992c); Takhtajan, (2009) classification, who put them under tribe Lavanduleae and Thonner (1962) who put under subfamily Lavanduloideae. The dissimilarity distance between *Lavandula coronopifolia* and *L. citriodora* was 17.2. Therefore, the latter species separated in single branch inside (G10). The result indicates that trichomes play important role in species delimitation among genera. In this study, clustering (G8) and (G9) in one branch supporting the views of the close relation between (two *Ocimum* varieties, *Origanum majorana*, *Mentha longifolia* and *Nepeta deflersiana*) this result in accordance with Cantino et al.(1992) classification who put them under one subfamily Nepetoideae.

In conclusion, the classification of most of the studied taxa of family Lamiaceae is in accordance with that of Bentham and Hooker, Briquet, Thonner classification, while, agrees with Cantino et al. and Takhtajan classification to some extent for some genera. More studies on many taxa still needed for accurate assignments.

Table (7). Quantitative macromorphological measurements of the studied taxa

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3.4
Table 8. Quantitative micromorphological measurements of the studied taxa

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Prob<0.05: 0.0001* 0.0001* 0.0001* 0.0001* 0.0001* 0.0001* 0.0001* 0.0001*

Table 8. Cont’d.

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Prob<0.05: 0.0001* 0.0001* 0.0001* 0.0001* 0.0001* 0.0001* 0.0001* 0.0001*

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Fig(1): Dendrogram based on 298 attributes of micro- & macromorphological characters using *jmp* program, showing the interrelationships between 16 Lamiaceae taxa based on dissimilarity distance.
References


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