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Systematic importance of pollen morphology of some plants of Lamiaceae

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ABSTRACT

Pollen morphology of seven taxa belonging to Lamiaceae and common used as medicinal plants were examined by light microscopy and scanning electron microscopy - *Thymus vulgaris* L., *Mentha longifolia* (L.) Huds., *Mentha spicata* L., *Ocimum basillicium* L., *Salvia elegans* Vahl., *Salvia farinacea* Benth, and *Salvia splendens* Sellow ex Roem. and Schult. Pollen morphological characteristics are provided for these taxa. Among the studied taxa, *M. longifolia* has the smallest pollen grains, and *O. basillicium* possesses the largest ones. The main shape of pollen grains in the most taxa suboblate, oblate-spheroidal or prolate-spheroidal. However, subprolate are recorded for *S. elegans*. The grains are hexacolpate in all taxa, but in S. *farinacea* octacolpate pollen was found. Three distinct exine sculpturing exist, reticulate-perforate the common type and also reticulate, granulate, and bireticulate. However, the fine details are characteristic to differentiate between the pollen species. Exine is microreticulate in case of *S. elegans* and S. *farinacea*, but reticulate-foveolate in the case of *S. splendens*. As well as, the presence of holes on colpus membrane ornamentation can be used as a taxonomic tool for differentiate between *S. elegans* and *S. splendens.*

KEY WORDS: Lamiaceae, medicinal plants, pollen morphology, pollen ultra-structure (scanning electron microscopy), taxonomy

INTRODUCTION

Lamiaceae or Labiatae, called as the mint family, is a family of flowering plants. It had traditionally been considered closely related to Verbenaceae (Harley *et al.*, 2004), but in 1990, phylogenetic studies showed that many genera classified in Verbenaceae belong instead in Lamiaceae (Cantino *et al.*, 1992) and (Wagstaff *et al.*, 1998), mostly aromatic herbs, shrubs, and few trees. The mints family Labiatae or Lamiaceae includes over 6500 species of herbs, shrubs, and trees of cosmopolitan distribution. This family is characterized by square stems and whorled inflorescences. The herbs often covered with hairs and aromatic, being rich in essential oils.

Lamiaceae almost cosmopolitan but absent from coldest regions (Erdtman, 1945) divided pollen of Lamiaceae into two main groups based on aperture number; the first group has tri-colpate pollen grains and comprises the subfamily Lamioideae, while second has hexacolpate pollen grains and comprises the sub-family Nepetoideae (Cantino and Sanders, 1986). Sub-family Nepetoideae has often strongly aromatic species with volatile terpenoids. They are mainly grasses and shrubs, fragrant and rich in medicinal properties. Some medicinally potential plants on Lamiaceae family are following like volatile oil of cineole, menthol, and limonene having peppermint (Raja, 2012).

Pollen grain features are taxonomically significant. A large number of studies on pollen morphology of Lamiaceae recorded in literature (Celenk *et al.*, 2008; Moon *et al.*, 2008a; Moon *et al.*, 2008b; Hassan *et al.*, 2009; Aytaç *et al.*, 2012). Investigations of pollen morphology in the Lamiaceae have proven essential as an aid to classification within the family (Abu-Asab and Cantino, 1992). Pollen morphology is generally supported segregation of some genera of Lamiaceae (Abu-Asab and Cantino, 1994).

In this study, we try to elucidate light on pollen morphological studies, on seven taxa of the Lamiaceae medicinally important plants. The main objectives of this study are to provide an additional tool of the pollen morphology of the selected plants which can be used as a taxonomic character to differentiate among the selected studied taxa specially when they used in powdered form

to medical or food processing as an additional tool, also, to revealed adulteration in herbal mixtures. This study can be useful in other areas such as archeobotanical researches since many of the Lamiaceae species are commonly used by humans as food and in medicine.

MATERIALS AND METHODS

Pollen grains of the seven recognized taxa of the Lamiaceae were taken from plant specimen vouchers herbarium material housed at the Herbarium of the Environmental Studies and Research Institute University (ESRI), Sadat City University and the herbarium of Cairo University, Egypt. However, the reference pollen representing these seven taxa have often been extracted from fresh flowers of plant specimens collected from Royal Farms for Medicinal plants (Fayoum Governorate, Egypt) during spring season 2015, and compared with the voucher herbarium specimens. For light microscopy (LM) examination, pollen grains were prepared according to the method of (Erdtman, 1960). Pollen morphological descriptions are based on features recognized at ×1000 magnifications and photographs of pollen grains were taken with an Olympus BX21 light microscope while, pollen slides prepared were kept in special slide holders at the herbarium of ESRI, Surveys of Natural Resources Department, Sadat City University, Egypt. For LM, pollen grains were mounted on slides in glycerin jelly. The polar and equatorial axes of 20 grains were measured for each specimen, making 100-160 pollen grains per species. The ratio of polar to equatorial axis length (P/E) was determined for each measured grain and the mean P/E ratio was calculated from these individual values. Pollen grains with ruptured colpus membranes were excluded from the measurements (Sebsebe and Harley, 1992) although some cases with expanded grains, but with intact colpus membranes could be included. For scanning electron microscopy (SEM), pollen air dried using 95% ethanol was mounted on a glass coverslip attached to an aluminum stub and then coated with gold, to a thickness of 300 A, using a Jeol Jfc 1100 sputter coater. The subsequent examination was with a Tesla BS 340 SEM. Selected SEM micrographs were then digitized and classified using image analysis software. The rectangular area for analysis was outlined in the mid-mesocolpium of each pollen grain, following method of (Vezey *et al.*, 1992). While analysis of variance and Scheffe's test were used for statistical evaluation (Sokal and Rohlf, 1981), results are expressed as mean ± standard deviations. The measured polar axis and equatorial diameter were based on at least 30 samples and other characters on approximately 20 under the LM. All of the measurements were done using Caranoy 2 (Schols *et al.*, 2002).

Non-parametric tests (Kruskal–Wallis and Mann–Whitney *U*-test) were applied using SPSS 10.0. The pollen terminology follows (Faegri and Iversen 1975; Hesse et al., 2009).

RESULTS

The different palynological characters of the studied taxa were presented in Tables 1 and 2, Figures 1-3. Morphological variation of pollen grains was described for size and shape, the number, position and morphology of apertures, and exine ornamentation.

Pollen Morphology

Thymus vulgaris L.

The pollen grains of *T. vulgaris* are hexazonocolpate, suboblate to euprolate, polar axis: 28-36 µm; equatorial diameter: 21-31 µm. Sculpture: Reticulate. The statistical variations are given in Table 1. The pollen size variations and measurements are given in Table 1, Figure 1 - Photo 1 (LM), and Figure 2 - Photo 70 (SEM). Pollen is small to midium (P = [28-36] ± 2.20, E = [21-31] ± 3.26 µm). The shape of the pollen grains in equatorial view ranges from sub-oblate to perprolate (P/E = 1.3) (Table 1).

Mentha longifolia (L.) Huds.

Pollen unit monad, size: Medium-sized (26-50 µm), polarity: Isopolar, oblate dry pollen: Elliptic, aperture:

Table 1: Pollen morphological data (mean values, standard deviations, minimum- mean-maximum for E, P, E/P (dimensions, shape class, pollen shape, pollen class [LM and SEM]) of the studied taxa of Lamiaceae

Plant name	Dimension (LM)			Shape class	Pollen shape		Pollen class (LM and SEM)
	(µm) Polar axis P	(µm) Equatorial axis E	P/E		Polar	Equatorial	
Thymus vulgaris L.	(28-36)±2.20	(21-31)±3.26	1.3	Spheroidal	Suboblate	Euprolate	Colpzate
Mentha longifolia (L.) Huds.	(25-32)±2.04	(26-34)±2.3	0.90	Oblate-spheroidal	Elliptic	Suboblate	Zonocolpate
Mentha spicata L.	(27-31)±2.4	(29-32)±2.9	0.91	Oblate	Elliptic	Prolate	Isopolar
Ocimum basillicium L.	(64-69)±1.6	(66-71)±2.1	0.95	Oblate	Circular	Prolate	Colpate
Salvia elegans Vahl.	(33-48)±2.4	(36-55)±2.54	0.98	Circular	Oblate	Prolate	Prolate
Salvia farinacea Benth.	(34-48)±2.83	(37-57)±2.34	0.99	Circular	Oblate	Prolate	Prolate
Salvia splendens Van. Houttei.	(33-48)±2.70	(38-55)±2.74	0.98	Spheroidal	Circular	Prolate	Prolate

E: Equatorial axis, P: Polar axis, all measurements in um, LM: Light microscopy, SEM: Scanning electron microscopy

Table 2: The morphological and features of the studied taxa of Lamiaceae

Species	Pollen shape	Aperture type	Sculpture
Thymus vulgaris L.	Spheroidal	Hexazonocolpate	Reticulate
Mentha longifolia (L.) Huds.	Prolate	Hexacolpate	Reticulate
Mentha spicata L.	Prolate	Hexacolpate	Bireticulate
Ocimum basillicium L.	Spheroidal	Hexacolpate	Bireticulate
Salvia elegans Vahl.	Prolate	Hexazonocolpate	Reticulate
Salvia farinacea Benth.	Prolate	Hexazonocolpate	Reticulate
Salvia splendens Sellow ex Roem.	Spheroidal	Hexazonocolpate	Reticulate

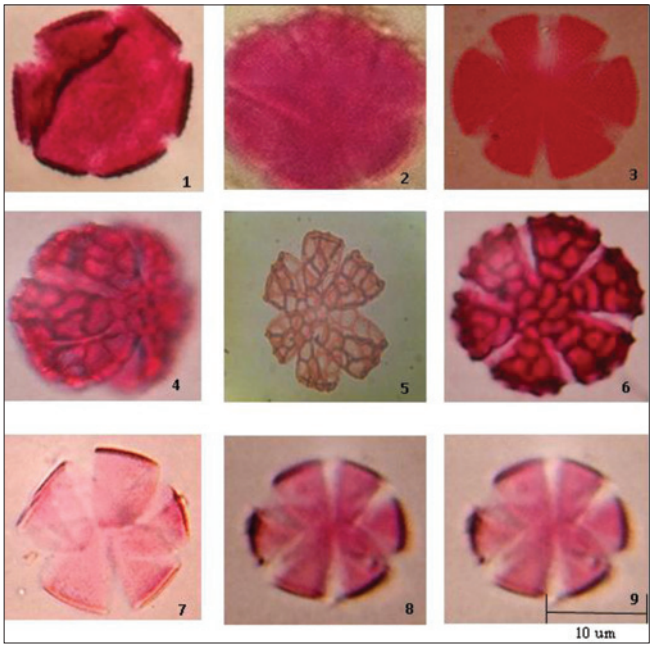

Figure 1: LM micrographs of pollen grains of 1- *Thymus Vulgaris* 2- *Mentha spicata* 3- *Mentha longifolia* 4,5,6- Ocimum *basilicum*, 7- *Salvia elegans*, 8- *Salvia frinacea* 9- *Salvia splendens*. Figs 4,5,6,7,8 and 9 Colpus shape. Figs 1,2. Polar view and low focus. Figs 5,6. Equatorial view and high focus. Fig. 4,7,8 and 9. Equatorial and low focus.

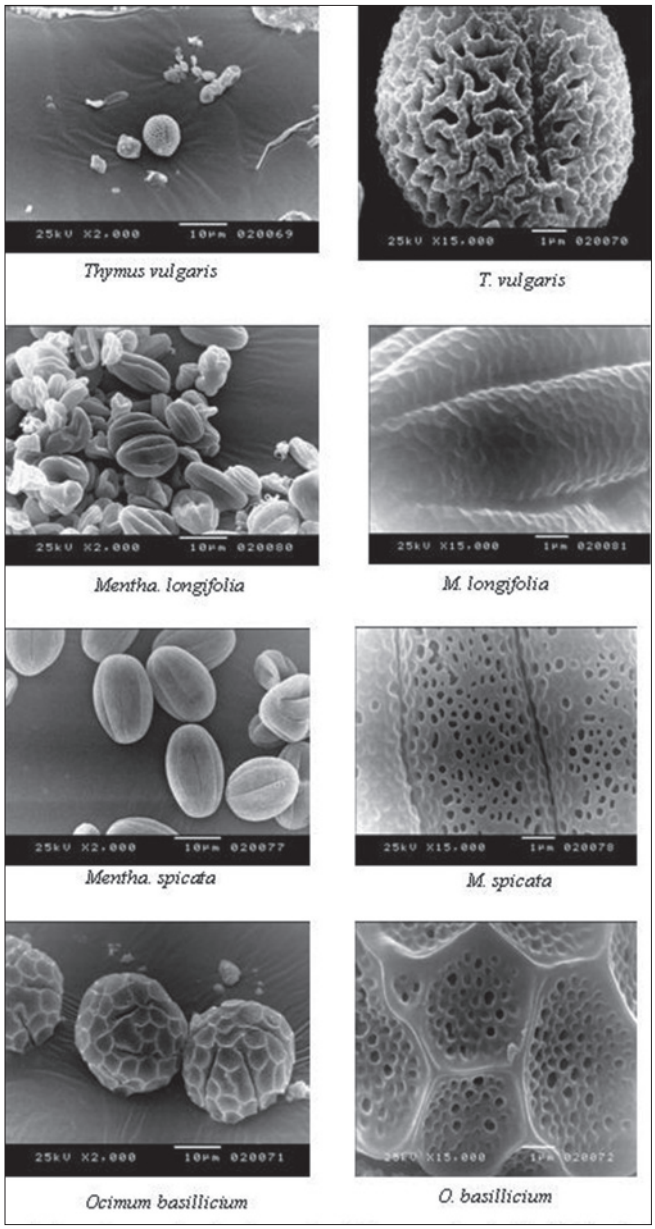

Figure 2: Scanning electron micrographs of pollen grains of *Thymus vulgaris*, Figs 69-70 (surface details, mesocolpium and colpus, *Mentha longifolia* Figs 80-81. *Mentha soicata* 77-78, *Ocimum basilicum* 71-72. (euqtorial view) and Sculpture types, Scale bar 10 um.

Colpate, aperture membrane ornamented. Data revealed that *M. longifolia* reported following pollen sizes: *P* = (25-32) ± 2.04 µm, E = (26-34) ± 2.3 µm, (P/E = 0.90) (Table 1).

Mentha spicata L.

The pollen grains are monads, six-zonocolpate (very rarely intermixed with eight or four colpate grains) or heterosyncolpate, prolate-spheroidal to suboblate, with a polar axis of (27-31) ± 2.4 µm and an equatorial diameter of (29-32) ± 2.9 µm of the species investigated (Tables 1 and 2, Figure 1 - Photo 2, and Figure 2 - Photo 77, 78).

Ocimum basilicum L.

Pollen grains of *O. basilicum* characterized with 6-colpate, zonocolpate, oblate-spheroidal in equatorial view, circular with indents at apertures in polar view (64-69) ± 1.6 µm, while (66-71) ± 2.1 µm equatorial view, (P/E = 0.95) (Table 1). Exine 3, sexine = nexine; reticulate.

Salvia elegans Vahl.

Representative pollen grains of each *Salvia* species are illustrated (Figure 3), and size and shape measurements are provided (Table 1). The pollen grains of all species examined in the genus *Salvia* are single, isopolar, and hexacolpate. The dimension micromorphological studies of *S. elegans* (Tables 1 and 2). Statistical description of quantitative micromorphological characters of *S. elegans* (Table 1), pollen grains of *S. elegans* characterized with, 6-colpate, zonocolpate, oblate, pollen shape prolate in

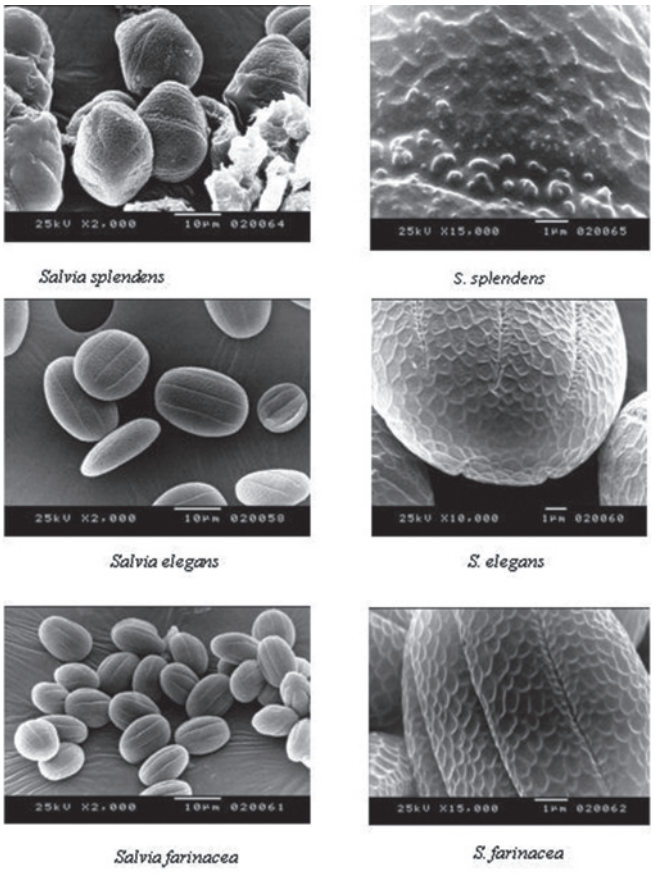

Figure 3: Scanning electron micrographs plooen grains of *Salvia splendens* Figs. 64-65. *Salvia aelegans* 58-60, and Salvia farinaces Figs 61-62 (surface details, msocolpium, and colpus margin). Fig. 65 surface ornamentation. Scale bars, 10 um

equatorial view and circular with indents at apertures in polar view (33-48) ± 2.4 µm ± while, (36-55) ± 2.54 µm equatorial view, (P/E = 0.98) (Table 1). Exine 3, sexine = nexine; reticulate in Table 2.

Salvia farinacea Benth.

The pollen grains of the genus *S. farinacea* are single, isopolar, and hexazonocolpate. The dimension micromorphological studies of *S. farinacea* (Tables 1 and 2).

Pollen grains of *S. farinacea* characterized with, 6-colpate, zonocolpate, oblate, pollen shape prolate in equatorial view, circular with indents at apertures in polar view (34-48) ± 2.83 µm. While (37-57) ± 2.34 µm equatorial view, (P/E = 0.99) (Table 1). Exine 3, sexine = nexine; reticulate Table 2. Statistical analysis of the quantitative micromorphological characters shows the differences in pollen characters between the three species (Table 1).

Salvia splendens Van. Houttei.

The pollen grains of the genus *S. splendens* are single, isopolar, and hexazonocolpate. The dimension micromorphological studies of *S. splendens* Tables 1 and 2.

Pollen grains of *S. splendens* characterized with 6-colpate, zonocolpate, oblate, pollen shape spheroidal in equatorial view, circular with indents at apertures in polar view (33-48) ± 2.70 µm, while, (38-55) ± 2.74 µm equatorial view, (P/E = 0.98) Table 1. Exine 3, sexine = nexine; reticulate in Table 2. Statistical analysis of the quantitative micromorphological characters shows the differences in pollen characters between the three species (Table 1).

DISCUSSION

Lamiaceae or Labiatae is divided into two main groups based on pollen characteristics such as aperture number and number of nuclei in shed pollen (Erdtman, 1945). The first group comprises the subfamily Lamioideae, and the second group contains the subfamily Nepetoideae (Cantino and Sanders, 1986). Aperture number has been considered a useful character to define the subfamily Nepetoideae. This subfamily is characterized by hexacolpate pollen grains with three nuclei at maturity.

T. vulgaris L.

The genus *Thymus* put into the subfamily Nepetoideae, tribe Mentheae (Cantino *et al.*, 1992). Based on a study of 128 species of this tribe, Wagstaff, 1992 produced a phylogenetic interpretation of pollen micromorphology. The apertures of pollen grains of Lamiaceae were observed as hexacolpate. Pollen grains of *T. vulgaris* as described in Tables 1 and 2, Figure 2 - Photo 70. The pollen size variations and measurements are given in Table 1 and Figure 1 - Photo 1 (LM) and Figure 2 - Photo 70 (SEM) conclude that pollen is small to medium (*P* = (28-36) ± 2.20, E = (21-31) ± 3.26 µm), while shape of the pollen grains in equatorial view ranges from sub-oblate to perprolate (P/E = 1.3). The exine is bireticulate so agree with (Harley *et al.*, 1992) and (Harley, 1992).

Mentha Species

Menthinae is a stenopalynous group. Their pollen is small to medium size (*P* = 13.0-43.3 lm), hexacolpate, with an oblate to prolate shape, and a perforate, microreticulate, or bireticulate exine ornamentation. The colpus membranes are beset with granules and the exine stratification typically shows simple columellae, orbicules are consistently absent. The literature showed that the size of pollen grains is greatly affected by different preparation treatments, and generally critical point-dried pollen is smaller than acetolysed pollen grains in SEM (Lens *et al.*, 2005), (Reitsma, 1969), and (Schols *et al.*, 2004).

The present results showed variation in sizes between taxa of menthinae (*M. longifolia* L. Huds. and *M. spicata* L.), and

it is notable that the range of size variation is more or less constant within the same genus (Table 1). In menthinae, (Trudel and Morton, 1992) stated that the hexacolpate is always dominant but mixed together with tetra-, penta- or octocolpate pollen (Figure 2 - Photos 78 and 81).

Our results show that taxa studied in menthinae (*M. longifolia* and *M. spicata*) has predominantly hexacolpate pollen, which supports hexacolpate pollen as a synapomorphy for Nepetoideae. Simple columellae are plesiomorphic and found in most gynobasic-styled Labiatae (Abu-Asab and Cantino, 1992). Furthermore, the taxa have simple columellae and a continuous or discontinuous foot layer with hardly observable endexine, in this connection (Gocmen *et al.*, 1997) provided brief pollen data of *Mentha* taxa using LM. Most of the taxa investigated had more or less similar pollen morphologies to those examined by us, except for the differences in size, ratio of the polar axis to equatorial diameter, and sculpture types. The values are a little different from those given in the present paper.

O. basilicum L.

The pollen grains of the *Ocimum* species and the variety studied looked similar, though there were some differences, especially in *O. basilicum* where more colpi were encountered. The pollen grains of all the species and the variety were spherical to ellipsoid in shape. While the morphologically different pollen grains like the concave in shape was encountered in *O. basilicum*. Tetrazonocolpate pollen grains with four colpi arranged in an equatorial zone were encountered in *O. basilicum*, according to the descriptions of (Moore and Webb, 1978).

Salvia Species

Erdtman, 1945 classified the Lamiaceae into two subfamilies based on number of apertures and nuclei in the mature pollen grains. The subfamily Nepetoideae had hexacolpate (rarely 8-12 colpate) pollen shed in a three-celled stage, and the subfamily Lamioideae had tricolpate pollen shed in a two-celled stage. In *Salvia* species, the exine of the pollen is uniform as semitecta reticulate (with small lumina) and could be described as reticulate-perforate, especially in *S. splendens* (Figure 3 - Photo 65), while in *S. elegans* and *S. farinacea* the exine shows mega reticulate (Figure 3 - Photos 60 and 62), respectively.

Our palynological findings were compared with those of the study of Ozler *et al.*, 2013; and according to their classification, *Salvia* classified as the common type in Lamiaceae. Due to it is a semitectate reticulate (with small lumina) and could be described as reticulate-perforate, our pollen showed similarities with *S. elegans* Vahl and *S. farinacea* Benth. However, *S. splendens* Sellow ex Roem pollen shape is different from the two taxa in being a spheroidal, with reticulate sculpture.

Cantino *et al.*, 992 revised the classification of certain genera in the Lamiaceae on the basis of palynological features. He also placed the genus *Salvia* L. within the subfamily Nepetoideae since it had hexacolpate pollen grains. This study showed that *S. splendens* had hexacolpate pollen grains. The pollen was suboblate, oblate-spheroidal, or prolate-spheroidal in the two *Salvia* species investigated previously, whereas it was oblate-spheroidal to prolate-spheroidal.

Exine Sculpturing

Exine sculpturing displays 2 distinct types of surface structures: Bireticulate (the common type) and reticulate-perforate (Hassan *et al.*, 2009), based on the detailed configuration of the exine sculpturing, reticulate-perforate and bireticulate patterns can be subdivided into 2 subtypes. Exine thickness is 1.0-2.2 µm, and intine thickness is 0.2-1.2 µm (Table 2).

CONCLUSION

Palynological and morphological studies of pollen grains, e.g., size, shape, sculpture, exine ornamentation, number, and size of apertures are important and deciding factor for the systematic study of various genera belonging the family Lamiaceae. Our study suggests further comparative studies on the other species belonging to other genera of Lamiaceae.

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