

VARIATION AND DISTRIBUTION OF CALCIUM OXALATE CRYSTALS IN SPECIES OF THE FAMILY ARACEAE

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The family Araceae includes 2500 species within 110 genera they protect themselves from weeds and animal predators by forming insoluble salts in the form of calcium oxalate crystals in specialized cells called idioblasts. This research investigates the types of calcium oxalate crystals in three species of the Araceae family, namely *Arum palaestinum*, *Arum dioscoridis*, and *Arisarum vulgare*. Based on the transverse section of different plant parts including tubers, leaves, spathe, appendix, male flowers, and female flowers five forms including raphide crystal, star crystal, prismatic crystal, styloid crystal, and sand crystal were identified. Raphides were uniformly present in all parts of the three species, but the positions of other crystal forms varied within their organs. This study also confirmed different types of specialized cells (idioblasts) containing raphid crystals. Idioblasts are unaltered cells with wide, elongated, tubular, and spindle shapes, housing simple, compound, and oblique-overlapping bundles. Additionally, certain plant parts displayed calcium oxalate crystals on their exterior surfaces, detached from the tissues.

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Keywords: Araceae; crystal patterns; druses; idioblasts cells; Raphides; taxonomy

تنوع و توزیع کریستال‌های اگزالات کلسیم در گونه‌های متعلق به خانواده Araceae

لاما جدید: گروه گیاه‌شناسی دانشکده علوم، دانشگاه تشرين، لاذقیه، سوریه

دینا حداد: گروه گیاه‌شناسی دانشکده علوم، دانشگاه تشرين، لاذقیه، سوریه

سمیر تاباش: گروه حفظ نباتات، دانشکده کشاورزی، دانشگاه تشرين، لاذقیه، سوریه

خانواده Araceae شامل ۲۵۰۰ گونه در ۱۱۰ جنس است که با تشکیل نمک‌های نامحلول به شکل کریستال‌های اگزالات کلسیم در سلول‌های تخصصی به نام Idioblasts از خود در برابر علف‌های هرز و حیوانات شکارچی محافظت می‌کنند. این تحقیق به بررسی انواع بلورهای اگزالات کلسیم در سه گونه گیاهی از خانواده Araceae به نام‌های *Arum palaestinum*، *Arum dioscoridis* و *Arisarum vulgare* می‌پردازد. براساس برش عرضی قسمت‌های مختلف گیاه شامل غده، برگ، اسپات، آپاندیس، گل‌های نر و گل‌های ماده، پنج شکل از جمله کریستال رافید، کریستال ستاره‌ای، کریستال منشوری، کریستال استیلوئید و کریستال ماسه‌ای شناسایی گردیدند. رافیدها در تمام بخش‌های هر سه گونه حضور داشتند درحالی‌که اشکال دیگر بلورها از نظر موقعیت در اندام‌های سه گونه متفاوت بودند. این مطالعه همچنین وجود تنوعی از سلول‌های تخصصی (ایدیوبلاست) حاوی بلورهای رافید را تایید کرد. ایدیوبلاست‌ها سلول‌های تغییر نیافته‌ای هستند که شکل‌های پهن، کشیده، لوله‌ای و دوکی از خود

نشان می‌دهند و حاوی انواع مختلفی از دسته‌های ساده، مرکب و مورب هم‌پوشان رافید هستند. به‌علاوه، بخش‌های خاصی از گیاهان، کریستال‌های اگزالات کلسیم را روی سطوح بیرونی خود، جدا از بافت‌ها، نشان می‌دهند.

INTRODUCTION

The family Araceae belongs to the phylum Angiospermae (Magnoliophyta), the class Liliopsida (Monocotyledons), the subclass Arecidae, and the order Arales according to the system of Cronquist (2018). It includes 2500 species within 110 genera worldwide (Boulos 2004). Plants of this family characteristically have a special defense ability; they protect themselves from weeds and animal predators by forming insoluble salts in the form of calcium oxalate crystals in specialized cells called idioblasts (Coté, & Gibernau 2012). The plant produces these crystals by a natural physiological process resulting from the interaction of calcium and oxalic acid (a two-carbon dicarboxylic acid) C₂H₂O₄. The accumulation of calcium oxalate crystals is a response to an excess amount of calcium in the plant. These crystals play a role in protecting plant parts from predation (Pearce & Pearce 2023), in addition to regulating calcium concentrations in plant tissues and organs, removing toxins and heavy metals, strengthening tissues, and maintaining ion balance (Islam & Kawasaki 2014; Raman & al. 2017). The composition and arrangement of calcium oxalate crystals within the plant exhibit considerable diversity across different species. They may occur systematically in the plant (roots, stems, leaves, flowers, fruits, and seeds), or they may be deposited in one or more organs of the plants. This is confirmed by the study of Akyol & al. (2018), which found that the formation of crystals within a cell is under genetic control. Hence, the specific type of crystals and their occurrence or absence can serve as distinctive taxonomic traits. It is suggested that the type and location of CaOx crystals constitute a taxonomically important character, which is indicated by Kartal (2016) in his study of Cardueae (Asteraceae).

Accordingly, identifying the type and localization of calcium oxalate crystals can significantly contribute to taxonomic investigations within plant studies. Genua & Hillon (1985) in their study of 14 species of aroids, indicated the specific location and type of calcium oxalate crystals within aroid species.

Araceae family has a wide range of applications, as some of its species are used as ornamental plants, others are grown as sources of carbohydrates for food, (Singla & al. 2020), and many are significant medicinal plants. Studies have shown that the genus *Arum* has great inhibitory activity against cancer cells (Abu-darwish & Efferth 2018). Many experiments using its extracts have shown a high inhibitory effect against many

pathogenic bacterial strains (Obeidat & al. 2012; Husein & al. 2014). Several aroid species in South America are traditionally used for malaria treatment. Moreover, members of this family exhibit efficacy against pathogenic fungi affecting humans and plants. Additionally, certain species within this family possess antiviral properties, with some proving effective against viruses like influenza (H5N1), (Farid & al. 2017).

This research aims to study the types of calcium oxalate crystals found in selected species of Araceae and their distribution in the different organs of the plant to identify the most toxic parts and determine the types of crystal bundles. This information is significant to supporting and documenting taxonomic studies to include them as diagnostic anatomical characters on which species of the Araceae are classified.

MATERIALS AND METHODS

Study area and plant material

Latakia is a coastal city in Syria overlooking the Mediterranean Sea, where four sites of different elevations above sea level were chosen. These sites are Beit Yashout, Banjaro, Jableh suburbs, and Tishreen University Park. Figure 1 shows study sites, and Table 1 summarizes the collected species, collection sites, and flowering periods. Three species belonging to two genera of the Araceae were collected: *Arum palaestinum* Boiss., *Arum dioscoridis* Sibth. et Sm., and *Arisarum vulgare* Targ. -Tozz. (Fig. 2). Plant species were identified using relevant taxonomic references (Moutterde 1966; Davis 1984; Dothan 1986; Mayo & al. 1997). Voucher specimens have been deposited in the herbarium of the Faculty of Science, Tishreen University, Latakia, Syria, (Table 1).

Preservation of specimens

After washing, Plant specimens were kept in the fixative F.A.A. (70 ml alcohol+20 ml formaldehyde+10 ml acetic acid) in plastic containers (Sass 1951). An identification card with the sample's name, location, and date of collection was placed on each container.

Microscopic observation of calcium oxalate crystals

Various plant parts (tubers, leaves, spathes, appendix, male and female flowers) from each FAA-preserved specimen were used to study crystals. Anatomical transverse sections were prepared, washed with water, placed on slides under a coverslip, and examined using a light microscope under 100x, 400x

magnification. The different types of crystals were identified using relevant references (Franceschi & Horner 1980; Horner & Wagner 1995).

The presence of idioblastic cells bearing calcium oxalate raphide crystals characteristic of Araceae, was studied in the different plant parts of the three selected

species and the types of cells and bundles have been identified according to the study of Keating (2004) on the Araceae species. Furthermore, the extracellular calcium oxalate crystals present on the surface of plant organs were studied.

Table 1. The collection data of studied species

Species	Collection data	Flowering period
<i>Arum palaestinum</i>	Beit Yashout, Banjaro, Lama Jdeed, A22-3-2022	March-April-
<i>Arum dioscoridis</i>	Beit Yashout, Tishreen University Park, Lama Jdeed, A14-5-2022	May
<i>Arisarum vulgare</i>	Banjaro, Jableh suburbs, & Tishreen University Park, Lama Jdeed, A18-12-2022	November-January



Fig. 1. Study area. 1, Tishreen University Park; 2, Jableh suburbs; 3, Banjaro; 4, Beit Yashout.



Fig. 2. The studied species. 1, *Arum palaestinum*; 2, *Arum dioscoridis*; 3, *Arisarum vulgare*.

RESULTS

Types of calcium oxalate crystals

This study confirmed the presence of five types of calcium oxalate crystals distributed in different parts of the selected species as follows:

Raphides: These crystal bundles were present in all parts of the three studied plant species (Figs. 3a, 3b).

Druses: Their occurrence varied among species, as summarized in Table (2). They were found in the spathes of the three species, in the female flowers of *A. dioscoridis*, *Arisarum vulgare*, and in the male flowers of *Arisarum vulgare* (Figs. 3c, 3d). The presence of this type in *Arum palaestinum* was restricted to the leaves and the appendix.

Prisms: This type was found in the leaves, appendix, and female flowers of *Arisarum vulgare* in rhombic and hexagonal shapes. It was also present in the leaves and spathe in a rhombic shape in *Arum dioscoridis* (Figs. 3e, 3f, 3g).

Styloids: It was only observed in the leaves, spathe, appendix, and male flowers of *Arisarum vulgare* (Fig 3h).

Crystal sands: Only the two species of the genus *Arum* contained this type of crystals, with a varied distribution. It was more common in *Arum palaestinum*, as it was found in all parts of the plant except the tubers. In *Arum dioscoridis*, these crystals were found in the leaves and spathe (Fig 3i).

Table 2. Types of calcium oxalate crystals and their distribution in plant parts (+: presence of the type in the studied plant part).

Types of crystals	Distribution					
	Tuber	Leaf	Spathe	Appendix	Male flowers	Female flowers
<i>Arum palaestinum</i>						
Raphides	+	+	+	+	+	+
Prisms						
Druses		+		+		
Styloids						
Crystal sands		+	+	+	+	+
<i>Arum dioscoridis</i>						
Raphides	+	+	+	+	+	+
Prisms		+	+			
Druses		+	+			+
Styloids						
Crystal sands		+	+			
<i>Arisarum vulgare</i>						
Raphides	+	+	+	+	+	+
Prisms		+	+	+	+	+
Druses			+		+	+
Styloids		+	+	+	+	
Crystal sands						

Types of raphide idioblasts

The results showed several types of idioblasts; cells bearing calcium oxalate raphide crystals (Tables 3, 4, and 5) and illustrated in Fig. 4. These types were as follows:

Unmodified cells (U): This type was observed in all parts of the two examined species within the genus *Arum*, excluding the tuber (Figs. 4a, 4b).

Wide cells (W): This type was common in the three studied species, but there were variations in the crystal bundle type between them (Figs. 4c, 4d).

Elongated cells (E): It was found only in *Arisarum vulgare*, in the leaves, spathe, and appendix (Fig. 4e).

Tubular cells (T): It was only found in the leaves, spathe, and the appendix of *Arisarum vulgare* (Fig. 4f).

Spindle-shaped cells (Sb): This type was found in the tuber, leaves, spathe, appendix, and female and male flowers of *Arum palaestinum* and *A. dioscoridis*, as well as in the female and male flowers and tubers of *Arisarum vulgare* (Figs. 4g, 4h).

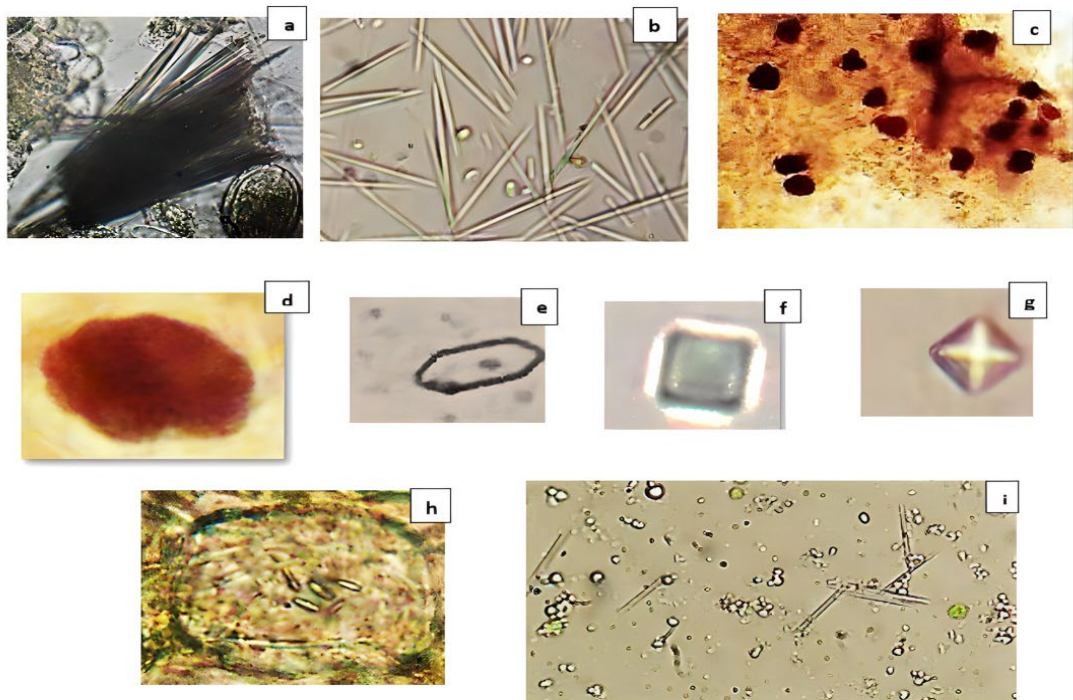


Fig. 3. Types of calcium oxalate crystals. a, Raphides at 200X (a raphide bundle in *Arum dioscoridis*); b, Scattered raphides at 400X (*Arum dioscoridis*); c, d, Druses at 100X and 400X (in the spathe of *Arum dioscoridis*); e, f, g, Prisms at 1000X rectangular, square and rhomboid (respectively in the leaf of *Arisarum vulgare*, the spathe of *Arisarum vulgare*, and the leaf of *Arum dioscoridis*); h, Styloids at 1000X (in the Spathe of *Arisarum vulgare*); i, Crystal Sands at 1000X (in the Appendix of *Arum palaestinum*).

Table 3. Types of raphide cells and crystal bundles in the studied parts of *Arum palaestinum* (+: presence of the type in the studied plant part).

Type of raphide cells and crystal bundles	Studied plant part					
	Tuber	Leaf	Spathe	Appendix	Male flowers	Female flowers
Unmodified cells and simple bundles		+	+	+	+	+
Unmodified cells and compound bundles						
Unmodified cells and oblique-overlapping bundles						
Wide cells and simple bundles					+	+
Wide cells and compound bundles		+			+	+
Wide cells and oblique-overlapping bundles			+	+		+
Elongated cells and simple bundles						
Elongated cells and compound bundles						
Elongated cells and oblique-overlapping bundles						
Tubular cells and simple bundles						
Tubular cells and compound bundles						
Tubular cells and oblique-overlapping bundles						
Spindle-shaped cells and simple bundles	+					
Spindle-shaped cells and compound bundles		+	+	+		
Spindle-shaped cells and oblique-overlapping bundles						

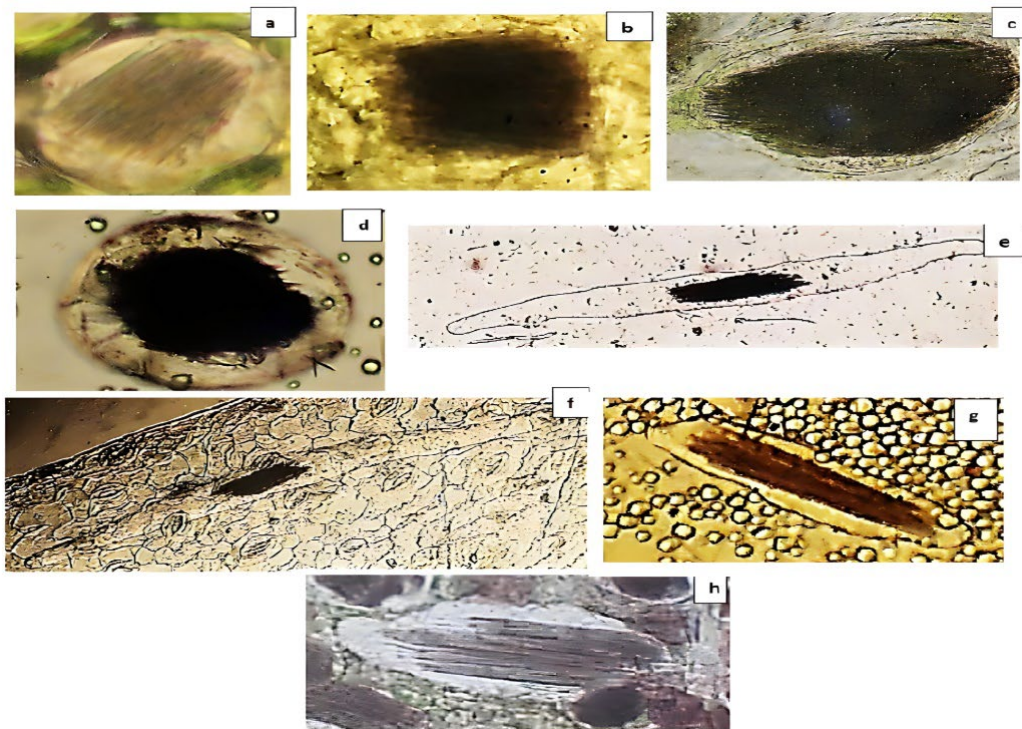


Fig. 4. Types of raphide cells. a, Unmodified cells at 400X (in the leaf of *Arum dioscoridis*); b, Unmodified cells at 400X (in the leaf of *Arum palaestinum*); c, Wide cells at 400X (in the appendix of *Arum dioscoridis*); d, Wide cells at 400X (in the spathe of *Arum palaestinum*); e, Elongated cells at 100X (in the leaf of *Arisarum vulgare*); f, Tubular cells at 100X (in the leaf of *Arisarum vulgare*); g, Spindle-shaped cells at 400X (in the tuber of *Arum palaestinum*); h, Spindle-shaped cells at 400X (in the leaf of *Arum dioscoridis*).

Table 4. Types of raphide cells and crystal bundles in the studied parts of *Arum dioscoridis* (+: presence of the type in the studied plant part).

Type of raphide cells and crystal bundles	Studied plant part					
	Tuber	Leaf	Spathe	Appendix	Male flowers	Female flowers
Unmodified cells and simple bundles		+	+	+	+	+
Unmodified cells and compound bundles						
Unmodified cells and oblique-overlapping bundles						
Wide cells and simple bundles					+	+
Wide cells and compound bundles		+	+	+	+	+
Wide cells and oblique-overlapping bundles			+	+		+
Elongated cells and simple bundles						
Elongated cells and compound bundles						
Elongated cells and oblique-overlapping bundles						
Tubular cells and simple bundles						
Tubular cells and compound bundles						
Tubular cells and oblique-overlapping bundles						
Spindle-shaped cells and simple bundles	+	+	+	+		
Spindle-shaped cells and compound bundles						
Spindle-shaped cells and oblique-overlapping bundles						

Types of crystal bundles within raphide cells

The study of raphide bundles confirmed the presence of three types of crystal bundles within idioblasts, as summarized in Tables 3, 4, and 5, and illustrated in Fig 5. These bundles are simple (Fig 5a)

and found in species with unmodified and spindle-shaped cells; compound (Fig. 5b) and found in all types of raphide cells except in unmodified cells; oblique-overlapping bundles (Fig. 5c). This type was found in tubular, elongated, and wide cells.

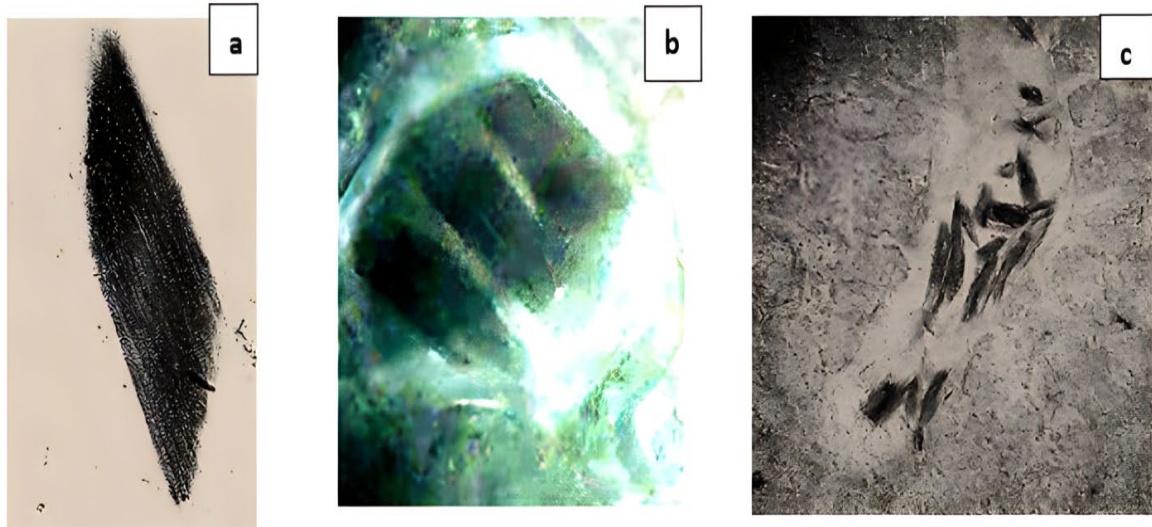


Fig. 5. Raphide crystal bundles at 200X. a, Simple bundles (in the leaf of *Arum palaestinum*); b, Compound bundles (in the spathe *Arum dioscoridis*); c, Oblique-overlapping bundles (in the spathe *Arisarum vulgare*).

Table 5. Types of raphide cells and crystal bundles in the studied parts of *Arisarum vulgare* (+: presence of the type in the studied plant part).

Type of raphide cells and crystal bundles	Studied plant part					
	Tuber	Leaf	Spathe	Appendix	Male flowers	Female flowers
Unmodified cells and simple bundles						
Unmodified cells and compound bundles						
Unmodified cells and oblique-overlapping bundles						
Wide cells and simple bundles						
Wide cells and compound bundles					+	+
Wide cells and oblique-overlapping bundles		+	+	+	+	+
Elongated cells and simple bundles		+	+	+		
Elongated cells and compound bundles		+	+	+		
Elongated cells and oblique-overlapping bundles		+	+	+		
Tubular cells and simple bundles		+	+	+		
Tubular cells and compound bundles		+	+	+		
Tubular cells and oblique-overlapping bundles		+	+	+		
Spindle-shaped cells and simple bundles	+				+	+
Spindle-shaped cells and compound bundles						
Spindle-shaped cells and oblique-overlapping bundles						

The presence of extracellular calcium oxalate crystals on the plant surface

The results of this study demonstrated that the presence of calcium oxalate crystals is not restricted to idioblasts; they could also be found extracellularly on male and female flowers. Raphides were found on these organs in *Arum palaestinum* and *A. dioscoridis*, and they may be stuck on the surface of the spathe, because of friction and proximity with the stamens and stigmas. Prisms, in addition to raphides, were also found on the surface of the stigmas and stamens of *Arisarum vulgare*.

DISCUSSION

All five patterns of calcium crystals were found in the three studied species of Araceae, one of the rare families that have all five patterns together (Prychid & Ruball 1999; Coté 2009).

The Raphides were distributed within idioblasts (specialized cells) in all studied plant parts in varying proportions in the three species (Çeçen, 2018). Druses were also found in the three species with variation in their distribution as summarized in Table 2, and this result is consistent with the study of Chairiyah & al. (2013, 2016) on Araceae species. This is consistent with Geçura & Winiarczyk's study (2016) on *Tinantia anomala* (Commelinaceae), which found three types of calcium crystals.

The styloids were only present in the leaves, spathe, appendix, and male flowers of *Arisarum vulgare*. The prisms were found in *Arisarum vulgare* in rhombic and hexagonal shapes, while limited to the leaf and spathe in a rhombic shape in *Arum dioscoridis*, and were absent in *Arum palaestinum*.

Sand crystals were observed in all parts of *Arum palaestinum*, except for the tuber, while limited in *Arum dioscoridis* to the leaves and spathe and absent in all parts of *Arisarum vulgare*.

There are several types of idioblasts: the unmodified cells (U) type remains common in the genus *Arum* (Tables 3 and 4). Wide cells (W) are common and prevalent. It is observed within the reproductive organs of the studied species (Arogundade & Adedeji 2019).

Arisarum vulgare was characterized by the presence of two types of raphide-bearing cells, which are elongated cells (E) and tubular cells (T), which are mostly concentrated in subfamilies branching at nodes earlier than the Aroideae, distributed within its different parts (the leaf, the spathe, and the appendix), as shown in Table 5, (Crowther 2009).

Spindle-shaped cells (Sb) type was found in the two studied species of the genus *Arum* in different parts of the plant (the tuber, the leaf, the spathe, and the

appendix), (Saadi & Mondal 2012; Jaradat & al. 2015) and this type was also found in the tuber, male, and female flowers of *Arisarum vulgare*. Interestingly, oblique-overlapping bundles were present in the different plant parts of the three studied species (Klimko & al. 2016), while simple and compound bundles were found within unmodified, wide, tubular, and elongated cells.

Crystals first develop inside the tissues, but when the stigmas and stamens mature, they are released on the exterior surfaces (Barabé & al. 2004). The presence of extracellular crystals is characteristic of Araceae, a rare occurrence in the plant kingdom (Barabé & al. 2004).

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