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Cystoliths and their Taxonomic Importance of *Ruellia* L. (Acanthaceae) in Kingdom of Saudi Arabia

Jawaher S Almuteri[1], Mona S Alwahibi[2], Ghada M.Al-Shuwaiman[3]

Department of Botany and Microbiology, King Saud University, Riyadh, Kingdom of Saudi Arabia

Abstract: ***This study deals with Ruelliaspp (Acanthaceae) from an anatomical point of view in the Kingdom of Saudi Arabia, especially the stony cell structures that are composed of vesicles consisting of calcium carbonate, spread on the surface of the leaf in two forms: rounded and elliptic, with the latter differing in the shape of its edges. The spherical shape was recorded in all studied species, while the oval shape was recorded in R. malacosperm, R sp.Jazan, R.sp.Taif.violet, R. patula, and the oval shape with blunt both end was observed only in R. malacosperma, while the oval shape with blunt one end was observed in R.patula, R.sp.Aseer, R.sp.Jazan, R. sp.Taif.white.***

Keywords: *Ruellia*, Acanthaceae, Rounded, Elliptic, Saudi Arabia

1. Interaction

Stone vesicles are secondary outgrowths of cell wall material with stony sacs containing calcium oxalate or silicon, in addition to calcium carbonate. They are found in the leaves, stems, and roots of some plant species, including the Acanthaceae family. Each vesicle is contained within a cell called the stony cell. Stylocytes can be found in the mesoderm or epidermis of leaves (Koch et al., 2009). And are characteristic of the leaf surfaces of some Rulea species (Gabel et al., 2020). It's more of a unique and interesting trait that could be very important from a taxonomy point of view. The presence of cystoliths (calcium oxalate or calcium carbonate) in the vegetative organs is one of the diagnostics useful in identifying Acanthaceae species (Tripp et al., 2014). Crystals are usually easy to see as short white lines in dried herbaceous specimens, especially in leaves (Choopan and Grote, 2015). Their type and location are often used in plant classification (Solereder, 1908). Their nature and distribution are of important value for the identification of genera and species. (Metcalfe and Chalk, 1950). Their nature and where they live are very important for figuring out what genus and species something is. (Metcalfe and Chalk, 1950). A study by Tripp et al. (2014) indicated that leaves of Ruellia species on the lower and upper surfaces contain ductal cells, either spherical or elongated, containing cystoliths of calcium carbonate CaCO3 or calcium oxalate CaC_2O_4, but they are present in abundance on the upper surface. It also varies in size within the same species (Inamdar et al., 1990), in addition to being a feature that makes each genus of the Acanthus family different (Holm, 1907). The occurrence of cystoliths in Ficus was first discovered by the scientist Meyen in 1827, and since then they have been observed in a number of plant families, including the Acanthus, and these, under certain conditions, can act as calcium deposits for the requirements of metabolism and growth, regardless of which the latter can store calcium temporarily (Smith, 1982).

2. Material and Methods

Samples collected of *Ruellia*spp from their original environments were given a number as shown in Table (1).the leaves were cut from them and washed with running water to remove the dust attached to them. After Cut the epidermal layer was carefully separated from the leaf and placed on a microscope slide.According to the method of Al-Daiji et al. (1997) and (Kiran, et al., 2011) with some modifications to suit the leaves of the studied plant,and the leaves were placed in a solution of 88 ml of lactic acid and 12 ml of distilled water in a water bath at 70-100 ° C for 20 minutes, because the plant's leaves contain a large amount of stone giblets, making obtaining a clear epidermal layer that requires more materials to dissolve it difficult. After removing it from the solution, a simple incision is made from the surface of the leaf with a sharp razor, then using pointed forceps and very carefully, a thin layer is removed from the leaf. The layer placed on a glass slide, making sure that the upper part is outward, and the skin is covered with drops of ethyl alcohol for 3-5 minutes to liquefy the skin and make it as transparent as possible, then the skin is temporarily loaded with a drop of glycerin to preserve the skin, then covered with a slide cover, then the glass slides are examined by light microscope and photographed.

Table 1: List of the Ruellia species studied and their localities in Saudi Arabia

Taxa	collection place	date of collection	GPS coordinates
Ruelliabrittoniana	Abha	December, 2021	18°16'37.6"N 42°43'23.5"E
Ruellia.sp.sh2010	Aseer	December, 2021	19°07'44.5"N 41°55'42.2"E
Ruelliacarolinensis	Jazan	September, 2021	16°59'30.7"N 42°42'59.0"E
Ruelliamalacosperma	Riyadh, Jazan, Madinah	September, 2021	24°43'07.9"N 46°37'24.4"E
Ruelliapatula	Jazan	September, 2021	16°54'50.1"N 42°33'20.4"E
Ruelliatweediana.rose	Taif	December, 2021	21°30'34.8"N 40°29'17.2"E
Ruelliatweediana. Violet	Taif	December, 2021	21°30'34.8"N 40°29'17.2"E
Ruelliatweediana. White	Taif	December, 2021	21°30'34.8"N 40°29'17.2"E

3. Results dissertation

The stony galls are spread on the surface of the leaf in two forms: rounded and elliptic, with the latter differing in the shape of its edges, it is a large outgrowths of cell wall material and calcium carbonate with a silicon-containing stalk found in the leaves, stems and roots of only a handful of plant families such as Cannabaceae, Urticaceae and Moraceae (e.g., Kuo-Huang & Yen 1996; Wu & Kuo-Huang 1997; Scotland &Vollesen 2000).. The spherical shape was recorded in all studied species, while the oval shape was recorded in each of the species *R. malacosperm*, *R sp*.Jazan, *R.sp*.Taif.violet, *R. patula*. The oval shape with blunt both end was observed only in *R.malacosperma*, while the oval shape with blunt one end was observed in *R.patula*, *R.sp*.Aseer, *R. sp*.Jazan, *R. sp*.Taif.white, as shown in Figures (1&2).

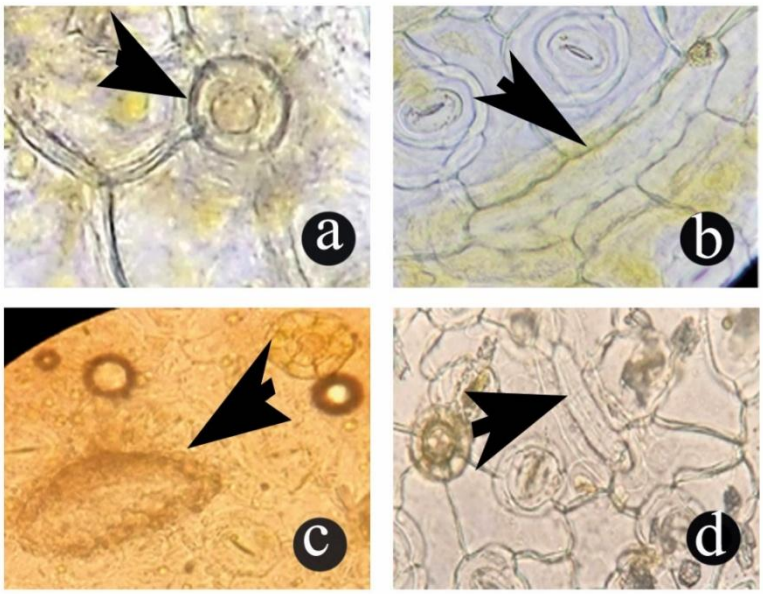

Figure 1: Stone vesicles of *Ruellia* species studied in Saudi Arabia using a light microscope with (40X) . (a) Spherical stone cell (b) acute-end oval stone ((c) acute-end oval stone (d) oval stone cell

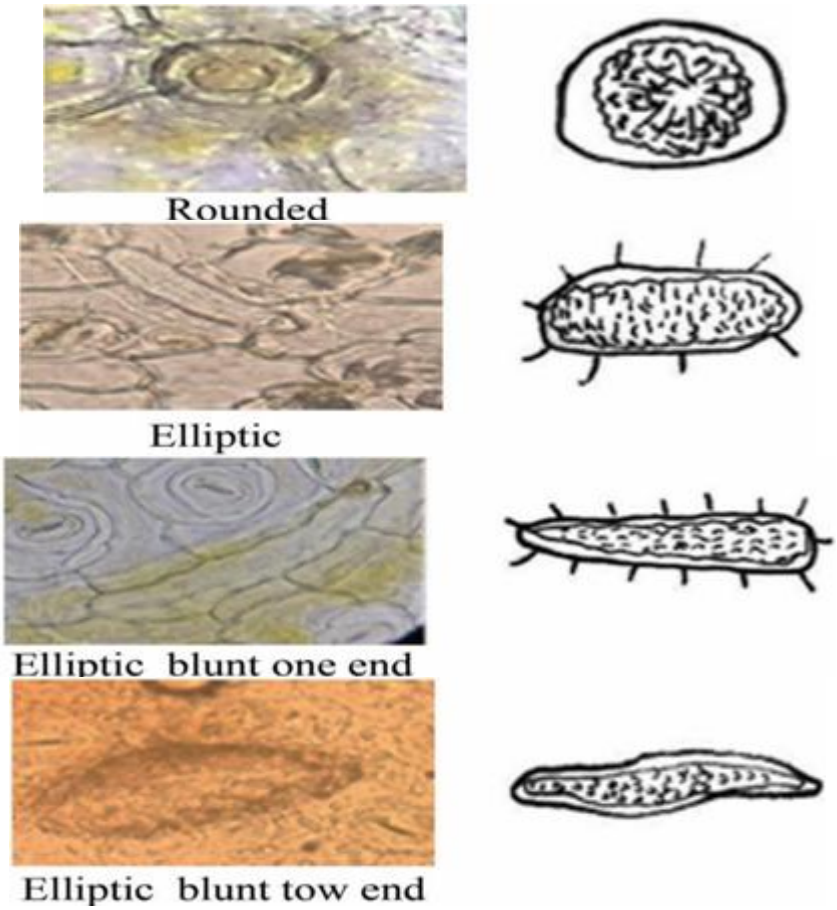

Figure 2: Types of stone cells in *Ruellia* species in Saudi Arabia using a light microscope (40X).

From the foregoing, the leaves of the species belonging to *Ruellia* species contained different patterns of stony vesicles and stony cysts, and they took several forms, according to what was mentioned in the results: spherical, oval, and oval with sharp ends, in addition to the oval with sharp ends, and one or all of the shapes may be present in one species. The current results are consistent with the study (Zakaria, et al., 2020; Patil and Patil, 2011; Inamdar et al., 1990).

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