

*Full Length Research Paper*

# Occurrence of calcium oxalate crystals in some wild plants used in traditional medicine in Saudi Arabia

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Plants are widely used in traditional medicine, but could be detrimental to patients, due to the presence of some harmful substances such as calcium oxalate (CaOx)  $C_2CaO_4$  crystals. The presence, morphology and distribution of CaOx crystals were investigated in 60 wild, herbal plant species from the Al Qussim Region, Saudi Arabia. This was done via light microscopy of transverse sections of stems and leaves using light microscopy. Different types of CaOx crystals were found in only 24 of the plant species studied, the rest had no CaOx crystals. The stems of 12 plant species under study contained different types of CaOx crystals, with crystal sand being the most common. In the leaves of four plant species, druses and solitary crystals were the most frequently found types. In addition, eight species had different types of crystals in their respective stems and leaves. *Rumex vesicarius* had the most crystals in both its stems and leaves from all investigated species, followed by *Asphodelus tenuifolius*.

**Key words:** Ca-oxalate crystals, light microscope, plant leaves, stems, Al Qussim Region.

## INTRODUCTION

Calcium oxalate (CaOx) crystals frequently occur in higher plants (plants of relatively complex or advanced characteristics, especially vascular plants, including flowering plants and gymnosperms (Hudgins et al., 2003). Calcium oxalate crystals are present in nearly all plant organs including roots, stems, leaves and seeds (Ilarslan, 2001). These crystals were generally widely distributed in the mesophyll and epidermal cells of leaves (Franceschi and Horner, 1980), as well as in the stem cortex and pith cells (Doaigey, 1991). Furthermore, these crystals occur in a variety of defined shapes. For example,

as druse, raphides, prismatic, styloid, and sand crystals (Gębura and Winiarczyk, 2016). The shape and distribution of CaOx crystals is characteristic to each plant family and can be used in taxonomical identification (Molano-Flores, 2001). For example, Druses and styloids were observed in stem epidermal cells, pith cells, and leaf mesophyll cells of *Vernonia amydaline* (Asteraceae) (Nwosu et al., 2013), while rhomboidal crystals were found in axial parenchyma and multiseriate ray cells of the secondary xylem of *Quercus* species (Fagaceae) (Serdar and Demiray, 2012).

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Calcium oxalate crystals are reported as a storage form of both calcium and oxalic acid function in the regulation of calcium levels in plant tissues (Franceschi and Nakata, 2005), protecting plants against herbivory (Molano-Flores, 2001), provide mechanical support to structures, facilitate light gathering and reflection (Franceschi and Horner, 1980), and can detoxify heavy metals in plant tissues (Nakata, 2003).

Calcium oxalate crystals have a diagnostic value, in that their presence or absence and dimension are of significant value in the identification of crude drugs (Jackson and Snowdon, 1968). Furthermore, they help in the detection of adulterants in herbal drugs (Lersten and Horner, 2006). For example, Tripathi et al. (2015) noted that CaOx crystals in edible plants can lead to kidney stones in humans. Saganuwan (2010) identified 150 medicinal plant species used in the treatment of various human diseases in the Arabian Peninsula. However, as previously stated these plants could be detrimental to patients due to the presence of harmful substances, such as CaOx crystals. As a result, this study was conducted to investigate the occurrence of CaOx crystals in several wild medicinal herbal plants from the Al Qussim Region in Saudi Arabia, which has a high level of herbal plant use and recent studies do not exist in this research topic. This study will be of value to determine which plants contain these crystals that may have harmful effects on patients.

## MATERIALS AND METHODS

### Study area

The province Al Qussim (Figure 1) is located in the center of Saudi Arabia approximately 400 km northwest of Riyadh, the capital. The climate is desert continental, hot and dry in summer, cold and rainy in winter, the summer temperature is 45°C, which reaches 3°C below zero in winter, and the average annual rainfall is 145 mm (Wikipedia, 2020).

### Plant collection

Sixty herbal plant species from the Al Qussim Region in Saudi Arabia were randomly selected from 150 plant species collected in spring of 2018 to be screened for the presence of CaOx crystals. They were taxonomically identified by a King Saud University Herbarium (KSUH) staff member. Voucher specimens were also deposited at this herbarium. The whole plant was collected.

### Plant material preparation

A few representative mature fresh samples of leaves and stems of each species were fixed in FAA solution (10% formaldehyde, 50% ethyl alcohol, 5% acetic acid, and 35% distilled water) overnight at room temperature and then samples were kept in 70% ethyl alcohol until prepared for microscopy (Doaigey et al., 1997).

### Plant tissue preparation

Permanent cross sections of leaves and stems were prepared

using the paraffin wax method of Doaigey et al. (1997). A rotary microtome (Leica RM 2135) was used to prepare 15 to 20 µm sections. The components of crystals were confirmed using the method of Pizzolato (1964). Samples were examined and photographed under a light microscope (Leitz Labor Lux D with camera Moticam 2000).

## RESULTS AND DISCUSSION

### Species containing no CaOx crystals

Screening the transverse sections of stems and leaves revealed that 36 (60%) of the 60 examined species did not contain any type of CaOx crystals. These plant species can be used with safety precautions based on the results indicated by the references mentioned in Table 1.

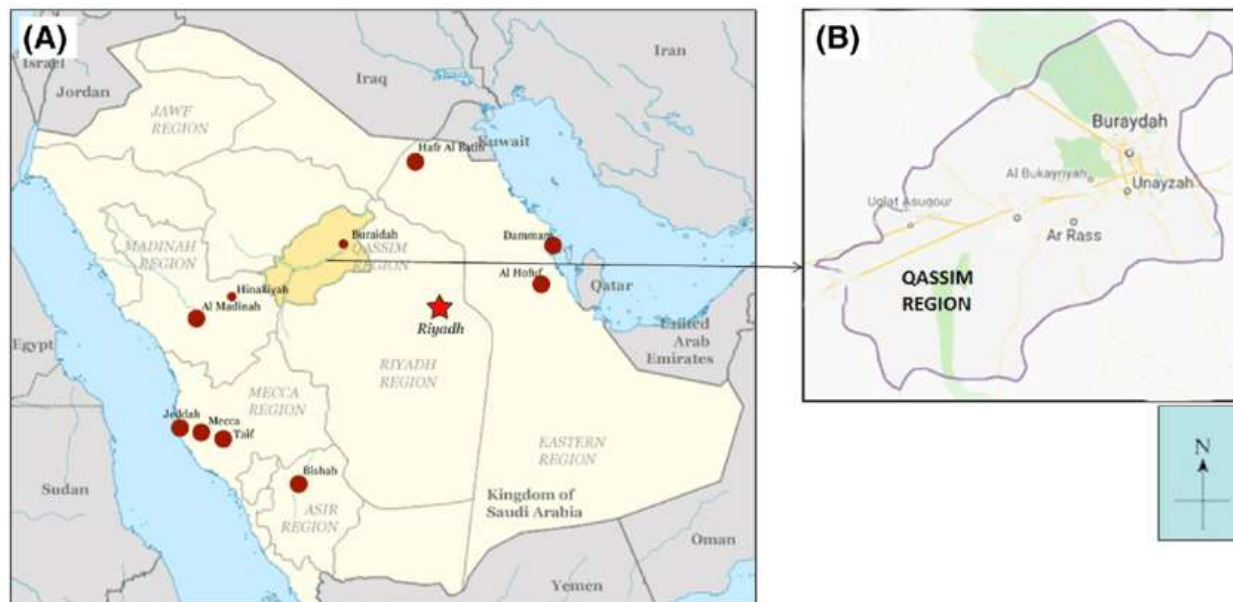
### Species containing different types of CaOx crystals

The 24 species were found to have one or more types of CaOx crystals in the transverse sections of stems (Table 2 and Figures 2 and 3), transverse sections of leaves (Table 3 and Figure 4), and in the transverse sections of stems and leaves (Table 4 and Figures 5 to 7). This agrees with the results obtained by Doaigey (1991), Gębura and Winiarczyk (2016) and Konyar et al. (2014). Their results showed that the most dominant type of crystal found in the transverse sections of stems and leaves were Druses.

### Species containing different types of CaOx crystals in stems

From the results mentioned in Table 2, there were 12 herbal plant species found to contain different types of CaOx crystals in the transverse sections of the stems only. This may be due to the nutrient content of the plant organ and the presence of mechanisms that prevent the formation of calcium oxalate in a plant organ, and it does not form in the other (Coté, 2009). The morphology and distribution of crystals is constant within a species. This indicates that their presence, morphology and distribution in a species are under genetic control (Ilarslan, 2001; Franceschi and Nakata, 2005); and the location, size and other properties of crystals may be effected by physical, chemical and biological conditions such as light, temperature, pH, ion concentration and herbivory (Franceschi and Horner, 1980; Molano Flores, 2001; Kuo-Huang et al., 2007).

Crystal sand (Figures 2A, C, G and 3C, D, H) and solitary crystals (Figures 2B, 3A and E) were the most common in this set of plant species. The distribution of crystal sand idioblasts was very rare in *Aizoon hispanicum*, *Anastatica hierochuntica*, *Bassia eriophora*, *Picris babylonica*, *Reseda lutea* and *Tripleurospermum*



**Figure 1.** (A) Map of Saudi Arabia showing (B) location of collection sites at Al Qassim region in yellow (B). (Rasheed et al., 2019).

*auriculata* species. In addition, the distribution of solitary crystals idioblasts was very rare in *Arnebia decumbens*, *Astragalus schimperi*, *B. eriophora* and *T. auriculata*, and rare in *Erucaria hispanica* and *Sclerocephalus arabicus*. This indicates that their occurrence, morphology and spreading in a species are under genetic mechanism (Ilarslan, 2001; Franceschi and Nakata, 2005). Druse type crystals were also found to be very rare in *A. hispanicum* and *S. arabicus*, and rare in *Herniaria hirsuta* (Figures 2D, E, F, H and 3F, G), which corresponds with that observed by Nwosu et al. (2013) in stem of *V. amygdalina*. The last type of crystal found commonly was of raphides (Figure 3B). Idioblasts was very frequent in *Lepidium sativum*, a result that is contrary with Wu and Kuo-Huang (1997), who examined calcium crystals in the leaves of *Humulus scandens* and found calcium carbonate crystals only in its leaves. The leaves of the above mentioned plant species can be used safely by humans and grazing animals as they do not contain any CaOx crystals, and stems can also be used without adverse effects for the patients if used moderately based on the references indicated in the Table 2.

#### **Species containing different types of CaOx crystals in leaves**

Leaves of four species (*Agaricus arvensis*, *Medicago laciniata*, *Reichardia tingitana* and *Savignya parviflora*) were studied. These plant species showed different types of crystals in the transverse section of their leaves. Although druse crystals idioblasts were the most common crystals in this set of plant species, they were very rare in

*M. laciniata* and *R. tingitana*, and rare in *S. parviflora* plant (Figure 4B, C and E). These results agreed with those of Nwosu et al. (2013), who observed druses in the leaf mesophyll cells of *V. amygdalina*. The solitary crystals idioblasts were less common, and found to be very rare in *A. arvensis* and *S. parviflora* (Figure 4A, F). Crystals sand were the least common type with a rare distribution in *R. tingitana* (Figure 4D). All types of crystals occurred relatively infrequently, except for druses in the leaves of the *S. parviflora* that is not found in other species investigated. Patients can use leaves of *S. parviflora* moderately and animals because druses and solitary crystals showed rare and very rare distribution and the stems can be used safely because they were CaOx free based on the references indicated in Table 3.

#### **Species containing different types of CaOx crystals in leaves and stems**

Eight species (Table 4) contained all types of CaOx crystals in their leaves and stems. Crystal sand was the most common type in the leaves and stems in these 8 species (Figure 6A, C, F and 7C, G, F, H), followed by solitary (Figures 6B and 7B, E) and druses crystals (Figure 6D and 7A, D). These results are similar to Doaigey (1991), Nwosu et al. (2013), and Konyar et al. (2014) who observed druse crystals in the stem and leaves of *Saponaria officinalis*. Least were the raphide crystals (Figure 6E). The idioblasts distribution of CaOx crystals in this group of eight plants was very rare, except in *Chenopodium album* (many idioblasts) and *Rumex vesicarius* (very idioblasts).

**Table 1.** Plants containing no CaOx crystals in transverse sections of both leaves and stems.

Species	Economic importance
<i>Ammi majus</i>	Traditional and folk medicine (Batanouny, 1999). Poison (Doaigey, 1991).
<i>Anisosciadium lanatum</i>	Edible and additive (Alaried and Al-Faraj, 1995), grazed.
<i>Anthemis scrobicularias</i>	Traditional and folk medicine (Hassan, 1995), grazed.
<i>Calendula micrantha</i>	Traditional and folk medicine (Boon and Smith, 2004), additive grazed.
<i>Chrozophora plicata</i>	Traditional and folk medicine (Al-Shanawani, 1996), grazed poison.
<i>Cotula anthemoides</i>	Traditional and folk medicine (Al-Zoght and Al-Sheikh, 1999).
<i>Diplotaxis acris</i>	Traditional and folk medicine (Al-Qura'n, 2010), edible grazed.
<i>Eremobium aegyptiacum</i>	Grazed (Dossari, 2010).
<i>Euphorbia arabica</i>	Traditional, folk medicine and poison (Al-Shanawani, 1996).
<i>Euphorbia granulata</i>	Traditional and folk medicine (Al-Sayd, 2009).
<i>Fagonia indica</i>	Traditional and folk medicine (Al-Zoght, 1990), grazed (Al-Ataa, 2011).
<i>Gastrocotyle hispida</i>	Traditional, folk medicine and grazed (Boulus, 1983).
<i>Gymnarrhena micrant</i>	Traditional, folk medicine and grazed (Algamal, 1995).
<i>Hippocrepis constricta</i>	Traditional, folk medicine and grazed (Al-Sodany et al., 2011).
<i>Horwoodia dicksoniae</i>	Traditional, folk medicine and edible (Fawzy et al., 2012), additive grazed (Omar, 2007).
<i>Koelipinia linearis</i>	Traditional and folk medicine (Smanla, 2013), edible (Al-Ataa, 2011), and grazed (Koul et al., 2000).
<i>Lepidium aucheri</i>	Traditional and folk medicine (Akbar and Al-Yahya, 2011), edible and additive (Al-Takroui et al., 2008).
<i>Linaria haelava</i>	Traditional and folk medicine and grazed (Lahlob, 1992).
<i>Malva parviflora</i>	Traditional, folk medicine and poison for cattle (Abu Rumaila, 1988), edible (Hashim and Mahmud, 1988).
<i>Matthiola livida</i>	Traditional and folk medicine.
<i>Matthiola longipetala</i>	Traditional and folk medicine (Chaieb, 2011).
<i>Neurada procumbens</i>	Traditional and folk medicine edible (Qureshi, 2012), grazed (Dossari, 2010).
<i>Picris abyssinica</i>	Traditional and folk medicine (Al-Shanawani, 1996), grazed.
<i>Plantago amplexicaulis</i>	Traditional and folk medicine (Al-Shanawani, 1996; Akbar and Al-Yahya, 2011), grazed (Dossari, 2010).
<i>Pteranthus dichotomus</i>	Traditional and folk medicine (Alrawy, 1976), grazed and poison for cattle (Omar, 2007).
<i>Rumex pictus</i>	Traditional and folk medicine (Abutbul et al., 2005), edible (Doaigey and Wahibi, 2006); grazed (Omar, 2007).
<i>Reseda stenostachya</i>	Traditional and folk medicine (Al-Zoght, 1990; Al-Shanawani, 1996), edible (Al-Zoght, 1990).
<i>Salvia deserti</i>	Traditional and folk medicine (Pirbalouti et al., 2012).
<i>Scabiosa oliveri</i>	Foraged by bees (Hossain et al., 2012).
<i>Schimpera arabica</i>	Traditional, folk medicine, edible and grazed (Akbar and Al-Yahya, 2011).
<i>Silene arabica</i>	Traditional and folk medicine (Aljohany, 2007), grazed (Omar, 2007).
<i>Sonchus oleraceus</i>	Traditional, folk medicine and edible (El-Ghazali et al., 2010).
<i>Stipa capensis</i>	Grazed and poison for sheep (Mandaville, 2011).
<i>Stipagrostis plumosa</i>	Grazed (Daur, 2012).
<i>Trigonella hamosa</i>	Traditional and folk medicine (Bouaziz et al., 2009), grazed (Umadevi et al., 2012).
<i>Trigonella stellate</i>	Traditional and folk medicine (El-Ghazali et al., 2010; Al-Rumaih and Al-Rumaih, 2008), grazed (El-Ghazali et al., 2010), poison (Tawaha, 2006).

Results indicate that 62.5% of the plant species contained CaOx crystal idioblasts in the transverse sections of stems, while 37.5% of plant species contained crystals in their leaves. This is contrary to the results obtained by Anitha and Sandhiya (2014), who reported that the CaOx crystals were distributed more in the leaves of plants than in their stems, but in our results calcium oxalate crystals in this group are distributed in stems more than leaves.

## Conclusion

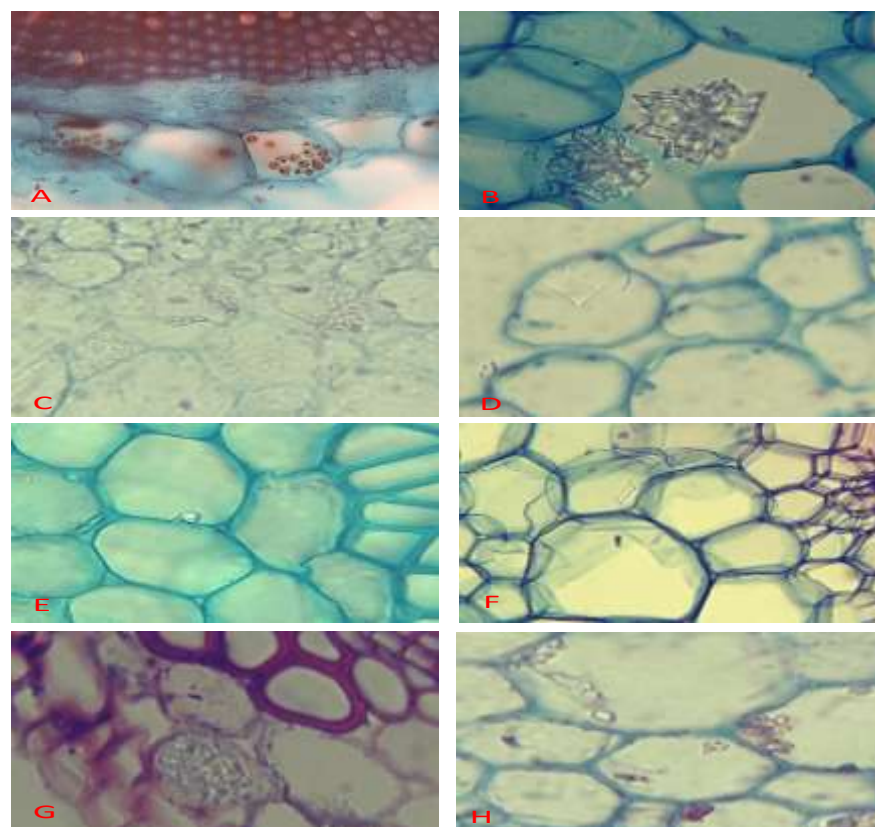
As there was no previous study explaining the presence

of CaOx crystal in plants used in folk medicine in Saudi Arabia except for the study of Doaigey (1991), which studied the presence of CaOx crystal in some known poisons plant, the results of the present study revealed that 60% of the 60 examined species did not contain any type of CaOx crystals, hence it can be used safely and completely by humans and livestock, while taking the required precaution. 20% of this 60 plants contained crystals idioblasts in their transverse sections of stems. The leaves of this group of plants can be used safely and completely by humans and livestock, and their stems are used with caution. 6.7% of plants contained crystals in their leaves, therefore, its leaves should be used with

**Table 2.** Herbal plants containing different types of CaOx crystals in transverse sections of stems.

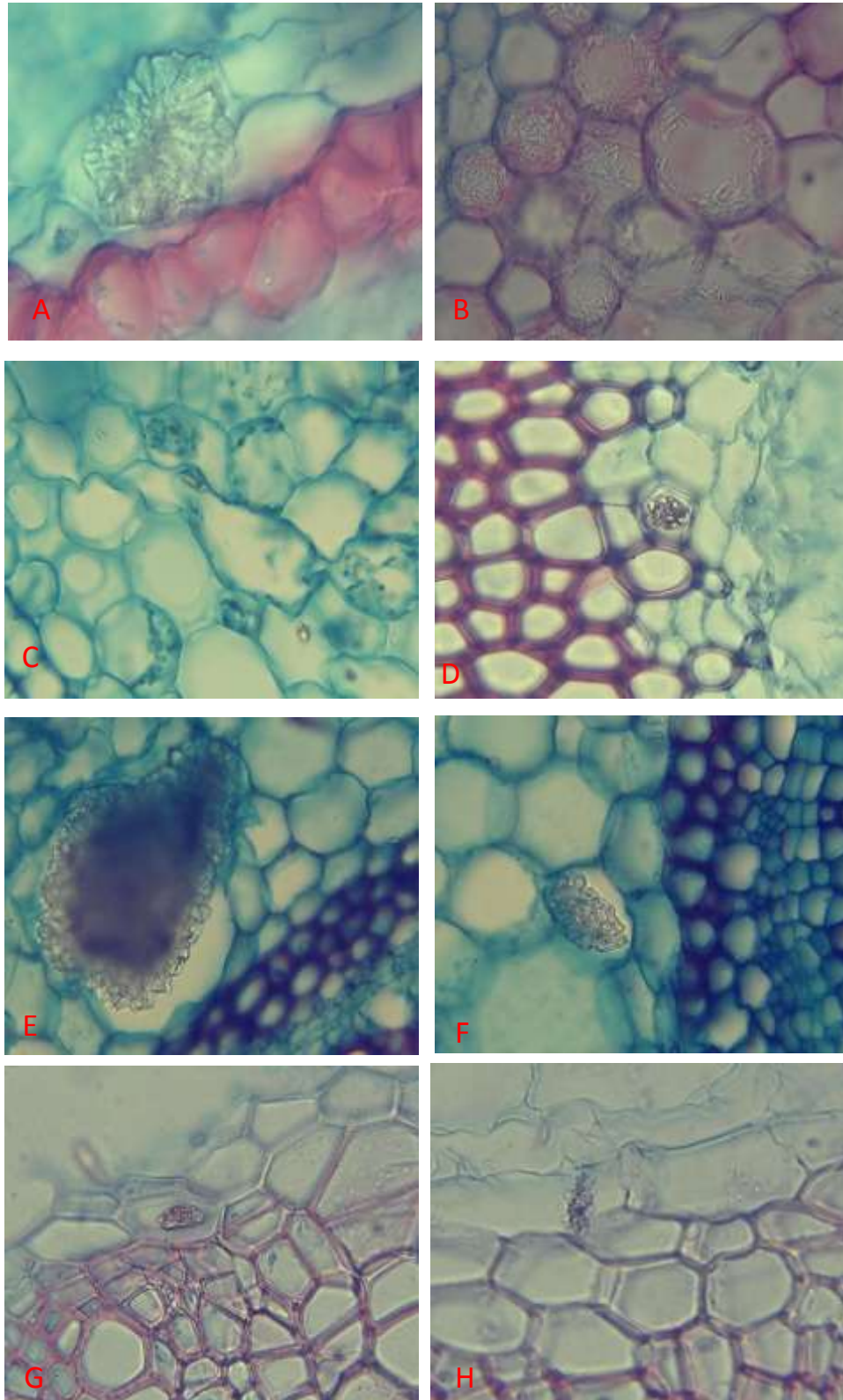
Species	Crystals		Economic importance
	Type	Distribution	
<i>Aizoon hispanicum</i>	D Cr	(vr)	Traditional, folk medicine and grazed (Middleditch, 2012), edible (Alali et al., 2007).
<i>Anastatica hierochuntica</i>	Cr	(vr)	Traditional and folk medicine (Ihsanullah, 2012).
<i>Arnebia decumbens</i>	S	(vr)	Traditional and folk medicine (Al-Shanawani, 1996; Omar, 2007), Grazed.
<i>Astragalus schimperi</i>	S	(vr)	Traditional, folk medicine and grazed (Abu-Dahab and Afifi, 2007).
<i>Bassia eriophora</i>	S Cr	(vr)	Traditional and folk medicine (Al-Shanawani, 1996).
<i>Erucaria hispanica</i>	S	(r)	Grazed (Dossari, 2010).
<i>Herniaria hirsuta</i>	D	(r)	Traditional and folk medicine (Al-Shanawani, 1996; Atmani and Khan, 2000).
<i>Lepidium sativum</i>	R	(vf)	Traditional and folk medicine (Qubaisi, 2007), edible (Adam et al., 2011).
<i>Picris babylonica</i>	Cr	(vr)	Traditional, folk medicine edible and grazed (Dossari, 2010).
<i>Reseda lutea</i>	Cr	(vr)	Traditional, folk medicine, edible, additive, grazed (Doaigey and Wahibi, 2006).
<i>Sclerocephalus arabicus</i>	D S	(vr) (r)	Traditional and folk medicine (Abutbul et al., 2005).
<i>Tripleurospermum auriculata</i>	S Cr	(vr)	Traditional and folk medicine (Tariq et al., 1987), edible, additive and grazed (Al-Saeed, 2001).

D = Druses, R = Raphides, Cr = Crystal sand, S = Solitary, vf = very frequent, f = frequent, r = rare, vr = very rare.



**Figure 2.** Plants contain different types of calcium oxalate crystals in stems. A- *Aizoon hispanicum* 25X Crystal sand. B- *Aizoon hispanicum* 40 X Druses. C- *Anastatica hierochuntica* 40 X Crystal sand. D- *Arnebia decumbens* 40 X Solitary. E- *Astragalus schimperi* 40 X Solitary. F- *Bassia eriophora* 40 X Solitary. G- *Bassia eriophora* 40 X Solitary. G- *Bassia eriophora* 40 X Solitary. H- *Erucaria hispanica* 40 X Solitary.



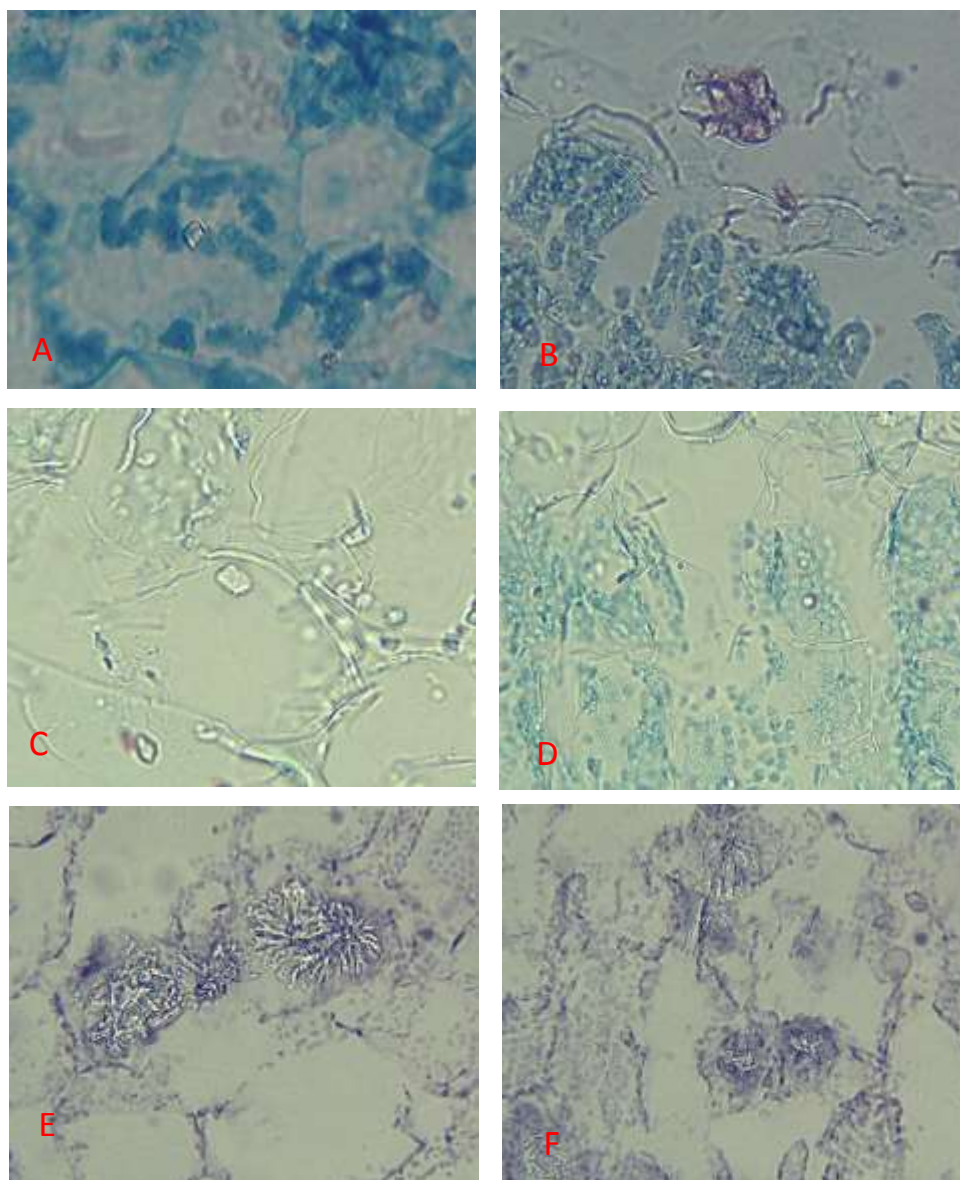


**Figure 3.** Plants contain different types of calcium oxalate crystals in stems. A- *Herniaria hirsuta* 40 X Druses. B- *Lepidium sativum* 40 X Raphids. C- *Picris babylonica* 40 X Crystal sand. D- *Reseda lutea* 40 X Crystal sand. E- *Sclerocephalus arabicus* 25 X Druses. F- *Sclerocephalus arabicus* 25 X Solitary. G- *Tripleurospermum auriculata* 25 X Solitary. H- *Tripleurospermum auriculata* 40 X Crystal sand.

**Table 3.** Herbal plants containing different types of CaOx crystals in transverse sections of leaves.

Species	Crystals		Economic importance
	Type	Distribution	
<i>Anagallis arvensis</i>	S	(vr)	Traditional and folk medicine (Al-Oudat, 1982), poison (Doaigey, 1991).
<i>Medicago laciniata</i>	D	(vr)	Traditional and folk medicine (Mahasneh, 2002), grazed (Villegas et al., 2006).
<i>Reichardia tingitana</i>	D	(vr)	Traditional and folk medicine (Sawsan, 2012), edible and grazed (Mohamed and Adam, 1980).
	Cr		
<i>Savignya parviflora</i>	D	(r)	Edible (Akbar and Al-Yahya, 2011), grazed (Middleditch, 2012).
	S	(vr)	

D = Druses, R = Raphides, Cr = Crystal sand, S = Solitary, vf = very frequent, f = frequent, r = rare, vr = very rare.

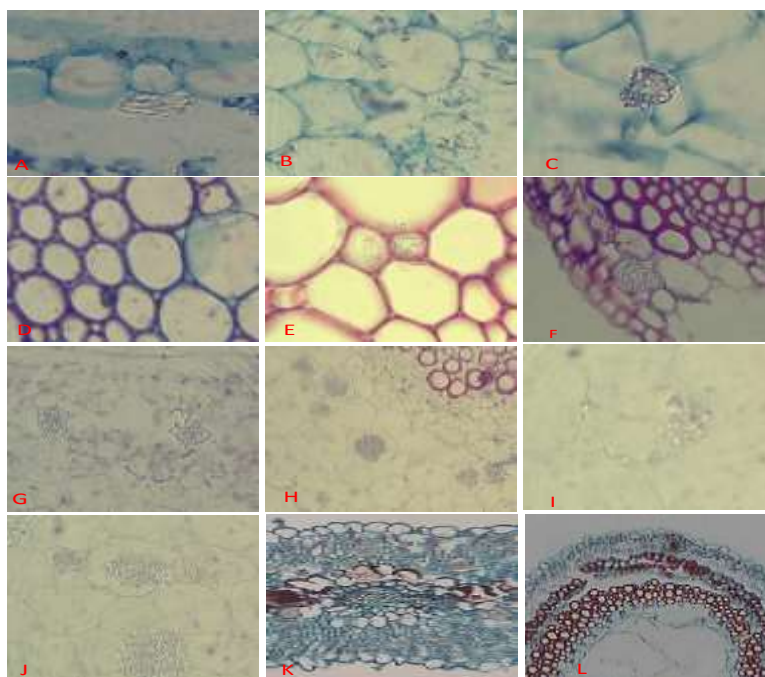


**Figure 4.** Herbal plants contain different types of calcium oxalate crystals in its leaves. A- *Anagallis arvensis* 40 X Solitary. B- *Medicago laciniata* 40 X Druses. C- *Reichardia tingitana* 40 X Druses. D- *Reichardia tingitana* 40 X Crystal sand. E- *Savignya parviflora* 25 X Druses. F- *Savignya parviflora* 25 X Solitary.

**Table 4.** Herbal plants containing different types of CaOx crystals in transverse sections of both leaves and stems.

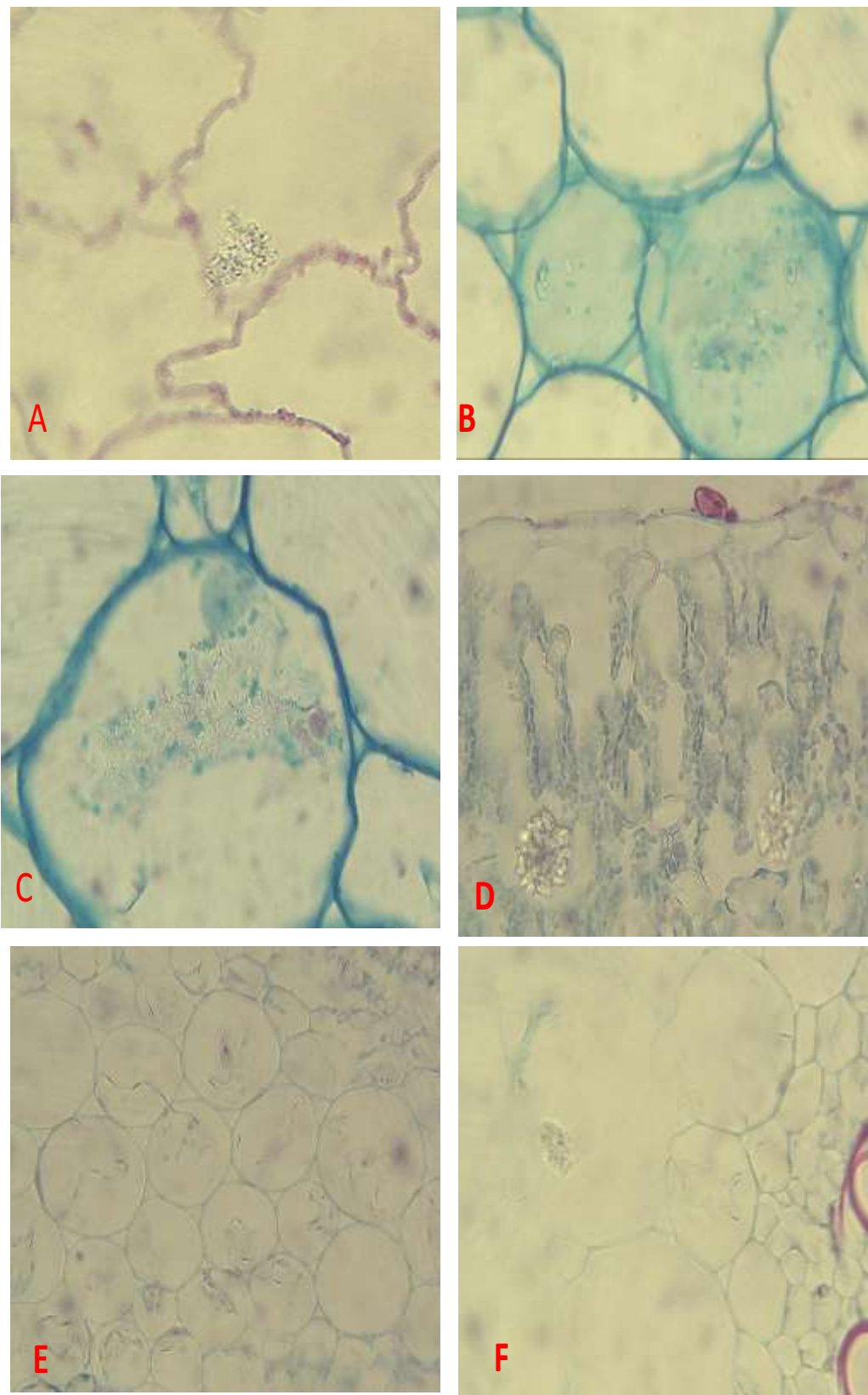
Species	Crystals				Economic importance
	Stems		leaves		
	Types	Distribution	Types	Distribution	
<i>Asphodelus tenuifolius</i>	S	(r)	R S Cr	(r) (vr)	Traditional and folk medicine (Qureshi et al., 2010; Al-Shanawani, 1996); additive and foraged by bees (Mandaville, 2013, 1990, 2011).
<i>Bassia muricata</i>	Cr	(vr)	S	(vr)	Traditional, folk medicine and grazed (Akbar and Al-Yahya, 2011).
<i>Chenopodium album</i>	S Cr	(vr)	D Cr	(f)	Traditional and folk medicine (Al-Shanawani, 1996; Abdel-Sattar et al., 2000); edible (Gesinski and Nowak, 2011; Yildirim et al., 2001) grazed.
<i>Echium horridum</i>	S Cr	(vr)	Cr	(vr)	Traditional, folk medicine and grazed (El-Shazly et al., 1999).
<i>Emex spinosa</i>	R Cr	(vr) (r)	D	(r)	Traditional and folk medicine (Al-Shanawani, 1996), edible and grazed (Al-Ataa, 2011).
<i>Paronychia arabica</i>	D	(r)	D	(r)	Traditional and folk medicine (Sakkir et al., 2012) grazed (Omar, 2007).
<i>Rumex vesicarius</i>	D S Cr	(r)	D S Cr	(vf) (vr) (r)	Traditional and folk medicine (Al-Zoght, 1990; Salamah et al., 1989), edible and additive (Al-Zoght, 1990).
<i>Solanum nigrum</i>	Cr	(vr)	Cr	(vr)	Traditional and folk medicine (Aboul-Enein et al., 2012), grazed and poison (Doaigey, 1991).

D = Druses, R = Raphides, Cr = Crystal sand, S = Solitary, vf = very frequent, f = frequent, r = rare, vr = very rare.

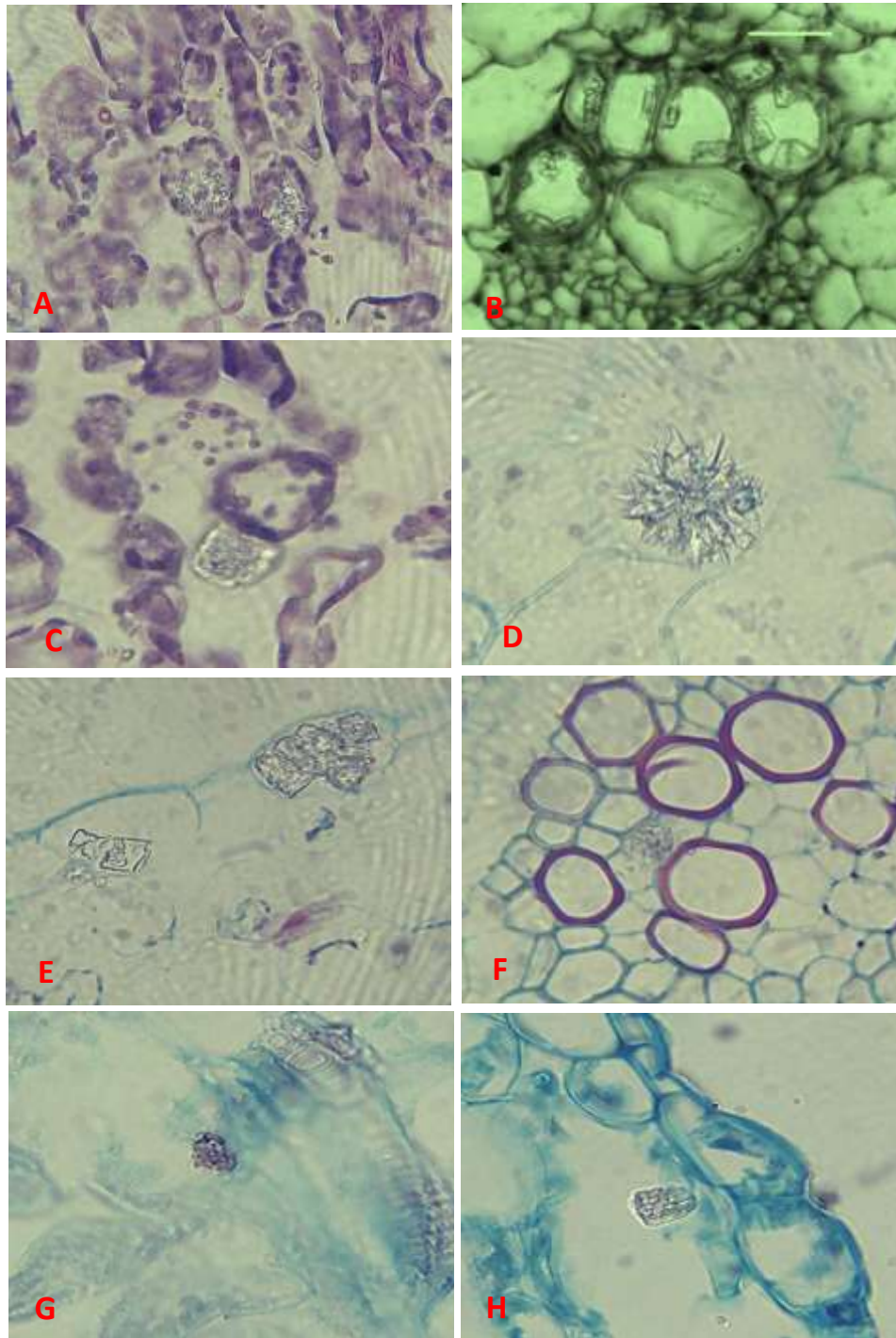


**Figure 5.** Herbal plants contain different types of calcium oxalate crystals in both its leaves and stems. A- *Asphodelus tenuifolius* leaves 40 X Raphids. B- *Asphodelus tenuifolius* leaves 40 X Solitary. C- *Asphodelus tenuifolius* leaves 40 X Crystal sand. D- *Asphodelus tenuifolius* stems 40 X Solitary. E- *Bassia muricata* leaves 40 X Solitary. F- *Bassia muricata* stem 40 X Crystal sand. G- *Chenopodium album* leaf 40 X Druses. H- *Chenopodium album* leaf 40 X Crystal sand. I- *Chenopodium album* stem 40 X Solitary. J- *Chenopodium album* stem 40 X Crystal sand. K- *Paronychia arabicum* Leaf 25 X Druses. L- *Paronychia arabicum* stem 5 X Druses.





**Figure 6.** Herbal plants contain different types of calcium oxalate crystals in both its leaves and stems. A- *Echium horridum* leaf 25 X Crystal sand. B- *Echium horridum* stem 25 X Solitary. C- *Echium horridum* stem 25 X Crystal sand. D- *Emex spinosa* Leaf 25 X Druses. E- *Emex spinosa* stem 25 X Raphids. F- *Emex spinosa* stem 25 X Crystal sand.



**Figure 7.** Herbal plants contain different types of calcium oxalate crystals in both its leaves and stems. A- *Rumex vesicarius* Leaf 40 X Druses. B- *Rumex vesicarius* Leaf 40 X Solitary. C- *Rumex vesicarius* Leaf 25 X Crystal sand. D- *Rumex vesicarius* Stem 25 X Druses. E- *Rumex vesicarius* Stem 25 X Solitary. F- *Rumex vesicarius* Stem 25 X Crystal sand. G- *Solanum nigrum* leaf 40 X Crystal sand. H- *Solanum nigrum* stem 40 X Crystal sand.

extreme caution in patients, and its stems can be safely used by humans and grazing animals. Further, 13% of plants contain CaOx crystal in their leaves and stems, hence its leaves and stems should be used with extreme

caution in patients. The types of crystals that were found include: druses, raphides, crystal sand and solitary crystals. It is recommended that more toxicological and biochemical studies be conducted to determine the

chemical components, active substances and their impact on humans.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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