# LEAF SURFACE MICROMORPHOLOGY AND PHYTOCHEMICAL ANALYSIS OF *TETRAENA QATARENSIS* (HADIDI) BEIER AND THULIN (FAMILY ZYGOPHYLLACEAE)

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**ABSTRACT**

This paper briefly describes the surface micromorphology of young stem and the succulent leaf of *Tetraena qatarensis* (Hadidi) Beier and Thulin. Qualitative phytochemical characterization of various part of the plant (root, mature stem, tender stem and leaf) has also been undertaken. The tender stem near the nodal region is densely trichomatous. The trichomes are less dense on the leaves. The anomocytic stomata are distributed all over the foliar surface but distantly. Stomatal density averaged to 38.33 ± 1.38 stomata per $mm^2$ varying from 19.66 to 58.97 stomata per $mm^2$. The phytochemical analysis indicated the presence of 16 secondary metabolites such as phenols, saponins, terpenoids, triterpenoids, steroids, phytosterols, tannins, Phlobatannins, flavonoids, alkaloids, anthraquinones, proteins, anthocyanin, glycosides, carbohydrates, and coumarins in substantial amounts which indicated great phytochemical richness of the plant and its potential medicinal significance.

**Key words:** *Tetraena qatarensis* (Hadidi) Beier & Thulin, surface micromorphology, plant phytochemistry

## INTRODUCTION

*Tetraena qatarensis* (Hadidi) Beier and Thulin *(Syn. Zygophyllum hamiense* var. qatarense (Hadidi) Jac. Thomas and Chaudhary*;* Z. qatarense Hadidi; vernacular name 'Haram' in Arabic) is an element of the flora of Eastern Saudi Arabia (Mandaville, 2013) and one of the constituent species of coastal low land vegetation of KSA (Shaltout *et al.* (1997). It is an element of the phytogenic hillocks (nabkhas) of Kuwait (El-Sheikh *et al.,* 2010). It also grows in sabkhas (coastal salt flats) in depression, on calcarious soils of non-salty loam, on slightly salty soil and clay margins of salt marshes (Sayed, 1996). It is, however, intolerant to frequent inundation of Seawater. It is distributed in Socotra Archipelago, the Galapágos of the Indian Ocean (Brown and Miles, 2012) and Bander Abbas, Iran (Barzegar *et al.,* 2018). It is a leaf-succulent $C_3$ shrubby halophytic species, widespread in Qatar - exhibiting enormous habitat diversity and plasticity (Abdul Fatih *et al.,* 2002; Yasseen and Al-Thani, 2013). Ghazanfar (1999) reported it from Oman. Zahran and Ansari (1999) reported it from Al-Samaliah island UAE. It is wide ecological range (Batanouny, 1981) and quite widespread in UAE. The first author collected it from Jumeirah, Dubai (UAE) in in a derelict road-side sandy land (coastal locality) in 2018.

This species may grow in sandy-rocky and silt-loamy terrain and dry areas which receive scanty winter rains and moist soils as well. It may tolerate salinity up to 11.8 $dS.m^{-1}$ (Abdul Fatih *et al.,* 2002). The leaf succulence of *T. qatarensis* (defined as leaf fresh weight to dry weight ratio) was estimated to be around 11.4 (Sayed, 1998). Abbas (2005) has described seasonal variation in ash contents of this halophyte from Bahrain and Abbas (2006) described protein contents in roots and leaves of this species in saline and non-saline conditions of Bahrain. Batanouny *et al.* (1985) have described its ecological physiology.

This paper briefly describes the surface micromorphology of young stem and the succulent leaf of this species. Qualitative phytochemical characterization of various part of the plant has also been undertaken in view of the medicinal importance of this plant (Tanira *et al.,* 1996; Mahasneh, 2002; Barzegar *et al.,* 2017).

## CLIMATIC FEATURES OF DUBAI

UAE is located in Middle East, situated on Arabia Peninsula between Oman and Saudi Arabia bordering the Gulf of Oman and the Persian Gulf. It covers an area of 83,600 Sq. km. Its largest city is Dubai. Dubai landscape is sandy – extreme hot. Days are sunny all the year around. Humidity is discomfortingly high in coastal region. According to Köppen classification, its climate is of Bwh type (Tropical desert climate) and bio-climate as given by Holdridge falls into the category of Tropical Desert Bush formation. Climate of Dubai is described in Table 1. The record high temperature is 52 $^oC$ and record low $9^oC$. Average high temperature is 33.4 and average low $23.5^oC$. Rainfall is low – around 103.7 mm which is largely in winter months (December, January – March). Annual seawater temperature averages to 28.3 $^oC$ –varying 13.69%. UV index is high (11+) in summer. Evapotranspiration for Wadi Ham catchment is presented by Al Mulla (2005) to be ≥ 6 mm per day for March through September and maximum in March and September – around 8.5 mm per day.

## MATERIALS AND METHODS

Plant material of *T. qatarensis* was collected from Jumeirah, Dubai (UAE). A small population of this plant was growing in a derelict road-side sandy plain of Umm Sequim 2 (Dubai) at some distance from the coast.

Leaf epidermal impressions of leaf were made by applying clear nail polish to the surface of leaf (Wang *et al.*, 2006). The imprints were studied under microscope. Stomatal nomenclature suggested by Prabhakar (2004) being simple and based upon structure of stomata and not their ontogenetic pathways was adopted to ascertain stomatal types. Prabhakar (2004) recognized eleven types of stomata. This nomenclature does not recognize actinocytic and stephanocytic stomata and categorize them as anomocytic type. As a basic criterion, all the cells abutting the guard cells are considered distinct by Prabhakar (2004) from the other epidermal cells by virtue of their position (i.e. abutting nature to the guard cells) hence he prefers to call them subsidiaries.

For scanning electron microscopy (SEM), air-dried plant material (young stem (near nodal region) and dry leaf) was mounted on brass stubs and coated with a 250 oA gold layer with JFC-1500 gold coater. SE micrographs were made at 15kV with JEOL JSM- 6380A electron microscope at various magnifications. Element detection system (EDS) attached to the SE microscope was employed to detect elements in the dry leaf.

Table 1. Climatic characteristics of Dubai. *

| Months | Temperature ($^oC$) | | | | Rain (mm) | Rainy Days | Seawater Temp. ($^oC$) | UV index |
|---|---|---|---|---|---|---|---|---|
| | Record High | Average High | Average Low | Record Low | | | | |
| J | 31.8 | 26.1 | 17.8 | 9.0 | 18.8 | 4.4 | 23.4 | 6 |
| F | 37.5 | 27.4 | 18.6 | 10.0 | 25.0 | 3.0 | 21.9 | 8 |
| M | 41.3 | 31.6 | 20.9 | 11.0 | 32.1 | 5.8 | 28.2 | 10 |
| A | 43.5 | 34.2 | 23.2 | 15.4 | 7.2 | 2.6 | 25.5 | 11 + |
| M | 47.0 | 38.6 | 26.1 | 18.1 | 0.4 | 0 | 29.8 | 11 + |
| J | 50.9 | 40.7 | 28.5 | 21.2 | 0.1 | 0 | 31.6 | 11 + |
| J | 52.0 | 41.2 | 31.2 | 23.4 | 0.1 | 0 | 32.7 | 11 + |
| A | 51.5 | 41.8 | 31.5 | 25.1 | 0 | 0 | 33.5 | 11 + |
| S | 48.12 | 39.8 | 28.7 | 23.5 | 0 | 0.2 | 33.1 | 11 |
| O | 42.4 | 35.6 | 24.3 | 17.0 | 1.1 | 0.5 | 31.3 | 8 |
| N | 38.0 | 32.2 | 21.0 | 14.8 | 2.7 | 1.0 | 28.6 | 6 |
| D | 33.2 | 28.3 | 20.3 | 12.2 | 16.2 | 2.8 | 25.4 | 5 |
| Year | 52.0 | 33.4 | 23.5 | 9.0 | 103.7 | 20.3 | 28.3 | 9.1 |

*, Data source: (*http://en.wikipedia.org/wiki/Climate_of_Dubai#Climate_data)* –date of retrieval – June 29, 2018. (Data being mainly the courtesy of Dubai Meteorological office, Seatemperature.org, Climatebase.ru, Weather Atlas; Gulf News, etc.

**Phytochemical Methods**

**Collection of different parts of Plant Samples**

Fresh leaves, stems, tender stem and roots of *T. qatarensis* were separately air dried in shade for a month. The dried samples were ground into powder in a Grinder machine.

**Preparation of Ethanolic Extracts**

Ten g powder of a plant part (Leaf, woody, tender stems or roots) was immersed in 100 mL of ethanol for 15 days, after which it was filtered out three to four times by alcohol washings and concentrated by rotary evaporation. The dried crude residue of each component part was kept in sterile vial and stored at $4^oC$ for qualitative phytochemical screening.

**PHYTOCHEMICAL ANALYSIS**

The residue left after evaporation was used for phytochemical analysis by using the following standard methods described in Harborne (1998), Trease and Evans (1989) and Sofowora (1993).

**Terpenoids (Salkowski test):** Residue extract was mixed with 2 mL of $CHCl_3$ and $H_2SO_4$ (concentrated) was added. A reddish-brown coloration at the interface indicated the presence of Terpenoids (Indumathi *et al.*, 2014)

**Steroids (Salkowski test):** Residue extract was dissolved in 2 mL $H_2O$. It was added with 2 mL $CHCl_3$ and 2 mL $H_2SO_4$ (concentrated). The presence of reddish-brown ring at the junction of two liquids indicated the presence of steroids.

**Saponins (foam test):** Residue extract was dissolved in 5 mL $H_2O$ and heated. Froth appearance in the test indicates presence of saponins. The permanence of froth forming of aq. Extract on shaking also indicated the presence of saponins.

**Proteins (Biuret test):** Crude extract was mixed with 40% NaOH solution and two drops of 1% copper sulphate solution was added. The appearance of violet colour indicated the presence of proteins.
**Coumarins:** Crude extract was mixed with 3 mL NaOH (10%). The presence of yellow coloration indicated the presence of Coumarins.
**Phenol:** Crude extract was mixed with few drops of 10% solution of Lead acetate. The appearance of white precipitates in the solution indicated the presence of phenol.
**Phlobatannins:** Crude extract was mixed with 2 mL HCl (1%) and heated. The appearance of red precipitates indicated the presence of Phlobatannins.
**Carbohydrates:** Crude extract was mixed with few drops of α- naphthol solution in alcohol and $H_2SO_4$ (concentrated) was added along with the test tube. Violet ring formed at the junction of two liquids in test tube indicated the presence of carbohydrates.
**Flavonoids**: Crude extract was mixed with 5 ml of dilute ammonia followed by addition of $H_2SO_4$ (concentrated). The solution in the test tube will turn yellow in color indicated the presence of flavonoids.
**Tannin:** Residue extract was mixed with 2 mL of $FeCl_3$. The presence of dark green coloration indicated the presence of tannin.
**Anthraquinones**
Crude extract mixed with 5 mL conc. $H_2SO_4$, 1 mL benzene was added to it and further 1 mL dilute ammonia was added. Pink coloration showed positive results of Anthraquinones.
**Cardiac glycosides (Keller-Killiani test):**
Crude extract was added to 2.5 mL distilled water. 1 mL glacial acetic acid containing a few drops of ferric chloride was added then 0.5 mL of concentrated sulfuric acid was added. The Presence of brown ring at the junction indicates the presence of deoxyribose sugar and a green-blue ring below the brown ring confirms the presence of Cardiac Glycosides (See Benmehdi *et al.*, 2012).
**Alkaloids (Mayer's test):**
One mL of Mayer's reagent was added to crude extract. The formation of creamy precipitate indicated the presence of alkaloids.
**Triterpenoids (Liebermann-Burchard's test):** Crude extract was mixed with the 1mL of chloroform. Few drops of acetic anhydride and concentrated $H_2SO_4$ were added along the side of the test tube. The appearance of Radish brown color indicated the presence of triterpenoids.
**Phytosterol (Liebermann-Burchard's test):**
Crude extract was dissolved in 2 mL of acetic anhydride, heated to boiling, cooled and then 1 mL of concentrated sulfuric acid was added along the side of the test tube. The brown ring formation at the junction and the turning of the upper layer to dark green color confirmed the test for the presence of phytosterols.
**Glycosides:** Crude extract was taken in 1 mL of water in a test tube and a few drops of aqueous NaOH were added. A yellow coloration indicated the presence glycosides.
**Emodin:** Crude extract was mixed in 2 mL $NH_4OH$ and add 3ml Benzene. The formation of red coloration confirmed the presence of Emodin.
**Anthocyanins:** Crude extract was mixed in 2mL of HCl (2N) and then few drops of Ammonia were added. Pinkish red or Bluish violet color formation confirmed the presence of Anthocyanins.

## RESULTS AND DISCUSSION

### Brief phytography

*Tetraena qatarensis* is a dwarf shrub with profusely branched stem which is woody below (Fig. 1) but tender green above. It has tiny, fleshy spherical / cylindrical leaves with paired leaflets which are deciduous, dropping off in stressful conditions. The flowers usually have five white or yellow-cream petals (sometimes four), ten projecting stamens and a minute style. The fruit is capsule, splitting into five parts when ripe.

### Surface micromorphology of young stem and leaf

The surface micromorphology of the leaf and tender stem is presented in Fig. 2 to 4. The tender stem near the nodal region is densely trichomatous (Fig. 2A). The trichomes are less dense on the leaves (Fig. 2B). The stomata are distributed all over the foliar surface but distantly. The stomata were anomocytic (Fig. 3B) and oval in shape. Cuticular encrustation is in form of sheet and granular epicuticular crystalloids are distributed all over the leaf which are probably waxy. *Zygophyllum* has been reported to have waxy cuticle.

Stomatal density is quite low averaging to 38.33 ± 1.38 stomata per $mm^2$ varying from 19.66 to 58.97 stomata per $mm^2$. Sayed (1998) has reported stomatal density in *T. qatarensis* to be 38 stomata per $cm^2$ of leaf which appears to be quite low in comparison to our estimate of 38.33 ± 1.38 stomata per $mm^2$ (Table 2). The stomata averaged to 28.32 ± 0.473 µm in length (N = 50, 18.04 – 34.66 µm, CV: 11.8%) and 20.90 ± 1.449 µm in width (N = 50, 16.40 –

29.52 µm, CV: 15.2%) as estimated in optical microscopically of the nail polish imprint of the leaf. The two stomata in SEM study appeared to have stomatal length of 17.6 and 15.5 µm, respectively and width of 12.6 and 7.11 µm, respectively (Fig. 4). The length of stomatal ledge aperture was 7.65 µm and width of the stomatal ledge aperture 3.91 µm. The stomata were situated in depression and protected with trichomes in the surrounding. Cuticular layer was thick. Real stomatal pore is quite sunken in the dome shaped cavity of the outer ledge. Strong cutinization of leaf surface covered with epidermal hairs and sunken stomata are the main adaptations of arid land plants to reduce water loss. These characteristics are seen in *Z. qatarense.*

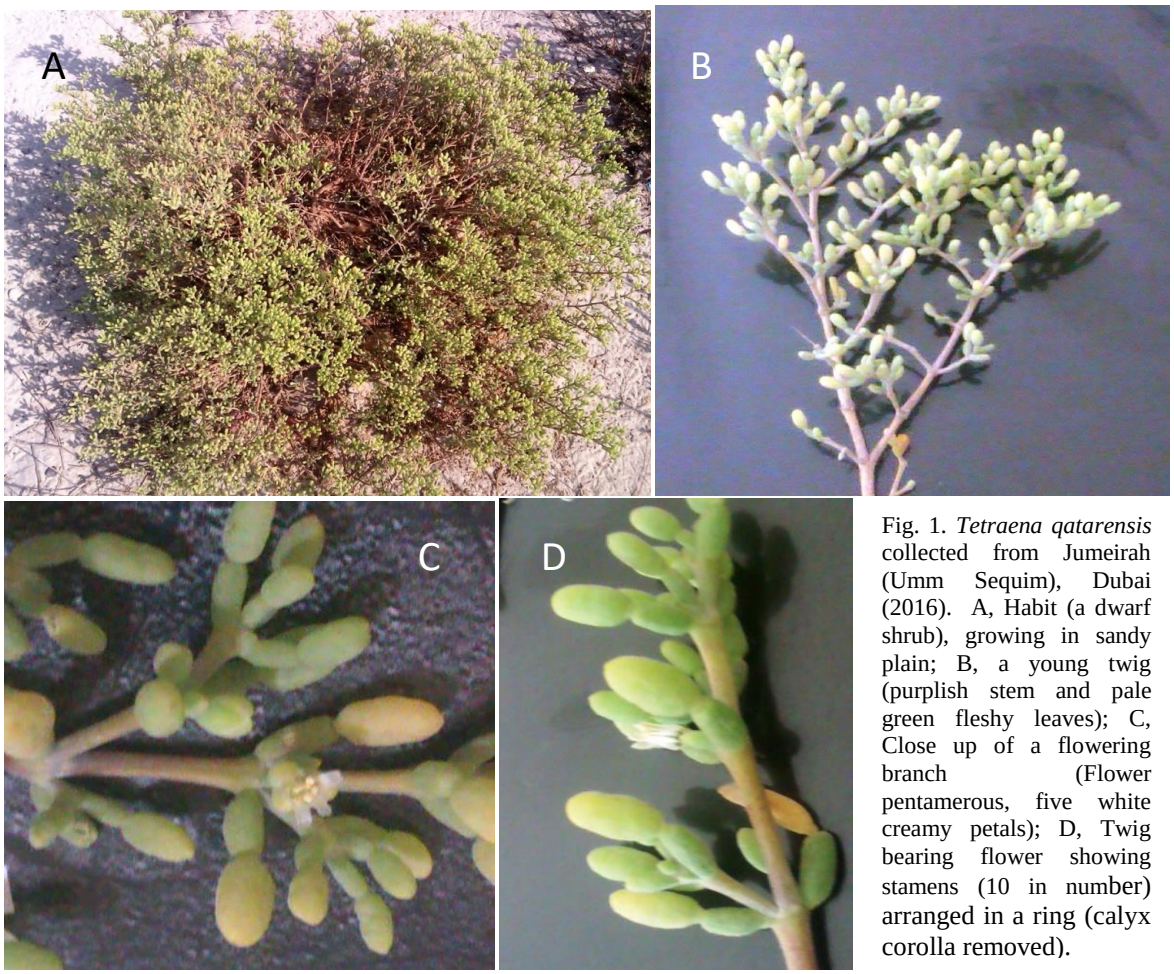



Fig. 1. *Tetraena qatarensis* collected from Jumeirah (Umm Sequim), Dubai (2016). A, Habit (a dwarf shrub), growing in sandy plain; B, a young twig (purplish stem and pale green fleshy leaves); C, Close up of a flowering branch (Flower pentamerous, five white creamy petals); D, Twig bearing flower showing stamens (10 in number) arranged in a ring (calyx corolla removed).

**Phytochemicals**

The results of phytochemical analysis of various components of *T. qatarensis* are presented in Table 3. Phytochemical analysis indicated the presence of 16 secondary metabolites such as phenols, saponins, terpenoids, triterpenoids, steroids, phytosterols, tannins, Phlobatannins, flavonoids, alkaloids, anthraquinones, proteins, anthocyanin, glycosides, carbohydrates, and coumarins in substantial amounts. Various phytochemicals were relatively more diverse and in higher concentrations in leaves followed by stems and roots. Emodin and cardiac glycosides were not detected in any of the parts of in-hand plant. Amal M.Y. Moustafa *et al.* (2007) have reported cardiac glycosides from *Zygophyllum album*. Phytosterols and saponins were only present in leaves and didn't occur in stems and roots and phenols were relatively in low amounts in leaves as compared to in stem and roots. Tender stem showed phytochemicals concentration comparatively higher than woody part of the stem.

Phytochemical composition of some common coastal halophytes of UAE has been reviewed by Cybulska *et al.* (2014). Barzegar *et al.* (2017) have reported some important phenolic acids from *Z. qatarense* such as Chlorogenic acid, Sinapic acid, Caffeic acid, Catachin, Trans-ferulic acid, Rosmarinic acid. According to them, methanolic extract of leaf of this plant have good potential against *Klebsiella pneumonia* and *Staphylococcus epidermidis*. Silver nanoparticles prepared in the presence of methanolic extract of leaf of *Z. qatarense* were found to be more effective against fungi, *Penicillium digitatum* and *Aspergillus niger* (Barzegar *et al.,* 2018). It was reported to contain alkaloids, sterols and coumarins (Taha and Al Sayed, 2000).

Table 2. Foliar stomatal density.mm$^{-2}$ and stomatal length and width (µm) in *T. qatarensis* as measured in light microscopy of nail polish imprint.

| Statistical Parameters | Stomatal Density Per mm$^2$ | Stomatal length (µm) | Stomatal Width (µm) |
|---|---|---|---|
| N | 60 | 50 | 50 |
| Mean | 38.33 | 28.32 | 20.90 |
| SE | 1.376 | 0.47278 | 0.44886 |
| CV (%) | 27.81 | 11.803 | 15.19 |
| Minimum | 19.66 | 18.04 | 16.40 |
| Maximum | 58.97 | 34.44 | 29.52 |
| Skewness (Sg1) | 0.287 (0.307) | 0.745(0.3370 | 0.711 (0.337) |
| Kurtosis (Sg2) | - 0.383 (0.906) | 1.144 (0662) | 0.126 (0.662) |
| K-S* test (p) | 0.213($p < 0.001$) | 0.180 (0.0001) | 0.240 (0.0001) |
| Shapiro-Wilk test (p) | 0.906 (0.0001) | 0.924 (0.003)) | 0.910 (0.0001) |

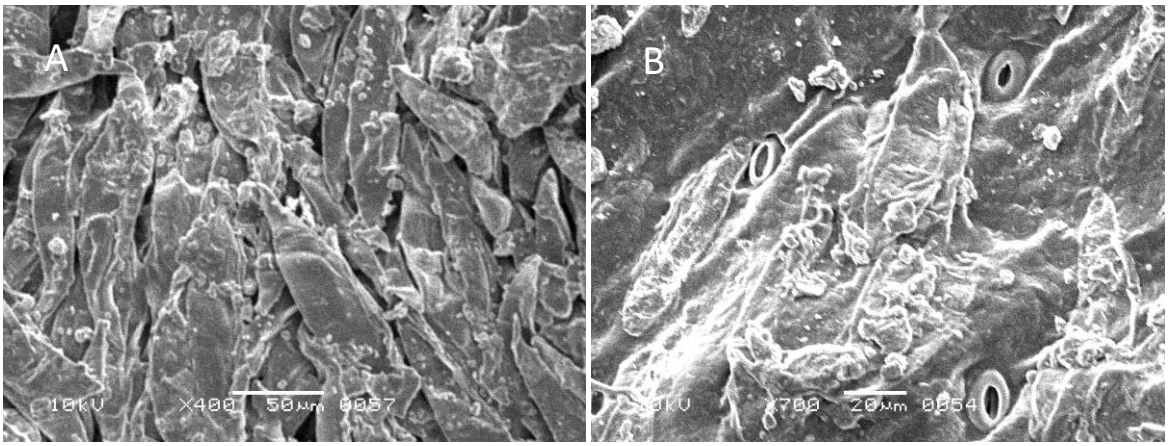



Fig. 2. A; SEM view of the tender stem just near the node showing a number of trichomes. The granules scattered all over are presumably the epicuticular granular type of wax crystalloids. B, SEM view of the leaf surface showing stomata and trichomes.

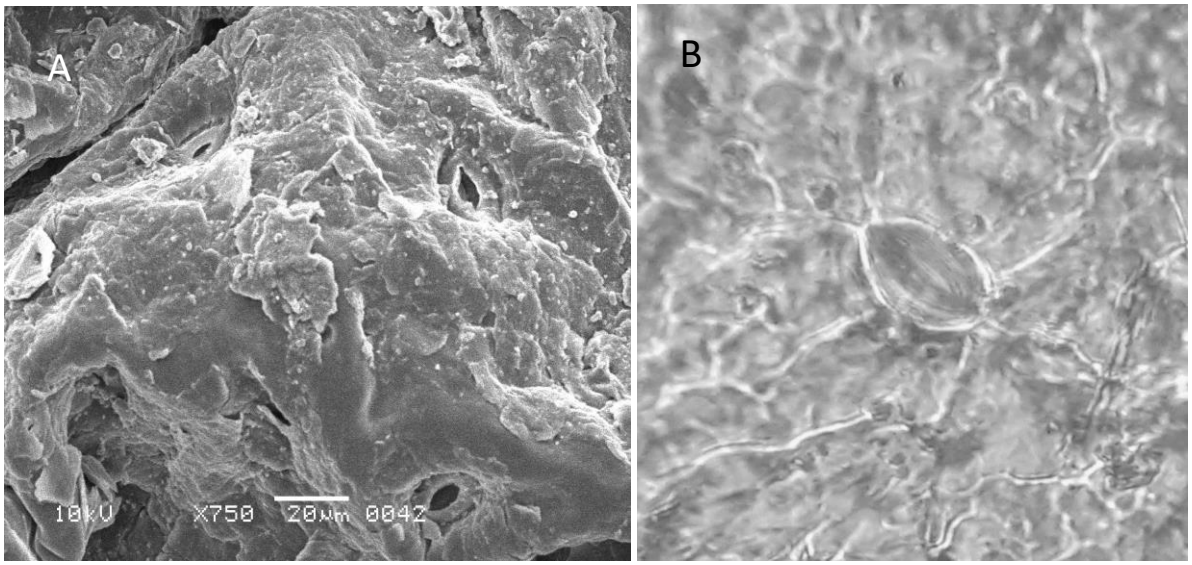



Fig. 3. A, SEM view of the leaf surface showing stomata scattered, cuticular encrustation in form of sheet and granular epicuticular crystalloids, presumably waxy. The undulations over the surface may have appeared during drying of the fleshy succulent leaves. Stomata are at level below the ground epidermal layer; B, Nail polish imprint leaf surface of *T. qatarensis* showing anomocytic stoma (magnification: 45 x 15X).

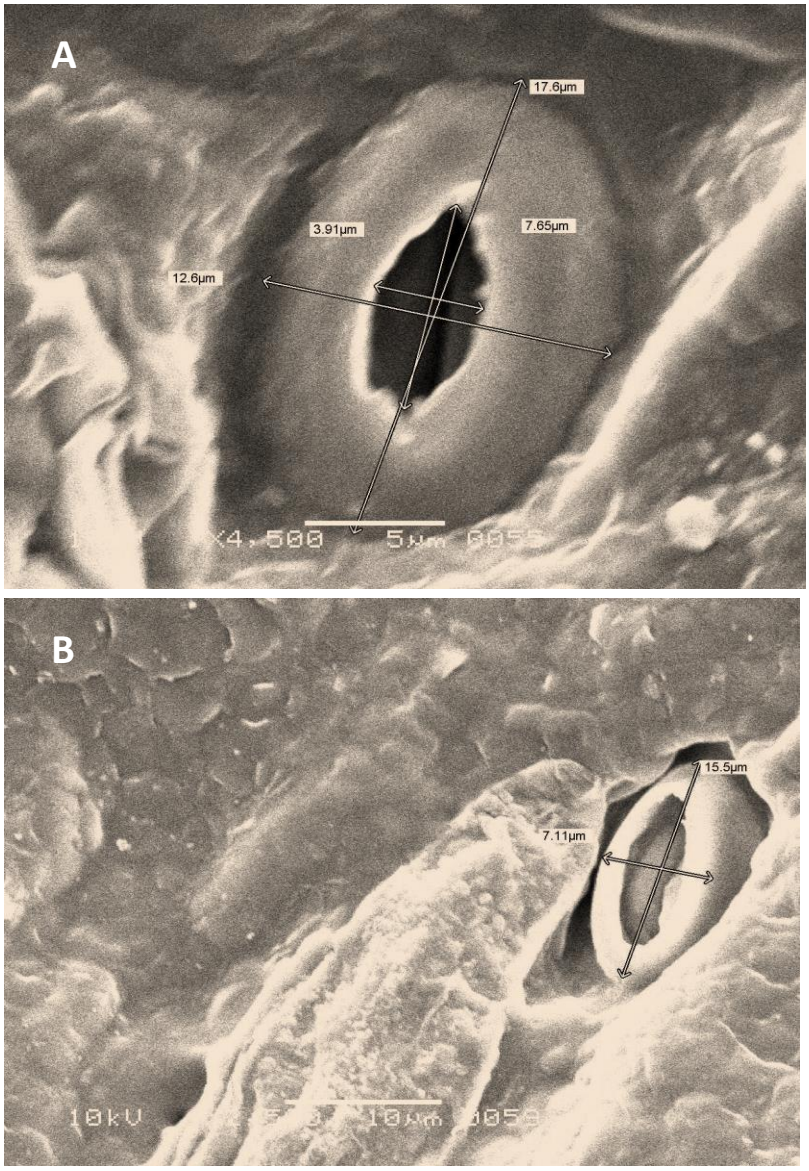



Fig. 4. Two SEM views of leaf surface of *T. qatarensis* – showing stomatal size (A and B). The two stomata showed the stomatal length of 17.6 and 15.5 µm, respectively and width of 12.6 and 7.11 µm in A and B, respectively. In upper stoma, the length of stomatal ledge aperture is 7.65 µm and width of the stomatal ledge aperture is 3.91 µm. The stomata are situated in depression and protected with trichomes. Cuticular layer is thick. Real stomatal pore is quite sunken in the dome shaped cavity of the outer ledge.

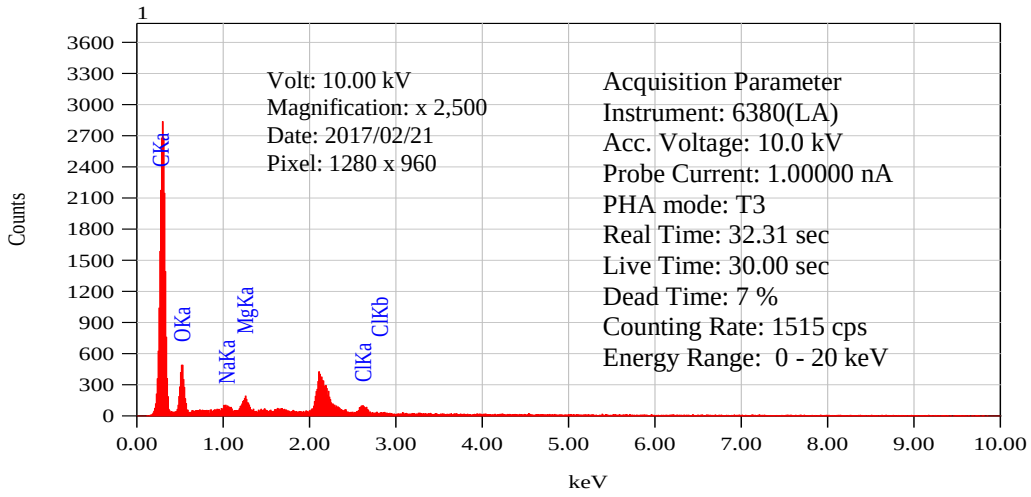
A comprehensive review on genus *Zygophyllum* (rich in bioactive principles has been published by *Shawky et al.* (2019) as traditional medicinal genus. Various species of *Zygophyllum*, being rich in bioactive compounds, have been reported to be used as antirheumatic, antigout, antidiabetic, antilipidemic, antimicrobial, antioxidant, anti-hypertensive, antiseptic, anti-eczema and anti-diarrheal by various researchers – reviewed in Shawky *et al.* (2019). There is a need to screen the biological activities of *T. qatarensis* (Syn: Z. qatarense) in detail in view of its richness in bioactive compounds known to be of medicinal value. This species is already reported to be antifungal (Barzegar *et al.*, 2018).

**Determination of Elements**

Elements detector system (EDS) based on Energy dispersive X-ray spectroscopy (EDS) attached to SEM was employed for element detection and quantitative elemental analysis. In spite of the fact this is a standard less quantitative analysis; the results of scan for a foliar region are presented in Fig. 5. In scan, the dominating element was carbon (45.33 ± 1.05%), followed by oxygen (40.54 ± 3.10%), Sodium (2.22 ± 2.77%), Magnesium (5.11 ± 2.62) and Chlorine (6.71 ± 4.59 %). No Calcium and Potassium could be detected in the analysis possibly due to their low concentration. The high percentages of Carbon and Oxygen indicated higher concentration of organic molecules. In EDS analysis, since single shelled elements are not detected, we have no estimate of Hydrogen.

Table 3. Phytochemicals observed in various components of *T. qatarense*. Acronyms: +, low concentration; ++, moderate concentration and +++, high concentration.

| S. No. | Phytochemicals | Leaves | Woody stem | Tender stem | Roots |
|---|---|---|---|---|---|
| 1 | Terpenoids | +++ | +++ | + | ++ |
| 2 | Steroids | +++ | +++ | +++ | ++ |
| 3 | Tannins | +++ | ++ | +++ | ++ |
| 4 | Coumarins | +++ | +++ | +++ | ++ |
| 5 | Saponins | +++ | - | - | - |
| 6 | Phenol | + | ++ | + | ++ |
| 7 | Carbohydrate | +++ | +++ | ++ | + |
| 8 | Proteins | +++ | + | ++ | + |
| 9 | Phlobatannins | +++ | ++ | +++ | - |
| 10 | Flavonoids | ++ | ++ | +++ | - |
| 11 | Alkaloid | +++ | + | ++ | ++ |
| 12 | Anthraquinones | ++ | ++ | ++ | + |
| 13 | Triterpenoid | +++ | + | ++ | - |
| 14 | Emodin | - | - | - | - |
| 15 | Cardiac glycosides | - | - | - | - |
| 16 | Anthocyanin | +++ | - | +++ | + |
| 17 | Glycosides | +++ | + | +++ | - |
| 18 | Phytosterols | + | - | - | - |




ZAF Method Standard less Quantitative Analysis Fitting Coefficient: 0.8401

| Element | KeV | Mass % | Error % | At % |
|---|---|---|---|---|
| Carbon | 0.277 | 45.33 | 1.05 | 55.42 |
| Oxygen | 0.527 | 40.54 | 3.10 | 37.30 |
| Sodium | 1.041 | 2.22 | 2.77 | 1.42 |
| Magnesium | 1.253 | 5.11 | 2.62 | 3.09 |
| Chlorine | 2.621 | 6.71 | 4.59 | 2.75 |
| Total | | 100 | | 100 |

JED-2300 *AnalysisStation* JEOL

Fig. 5. Quantitative element detection by EDS (SEM) in dry leaf of *T qatarensis*.

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