



Morpho-anatomical Characters of Leaves and Stems as a Tool for the Identification of Some Taxa of Zygophyllaceae of Eastern Saudi Arabia

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IN THIS STUDY the morphological and anatomical variation in stem and leaf tissues of three genera of Zygophyllaceae: *Fagonia* (4 taxa), *Tetraena* (4 taxa), and *Tribulus* (4 taxa) were investigated. The twelve examined taxa included nine species that grow in the eastern region of Saudi Arabia. Additional features for taxon identification and characterization can be developed because of our findings. The anatomical features investigated included, stem diagram, epidermal cell shape, structure of cortex and pith, the number of vascular bundles, petiole and blade outline in cross section, mesophyll type, and distribution of secretory tissues and crystals. The conclusions of this study show that anatomical features, particularly the petiole and blade outlines and the type of vasculature of the stem and petiole, are significant tools in identifying and distinguishing between the species examined and can provide a key for identification of the investigated taxa. Distinction of the investigated Zygophyllaceae species, based on the characters assessed in this study, agreed with previous classifications of these species.

Keywords: Anatomy, Eastern Saudi Arabia, Morphology, Zygophyllaceae.

Introduction

Zygophyllaceae (caltrop family) is a large, widespread family of plants adapted to semi-desert and Mediterranean climates (Hammoda et al., 2013). This family is found in parts of Europe, Asia, Australia, Africa, and Americas (Sheahan, 2007), and consists of approximately 285 species belonging to 22 genera (Mabberley, 2008). The family has been recently divided into five subfamilies (Zygophylloideae, Seetzenioideae, Tribuloideae, Morkillioideae, and Larreoideae) according to Sheahan & Chase (2000), Beier et al. (2003) and Bellstedt et al. (2008).

The list of taxa belonging to Zygophyllaceae has been changed through time, since there is substantial structural diversity in the relevant species, chiefly those of the genera *Balanites*, *Nitraria*, *Peganum*, and *Tetradiclis*. Originally, Engler (1931) included the latter four genera

within the Zygophyllaceae under different subfamilies; but Takhtajan (1969) separated species of *Nitraria*, *Balanites* and *Peganum* from Zygophyllaceae and allocated them to distinct families (i.e., Nitrariaceae, Balanitaceae, and Peganaceae, respectively). Based on the anatomical investigations of stems and leaves of 37 species belonging to 19 genera of Zygophyllaceae, Sheahan & Cutler (1993) found that there was sufficient micro-morphological evidence to separate *Balanites* into a distinct family, and to separate the genera *Tribulus*, *Kallstroemia*, and *Kelleronia* from the Zygophylloideae subfamily. Later, El-Hadidi & Fayed (1995) suggested adding the genera *Peganum*, *Tetradiclis*, *Seetzenia*; *Zygophyllum*, and *Fagonia* to Zygophyllaceae and considered *Tribulus*, *Nitraria*, and *Balanites* as distinct families (i.e., Tribulaceae, Nitrariaceae and Balanitaceae, respectively).

Based on thorough investigation including

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morphological, anatomical, and DNA data, Sheahan & Chase (1996) considered the genera *Nitraria* and *Peganum* to be distinct from Zygophyllaceae but more relevant to Sapindales families. Boulos (2009) agreed with Sheahan & Chase (1996) and showed that Zygophyllaceae includes the genera *Fagonia*, *Zygophyllum*, *Tribulus*, *Balanites* and *Seetzenia*; meanwhile, he separated *Nitraria* and *Peganum* under distinct families, that is Nitrariaceae and Peganaceae, respectively. Then, Sheahan (2011) placed the genera *Zygophyllum*, *Fagonia*, *Tribulus*, *Balanites*, *Seetzenia* and *Guaiacum* within the family Zygophyllaceae.

In 1978, Migahid detected five genera and 17 species from the Zygophyllaceae in Saudi Arabia. Recently, Chaudhary (2001) recorded seven genera (*Zygophyllum*, *Fagonia*, *Tribulus*, *Balanites*, *Seetzenia*, *Peganum*, and *Nitraria*) that included 24 species, two sub-species and 12 varieties. More recently, Thomas (2011) identified six genera of Zygophyllaceae (*Balanites*, *Fagonia*, *Seetzenia*, *Tetraena*, *Tribulus*, and *Zygophyllum*), which included 22 species and nine varieties. Concerning the eastern sector of Saudi Arabia, Mandaville (1990) recorded only 15 species, belonging to six genera (*Zygophyllum*, *Fagonia*, *Tribulus*, *Seetzenia*, *Peganum*, and *Nitraria*). Al-Yasi (2020) recorded *Fagonia* and *Tetraena* in the Unique Microhabitat of Al-Wahbah Crater at Taif Region.

The Zygophyllaceae species present in Saudi Arabia belong to the Zygophylloideae subfamily. Zygophylloideae are the largest subfamily of Zygophyllaceae, consisting of about 180 species of shrubs, subshrubs, and herbs which are currently grouped into four genera, monotypic *Augea*, *Fagonia*, *Tetraena*, and *Zygophyllum* as per Sheahan & Chase (1996, 2000). Recently, many phylogenetic studies have been performed on the Zygophylloideae. For example, Beier et al. (2003) investigated the phylogeny of the Zygophylloideae by combining morphological characters and noncoding *trnL* plastid data. Using this dataset, they proposed moving 35 species from *Zygophyllum* to *Tetraena*. This classification scheme was validated by subsequent studies, including Norton et al. (2009), Louhaichi et al. (2011), Mosti et al. (2012), Sakkir et al. (2012), Symanczik et al. (2014), and Ghazanfar & Osborne (2015).

The main characters used to distinguish the different taxa of Zygophyllaceae are foliate number, foliate shape, persistence of calyx with the fruit, the number of stamens, and fruit characters as shape and texture. Ahmed (1991) studied the petiole vasculature of *Fagonia* species of the Egyptian flora, while Sheahan & Cutler (1993) described the micro-morphology of 37 Zygophyllaceae species from 19 genera, and Ahmed & Khafagi (1997) investigated both macro- and micro-morphological leaf characters of *Fagonia* species found in Egypt. Another study by Khafagi (2004) examined the macro- and micro-morphological characters of spiny stipules in *Fagonia* species. Finally, Waly et al. (2011) studied the petiole and blade anatomy of eleven *Zygophyllum* species from Saudi Arabia, and Elkamali et al. (2016) studied the stem and leaflet anatomy of *Tribulus longipetalous*, *T. pentandrus*, and *T. terrestris* found in Khartoum State (Central Sudan). Given the difficulty encountered in the classification of Zygophyllaceae species, the main objective of this study is to identify additional characters that would be useful for the identification and differentiation between the Zygophyllaceae species by studying the morphological and anatomical characters of the vegetative organs of Zygophyllaceae taxa from eastern Saudi Arabia.

Materials and Methods

Twelve taxa belonging to five species and seven varieties representing three genera of the family Zygophyllaceae were collected fresh from different locations within the eastern sector of Saudi Arabia (Table 1). The plants collected in this study were identified using the keys of Mandaville (1990), Chaudhary (2001), Thomas (2011), and Alzahran & Albokhari (2017). Leaf surface was examined using a binocular stereo microscope. For micro-morphological investigation, specimens were fixed in the FAA mixture that consists of formalin: glacial acetic acid: alcohol 70% (5:5:90, v:v:v) (Nassar & El-Sahhar, 1998). Sections of stem and leaf specimens (i.e., petioles and blades) of 20–30 µm in thickness were stained with safranin (1% in 50% ethanol) and light green (1% in 96% ethanol) for microscopic observation (Dilcher, 1974). Stomata were examined by stripping and fixing the lower leaf epidermis in 70% ethanol, followed by clearing in 1% warm lactic acid (Nassar & El-Sahhar, 1998). The terminology concerning mesophyll type was according to Fahn (1974) and Metcalfe & Chalk (1979).

TABLE 1. Sampling data for species included in the present study. Nomenclature of species followed Chaudhary (2001), Beier et al. (2003) and Alzahrani & Albokhari (2017)

Species	Collection site and date
<i>Fagonia olivieri</i> DC.	Rayan, Dammam, 3/2017
<i>Fagonia indica</i> Burm.f.	Rayan, Dammam, 3/2017
<i>Fagonia ovalifolia</i> Hadidi. f.	Rayan, Dammam, 3/2017
<i>Fagonia bruguieri</i> DC.	Rayan, Dammam, 3/2017
<i>Tetraena migahidii</i> (Hadidi) Beier & Thulin.	Rawda, Dammam, 5/2017
<i>Tetraena hamiensis</i> (Scweinf) Beier & Thulin var. <i>hamiensis</i> . = <i>Zygophyllum hamiense</i> Schweinf.	Rayan, Dammam, 3/2018
<i>Tetraena hamiensis</i> (Scweinf) Beier & Thulin var. <i>qatarensis</i> (Hadidi) Alzahrani & Albokhari. = <i>Zygophyllum hamiense</i> var. <i>qatarense</i> (Hadidi) Thomas & Chaudhary	Rayan, Dammam, 3/2018
<i>Tetraena hamiensis</i> (Scweinf) Beier & Thulin var. <i>mandavillei</i> (Hadidi) Alzahrani & Albokhari. = <i>Zygophyllum hamiense</i> var. <i>mandavillei</i> (Hadidi) Thomas & Chaudhary	Rayan, Dammam, 3/2018
<i>Tribulus pentandrus</i> Forssk var. <i>pentandrus</i> . = <i>T. longipetalus</i> Viv., Pl. Aegypt.	Rawda, Dammam, 4/2017
<i>Tribulus macropterus</i> Boiss var. <i>arabicus</i> (Hosni) Al-Hemaid & J. Thomas. = <i>T. arabicus</i> Hosni	Second Industrial City, Dammam, 4/2017
<i>Tribulus terrestris</i> L. var. <i>terrestris</i> . = <i>T. terrestris</i> var. <i>robutus</i> (Boiss, and Noe) Boiss.	Rayan, Dammam, 3/2018
<i>Tribulus terrestris</i> L. var. <i>parvispinus</i> (Presl) Al-Hemaid & J. Thomas. = <i>t. parvispinus</i> Presl.	Rawda, Dammam, 4/2017

Results

In the present study, 12 taxa belonging to the Zygophyllaceae from the eastern region of Saudi Arabia were investigated for their morphological and anatomical characters

to find the most important characters for identification and discrimination between the studied taxa. The different morphological and anatomical characters are summarized in Tables 2-4 and Plates 1-4.

I-Macro-morphological characters

Growth form (Life span): The growth form of the studied taxa varied from annual herbs (*Tribulus* sp.), perennial shrubs (*Tetraena* sp.) and annual to perennial herbs (*Fagonia* sp.). The texture was either hairy (*Tribulus*) or glabrous (the other eleven taxa). Also, only the genus *Tetraena* contained succulent species.

Stem: The stems was erect in *Tetraena* taxa, prostrate in *Tribulus* taxa, and procumbent in *Fagonia* taxa. The internode length was short (1.5–2cm) in most of the taxa studied but long (2.1–2.5cm) in *Tetraena migahidii*, *Tribulus macropterus* var. *arabicus* and *Tribulus terrestris* var. *parvispinus*.

Leaf: Great variability was observed among the studied taxa in leaf attributes such as shape, type, texture, apex, and number of leaflets. Most of the focal taxa had petiolate leaves except *Fagonia olivieri*, *Fagonia indica*, and *Fagonia ovalifolia*, which had sessile leaves. In *Fagonia bruguieri*, while the lower leaves were petiolated the upper leaves were sessile. The leaves of most of the studied taxa were compound and unifoliate; some exceptions are *Tetraena migahidii*, which had compound and bifoliate leaves and *Tribulus* sp. which had multifoliate (>3) leaves. Two types of leaves had been observed in *Fagonia bruguieri*: the lower leaves were trifoliate while the upper leaves were unifoliate.

The leaves of all species are entire and exstipulate except in *Fagonia* sp., where they were spiny-stipulate. With respect to blade outline (leaflet shape) six main types were recorded: oblong with three sub-types, in *Tetraena hamiensis* var. *hamiensis*, *Fagonia indica*, *Fagonia ovalifolia* and *Tribulus* sp.; elliptic lanceolate, in *Fagonia olivieri* and *Fagonia bruguieri*; obovate in *Tetraena migahidii*; globose in *Tetraena hamiensis* var. *qatarensis*; and sub-globose in *Tetraena hamiensis* var. *mandavillei*. Leaflet apex ranged from acute in *Tribulus* sp. and *Fagonia* sp. to obtuse in *Tetraena* sp. A truncate apex was observed in *Tetraena hamiensis* var. *qatarensis*. Leaflets were glabrous in most of the studied taxa except in *Tribulus* sp., where they were pubescent. The leaflet length was short (0.5–0.9cm.) in most of the studied taxa, but was longer (1–1.5cm.) in *Fagonia*

bruguieri and *Tetraena migahidii*.

II- Micro-morphological characters

A. Stem Anatomy

The anatomical characters of stems of the studied Zygophyllaceae species are recorded in Table 3 and Plate 2.

Stem Cross Sections: The outline of the stem cross section is useful in distinguishing the studied taxa. It varies from terete in *Tribulus terrestris* var. *terrestris* and *Tribulus terrestris* var. *parvispinus*, terete with a wavy margin in *Tetraena hamiensis* var. *hamiensis*, *Tetraena hamiensis* var. *qatarensis*, *Tetraena hamiensis* var. *mandavillei* and *Tribulus pentandrus* var. *pentandrus*, ovoid with a wavy margin in *Tribulus macropterus* var. *arabicus*, rectangular with many ridges in *Tetraena migahidii*, obtriangle with many ridges in *Fagonia olivieri* and *Fagonia bruguieri*, hexagonal with many ridges in *Fagonia indica* and *Fagonia ovalifolia*.

Cortex: The structure of cortex also has value in distinguishing the twelve test taxa. The cortex consisted of palisade tissue followed by parenchyma in *Fagonia* sp. but consisted of parenchyma only in all other taxa. The number of cortex layers exhibited marked variation among species. A palisade zone of 3–5 layers was detected followed by two layers of parenchyma which was either irregular (in *Fagonia olivieri* and *Fagonia indica*) or round (in *Fagonia ovalifolia* and *Fagonia bruguieri*). The cortex consisted of 4–6 layers of parenchyma which was either round (in *Tribulus terrestris* var. *parvispinus*) or polygonal (in *Tribulus terrestris* var. *terrestris*), whereas it consisted of 5–6 layers of polygonal parenchyma in *Tetraena hamiensis* var. *hamiensis*, *Tetraena hamiensis* var. *qatarensis* and *Tetraena hamiensis* var. *mandavillei*, 7–8 layers of round parenchyma in *Tribulus macropterus* var. *arabicus*, 7–9 layers of polygonal parenchyma in *Tribulus pentandrus* var. *pentandrus*, or irregular parenchyma in *Tetraena migahidii*. Patches of stone cells were also detected in the cortex of some *Fagonia* sp.

Vascular system: The vascular system differed significantly between the test taxa. In most taxa it consisted of an ectophloic

siphonostele with complete phloem and xylem rings. However, in *Tribulus* sp. the vascular system was eustele with 15–20 vascular bundles. Phloem with ill-defined elements and Xylem with wide vessels in most studied taxa except in *Tetraena hamiensis* var. *hamiensis*, *Tetraena hamiensis* var. *qatarensis* and *Tetraena hamiensis* var. *mandavillei* have will defend phloem and narrow xylem vessels.

Pith: The Pith was narrow in most of the studied taxa except in *Tribulus pentandrus* var. *pentandrus*, *Tribulus terrestris* var. *terrestris*, and *Tribulus terrestris* var. *parvispinus*, where it was wide. The pith consisted mainly of thin-walled parenchyma that was either round (in *Tetraena hamiensis* var. *qatarensis* and *Tribulus terrestris* var. *parvispinus*), irregular (in *Tetraena migahidii* and *Tribulus terrestris* var. *terrestris*), round/polygonal (in *Fagonia olivieri*, *Tetraena hamiensis* var. *hamiensis*, *Tetraena hamiensis* var. *mandavillei* and *Tribulus pentandrus* var. *pentandrus*) or elongated/irregular in all other taxa. Schizogenous canals were detected in all the studied taxa except *Tetraena migahidii*; likewise, druses were detected in all taxa except *Fagonia* sp. and *Tetraena migahidii*.

B- Petiole anatomy

The anatomical characters of leaf petioles are presented in Table 4 and Plate 3.

Petiole outlines: The petiole outline was oblong in *Tetraena hamiensis* var. *hamiensis* and *Tetraena hamiensis* var. *mandavillei*, a half circle with a wavy margin in *Fagonia bruguieri* and *Tetraena migahidii*, terete with two ridges in *Tribulus pentandrus* var. *pentandrus*, *Tribulus macropterus* var. *arabicus*, and *Tribulus terrestris* var. *parvispinus*, and terete in the reminder.

Ground tissue: The ground tissue was either wholly parenchymatous (in *Fagonia olivieri*, *Fagonia indica*, *Fagonia ovalifolia*, *Tetraena migahidii*, and *Tribulus terrestris* var. *terrestris*), palisade followed by parenchyma (in *Fagonia bruguieri*, *Tetraena hamiensis* var. *hamiensis*, *Tetraena hamiensis* var. *qatarensis*, and *Tetraena hamiensis* var. *mandavillei*) or of parenchyma with palisade on the ridge in the other species. The stone cells were detected only in *Fagonia bruguieri*. Schizogenous

canals and druses were absent from most of the studied taxa except *Tetraena hamiensis* var. *qatarensis* and *Tribulus* sp. were present.

Vascular system: The number of vascular bundles in the petiole varies from: one main bundle in the middle as in *Fagonia olivieri*, *Fagonia indica*, and *Fagonia ovalifolia*; three bundles including one large bundle in the middle and two smaller lateral bundles as in *Fagonia bruguieri*; five bundles including one large bundle in the middle and four smaller lateral bundles as in *Tetraena migahidii*; six bundles including four main bundles in the middle and two smaller lateral bundles as in *Tribulus* sp.; more than 10 bundles, including one main bundle in the middle and many peripheral bundles, as in *Tetraena hamiensis* var. *qatarensis* and *Tetraena hamiensis* var. *mandavillei*, or one main bundle with two lateral and many peripheral bundles as in *Tetraena hamiensis* var. *hamiensis*.

C- Blade anatomy

The anatomical characters of the blade of the examined Zygophyllaceae taxa are shown in Table 4 and Plate 4.

Blade shape: The outline of the blade was either oblong (in *Tetraena hamiensis* var. *hamiensis*, *Tetraena hamiensis* var. *qatarensis*, and *Tetraena hamiensis* var. *mandavillei*) or duplicate in other taxa.

Mesophyll: The blades varied from isolateral, as in *Tetraena migahidii*, *Tribulus pentandrus* var. *pentandrus*, *Tribulus macropterus* var. *arabicus*, *Tribulus terrestris* var. *terrestris*, and *Tribulus terrestris* var. *parvispinus*; isobilateral in *Fagonia* sp.; and centric in the reminder. The mesophyll is continuous in all studied taxa except in *Tetraena migahidii*, where it is discontinuous.

Vascular System of the Midrib: The number of vascular bundles in the midrib varied from one collateral bundle as in *Fagonia olivieri*, *Fagonia ovalifolia*, *Fagonia bruguieri*, *Tribulus pentandrus* var. *pentandrus*, *Tribulus macropterus* var. *arabicus*, *Tribulus terrestris* var. *terrestris*, and *Tribulus terrestris* var. *parvispinus*; two collateral bundles as in *Fagonia indica*, and three collateral bundles with peripheral bundles as in *Tetraena* sp.

TABLE 2. Morphological characteristics of the twelve Zygophyllaceae taxa under study

Characters		Taxa											
		<i>Fagonia olivieri</i>	<i>Fagonia indica</i>	<i>Fagonia ovalifolia</i>	<i>Fagonia bruguieri</i>	<i>Tetraena migahidii</i>	<i>Tetraena hamiensis</i> var. <i>hamiensis</i> .	<i>Tetraena hamiensis</i> var. <i>qatarensis</i>	<i>Tetraena hamiensis</i> var. <i>mandavillei</i>	<i>Tribulus pentandrus</i> var. <i>pentandrus</i> .	<i>Tribulus macropterus</i> var. <i>arabicus</i>	<i>Tribulus terrestris</i> var. <i>terrestris</i> .	<i>Tribulus terrestris</i> . var. <i>parvispinus</i>
Whole Plant	Life span: 1- Annual, 2- Perennial, 3- Annual to perennial	3	3	3	3	2	2	2	2	1	1	1	1
	Habit: 1- Herb, 2- Shrub	1	1	1	1	2	2	2	2	1	1	1	1
	Texture: 1- Glabrous, 2- Hairy	1	1	1	1	1	1	1	1	2	2	2	2
	Nature: 1- Succulent, 2- Non-succulent	2	2	2	2	1	1	1	1	2	2	2	2
Stem	Nature: 1- Erect, 2-Prostrate, 3- Procumbent	3	3	3	3	1	1	1	1	2	2	2	2
	Internode Mean length (cm)	1.96	1.98	1.74	1.56	2.12	1.26	1.74	1.7	1.68	2.18	1.5	2.12
	±SD.	±0.71	±0.75	±0.56	±0.33	0.87	±0.40	±0.55	±0.53	±0.53	±0.95	±0.66	±0.88
	Internode Mean diameter (cm)	0.15	0.15	0.15	0.15	0.2	0.2	0.23	0.19	0.15	0.15	0.15	0.2
	±SD.	±0.05	±0.05	±0.05	±0.05	±0.07	±0.07	±0.08	±0.07	±0.05	±0.05	±0.05	±0.07
	Petiole: 1- Petiolate, 2- Subsessile	2	2	2	1&2	1	1	1	1	1	1	1	1
	Petiole Mean length (cm)	0.08	0.08	0.1	0.64	1.2	0.74	0.52	0.64	0.42	0.64	0.6	0.6
	±SD.	±0.02	±0.02	±0	±0.26	±0.57	±0.21	±0.15	±0.15	±0.12	±0.20	±0.18	±0.14
	Leaf length (cm)	0.86	0.94	0.66	1.92	2.26	1.48	1.02	1.28	2.16	2.94	3.84	3.2
	±SD.	±0.21	±0.22	±0.14	±0.43	±0.78	±0.22	±0.23	±0.23	±0.39	±0.83	±0.69	±0.89
Leaf	Base: 1- stipulate, 2- Normal	1	1	1	1	2	2	2	2	2	2	2	2
	Number of leaflets: 1- One, 2- Two, 3- Three, 4-more than three	1	1	1	3	2	1	1	1	4	4	4	4
	Shape: 1- Oblong, 2- Oblong lanceolate, 3- Elliptic, 4- Oblong ovate, 5- Obovate, 6- Globose or sub- globose	2	3	3	5	5	1	6	6	4	4	4	4
	Apex: 1- Acute, 2- Obtuse, 3- Truncate	1	1	1	1	2	2	3	2	1	1	1	1
	Texture: 1- Glabrous, 2- Pubescent	1	1	1	1	1	1	1	1	2	2	2	2
	Length Mean (cm)	0.72	0.84	0.56	1.26	1.28	0.72	0.51	0.64	0.53	0.77	0.88	0.86
	±SD.	±0.21	±0.22	±0.14	±0.31	±0.37	±0.15	±0.10	±0.10	±0.07	±0.08	±0.31	±0.10
	Width Mean (cm)	0.3	0.26	0.18	0.25	0.37	0.39	0.46	0.53	0.26	0.35	0.3	0.32
	±SD.	±0.07	±0.06	±0.05	±0.06	±0.11	±0.08	±0.12	±0.07	±0.06	±0.09	±0.09	±0.07

Table 3. Stem anatomy of the twelve Zygophyllaceae taxa under study

Taxa													
		<i>Fagonia olivieri</i>	<i>Fagonia indica</i>	<i>Fagonia ovalifolia</i>	<i>Fagonia bruguierei</i>	<i>Tetraena migahidii</i>	<i>Tetraena hamiensis</i> var. <i>hamiensis</i>	<i>Tetraena hamiensis</i> var. <i>qatarensis</i>	<i>Tetraena hamiensis</i> var.	<i>Tribulus pentandrus</i> var.	<i>Tribulus macropterus</i> var. <i>arabicus</i>	<i>Tribulus terrestris</i> var. <i>terrestris</i>	<i>Tribulus terrestris</i> var. <i>parvispinus</i>
Stem outline	1- Terete												
	2- Terete with wavy margin												
	3- Ovoid with wavy margin												
	4- Rectangular with many ridges	5	6	6	5	4	2	2	2	2	3	1	1
	5- Obtriangle with many ridges												
	6- Hexagonal with many ridges												
Cuticle	Thickening: 1- Thin												
	2- Thick	2	3	3	2	2	1	2	1	2	2	2	2
	3- Very thick												
Epidermis:	Surface: 1- Smooth	1	2	1	1	1	1	1	1	1	2	1	1
	2- Warty												
Cortex:	1- Tangential												
	2- Radial	3	3	3	3	2	2	2	2	1	2	2	3
	3- Mixed (Tangential and radial)												
Pericycle	Type: 1- Parenchyma 2- Palisade follow by parenchyma	2	2	2	2	1	1	1	1	1	1	1	1
	No. of layers: 1- 1-6 layers	1	1	1	1	1	1	1	1	2	2	1	1
	2- more than 6 layers												
	Type of parenchyma:												
	1- Irregular	1	1	2	2	1	3	3	3	3	2	3	2
	2- Round												
Stone cell in cortex:	3- Round to polygonal												
	1- Present 2- Absent	1	1	1	1	2	2	2	2	2	2	2	2
Phloem:	Form: 1 - Cyclic 2- Patchy	2	2	2	2	1	2	2	2	2	2	2	2
	Type: 1- Parenchyma 2- Sclerenchyma	2	2	2	2	1	2	2	2	2	2	2	2
Xylem:	Type of stele: 1- Siphonostele 2- Eustele	1	1	1	1	1	1	1	1	2	2	2	2
	1- Well-defined 2- Poorly defined	2	2	2	2	2	1	1	1	2	2	2	2
No. of bundles:	1- Wide 2- Narrow	1	1	1	1	1	2	2	2	1	1	1	1
	1- Cycle 2- 15-20	1	1	1	1	1	1	1	1	2	2	2	2
Pith	1-Narrow 2- Wide	1	1	1	1	1	1	1	1	2	1	2	2
	Type: 1-Round parenchyma												
	2- Irregular parenchyma	3	4	4	4	2	3	1	3	3	4	2	1
	3- Round to polygonal												
Schizogenous canals:	4-Elongated to irregular												
	1-Present in cortex only												
Druses:	2-Present in Pith only	2	2	2	2	4	3	3	3	3	3	1	3
	3-Present in cortex and pith												
	4- Absent												
	1- Present 2- Absent	2	2	2	2	2	1	1	1	1	1	1	1

TABLE 4. Petiole and blade anatomy of the twelve Zygophyllaceae taxa under study

Character		Taxa														
		<i>Fagonia olivieri</i>	<i>Fagonia indica</i>	<i>Fagonia ovalifolia</i>	<i>Fagonia bruguieri</i>	<i>Tetraena migahitii</i>	<i>Tetraena hamiensis</i> var. <i>hamiensis</i> .	<i>Tetraena hamiensis</i> var. <i>qatarensis</i>	<i>Tetraena hamiensis</i> var. <i>mandavillei</i>	<i>Tribulus pentandrus</i> var. <i>pentandrus</i> .	<i>Tribulus macropterus</i> var. <i>arabicus</i>	<i>Tribulus terrestris</i> var. <i>terrestris</i> .	<i>Tribulus terrestris</i> var. <i>parvispinus</i>			
Petiole	Petiole outline:	1- Terete														
		2- Pentagonal														
		3-± Pentagonal with two ridges	2	1	1	5	5	4	1	4	2	2	1	3		
		4- Ovoid														
		5- Half circle with wavy margin														
	Cuticle	Thickness	1- Thin	2	2	2	2	2	1	1	1	2	2	2	2	
			2- Thick													
	Surface:	1- Smooth	1	1	1	1	1	1	1	1	1	2	1	2		
		2- Warty														
	Epidermis		1- Tangential													
			2- Radial	3	3	3	3	3	1	3	1	3	2	3	2	
			3- Mixed													
	Ground tissue	Type	1- Parenchyma													
			2-Palisade follow by parenchyma	1	1	1	2	1	2	2	2	3	3	1	3	
			3-Parenchyma and palisade at ridges													
Stone cell:		1- Present	2	2	2	1	2	2	2	2	2	2	2	2		
		2- Absent														
Vascular bundles	Main V. B.:	1- One	1	1	1	1	1	1	1	1	2	2	2	2		
		2- Four														
Lateral V B.:		1- Absent	1	1	1	2	3	2	1	1	2	2	2	2		
		2- Two														
		3- Four (2,2)														
Peripheral V. B.:		1- Present	2	2	2	2	2	1	1	1	2	2	2	2		
		2- Absent														
Schizogenous canals		1- Present	2	2	2	2	2	2	1	2	1	1	1	1		
		2- Absent														
Druses		1- Present	2	2	2	2	2	2	1	2	1	1	1	1		
		2- Absent														
Leaflet blade	Outline	1- Duplicate	1	1	1	1	1	2	2	2	1	1	1	1		
		2- Oblong														
	Cuticle Thickening	1- Thin	2	3	2	2	3	2	2	2	2	2	2	1		
		2- Thick														
		3- Very thick														
	Surface	1- Smooth	1	1	1	1	1	1	1	1	1	2	1	1		
		2- Warty														
	Epidermis:		1- Tangential	2	2	1	1	2	2	2	2	2	1	1		
			2- Mixed													
	Mesophyll Type:		1- Isolateral	2	2	2	2	1	3	3	3	1	1	1	1	
			2-Isobilateral													
			3- Centric													

TABLE 4 Cont. Petiole and blade anatomy of the twelve Zygophyllaceae taxa under study

Characters		Taxa											
		<i>Fagonia olivieri</i>	<i>Fagonia indica</i>	<i>Fagonia ovalifolia</i>	<i>Fagonia bruguieri</i>	<i>Tetraena migahidii</i>	<i>Tetraena hamiensis</i> var. <i>hamiensis</i> .	<i>Tetraena hamiensis</i> var. <i>qatarensis</i>	<i>Tetraena hamiensis</i> var. <i>mandavillei</i>	<i>Tribulus pentandrus</i> var. <i>pentandrus</i> .	<i>Tribulus macropterus</i> var. <i>arabicus</i>	<i>Tribulus terrestris</i> var. <i>terrestris</i> .	<i>Tribulus terrestris</i> var. <i>parvispinus</i>
Mesophyll in midrib	1- Continuous 2- Discontinuous	1	1	1	1	2	1	1	1	1	1	1	1
Vascular Bundles	Main V B	1- One	1	2	1	1	1	1	1	1	1	1	1
		2- Two											
	Lateral V B	1- Absent	1	1	1	1	2	2	2	2	1	1	1
		2- Two											
Peripheral V B	1- Present	2	2	2	2	2	1	1	1	2	2	2	
	2- Absent												
Bundle sheath:	1- Present	2	2	2	2	2	2	2	2	1	1	1	
	2- Absent												
Schizogenous Canals:	1- Present 2- Absent	2	2	2	2	1	1	1	1	2	2	2	
Druses	1- Present	2	2	2	2	1	1	1	1	1	1	1	
	2- Absent												
Stomata	Level: 1- Superficial 2- At level 3- Depressed		1	1	2	1	1	2	3	3	2	2	3

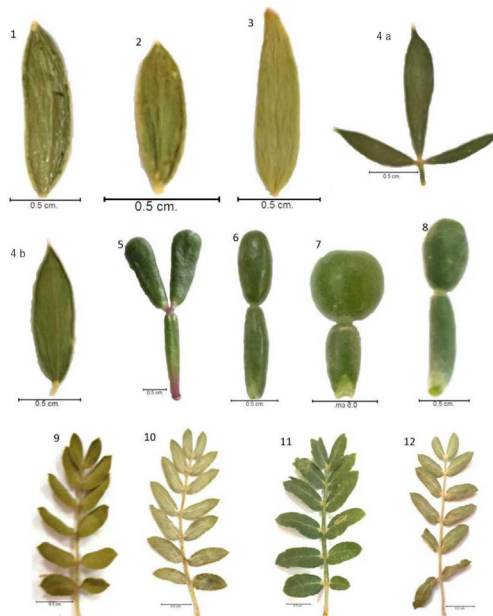


Plate 1. Leaf morphology of the twelve Zygophyllaceae taxa under study: 1. *Fagonia olivieri*; 2. *Fagonia indica*; 3. *Fagonia ovalifolia*; 4. *Fagonia bruguieri* (a- Lower leaf, b-Upper leaf); 5. *Tetraena migahidii*; 6. *Tetraena hamiensis* var. *hamiensis*; 7. *Tetraena hamiensis* var. *qatarensis*; 8. *Tetraena hamiensis* var. *mandavillei*; 9. *Tribulus pentandrus* var. *pentandrus*; 10. *Tribulus macropterus* var. *arabicus*; 11. *Tribulus terrestris* var. *terrestris*; 12. *Tribulus terrestris* var. *parvispinus*

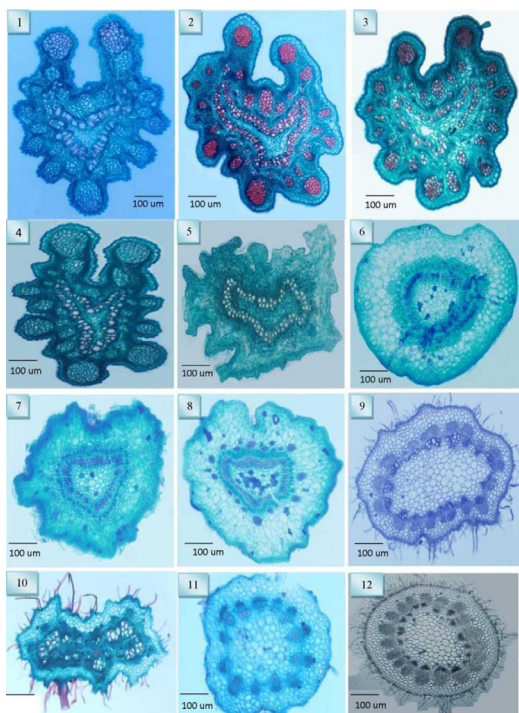


Plate 2. Stem anatomy of the twelve test Zygophyllaceae taxa: 1. *Fagonia olivieri*; 2. *Fagonia indica*; 3. *Fagonia ovalifolia*; 4. *Fagonia bruguieri*; 5. *Tetraena migahidii*; 6. *Tetraena hamiensis* var. *hamiensis*; 7. *Tetraena hamiensis* var. *qatarensis*; 8. *Tetraena hamiensis* var. *mandavillei*; 9. *Tribulus pentandrus* var. *pentandrus*; 10. *Tribulus macropterus* var. *arabicus*; 11. *Tribulus terrestris* var. *terrestris*; 12. *Tribulus terrestris* var. *parvispinus*

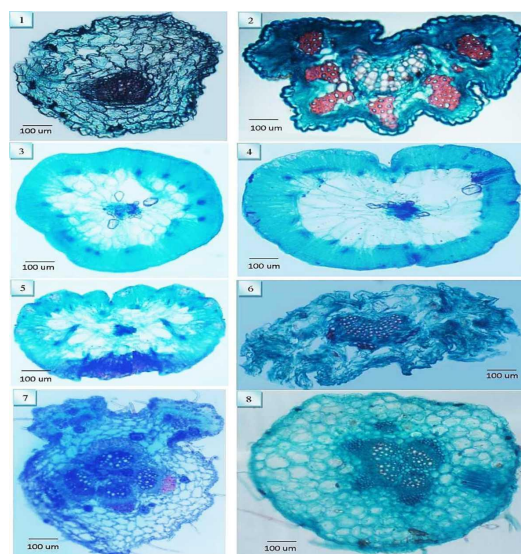


Plate 3. Main different Petiole anatomy of the Zygophyllaceae taxa under study: 1. *Fagonia olivieri*; 2. *Fagonia bruguieri*; 3. *Tetraena hamiensis* var. *qatarensis*; 4. *Tetraena hamiensis* var. *mandavillei*; 5. *Tetraena hamiensis* var. *hamiensis*; 6. *Tetraena migahidii*; 7. *Tribulus terrestris* var. *parvispinus*; 8. *Tribulus terrestris* var. *terrestris*

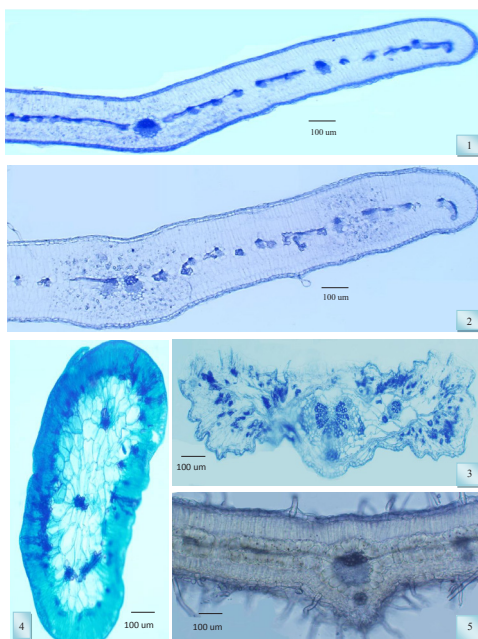


Plate 4. Main different leaflet blade anatomy of Zygophyllaceae taxa under study: 1. *Fagonia olivieri*; 2. *Fagonia indica*; 3. *Tetraena migahidii*; 4. *Tetraena hamiensis* var. *mandavillei*; 5. *Tribulus terrestris* var. *parvispinus*

II-Key

The data recorded in Tables 2-4 was used to construct the following bracketed key to the twelve Zygophyllaceae species examined in this study.

- 1- The plant succulent perennial shrub with erect stem.....2
- 1- The plant not succulent annual herb with prostrate or procumbent stem5
- 2- Leaf bifoliate with obovate shape and duplicate outline in cross section, stem outline rectangular with irregular parenchyma cortex, pericycle cycle of parenchyma tissue and peripheral vascular bundles absent *Tetraena migahidii*
- 2- Leaf one-foliate with other obovate shape and oblong outline in cross section, stem outline \pm terete with wavy margin and round to polygonal parenchyma cortex, pericycle patches of sclerenchyma tissue and peripheral vascular bundles present.3
- 3- Leaflet globose with truncate apex and \pm terete petiole outline.....*Tetraena hamiensis* var. *qatarensis*
- 3- Leaflet not globose with obtuse apex and petiole outline \pm oblong.....4
- 4- Leaflet oblong and the number of vascular bundles in petiole are three
.....*Tetraena hamiensis* var. *hamiensis*
- 4- Leaflet subglobose and the number of vascular bundles in petiole are one.....
.....*Tetraena hamiensis* var. *mandavillei*
- 5- Stem procumbent and not hairy, leaf uni - trifoliate, stem cortical palisade present, siphonostele stem, leaf bundle sheath absent and druses absent..... 6
- 5- Stem prostrate and hairy, leaf paripinnate, stem cortical palisade absent, euostele stem, leaf bundle sheath present and druses present 9
- 6- Leaf uni - trifoliate; lower petiolate, trifoliate and upper sessile, unifoliate, petiole outline half circle with wavy margin, ground tissue two types, stone cell present on petiole and the number of vasculatures in petiole are three..... *Fagonia bruguieri*
- 6- Leaf unifoliate, sessile, one-foliate, petiole outline \pm terete, ground tissue one type, stone cell absent in petiole and the number of vasculatures in petiole are one 7
- 7- Leaflet elliptic lanceolate shape and stem outline \pm obtriangle with wavy margin..... *Fagonia olivieri*
- 7- Leaflet oblong lanceolate or linear ovate in shape and stem outline \pm hexagonal with wavy margin.....8
- 8- Leaflet oblong lanceolate and the main vascular bundle in Madrid region is two.....*Fagonia indica*
- 8- Leaflet linear ovate and the main vascular bundle in midriff region is one.....*Fagonia ovalifolia*
- 9- Stem outline is ovoid with wavy margin with warty cuticle and round to irregular parenchyma cortex, pith elongated to irregular parenchyma.....*Tribulus macropterus* var. *arabicus*
- 9- Stem outline terete or \pm terete with smooth cuticle and round or round to polygonal parenchyma.....10
- 10- Stem outline \pm terete with wavy margin and pith have round to polygonal parenchyma
.....*Tribulus pentandrus* var. *pentandrus*
- 10- Stem outline terete and pith tissue irregular parenchyma or round parenchyma.....11
- 11- Cortex round to polygonal, pith irregular parenchyma, schizoncanal present in Cortex only, petiole outline terete, ground tissue one Type..*Tribulus terrestris* var. *terrestris*
- 11- Cortex round, pith round parenchyma, schizoncanal present in Cortex and pith, petiole outline terete with 2 ridges and ground tissue two types *Tribulus terrestris* var. *parvispinus*

Discussion

Identification and classification of the studied twelve Zygophyllaceae taxa depend greatly on morphological characteristics, where they showed marked variation in growth form, stem characters, and leaf characters (including leaf shape, type, texture, apex, and the number of leaflets).

In relations of micro-morphological characters, the present study show that for most of the studied taxa, the stem epidermis is covered

by a thick or very thick cuticle, the cortex has parenchyma associated with mechanical tissue, and the vascular system has poorly-defined phloem and narrow xylem vessels. *Fagonia* sp. are characterized by the presence of stone cells in the cortex and the ectophloic siphonostele vascular system with a complete phloem and xylem ring. This finding agrees with the report of Taia et al. (2017). *Fagonia bruguieri* is distinguished from congeneric taxa by the uni-trifoliate, obovate shape of its leaves. Similarly, *Fagonia ovalifolia* is distinguished by possessing simple leaves. For

the taxa of *Tribulus*, the cortex consists only of parenchyma and the vascular system is eustele, this agrees with Nikolova & Vassilev (2011). The presence of druses in some of the studied taxa agrees with the study of Abd Elhalim et al. (2016). *Tetraena* sp. are characterized by succulent and erect plant form, great number of oblong leaflets of acute to obtuse apex. Within this genus, *Tetraena migahidii* is distinguished from *Tetraena hamiensis* by possessing bifoliate leaves with long petiole (~1.2cm), long blade (~2.26cm), isolateral leaf mesophyll, and presence of druses and schizogenous canals.

The anatomical characters of leaves show many characters; the petiole outline recorded four types (oblong, half circle with wavy margin, ±terete and terete with two ridges). The ground tissue consists of parenchyma only or palisade and parenchyma. The numbers of vascular bundles in the petiole vary from: one main vascular bundle, three vascular bundles; five vascular bundles, six vascular bundles or more than 10 vascular bundles. The blades of the investigated studied taxa of Zygophyllaceae show three types of mesophyll; isolateral, isobilateral and centric. The number of vascular bundles in the midrib vary from; one main bundle, two collateral vascular bundles and three collateral vascular bundles with druses crystals in most studied taxa, all these characters are agreed with different studies for some species of Zygophyllaceae (Nikolova & Vassilev, 2011; Waly et al., 2011; Abd Elhalim et al., 2016; Elkamali et al., 2016; Taia et al., 2017).

Conclusion

The morphological characteristics of the plant such as duration, habit, texture, type and shape of leaves, floral and fruit characters are play an important role in identification and differentiation between the genus and species and used by the scientist of flora in all the world. The anatomical characters also play an important role. The most important anatomical characters used to differentiate between the different genus in this study are the plant's vascular system and type of mesophyll. Some anatomical characters are important in differentiating between species of the same genus, such as the outline of the stem and petiole, thickening of the cuticle, the types of cortex and pith tissue, the presence or absence of crystal, and the secretory canal.

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Authors' contributions: Proposed the idea of this study D. G. G. and O. G. R.; material preparation and data collection were performed by D. G. G.; All authors contributed in designing the experimental work, making the measurements, interpreting the data, and writing the manuscript; writing- review and editing, D. G. G. and O. G. R. All authors have read and agreed to the published version of the manuscript.

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الخصائص التشريحية للأوراق والسيقان كأداة لتحديد بعض الأصناف من *Zygothelloaceae* في شرق المملكة العربية السعودية

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في هذه الدراسة تم فحص التباين المورفولوجي والتشريحي في أنسجة الساق والأوراق لثلاثة أجناس من *Zygothelloaceae*: *Fagonia* (4 أصناف)، *Tetraena* (4 أصناف) و *Tribulus* (4 أصناف).

اشتملت الأصناف الإثنا عشر التي تم دراستها على تسعة أنواع تنمو في المنطقة الشرقية من المملكة العربية السعودية. يوجد صفات إضافية لتحديد الأصناف وتوصيفها بسبب النتائج التي توصلنا إليها. تضمنت السمات التشريحية التي تم فحصها، شكل الساق، شكل خلية البشرة، تركيب القشرة والنخاع، عدد الحزم الوعائية، شكل المقطع العرضي للورقة والعنق، نوع النسيج الوسطي، توزيع الأنسجة الإفرازية والبلورات.

تظهر استنتاجات هذه الدراسة أن السمات التشريحية، ولا سيما القطاع العرضي للورقة والعنق ونوع الأوعية للساق والعنق، هي أدوات مهمة في تحديد والتمييز بين الأنواع التي تم دراستها ويمكن أن توفر مفتاحًا لتحديد الأصناف التي تم دراستها. إن تمييز أنواع *Zygothelloaceae* التي تم فحصها، بناءً على الصفات التي تم تقييمها في هذه الدراسة، يتفق مع التصنيفات السابقة لهذه الأنواع.