

ORIGINAL ARTICLE

Morphological, Chemical, the pollen grain by Scanning electron microscopy (SEM) studies for the species *Tribulus terrestris* L. (Zygophyllaceae)

¹Lubab Gatea Al-Azerg, ^{2*}Khansaa Rasheed Al-Joboury, Sukeyna Abass Aliwy³

^{1,3} Department of Botany, University of Baghdad, Baghdad, Iraq.

² Iraq Natural History Research Center and Museum, University of Baghdad, Baghdad, Iraq.

E-mail of Corresponding author: mkansaa@yahoo.com

ABSTRACT

Morphological, chemical and pollen grain by scanning electron microscopy (SEM) for the species *Tribulus terrestris* L. (Zygophyllaceae). Different external and internal morphological, anatomical and pollen grain characteristics were found in the examined species. Also the chemical analysis suggested for total fatty acids by gas-liquid chromatography and seven fatty acids component identified which are they: palmitic acid, arachidic acid, pentadecylic acid, oleic acid, myristic acid, stearic acid and linoleic acid. The detection of flavonoids by HPLC (High performance liquid chromatography) about eight compounds of the aerial parts of this species were found, which are they: quercetin-3- glucoside, kampferol, hesperitin, luteolin-7- glucose, rutin, rhamnetin, apigenin-7- glucoside, acacetin. The pollen morphological analysis by light microscopy (LM) and scanning electron microscopy (SEM) done.

Keywords: *Tribulus terrestris* – Zygophyllaceae – Morphologic - pollen grain - electron microscopy

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INTRODUCTION

The herbaceous plant *Tribulus terrestris* (puncturevine) is annual of worldwide distribution that is characterized by having spiny mericarps of fruit from the Zygophyllaceae family which contains about 25 genera and 240 species [1,2]. It is considered a very important medicinal plant. Townsend and Guest have reported two species in Iraq that *Tribulus terrestris* is one of them. There are many studies in the world dealing with this species in many biological or environmental aspects, but in Iraq, the studies are few, such as the study in Bulgaria which dealt with the environmental and anatomical aspects of the species, and also the chemical study which carried out in Iran for the flavonoids in root, leaf and fruit of this species [3,4,5]. Therefore, a valuable study had been carried out on *Tribulus terrestris* in morphological, chemical, geographical and the pollen grain by scanning electron microscopy (SEM) studies. The study aimed to identify the characteristics that distinguish the species from the rest of the plant species.

MATERIALS AND METHODS

Morphological characteristics: Samples of mature leaves, stems, and roots of *Tribulus terrestris* were collected from the garden of Iraq Natural History Research Center and Museum in Iraq. So the species was identified by using available references [6].

Anatomical characteristics: The anatomical sections of fresh samples of leaves and stems done at Iraq natural history research center and museum, university of Baghdad, using hand sectioning method. [6]

Chemical analysis: a: total fatty acids:- GLC technique (gas-liquid chromatography used to analyze fatty acids) [7].

b: detection the flavonoids: according to [8].

Pollen grain: The materials for the study were collected from the garden of Iraq Natural History Research Center and Museum, Pollen grain were studied by means of light microscopy (LM).Its described by [9]and scanning electron microscopy (SEM) DSM 960A of an accelerating voltage of 10–15 kV..

RESULTS AND DISCUSSION

Table 1: Systematic Classification of *Tribulus terrestris*

Kingdom	Plantae
Division	Angiospermsae
Class	Rosids
Order	Zygophyllales
Family	Zygophyllaceae
Genus	Tribulus
Species	terrestris



Figure 1:*Tribulus terrestris* L.

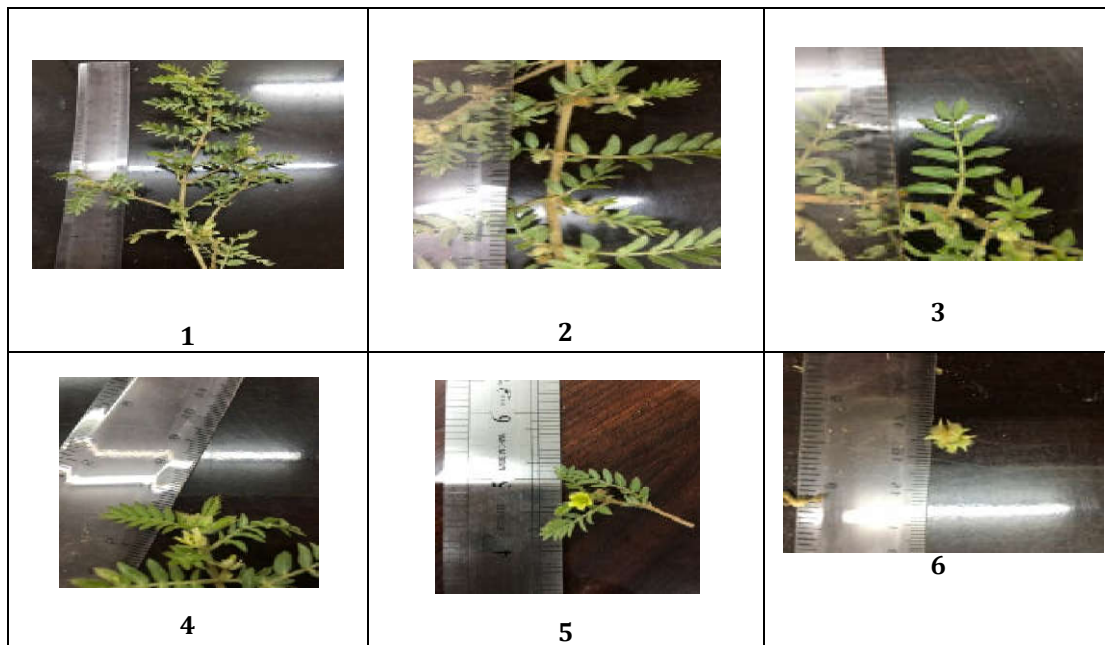


Figure 2:Morphological features of *Tribulus terrestris*

The morphological features In our studies for the species are summarized in table (1).Different external and internal morphological characteristics were found In the examined species *Tribulus terrestris* which is prostrate annual herb, also the measurements for the species in length of stem, leaves, pedicels, sepals, petals stamen and fruit were recorded. The species recorded the range from 90 mm to 120 mm in the stem length while the species recorded the range from 4 to 7 in leaflet pairs, oblong, mucronate white-sericeous on the lower side. So the stamen ranging from 8 to 10. Flowers solitary, pedicels ranged from 8 mm to 12

mm. This data agree with [10] hairy stem has circular shape in cross sections, So the cuticle layer thickness recorded the range from 5mm to 8mm and the Single layer of ovate and semi- rectangular cells of epidermis layer showed, Also thickness recorded the range form 30mm to 40mm while the cortex layer has ovate and oblong ovate parenchymatous cells with range of thickness from 88mm to 95mm. Pericycle containing continuous ring of sclerenchyma cells. Collateral vascular bundles was separated with medullary rays represented the stele of this plant and the pith thickness ranged from 300mm to 350mm which consisted of thin walled parenchymatic cells. The cross sections of *Tribulus terrestris* leaflets showed that a thin layer of cuticle which ranged from 2mm to 3mm covered the polygonal or rectangular uniseriate epidermal cells which ranged from 16mm to 20mm, So the adaxial, abaxial and stem epidermis of *Tribulus terrestris* have anomocytic stomatal complex. Mesophyll was bifacial of palisade layer thickness from 70mm to 80mm and spongy layer which has thickness ranged from 55mm to 60mm. The cross section of *Tribulus terrestris* petiole has subrectangular shape. The thin layer of cuticle which ranged from 2mm to 3mm covered the ovate and oblong ovate uniseriate epidermal cells which ranged from 20mm to 25mm, So cortex thickness ranged from 60mm to 64mm. *Tribulus terrestris* trichomes observed on All the observed trichomes on the leaf, petiole and stem surfaces of *Tribulus terrestris* were glandular unicellular type with acute apexes and thin walls This data agree with [11,12].

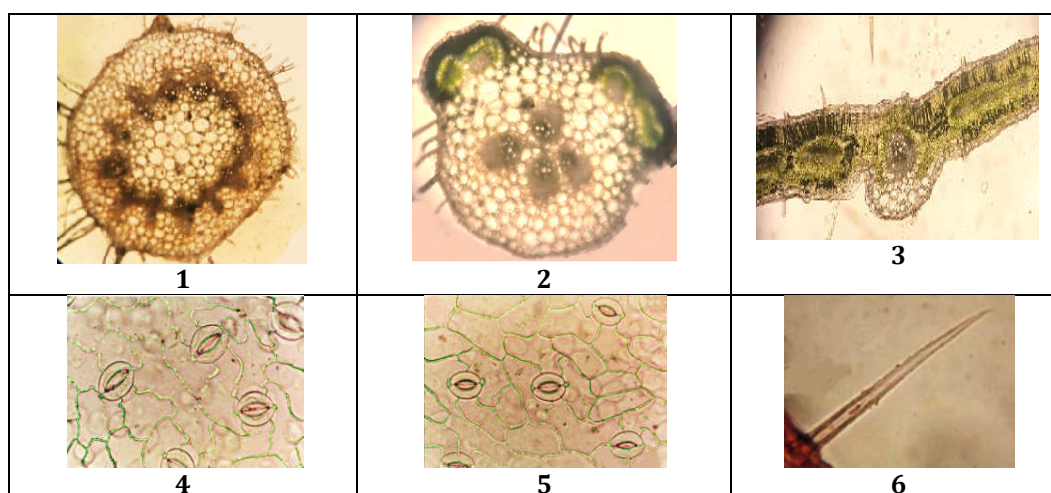


Figure 3: Anatomical features of *Tribulus terrestris* (520X)

Table 2: The features of *Tribulus terrestris*

Morphological features of <i>Tribulus terrestris</i>								
habit	Stem length cm	Leaflets pairs	Leaflets mm	Pedicels length mm	Sepals length mm	Petals length mm	Stamens	Fruit length mm
annual	90-120	4-7	4-6X 6-8	8-12	3-4	5-8	8-10	7-8
Anatomical features of <i>Tribulus terrestris</i> stem								
Cuticle thickness µm	Epidermis thickness µm	Cortex thickness µm	vascular tissue form	vascular tissue thickness µm	Pith Thickness µm			
7-5	30-40	88-95	obovate	110--120	300-350			
Anatomical features of <i>Tribulus terrestris</i> petiole								
Cuticle thickness µm	Epidermis thickness µm	Cortex thickness µm	vascular tissue form	vascular tissue thickness µm	Pith Thickness µm			
4-5	20-25	40-45	ovate	60-64	100-120			
Anatomical features of <i>Tribulus terrestris</i> leaf								
Cuticle thickness	adaxial epidermal thickness	abaxial epidermal thickness	mesophyll		vascular tissue+ sclerenchyma			
			palisade cells	spongy cells				
			2-3	16-20	17-22	70-80	55-60	300-400

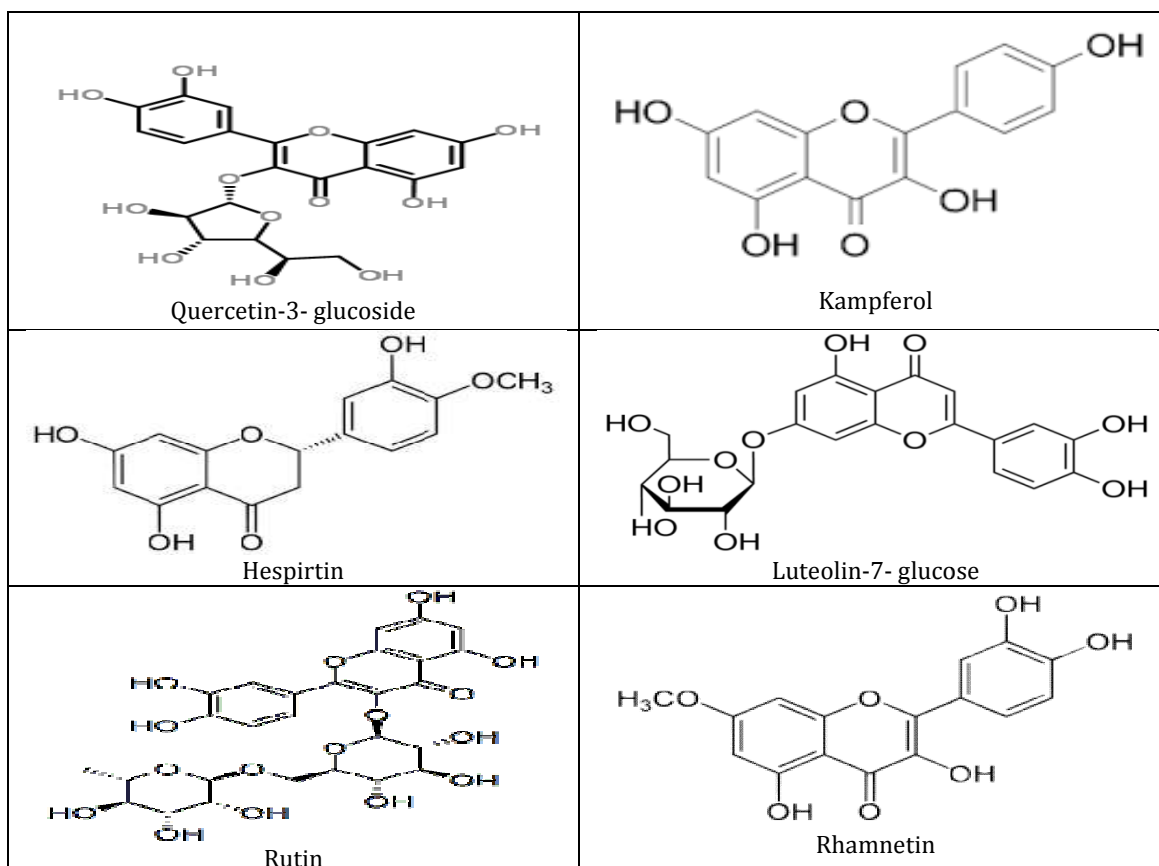
In chemical studies about seven fatty acids for different types identified. They are: palmitic acid, arachidic acid, pentadecylic acid, oleic acid, myristic acid stearic acid and linoleic acid, and the maximum percentage for the separated fatty acids was palmitic acid which was 20.65% while the minimum percentage for the separated fatty acids was linoleic acid which was 3.61%. These data in agreement with result of [9,10]The detection of flavonoids by HPLC about eight compounds of the aerial parts of this species were found, which are they: quercetin-3- glucoside, kampferol, hespirtin, luteolin-7- glucose, rutin, rhamnetin,apigenin-7- glucoside, acacetin. these data in agreement with result of [13,14,15].

Table 3: The component of fatty acid in *Tribulus terrestris*

No	fatty acids	Total amino acids (%)
1	Palmitic acid	20.65
2	Arachidic acid	10.77
3	Pentadecylic acid	8.38
4	Oleic acid	7.93
5	Myristic acid	16.46
6	Stearic acid	6.69
7	Linoleic acid	3.61

Table 4: The component of flavonoids in aerial parts in *Tribulusterrestris*

No.	Flavonoids	Mg/l
1	Quercetin-3-glucoside	1.87
2	Kampferol	2.66
3	Hespirtin	12.94
4	Luteolin-7- glucose	5.44
5	Rutin	3.72
6	Rhamnetin	0.58
7	apigenin-7- glucoside	4.36
8	Acacetin	6.31



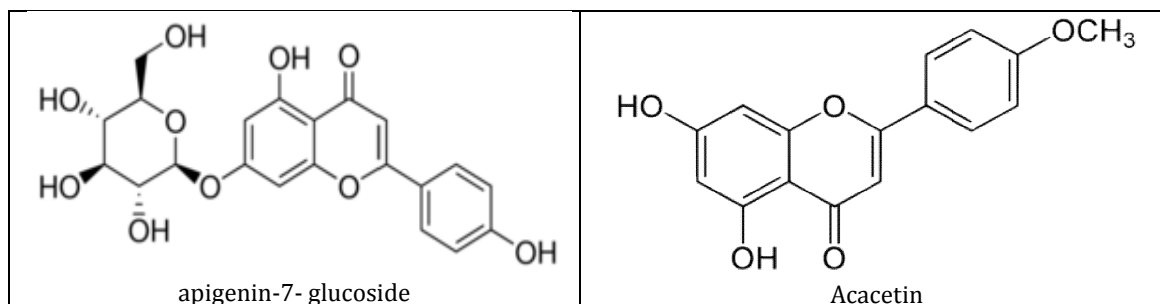


Figure 4: The structure of the investigated flavonoid

The pollen morphological analysis give a proved taxonomical significance and successfully using as an additional criterion for delimitation of the taxa, especially the polymorphous one. The results of pollen grains showed that the species *T. terrestris* in LM are spheroidal, exine pattern is reticulate, pantoporate, oblate-spheroidal. The value of the exine size ranged from 5µm to 6µm. dimensions for the exine reticulate structures ranged from 7µm to 8µm. magnitude for the bronchi ranged from 6µm to 10µm these data in agreement with result of [16,17,18].

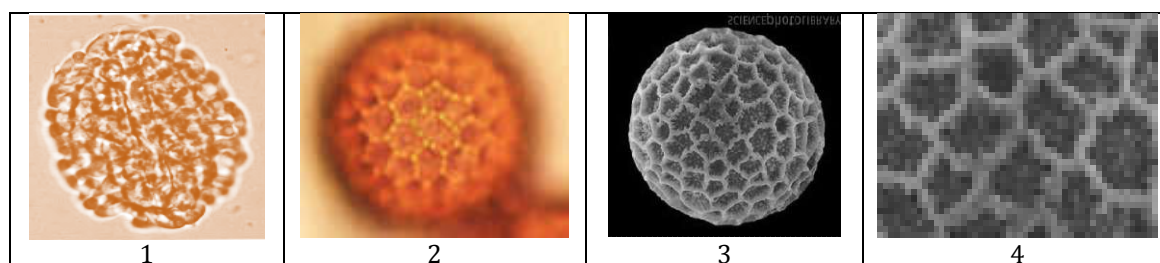


Figure 5: General view of pollen grains (1,2) (LM) ; (3,4) (SEM) 540X

REFERENCES

- Ahmed, Q. , Declan, P. and Andrea, P. (2014). A Systematic Review on the Herbal Extract *Tribulus terrestris* and the Roots of its Putative Aphrodisiac and Performance Enhancing Effect. *Journal of Dietary Supplements*, 11,(1).
- AL-Bayati, F. and AL-Mola, H. (2008). Antibacterial and antifungal activities of different parts of *Tribulus Terrestris* L. growing in Iraq. *Journal of Zhejiang University Science B*, 9 (2): 154-159.
- Mamdouh N., Mokhtar M. , Ahmed A. , Hanaa M. and Mohamed S. (2013). Pharmacognostical Studies on Flower of *Tribulus terrestris* L. *Journal of Pharmacognosy and Phytochemistry*, 1(5):18-22.
- Saima, A. , Hashimi, A. , Tamana B. , Khan, B. and Asad, J. (2014). Medicinal properties phytochemistry and pharmacology of *Tribulus terrestris* L. (*Zygophyllaceae*). *Pakistan Journal of Botany*, 46(1): 399-404.
- Sarwat, M., Das, S. and Srivastava, P. (2008). Analysis of genetic diversity through AFLP, SAMPL, ISSR and RAPD markers in *Tribulus terrestris*, a medicinal herb. *Plant Cell Reports*, 27: 519-528.
- Townsend, C. and Guest, E. (1980). *Flora of Iraq* .Vol.4. Ministry of Agriculture and Agrarian reform. Baghdad. Iraqi.
- Ackman, R. (1972). The analysis of fatty acids and related materials by gas-liquid chromatography. *Progress in the Chemistry of Fats and other Lipids*, 12: 165-284.
- Mattila, P.; Astola, J. and Kumpulainen, J. 2000. Determination of flavonoids in plant material by HPLC with diode-array and electro- array detections. *J. Agric. Food Chem.* 48: 5834-5841.
- Erdtman G. (1969): *Handbook of palynology*. – København: Munksgaard. 99p.
- Varghese, M. , Yadav, S. and Thomas, J. (2006). Taxonomic status of some of the *Tribulus* species in the Indian sub-continent. *Saudi International Journal of Biological Sciences*, 13: 7-12.
- Perveen, A. , Abid, R. and Fatima. R. (2007). Stomatal types of some dicots within flora of Karachi, *Pakistan Journal of Botany*, 39(4): 1017-1023.
- Al-Ali, M. , Wahbi, S. Twajj, H. and Al-Badr, A. (2003). *Tribulus terrestris*: preliminary study of its diuretic and contractile effects and comparison with *Zea mays*. *Journal of Ethnopharmacol*, 85(3):257-60.
- Kostova, I. and Dinchev, D. (2005). Saponins in *Tribulus terrestris*-chemistry and bioactivity. *Phytochemistry Reviews*, 4 (2-3): 111–137.
- Yang, M. Yang, C, Zhao, M. and Zhu, M. (2011). *Tribulus terrestris* Extraction of total flavonoids, Posted: 2011-4-27 16:01:00 views: 15342 Author (in Chinese).
- Temraz, A. , EL-Gindi, O. , Tommasi, N. and Braca, A. (2006). Steroidal saponins from the aerial parts of *Tribulus terrestris*. *Phytochemistry*, 67: 1011-1018.

16. Ivanka, S., Elina, Y., Georgi B.Yurukova, G.(2011).Pollen and Seed Morphology of *Tribulus Terrestris* L. (Zygophyllaceae). *Biotechnology & Biotechnological Equipment*, 25:2, 2379-2382.
17. Mahmood, A. and Mohammad, A.(2006). Scanning Electron Microscopic Observations on Micro-organisms in the Root Nodules of *Tribulus terrestris* L. (Zygophyllaceae) . *Scanning* 28, (4)233-235.
18. Yunus, D. and Nair, P. (1988). Pollen morphology of Indian Geraniales. V. XV-XVI.1-22.Today and Tomorrow's Printer. Publishers. New Delhi.

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