



**PHARMACOGNOSTICAL AND DNA FINGERPRINT STUDIES ON
DEVERRA TORTUOSA (DESF.) DC. FROM SADAT AREA**

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ABSTRACT

Medicinal plants have been widely used for the treatment of various ailments, about 80 % of the people from developing countries depend on traditional remedies for their primary health. These remedies used in alternative medicines derived from natural sources has been also spread to the industrialized nations. A variety of herbal plants has been the aim of this pharmacognostical work is to assess the essential oil GC-MS analysis of the volatile oil of roots, stems and flowers of *Deverra tortuosa* as well as to investigate the Anatomy of root, stem and flowers and DNA finger print.

INTRODUCTION

Flora of Egypt includes about 2000 species of plants distributed in its different localities. *Deverra tortuosa* (Desf.) DC, syns: *pituranthos tortuosus* Benth, is one among more than 20 species of the genus *Deverra* that grow naturally in almost all phytogeographical regions in Egypt especially desert wadis, sandy and stony plains. It is a perennial bushy plant (30-80 cm tall) with bluish green-branched stem and caduceus leaves. (Boulos, 2000) It is widely distributed in non- reclaimed zone in Sadat city. Deserts provide many benefits that can supply the demands of both the local inhabitants and other surrounding communities. These benefits include water, food supply, medicine and raw materials for industry. Consequently, deserts have been overlooked in most of ecosystem valuation studies (De Groot et al., 2012). A key component in desert ecosystems is the vegetation. Its structure and dynamics control the provision of ecosystem services (Peters et al., 2006). Many plant domestications originally occurred in the Middle-East, and many of the wild relatives of domesticated species are still extant in their centers of origin in desert areas (Batanouny, 1999).

Medicinal plants have been widely used for the treatment of various ailments, about 80 % of the people from developing countries depend on traditional remedies for their primary health care Ridditid et. al, (2008). These remedies used in alternative

medicines derived from natural sources has been also spread to the industrialized nations **Verma, et. al. (2008)**. A variety of herbal plants has been extensively used for the extraction of pure compounds used in the development of new and save drugs. One of the most important therapeutic priorities in handling pain involves pain control **Rang et. al (2003)**.

II. REVIEW OF LITERATURE

1. *Deverra tortuosa* (Desf) DC syns: *pituranthos tortuosus* Benth.

Deverra tortuosa is known in Arabic, as "Guezzah" and "Shabbat El-Gable". It is a perennial bushy plant belongs to family apiaceae, strongly aromatic shrub of 30–80 cm height. The plant has bluish green-branched stem with caduceus leaves (Figure 1). *D. tortuosa* grows naturally in almost all the phytogeographical regions of Egypt especially desert wadis, sandy and stony plains (Boulos, 2000). The aerial parts of plant used in this study had been collected from non-reclaimed zone in Sadat city.

Arbi Guetat et al. (2018), describes the chemical composition of the essential oil of different plant parts of *Devrra tortuosa*; in vivo and in vitro biological activities of plant extract and essential oils. Apiol was found to be the major component of the oil (between 65.73% and 74.41%). The best antioxidant activities were observed for the oil of flowers (IC₅₀ = 175 µg/ml). The samples of stems and roots exhibit lower antioxidant activity (IC₅₀ = 201 µg/ml and 182 µg/ml, respectively). The values of IC₅₀ showed that the extracts of methanol exhibit the highest antioxidants activities (IC₅₀ = 64.8 102 µg/ml). EOs showed excellent antifungal activity against yeasts with low azole susceptibilities (i.e. *Malassezia* spp. and *Candida krusei*). The MIC values of oils varied between 2.85 mg/mL and 27 mg/mL. The obtained results also showed that the plant extracts inhibited the germination and the shoot and root growth of *Triticum aestivum* seedlings.



Figure 1: *Deverra tortuosa* DC.

2.1. Traditional uses of *Deverra tortuosa* syns: *pituranthos tortuosus* Benth.

The plant is used traditionally in Egypt as carminative, diuretic, analgesic, it is also used to relief stomach pain and against intestinal parasites (Mahran *et al*, 1989). In Tunisia, Boukef, (1982) mentioned that *D. tortuosa* is used traditionally as an anti-asthmatic and against scorpion stings. **Hossam and Shahira, (2014)** reported that, The oil prepared in each case was tested for

its cytotoxic activity on three human cancer cell lines, i.e. liver cancer cell line (HEPG2), colon cancer cell line (HCT116), and breast cancer cell line (MCF7). The DE sample showed the most potent activity against the three human cancer cell lines (with IC₅₀ values of 1.67, 1.34, and 3.38 µg/ml against the liver, colon, and breast cancer cell lines, respectively). Terpinen-4-ol, sabinene, γ-terpinene, and β-myrcene were isolated from the DE sample and subjected to a similar evaluation of cytotoxic potency; significant activity was observed.

MATERIALS AND METHODS

1 Material

1.1 Fresh wild plants of *Deverra tortuosa* (Desf) DC.

2. Method

2.1 Collection of Plant material.

PLANT SPECIES

Deverra tortuosa (Desf.) DC. Synonym: *Pituranthos tortuosus* (Desf.) Asch. and Schweinf. Strongly aromatic glabrous shrub, 30-80 cm; stems dichotomously branched, striate; leaves caducous; basal leaves 3-8 cm, 2-pinnatisect into linearsubulate, acute lobes; petiole sheathing, with broad scarious margin; lower cauline leaves with sheaths to 1.5 cm; blade 1-2.5 cm, ternatisect, the lobes linearsubulate; upper leaves reduced to sheaths with filiform apices; umbels mostly terminal; peduncle 1.54cm, stout; umbel-rays 6-10, 1-2 cm, subequal; bracts 2-3 x 1-1.5 mm, triangular, margin scarious, apex mucronate; bracteoles minute; bracts and bracteoles persistent; flowers hardly opening; petals almost glabrous; styles longer than the depressed stylopodium; the fruit 1-1.5 mm, globose, hirsute Tackholm (1974) and Boluos (1999 and 2002).

Fresh plants of *Deverra tortuosa* DC were collected from the desert of Sadat city, Menofiya governorate at flowering stage on April 2014. The taxonomic identification of the plant material was confirmed using well identified herbarium specimens of Environmental studies and Research Institute and the Herbarium of Mansura Faculty of Science botany department. A voucher specimen (ESRI Hr221) was deposited at the Herbarium of of Environmental Studies and Research Institute (ESRI), University of Sadat city. Fresh plant material was freed from foreign materials, washed, shade dried and then powdered using electrical blender and stored in air tight bottles.

2.2. LOCATION OF PLANT COLLECTION (SADAT CITY).

Sadat City is located in a semi arid area in the western desert fringes of the Nile delta in the north west of Egypt. It is located between 30° 21' E and 30° 41' E longitude and 30° 19' N and 30° 34' N Latitude (figure 3). It lies at the kilometer 93 on Cairo – Alexandria highway north-west of Cairo in Egypt. Its total area is 523.5 km² and the total inhabitant area is 18 km². The city is surrounded by a green area of 126 km². (Riad *et al*, 2011)

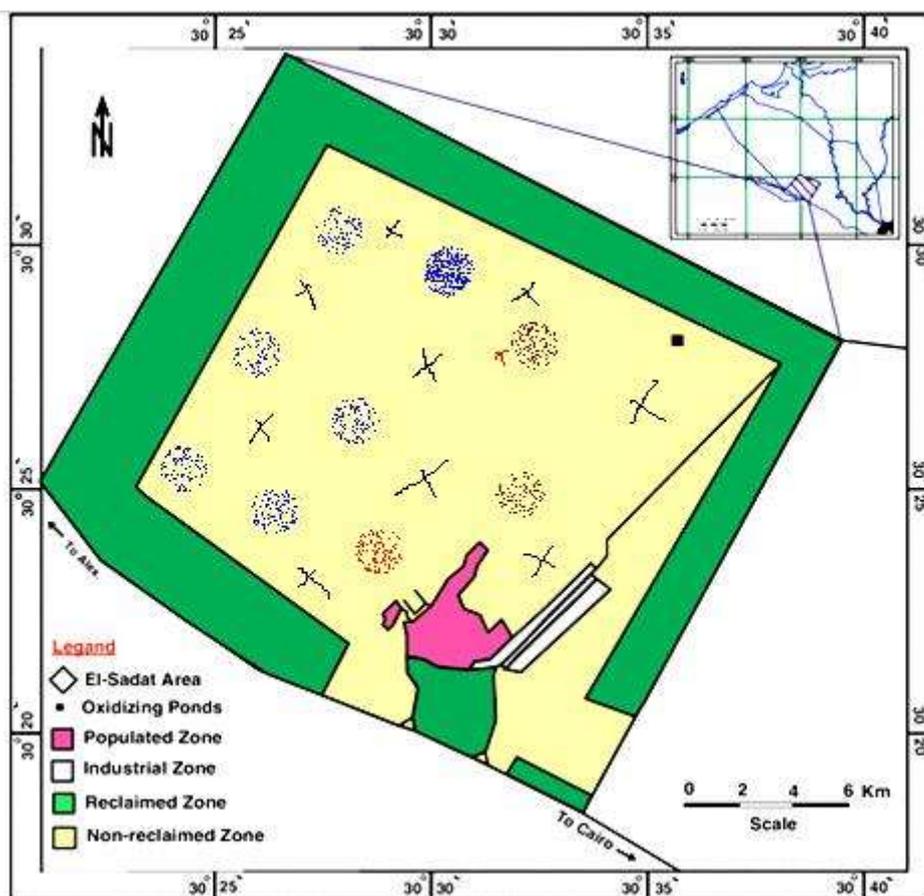
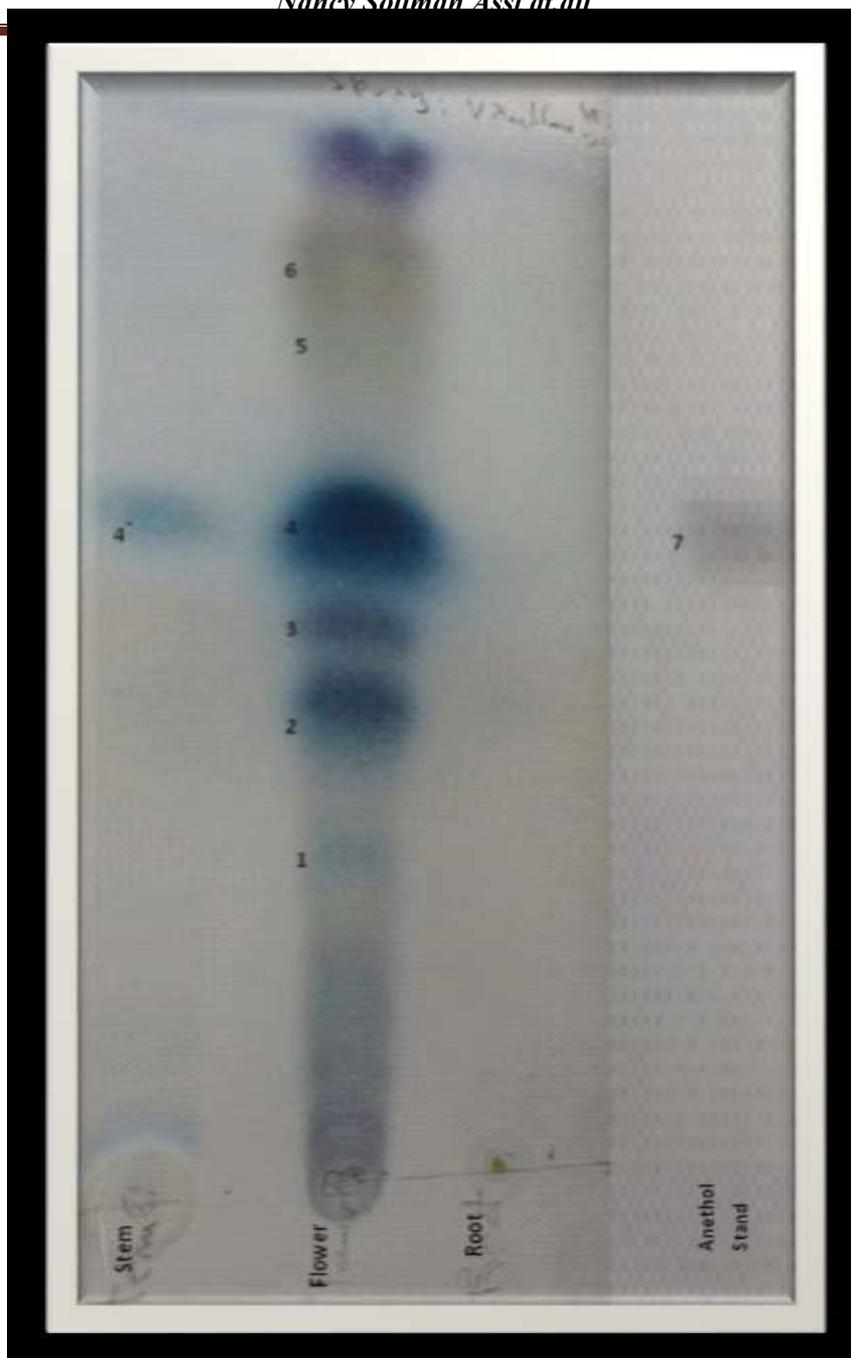


Figure 3: Sadat city map (Fattah, 2012)

(X sites of collection plants *Deverra tortousa*- non reclaimed zone)

Thin Layer Chromatography (Tlc) *Devera Tortusus* Essential Oil

Analysis was performed on 20 cm × 5 cm pre-coated aluminum-backed Silica gel 60-254 plates (Merck, Darmstadt, Germany). Before use, the plates were pre-cleaned by development with methanol and dried in fume hood. Standard (Artemisinin) and sample solutions were applied to the plates automated spray-on band applicator equipped with a 20- μ L syringe and operated with the settings band length 6 mm, application rate 10 s μ L⁻¹, distance between bands 1.5 cm, distance from the plate edge 1.5 cm, and distance from the bottom of the plate 17 cm.



(Fig.1). T.L.C Chromatogram of Steam distillation volatile oil (stem, flower and root) of *Deverra tortuosa*

Results and Discussion

Thin Layer Chromatography for *Deverra tortuosa* essential oil

The partitions on the TLC plate of the crude steam distillation volatile of the (stem, flower and root) are as shown subsequently. 6 spots were detected after spotting the volatile oil. Six spots were observed with naked eyes after spraying with 1% vanillin in sulphuric acid Fig.1, while two spots were observed under the UV light Fig.2. The Solvent system was (Toluene –Ethyl acetate 93-7), and the detection as following:

- Without chemical treatment, UV-254nm and UV-365nm.
- Using chemical treatment, Vanillin Sulphoric acid reagent, (Wagner et.al, 1984).

Spot no. 6 with vs reagent reveals that the characteristic main red brown zone (Rf. ca 0.95) of Methyl chavicol mixture.

Spot no 1 and 2 (blue green) with Rf 0.2 – 0.6.

Spots 3 and 4(violet and dark blue Rf 0.4 - 0.6) the violet red zone with conc H₂SO₄ may anisaldehyde and may be fenchone.

On the other hand the spot 4* of stem volatile oil is the same colour (blue and Rf 0.6 may be fenchone like spot 4 of the flower volatile oil.

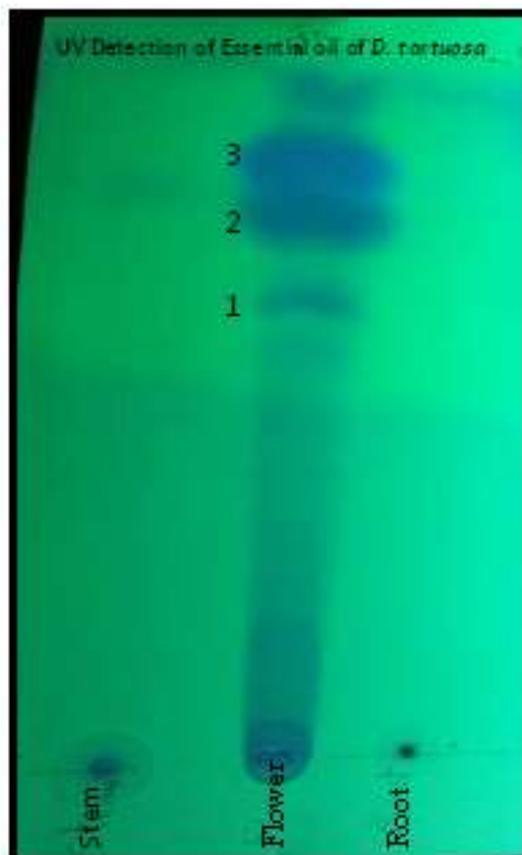


Fig.2. Thin Layer Chromatography for *Deverra tortuosa* essential oil UV Detection

The violet red zone with conc H₂SO₄ may anisaldehyde with UV 254nm.

(Wagner et.al, 1984).

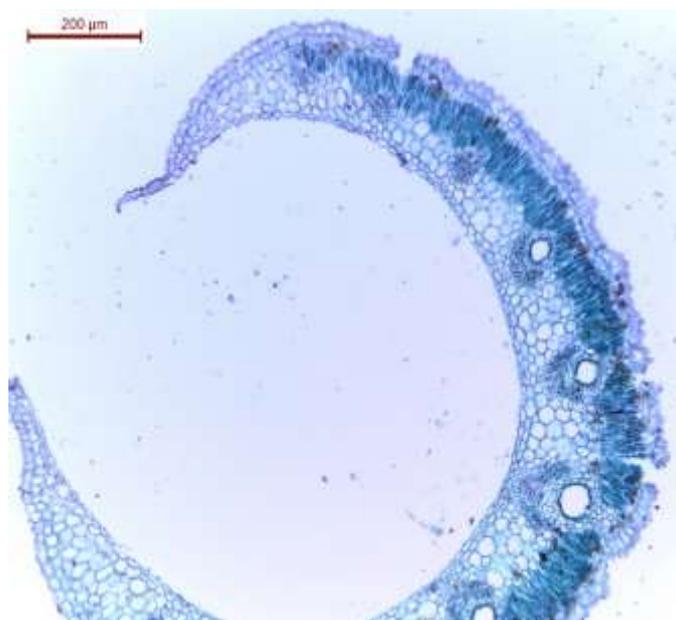
ANATOMY AND BOTANY

The distinguishing characters of stem were the presence of parenchyma, cork cells, irregular shape calcium oxalate crystals, simple and compound starch granules and fusiform fibers with pits. Root microscopic characters were presence of simple and spherical starch granules with rounded or slit hilum, groups of lignified xylem fibers, reticulate vessels, and sieve tissues. Leaves microscopy indicated the presence of paracytic stomata, lignified fibers having pits, spiral and annular vessels, numerous sclereids while in fruit microscopy epicarp, thin walled cells endocarp, thin walled parenchyma and collenchyma of mesocarp and abundant thick walled endospermic cells containing aleurone grains and micro rosette crystals.

Microphotographs showing anatomical elements of *Deverra tortuosa*



(a)



(b)

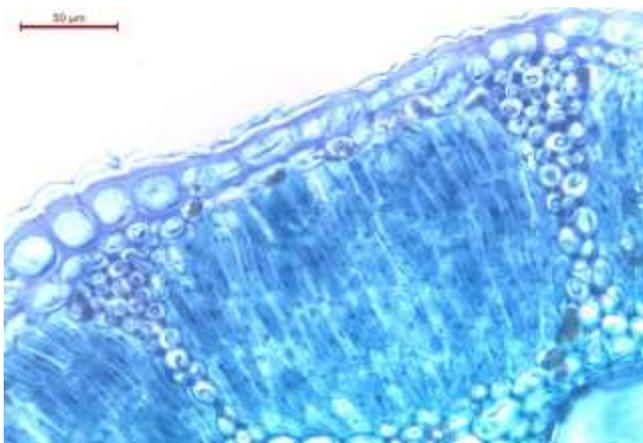


Fig3. (c)



Fig.3 (d)

Fig.3 General View of stem cross section (a, b)

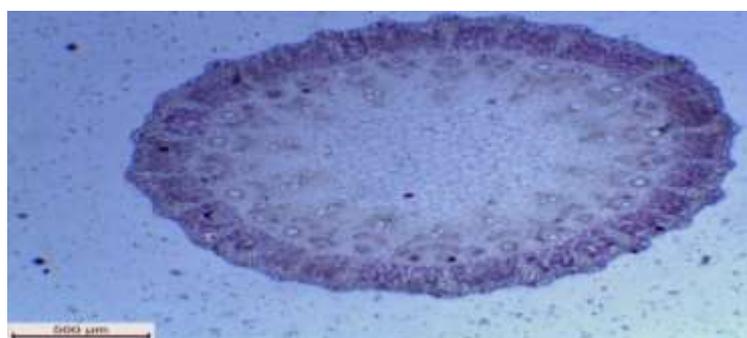
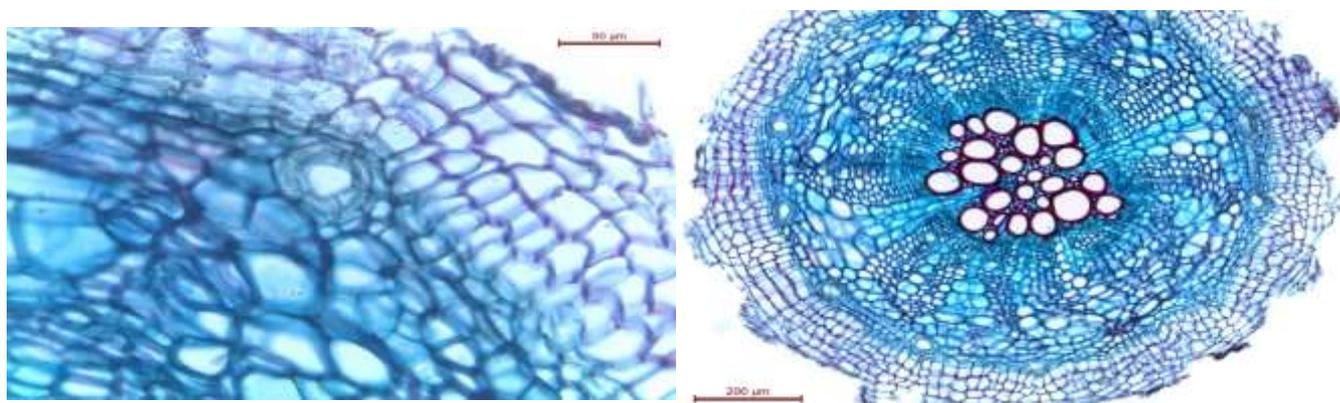


Fig.3 (e)

Fig.3 General View of stem cross section (c, d and e)

1. Primary vascular tissues principally comprising a ring of bundles
2. Medullary bundles
3. vessels very small to medium, reticulately perforated and The vessels without spiral thickening
4. The parenchyma paratracheal
5. Secretory cavities
6. ring of bundles of vascular tissue
7. Cortex is rather wide and consists of parenchymatic cells. Vascular bundles exist in groups and surrounded by sclerenchymatous cells.



(f)

(g)

Fig. 4 General View of root cross section (f and g)

Root Has an exodermis, which is not so very thick.

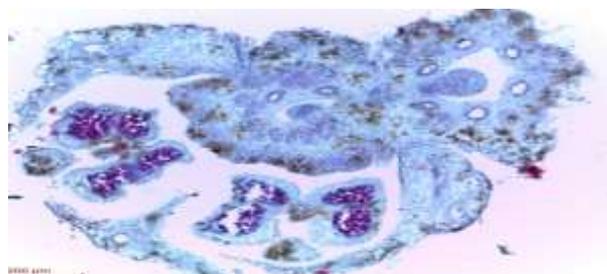
Cortex consists of parenchymatic cells and carries secretory canals which are small size.

The phloem does not carry secretory canals.

The cambium is distinguishable in the form of 1-2 layered.

Xylem elements extend towards the pith.

Trachea are small cells. The rays are distinguishable with 2-3 cells wide (Figure. 4 f and g).

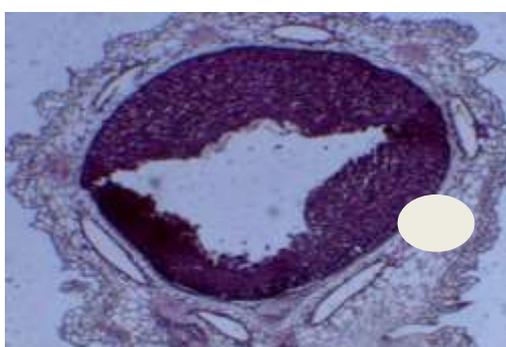
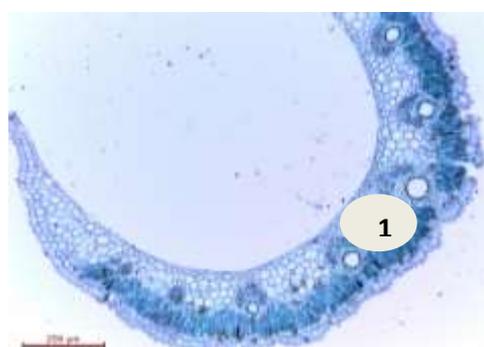
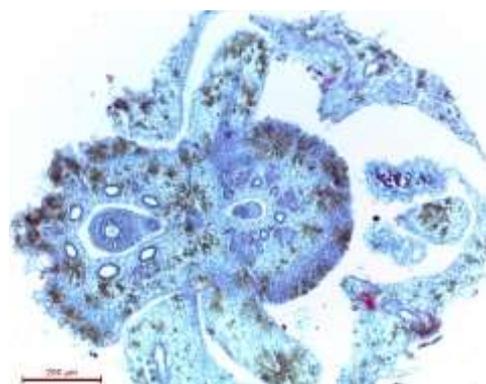
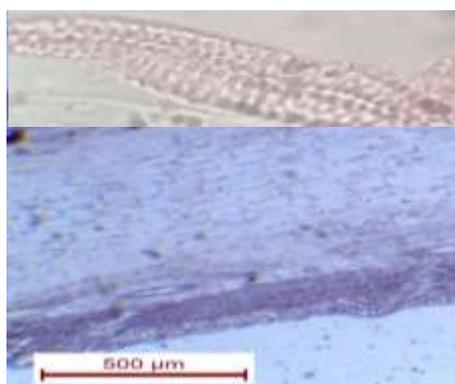


(h)

(i)

Fig. 5 General View cross section h and i of flower

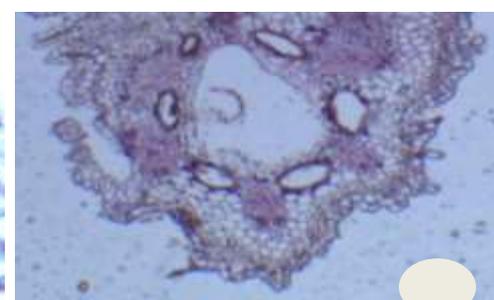
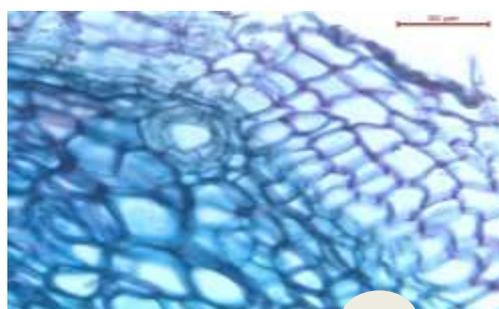
Microscopical examination of *Deverra tortuosa* plant powder and Transver section or stem, flower, leaf, fruit and root



4

5

6



7

8

9

- 1- Showing spiral vessels of wood
- 2- T.s of flower showing small; regular 5 merous (except for the gynoecium, ovary (1–) 2 locular. Gynoecium median, stamens 5, pollen grains, mostly (tri-) colpate
- 3- vascular elements in thick, cleared and stained tissue Fibers and trachieds, pollen grains
- 4- The mesophyll of the leaf consist of large paranchymatic cells, the secretory cavities containing oil, existing between the phloem and lower epidermis, secretory cavities containing oil,
- 5- T.s of fruit, endospermic
- 6- showing rostiac of calcium oxalate crystals

- 7- Root tissue, Xylem elements extend towards the pith
- 8- showing five vittae characteristic of Apiaceae members.
- 9- Dumbbell shaped pollen grains of *Deverra* with micro reticulate sculpture and tri colporate aperture.

We consider that the specific anatomical elements for the *Deverra tortuosa* species are represented by: aspect of pollen grains, crystallization state of calcium oxalate (rosittae), this has been demonstrated for the first time, also the anatomical study of *Deverra tortuosa* has not been done before.

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