

# Morphology and Histochemistry of the Glandular Trichomes of *Trigonella foenum-graecum* (Fabaceae)

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## ABSTRACT

Morphological studies of the trichome on stems of *Trigonella foenum-graecum* were carried out using light and scanning electron microscopy, concurrently to this their secreted material were also characterized using histochemical reagents. One type of non-glandular and two types of glandular trichomes were distinguishable. The non-glandular trichomes were uniseriate with warty surface whereas the glandular trichomes were capitate and divided into two types according to the shape of their secretory head. Both of the glandular trichomes displayed a high accumulation of alkaloids which could suggest that they are probably main accumulation sites. Results of histochemical tests confirmed that secondary metabolites including lipids, mucilaginous polysaccharides and phenolic compounds are present in glandular trichome. The glandular cells do not exhibit of that is involved in the secretion of terpenoids. The secretion of capitate was extruded through the rupture, micropore or channel the cuticle of the head cell.

Key words: *Trigonella foenum-graecum*, glandular trichome, morphology, histochemistry.

## INTRODUCTION

*Trigonella foenum-graecum* (Fenugreek) is an annual herb and one of the oldest known medicinal plants belonging to the family Fabaceae (Faboideae) is commonly used as traditional food, nutraceuticals and medicine (Acharya et al., 2008). Fenugreek have a strong aroma and somewhat bitter in taste. The whole aerial parts of plant are edible and are used as condiments and as folk medicine for treat diabetes, high cholesterol, inflammation, and gastrointestinal ailments (Srinivasan, 2006).

The multi-functional activities of fenugreek have been attributed to diverse chemical constituents of its stem, leaf and seeds such as alkaloids (trigonelline), terpenoids (diosgenin), amino acid 4-hydroxyisoleucine and various phenolic compounds (Thomas, 2011). Secretory structures are known to be primary sites of secondary metabolite biosynthesis, secretion and storage (Weiss, 1997). A great variety of important chemical compounds are produced by specialized secretory cells, on many plants in the form of glandular trichomes, which are involved in an array of functions (Fahn, 2000). Glandular trichomes are an important source of essential oils, *i.e.*, natural fragrances or products that can be used by the pharmaceutical industry, although many of these substances have evolved to provide the plant with protection against herbivores and pathogens

(Duke et al., 2000). Two main types of glandular trichome, peltate and capitate, occur which can be distinguished by head size and stalk length. As a rule, in acapitate trichome, the length of the stalk should be more than half the height of the head (Abu-Asab and Cantino, 1987), whereas peltate trichomes are short with a uni- orbicellular stalk and a large secretory head with four to 18 cells arranged in one or two concentric circles. Unlike peltate trichomes, that have a rather uniform morphology, capitates trichomes differ in terms of morphological characters, and can be subdivided into various types (Werker et al., 1985). The storage compartment of glandular trichomes usually is located on the tip of the hair and is part of the glandular cell, or cells, which are metabolically active (Fahn, 2000). Trichome secretions may have economic value, e.g. the bulk of the essential oil of *Mentha piperita* occurs in peltate trichomes (Sharma et al., 2003). Moreover, artemisinin is produced in the glandular trichomes of *Artemisia annua*, is used for the treatment of malaria (Weathers et al., 2011). In addition, gossypol produced by *Gossypium hirsutum* trichomes, have strong antifungal activity (Mollen et al., 2012) and are potential natural pesticides (Dayan and Duke, 2003). It is for these kinds of specialized metabolic properties, and for the opportunities to modify these properties via genetic engineering, e.g., (Lange et al., 2011), that trichomes have received increased attention over the past years (Tissier, 2012). Furthermore, the locality of secondary metabolites important in medicinal applications needed to be ascertained. This information would be useful in deciding the protocol required for isolation of such compounds. Most studies on glandular trichomes apply histochemical methods (Ascensao et al., 1999; Bottega and Corsi, 2000; Combrinck et al., 2007; Liu et al., 2011) because they are considered useful for an initial investigation of the presence of main chemical classes of metabolites present in plant secretions.

Many phytochemical studies have been carried out for identify chemical constituents of fenugreek (Warshney and Beg, 1978), but in terms of the histochemical and distribution these substances in various tissues has not yet been reported. In this work, morphology and histochemical test are applied for first time to the stems glandular trichomes of *T. foenum-graecum*. Identification of these compounds in fenugreek glandular trichomes and the application of these information for commercial exploit ion of this available external storage compartment and to open future investigation on medicinal implications of their products, is the focus of this research.

## MATERIALS AND METHODS

### *Plant material*

Fresh aerial plant parts of *T. foenum-graecum* were collected in during the vegetative growth period prior to the flowering period from plant growing in the experimental field of Tarbiat Modares University, Tehran, Iran (35° 43' N, 51° 22' E, 1283.7 m), (pH, 7.4). For the purpose of these investigations, stems were selected at the second or third internode from the shoot apex.

### *Microscopical investigations*

For light microscopy (LM), free-hand transverse sections of stems were prepared and mounted in glycerin on glass slides, and then examined with an Olympus BH2 light microscope. While for SEM, small pieces of stems were fixed in FAA (formalin, acetic acid, 70% alcohol) for 24 h, stored in 70% ethanol (Johansen 1940). After dehydration in a graded ethanol series, the material

was critical point dried with CO<sub>2</sub>, sputter-coated with a thin layer of gold and, finally, examined in a KYKY-EM 3200 scanning electron microscope at 26 kV.

### ***Histochemistry***

Free hand-section of fresh stem tissue were subjected to the following histochemical test: Sudan Black B (Lison, 1960) for total lipids ; NileBlue A (Jensen, 1962) for neutral and acidic lipids; Acetone- Nile Blue ((Dunnigan, 1968) for phospholipids; Nadireagent (David and Carde, 1964) for essential oils; concentrated H<sub>2</sub>SO<sub>4</sub> (Cappelletti et al., 1986) for sesquiterpenes; antimony trichloride (Hardman and Sofowora, 1972) for terpene-containing steroids; Dragendorffreagents (Svendsen and Verpoorte, 1983) for alkaloids; periodic acid-Schiff (PAS) reagent (Jensen, 1962)for polysaccharides; Ruthenium Red (Johansen, 1940)for pectins; potassium dichromate (Gabe, 1968) and ferric trichloride (Johansen, 1940), for phenolic compounds. Flavonoids were detected by Neu's reagent (Neu, 1957) under UV. Standard control procedureswere carried out simultaneously.

## **RESULTS**

### ***Morphology and distributions of the trichomes***

Morphological studies revealed one type of non-glandular and two types of glandular trichomes on the stems of *T. foenum-graecum*. Trichomes areoften curvedleaning towards the organ tip and the long axissometimes roughly parallel with the subtending surface(Fig. 1A). Non- glandular trichomes are uniseriate, multicellular (Fig. 1B)thatconsists ofprotruding basal cell,uni or multicelled stalk (Fig. 1E)and a long pointed apical cell with tuberculate surface (Fig. 1F). Two types of glandular trichomes are reported both of them are capitate, which can be divided into two subtypes according to the morphology of the glandular head. Clavate trichomes have an oval or ovoidhead, sometimes described as club-shaped. (Fig. 1C, G). Elongated trichomes possess a long and thin head, and stalk roughly of the same size as compared to the base of the head (Fig. 1D, H).In both of the glandular trichomes, multicellular headare supported by one to three short or long stalk cells and a prominent basal cell.The heads of the gland are uni- or multilayered, with one to four cells in each layer.In contrast to non-glandular trichomes, the surface of glandular trichomes is smooth and lack a micro-ornamentation.

Emergent stems aredensely coverd with trichomes that non-glandular trichomes partially obscured the glandular trichomes (Fig. 2A) and it appeared they matured at in early stage of stem development. These trichomes are also found in the young (Fig. 2B) and mature (Fig. 2C) stems, but their distribution frequency is lower in comparison to the emergent stems. Distribution of glandular trichomes is un-uniform and clavate glandular trichomes are more frequent than elongated trichomes on the stems surfaces (Fig. 2B).

### ***Secretion mode***

The clavate and elongated trichomes display similar secretory behavior. Pressure exerted by the secretion caused the cuticle separate from the underlying cell wall, creating a subcuticular space in the gland apex. The secretion release occur through micropore (Fig. 3A) and repture (Fig. 3B)

in the cuticle covering the head cells. As well as, it is possible that secretion rise through the trichome<sup>s</sup> channel to the top and subsequently released (Fig. 3C and D). Following the secretion release, shrinkage of the head cells generally observed in mature stems, and then glandular cells degenerate.

### **Histochemistry**

The secretion of both type glandular trichomes stained positively for lipophilic and hydrophilic substances (Table 1). Polysaccharides were present in the head cell, indicated by light pink color after using PAS (Fig. 4A, B). Ruthenium Red showed mucilaginous polysaccharides within the head cells, which became intensively red (Fig. 4C). Staining with Sudan Black B showed total lipid in basal cell, stalk and head cells. The secretory product can be observed stained in dark blue to black with Sudan Black B (Fig. 4D). Acid lipids indicating by a blue color with Nile blue in secretory cells (Fig. 4E). Dragendorff reagent gave a strong positive response only for glandular trichomes (Fig. 4L), showing intense reddish brown coloration of the head cell cytoplasm and secreted droplets on the subcuticular space (Fig. 4J, K); these result clearly indicated the presence of alkaloids. Phenolic compounds were evidenced by a black color when stained with ferric trichloride (Fig. 4M) or by brown color with potassium dichromate. Flavonoids stained yellow with Neu' s reagent under UV light (Fig. 4O). The histochemical tests carried out to detect terpenoids gave negative results (Fig. 4G, H), except for stalk cell of non-glandular trichomes which reacted positively for terpene-containing steroids; the test with conc. H<sub>2</sub>SO<sub>4</sub> is shown (Fig. 4I).

### **DISCUSSION**

The trichomes observed in *T. foenum-graecum* are morphologically similar to other trichomes described in the Faboideae. The stems surface of this plant is covered by one type of non-glandular trichome and two types of capitate glandular trichomes. The non-glandular trichomes are uniseriate, multicellular, with long apical cell. The glandular trichomes lack typical capitates trichomes which is consistent with what was previously reported by Werker (2000) The surface of the glandular trichomes is smooth, while the non-glandular trichomes covered by warty ornamentation.

The trichomes, with similar morphology, reported for several genera within Faboideae; *Medicago* sp. (Aziz *et al.*, 2005; Ragnerand Hower, 2001; Danielson *et al.*, 1989), *Trifolium* sp. (Willson and Retalack, 1988; Gupta and Murty, 1977), and *Lathyrus* sp.

Morphological studies revealed a high distribution of trichomes on the emergent stems. As the stems expands, the density of both non-glandular and glandular trichomes decreases progressively. It appeared that the high distribution of trichomes may provide a protective barrier on emergent stems which are more susceptible to biotic and abiotic pressures (Fernandes 1994; Ascensao *et al.*, 1999).

The observations made in the present work revealed the secretion of both trichomes types is released through micropore and cuticular repture, mainly due to the pressure exerted by the copious amounts secretion in the head cells. In addition, cuticle with its channels, could allow the

release of secretion components. The accumulation of secretion in a subcuticular space and its release following the micropore or breaking of the cuticle is common feature of many glandular trichomes (Nadio *et al.*, 2014; Ciccarelli *et al.*, 2007; Ascensao *et al.*, 1999; Werker *et al.*, 1993, 1985; Bruni and Modensi, 1983). In *Caesalpinia crista* (Fabaceae), secretions rise through the channel, either by a permeable cuticle or the trichome breaks (Diaz-Castelazo *et al.*, 2005).

Fenugreek as a medicinal plant, is a source of valuable constituents such as trigonelline (a pyridine alkaloid with therapeutic properties), and diosgenin (a steroid saponin, is a precursor of various synthetic steroidal drugs) (Radwan *et al.*, 1980; Djerassi *et al.*, 1952). One of the most remarkable features of glandular trichomes is their capacity to synthesize and store varied types of compounds which have significant commercial value. Detailed descriptions of trichomes are available in the literature for many commercially important genera, e.g. *Ceratotheca* (Nadio *et al.*, 2012), *Lippia* (Combrinck *et al.*, 2007), *Origanum* (Bosabalidis, 2002), *Pelargonium* (Oosthuizen and Coetzee, 1983) and *Cannabis* (Furr and Mahlberg, 1981). There is a lack of information on the histochemical aspects of trichomes of *Trigonella* species. Combination of staining and microscopy techniques provided valuable leads to the characteristics of main chemical classes of possible medicinal compounds produced by *T. foenum-graecum*.

These histochemical results indicate that capitate trichomes of *T. foenum-graecum* produced a heterogeneous secretion, containing polysaccharides, pectin, lipids, alkaloid and phenolic compounds but not reducing sugars, and terpenoids. Staining with PAS and Ruthenium Red indicated that the head cells of capitate trichomes secrete copious amounts of polysaccharides. The presence of these substances could act as an energy source (Gang *et al.*, 2001) and may have a lubricant role to facilitate the expansion of the organ in which they are located (Modenesi *et al.*, 1984).

Different types of histochemical tests for detecting lipids indicate the presence of fatty acids, but the phospholipids and natural lipids are absent in the secretions. Many specialized metabolites in trichomes are derived from fatty acids or have fatty acid moieties. Analysis of type VI trichomes of tomato (*Lycopersicon hirsutum*, Solanaceae) showed that the genes encoding enzymes of fatty acid biosynthesis in the plastids are highly expressed (Fridman *et al.*, 2005). Ranger *et al.* (2005) have reported that the stem trichomes of an alfalfa (*Medicago sativa*, Fabaceae) line that is resistant to potato leafhopper contain a series of saturated C14, C15, C16 and C18 fatty acids, and that these compounds may contribute to the observed resistance.

One of the most important compounds in fenugreek is alkaloids. Histochemical analyses with free-hand transverse sections of stems showed that alkaloids are absent from other tissues other than capitate trichomes and also appear to be the site of synthesis and/or storage of alkaloids. This constitutes a new finding for glands in the Fabaceae. Although further research is needed to evaluate their site of synthesis. Alkaloids are under-represented among specialized metabolites found in glands, and there have been no reports on the *de novo* synthesis of alkaloids in glands. However, tobacco species are known to contain the toxins nicotine and related alkaloids. Nicotine itself is synthesized in the roots and is mobilized to all aerial parts of the plant, including trichomes, upon herbivory (Zador, 1986).

The occurrence of polyphenols in the glandular trichomes was indicated by the positive reaction with ferric trichloride and potassium dichromate test. The only class of phenolic compounds histochemically identified in these trichomes, by fluorochromes under UV light, was the flavonoids. These results are consistent with the phytochemical data obtained from other analyses of the *T. foenum-graecum*. Three flavonoid glycosides were already isolated and

identified instems of this plant (Han et al., 2001). Approximately 8,000 phenolic compounds were already identified in plants, having biological role generally related to antifungal, antibacterial and antifeedant activities of some structures (Harbone 1997).

The lack of terpenoids (essential oils, steroids and sesquiterpenes) in the exudates confirm that the glandular trichomes on the stems of *T. foenum-graecum* are not involved with either terpenes biosynthesis or terpenes secretion, respectively. Only stalk cell of non-glandular trichomes reacted with conc. H<sub>2</sub>SO<sub>4</sub> for sesquiterpenes, which seems probable the site of this compounds accumulation. Aziz et al. (2005) reported in glandular trichomes of alfalfa no expressed sequence tag corresponded to enzymes of cyclized terpenoids biosynthesis.

## Conclusions

The stems of *Trigonella foenum-graecum* bear uniseriate non-glandular and two types of capitate glandular trichomes, namely the clavate and elongated glandular trichomes. Secretory products of capitate glandular trichomes consisted of polysaccharides, lipids, phenolic compounds and alkaloids. In summary, the results of histochemical study of the secretory product indicate that the capitate trichomes of *T. foenum-graecum* secrete compounds that are medicinally important and may be useful commercially. The results regarding morphological characteristics of glandular trichomes and histochemical analysis could be applied in the studies of their medicinal properties. Future studies should focus on isolating and quantifying the compounds in the secretory product.

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