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Research article/Araştırma makalesi

Pharmacognostic value of leaf anatomy and trichome morphology for identification of forskolin in a novel medicinal plant *Coleus forskohlii*

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Abstract

Forskolin, a labdane diterpene is the active principle of the indigenous medicinal plant *Coleus forskohlii* Briq**.** It is the only known natural source of the diterpenoid forskolin. Forskolin has a unique property of activating almost all hormone sensitive adenylate cyclase enzymes in a biological system. The pharmacological and biochemical investigations established that forskolin possesses multifaceted biological activities. This research paper have highlighted on phytochemical vis-a-vis histochemical localization of other terpenoids and forskolin in leaves of *C. forskohlii.* Qualitative analysis showed that terpenoids were present in leaves of the herb. Histochemical analysis of leaves of *C. forskohlii* shows that forskolin was found in the cells of palisade parenchyma, spongy parenchyma, and glandular trichomes of leaf in both the upper and lower epidermis. Thin layer chromatography of chloral hydrate washings showed presence of other terpenoids and forskolin in leaves of *C. forskohlii.* Rf value of forskolin was 0.6 and Rf values of other terpenoids were 0.62, 0.66, 0.86. This confirms that the yellowish-violet coloured vesicles seen in the sections of leaf contain the terpenoids. The leaves of the Indian drug plant are needed very badly to identify, isolate, design, develop, modify or to prepare new pharmacologically active compounds from the other terpenoids than forskolin. The mechanisms of action of various secondary metabolites isolated from this potential medicinal herb are yet to be elucidated. Leaves are significant ($p<0.05$) richer sources of antioxidant comparable with roots or tubers. The leaves can be used in place of tubers because they are significantly potent comparable with tubers and also we can ensure a continuous supply of the leaves without uprooting the plant for the tubers. This new finding of getting forskolin from leaves rather than sacrificing whole plant and tuber will help to conserve dwindling population of endangered important medicinal plant *C. forskohlii* vis-à-vis sustainable use of forskolin.

Key words: *Coleus forskohlii*, Forskolin, Phytochemical analysis, Leaf anatomy, Trichome morphology

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Yeni keşfedilen *Coleus forskohlii* tıbbi bitkisinde forskolinin teşhisi için yaprak anatomisi ve tüy morfolojisinin farmakognozik değeri

Özet

Labdan yapılı diterpen olan forskolin, doğal olarak yetişen tıbbi bir bitki olan *Coleus forskohlii* Briq**.'**in etken maddesidir. Bilinen tek doğal diterpenoid forskolin kaynağıdır. Forskolin, bir biyolojik sistemdeki hemen hemen bütün hormona duyarlı adenilat siklaz enziminlerini aktive etme özelliğine sahiptir. Farmakolojik ve biyokimyasal çalışmalar forskolinin çok yönlü biyolojik aktivitelere sahip olduğunu göstermiştir. Bu çalışmada *C. forskohlii* yapraklarında diğer terpenoidlerin ve forskolinin histokimyasal ve fitokimyasal lokalizasyonu karşılıklı olarak açıklanmıştır. Yapılan nitel araştırma, bitkinin yapraklarında terpenoid bulunduğunu göstermiştir. *C. forskohlii* bitkisinin yaprakları üzerinde yapılan histokimyasal inceleme palizat parenkiması, sünger parenkiması hücrelerinde ve hem üst hem alt epidermisteki glandüler tüylerde forskolin bulunduğunu göstermiştir. Kloral hidratla yıkanan (materyallerin) ince tabaka kromatografisi *C. forskohlii* yapraklarında forskolin ve diğer terpenoidlerin bulunduğunu ortaya koymuştur. Forskolin için Rf değeri 0.6; diğer terpenoidler için Rf değerler ise 0.62, 0.66, 0.86 olarak bulunmuştur. Bu sonuç, yaprak

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seksiyonlarında görülen sarımsı-mor renkli keseciklerin terpenoid içerdiğini doğrulamaktadır. Forskolin dışında diğer terpenoidlerden farmakolojik olarak aktif bileşiklerin teşhis edilmesi, izole edilmesi, tasarlanması, geliştirilmesi, değiştirilmesi veya hazırlanması için Hindistan da tıbbi bitki yapraklarına büyük ölçüde ihtiyaç duyulmaktadır. Bu potansiyel tıbbi bitkiden izole edilen çeşitli sekonder metabolitlerin etki mekanizmaları henüz açıklığa kavuşmamıştır. Kök ve yumrulara kıyasla yapraklar önemli ölçüde daha zengin antioksidan kaynağıdır ($p<0.05$) Yapraklar yumrulara göre önemli ölçüde etkili oldukları için yumru yerine kullanılabilir. Ayrıca, yumru elde etme amacıyla bitkiyi kökünden sökmeden sürekli olarak yaprak elde edilebilmektedir. Bitkinin tamamının veya yumrularının feda edilmeden yapraklardan forskolin elde edilmesi ile ilgi bu yeni bulgu, sayıları giderek azalan soyu tükenmekte olan önemli bir tıbbi bitki olan *C. forskohlii*'nin sürdürülebilir şekilde forskolin kullanımına karşılık korunmasına yardımcı olacaktır.

Anahtar kelimeler: *Coleus forskohlii,* Forskolin, Fitokimyasal analizi, Yaprak anatomisi, Tüy morfolojisi

1. Introduction

Throughout history, humans have derived many uses and benefits from the plants found in their own region. Initially, wild plants were collected from their natural habitat, followed by the cultivation of those that were used most commonly (Akan et al., 2008). Today the value of the plants is acknowledged and a number of studies are conducted on the plants. There is a growing body of research particularly concentrating on taxonomy, ethnobotanics, plant morphology, anatomy and plant chemistry (Kıvcak et al., 2009; Cabi et al., 2010; Duran et al., 2010; Koyuncu et al., 2010; Bani et al., 2011; Cakilcioglu and Civelek, 2011; Jabeen and Aslam, 2011; Korkmaz and Ozcelik, 2011; Ozudogru et al., 2011).

Forskolin, a labdane diterpene is the active principle of the medicinal plant *Coleus forskohlii* (Willd.) Briq. [synonym *C. barbatus* (Andr.) Benth.] of family Lamiaceae is an ancient root drug of Indian origin in Ayurvedic material medica (Valdes et al., 1987; Shah, 1996; Shan et al., 2008). The tuberous roots of the plant produce labdane diterpenoid forskolin. Forskolin has a unique property of activating almost all hormone sensitive adenylate cyclase enzymes in a biological system (De Souza and Shah, 1888). Forskolin is reported to be useful in the treatment of asthma (Lichey et al., 1984), glaucoma (Caprioli, 1984), hypertension (Dubey et al., 1981; De Souza et al., 2006), cancer (Agarwal and Parks, 1983; Bhat et al., 1993; Li and Wang, 2006), heart diseases (Kramer et al., 1987), diabetes (Ammon and Muller, 1984; Gold et al., 1988), and obesity (Allen, 1986). It also showed inhibition of platelet activating factor (Nourshargh and Hoult, 1986), increase in the rate of sensory nerve regeneration in freeze-lesioned sciatic nerves (Kilmer and Carlsen, 1984), stimulation of water and cation permeability in aquaporin 1 water channels (Yool et al., 1996) and direct alteration of gating of a single class of voltage-dependent potassium channels from a clonal pheochromocytoma (PC12) cell line independent of adenylate cyclase activation (Hoshi et al., 1988). It is an important plant used against various disorders in indigenous systems of medicine such as anti-aging, antioxidant (Khatun et al.2011) as a remedy for heart, abdominal and respiratory disorders. In addition, it has been shown to have anti-inflammatory property (Rupp et al., 1986). In Egypt and Africa, the leaf is used as an expectorant, emmenagogue and diuretic. In Brazil, it is used as a stomach aid and in treating intestinal disorders (Valdes et al., 1987). *C. forskohlii* is the only source for this compound. Indiscriminate collection of tuberous roots of *C. forskohlii* has led to rapid depletion of wild populations resulting in its listing as a plant vulnerable to extinction in India (Gupta, 1988). However, to our knowledge, phytochemical vis-a-vis histochemical localization of forskolin and other terpenoids in leaves of *C. forskohlii* is not reported so far. This prompted us to analyze phytochemical vis-a-vis histochemical localization of other terpenoids and forskolin in leaves of *C. forskohlii.*

2. Materials and methods

The fresh and healthy leaves of *Coleus forskohlii* (Willd.) Briq. [synonym *C. barbatus* (Andr.) Benth.] were collected from the experimental medicinal garden of the Department of Botany, The University of Burdwan University, Burdwan, India that have been cultivated of 30×30 cm spacing in field condition with soil pH 5.6; organic carbon 0.6%, phosphorus content 45 ppm (Figure 1). The plant materials were processed and analyzed. *C. forskohlii* field was photographed using a digital camera (Canon Power Shop S5IS, USA Inc.) having 8.0 mega pixels of 12 X optical zoom.

2.2. Processing of plant samples

The leaves of this plant are properly washed in tap water and then rinsed in distilled water. The rinsed leaves were dried in an oven at a temperature of 35–40 °C for 1 day. The dried leaves of this plant were pulverized, using a sterile electric blender, to obtain a powered form. The powdered form of leaves of this plant was stored in airtight glass containers, protected from sunlight until required for analysis.

2.3. Preparation of aqueous extract of plant samples

The aqueous extract of this plant sample was prepared by soaking 10 g of powdered leaves sample in 200 ml of distilled water for 12 h. The extracts were then filtered using Whatman no. 1 filter paper.

Figure 1. *Coleus forskohlii* (Willd.) Briq. of family Lamiaceae

2.4. Qualitative analysis on phytochemical constituent

Chemical tests were conducted on the aqueous extract of leaves sample and also of the powdered form of the plant samples using standard methods (Edeoga et al., 2005).

2.5. Histochemical localization of other terpenoids and forskolin

Hand-sections of fresh leaves of *C. forskohlii* were cut and observed Lieca Bright field microscope. Forskolin is reported to give violet colouration with 10% vanillin in acetic acid and perchloric acid, which has been used as a spectrophotometric method for detection and quantification (Inamdar et al., 1984). This colour reaction was tried directly on transverse sections of the leaves of *C. forskohlii*. Sections of the leaves of *C. forskohlii* were first placed in 2 ml of 10% vanillin in acetic acid to which 2–3 drops of perchloric acid (70%) was added and placed on water bath (70 ^{o}C) for 2–3 min (Abraham et al., 1988; Narayanan et al., 2002; Khatun et al., 2010). It was found that yellowish-red masses were stained violet and photographed using a bright field microscope (Lieca DFC295, version V3, Germany). In another study the sections of leaves of *C. forskohlii* were cleared with 75% chloral hydrate solution for 2 h. These sections were then stained with the reagent (10% vanillin in acetic acid and perchloric acid) as above. They did not get stained, indicating that the terpenoids have been washed away by choral hydrate. Thin layer chromatography (TLC) of choral hydrate (75%) washings of these sections was done using standard forskolin (HiMedia Chemicals, Mumbai, India).

2.6. Thin layer chromatography (TLC)

The thin layer separation was carried out using precoated TLC plastic sheets of $60F_{254}$ silica gel (Merck Chemicals, Mumbai, India). TLC plate size 20 x 20 cm and toluene: ethyl acetate (80:20, v/v) solvent system by using sample with capillary tube and Rf values were compared with standard forskolin (HiMedia Chemicals, Mumbai, India). After developing, the plate was sprayed with anisaldehyde sulphuric acid reagent (1 ml concentrated H_2SO_4 is added to 0.5 ml anisaldehye in 50 ml acetic acid) and heated at 100–105 °C and the Rf values calculated.

3. Results and discussion

3.1. Qualitative analysis on phytochemical constituent

Qualitative analysis showed that terpenoids were present in leaves of *Coleus forskohlii.* TLC profile of *Coleus forskohlii* leaves extract confirmed the presence of forskolin which is a major bioactive compound isolated from the leaf. Engprasert et al., (2004) proposed that froskolin is synthesized from isopentenyl-diphospate, a common biosynthetic precursor via a non-mevalonate pathway by geranyl geranyl pyrophosphate synthase is thought to be involve in the biosynthesis of forskolin which is primarily synthesized in the leaves and subsequently in the stem and root.The mevalonate pathway occours in the cytoplasm and an alternative mevalonate -independent pathway occurs in plastid (Rohmer et al., 1996).

3.2. Histochemical localization of other terpenoids and forskolin

Histochemical observations and analyses of leaves of *Coleus forskohlii* proved that forskolin was found in the cells of palisade parenchyma, spongy parenchyma and glandular trichomes of leaf in both the upper and lower epidermis (Figure 2, 3, 4). Transverse sections of leaves of *C. froskohlii* stained with 10% vanillin- perchloric acid revealed accumulation of compound terpenoid in nature are probably the site of froskolin accumulation in the palisade and spongy parenchyma tissues as brown colour. It is keeping with the current trend to use *in vivo* staining technique in morphology research because it is useful to link glandular morphology and chemical functionality in *Lippia scaberrima* (Combrinck et al., 2007) and in *Exocaria agallocha* (Satyan et al., 2010).

TLC of chloral hydrate washings showed presence of forskolin and other terpenoids in leaves of *C. forskohlii.* Rf value of forskolin was 0.6 and Rf values of other terpenoids were 0.62, 0.66, 0.86. This confirms that the yellowish-violet coloured vesicles seen in the sections of leaf contain the terpenoids. These histochemical results confirm earlier observations of presence of terpenoid phytochemically in stem of *Coleus forskholii* (Menon and Latha, 2011) and leaves of *C. aromaticus* (Rout et al., 2010). Our histochemical observations regarding forskolin present in leaves of *C. forskholii* validate the proposed idea of froskolin primarily synthesised in leaves Engprasert (2004) and in plastid (Rohmer et al., 1996).

In spite of the tremendous promise *C. forskohlii* holds in, medicinal formulation, very few attemts have been made for its replenishment on cultivation and it is currently listed as one of the threatened plant species vulnerable to extinction in India (Gupta, 1988; Sharma et al, 1991; Krishna et al, 2010) due to unsustainable use of this important, significant potential medicinal crop of the future with its therapeutic properties being scientifically authenticated recently (Kavitha et al, 2010; Khatun et al. 2011a). Better understanding of cellular effect is vital to properly utilize the phytochemicals,as promising agents for promoting health and preventing disease.

The leaves of this Indian drug plant are needed very badly to identify, isolate, design, develop, modify or to prepare new pharmacologically active compounds from the other terpenoids than forskolin. The mechanisms of action of various secondary metabolites isolated from this potential medicinal herb are yet to be elucidated. Our previous work (Khatun et al., 2011) showed that leaves are significant ($p<0.05$) richer sources of antioxidant comparable with roots or tubers. This throws open very exciting possibilities because, when the roots or tubers are used, the plant needs to be uprooted and therefore, more plants are needed to meet the medicinal demand. As the leaves of *C. forskohlii* contain terpenoid vis-à-vis forskolin – this allows prediction that the medicinal use of leaves is a valid option for sustainable use and conserving this novel potential medicinal plant which is threatened and vulnerable to extinction. This new finding of getting forskolin from leaves rather than sacrificing whole plant and tuber will help to play an important role in quality control and prevention of adulteration as well as to conserve dwindling population of endangered important medicinal plant *C. forskohlii* by its sustainable use.

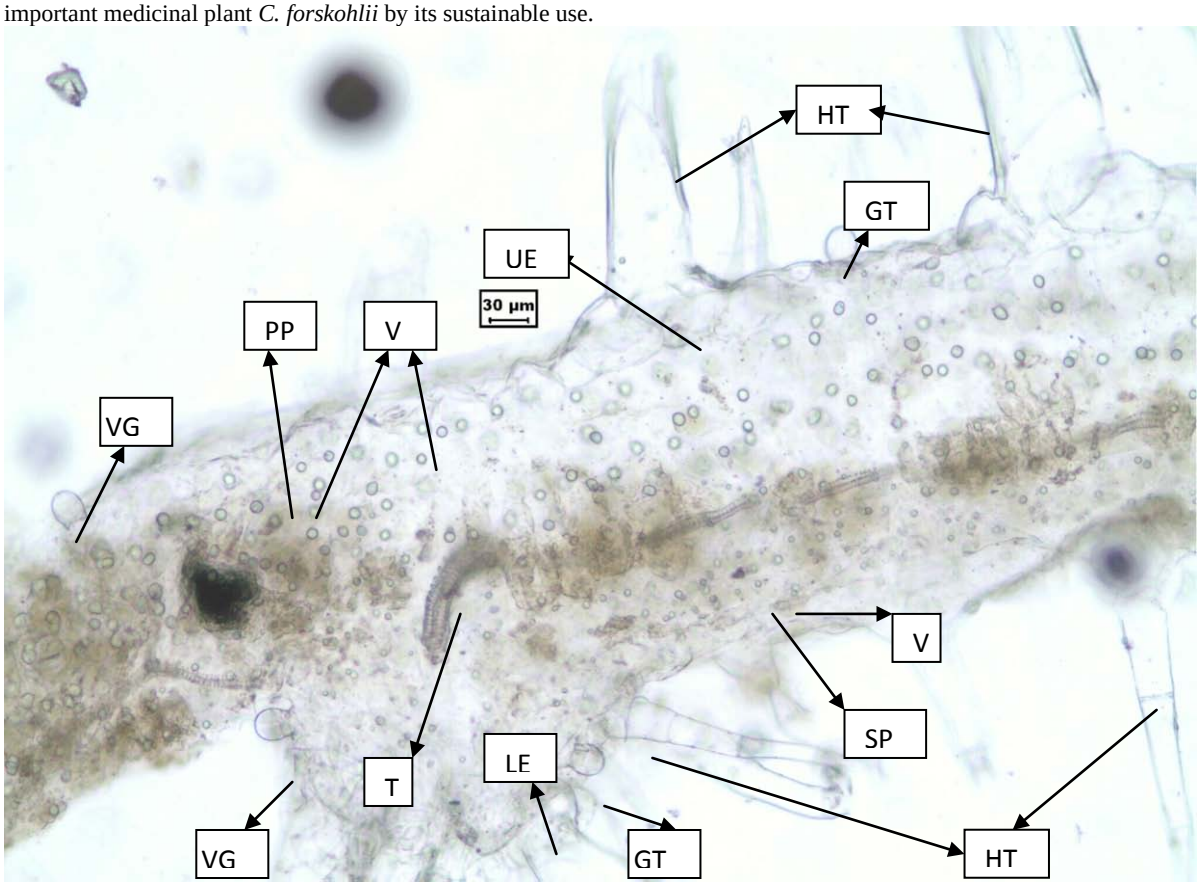

Figure 2. Transverse section of leaf of *C. forskohlii*. Violet stained vesicles were observed within the cells of palisade parenchyma, spongy parenchyma, and glandular trichomes of leaf in both the upper and lower epidermis of *C. forskohlii* (30µm) (**UE:** Upper epidermis, **LE:** Lower epidermis, **HT:** Hairy trichome, **GT:** Glandular trichome, **V:** Violet stained vesicle, **VG:** Violet stained gland, **PP:** Palisade parenchyma, **SP:** Spongy parenchyma, **T:** Trachea)

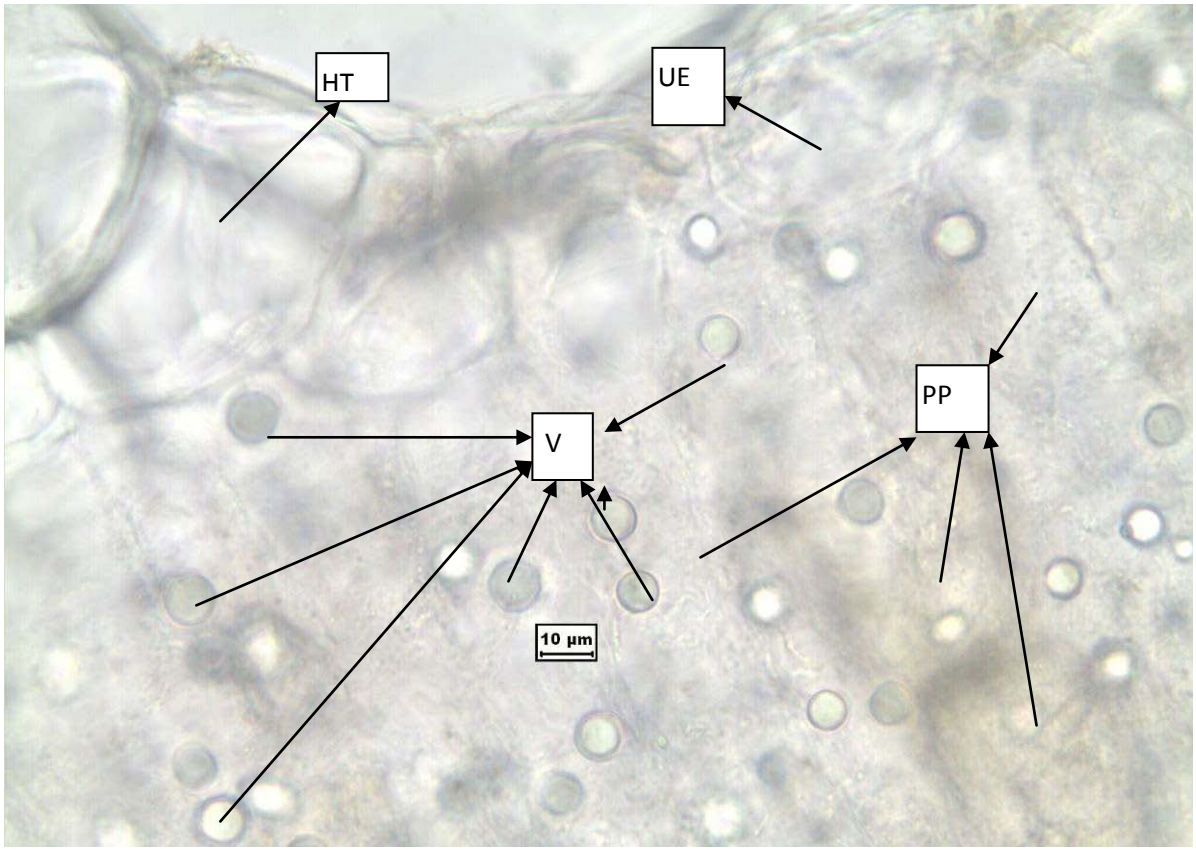

Figure 3. Transverse section of leaf of *C. forskohlii*. Violet stained vesicles were observed within the cells of palisade parenchyma of leaf of *C. forskohlii* (10µm) (**UE:** Upper epidermis, **HT:** Hairy trichome, **PP:** Palisade parenchyma, **V:** Violet stained vesicle)

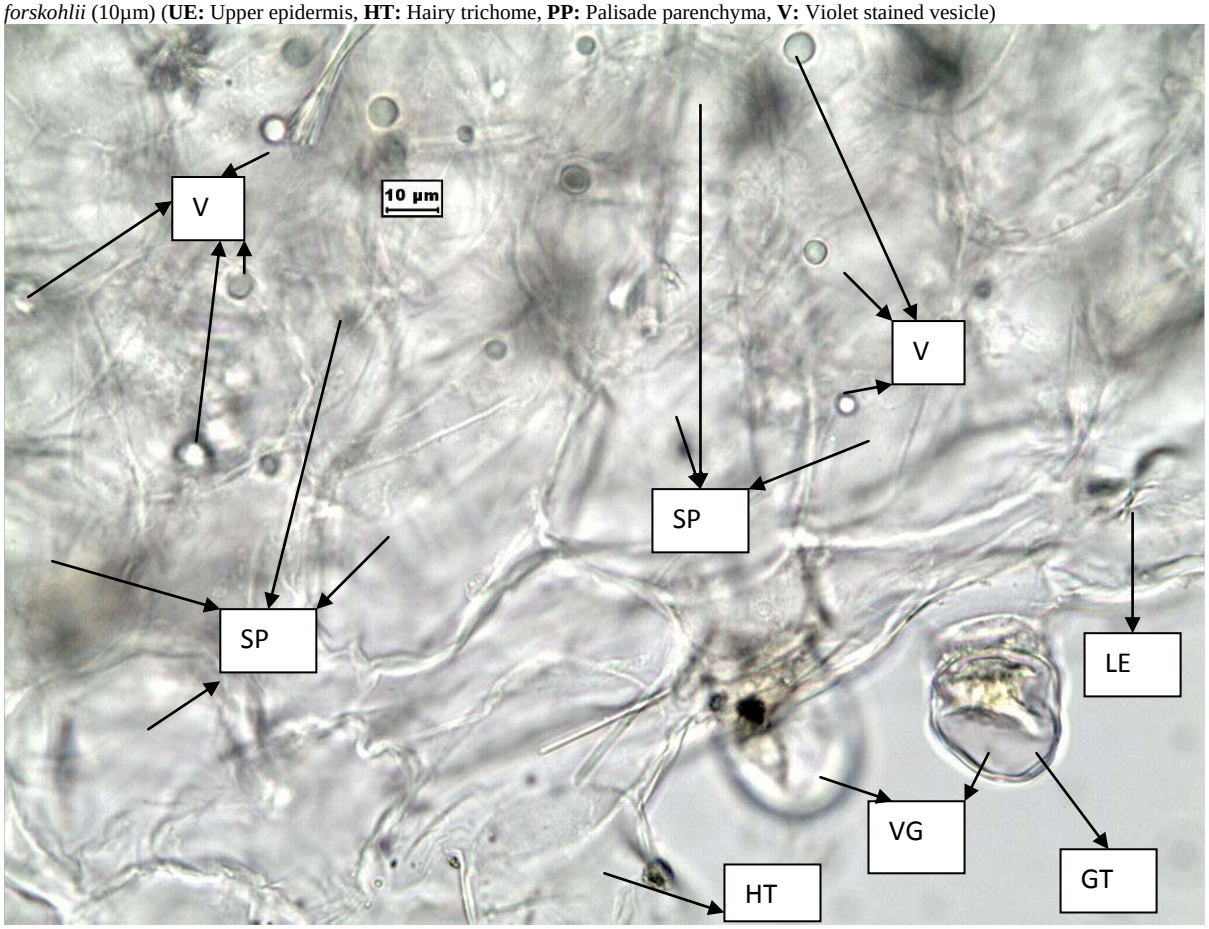

Figure 4. Transverse section of leaf of *C. forskohlii*. Violet stained vesicles were observed within the cells of spongy parenchyma and glandular trichomes of leaf of *C. forskohlii* (10µm) (**LE:** Lower epidermis, **SP:** Spongy parenchyma, **V:** Violet stained vesicle, **GT:** Glandular trichome, **VG:** Violet stained gland, **HT:** Hairy trichome)

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