

Full Length Research Paper

# Phytochemical constituents vis-a-vis histochemical localization of forskolin in a medicinal plant *Coleus forskohlii* Briq.

Selima Khatun<sup>1\*</sup>, Uğur Çakılcıoğlu<sup>2</sup> and Narayan Chandra Chatterjee<sup>1</sup>

<sup>1</sup>UGC Centre of Advanced Study, Department of Botany, Burdwan University, Burdwan 713104, West Bengal, India.

<sup>2</sup>Elaziğ Directorate of National Education, Elaziğ 23100, Turkey.

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**Tannins, phlobatannins, saponins, flavonoids, terpenoids, cardiac glycosides and alkaloids distribution in *Coleus forskohlii* belonging to the family Lamiaceae a well known medicinal plant in India was examined. Qualitative analysis carried out on this plant shows that terpenoids, tannins, flavonoids, phlobatannins, saponins and cardiac glycosides were present in *C. forskohlii*. The phytochemical constituents with respect to the role of this plant in traditional medicine treatment have well been established. Forskolin, a diterpene compound is the active principle of the *C. forskohlii*. Histochemical test was done to locate the forskolin in roots and tubers of *C. forskohlii*. Forskolin was found in the cells of cork, cortex, medullary rays and xylem in roots and tubers of *C. forskohlii*. These yellowish and reddish-brown masses are of diagnostic importance for this drug plant and can be used for its characterization.**

**Key words:** *Coleus forskohlii*, phytochemical constituents, forskolin, histochemical localization.

## INTRODUCTION

Phytochemical is a natural bioactive compound in plants, such as vegetables, fruits, medicinal plants, flowers, leaves and roots that work with nutrients and fibers that lower blood pressure (James and Anderson, 1983) and have hypocholesterolemic effects (Chen and Anderson, 1981) to act as a defense system against diseases or more accurately, to protect against disease. Phytochemicals are divided into two groups, which are primary and secondary metabolites. Primary metabolite is directly involved in normal growth, development, and reproduction while secondary metabolites are organic compounds that are not directly involved in the normal growth, development, or reproduction of organisms (Fraenkel, 1959); secondary metabolites often play an important role in plant defense against herbivory (Stamp, 2003) and other interspecies defenses; according to their

functions in plant metabolism. Primary constituents comprise common sugars, amino acids, proteins and chlorophyll while secondary constituents consists of alkaloids, terpenoids and phenolic compounds (Krishnaiah et al., 2007).

Complementary and Alternative medicine (CAM) represents a group of diverse medical and health care system, practices and products that are not considered to be the part of conventional medicine. Biofeedback, acupuncture, herbal medication, massage, bioelectromagnetic therapy, meditation and music therapy are example of CAM treatments. Complementary medicines include herbal remedies and homeopathic medicine. Medicinal crops are valued for the active principle they contain. Medicinal *Coleus forskohlii* Briq. contains forskolin- a labdane diterpenoid. Forskolin is concentrated in tubers and base of the stem than other plant parts (Yanagihara et al., 1995). The drug made of forskolin are used for treating hypertension (Dubey et al., 1981), congestive heart failure (Kramer et al., 1987), asthma (Lichey et al., 1984), glaucoma (Caprioli and

\*Corresponding author. [shelly\\_selima\\_india@yahoo.com](mailto:shelly_selima_india@yahoo.com). Tel: +919474916341.

Sears, 1983) and certain types of cancers (Agarwal and Parks, 1983). It is reported on morphology, phytochemistry and pharmacological aspects of *C. forskohlii* (Kavitha et al., 2010). There has been an increase in the use of herbal medicines in US over the last 15 to 20 years (James and Little, 2004).

*Coleus forskohlii* (Willd.) Briq. (synonym *C. barbatus* (Andr.) Benth.), family Lamiaceae (Labiatae) is an ancient root drug recorded in Ayurvedic materia medica under the Sanskrit name 'Makandi' and 'Mayini' (Shah, 1996). It is distributed over the subtropical warm temperate climatic zone on mountains of India, Nepal, Myanmar, Srilanka, Thailand and Africa. In India, it is reported to be distributed in dry hills of Western Uttarpradesh, Gujarat, parts of Orissa, Western ghats, Tamil Nadu (Somnath et al., 2005) and in kitchen gardens of Northern Karnataka (Belgaum and Dharwad districts) for its carrot like tubers, which are used as condiments in the preparation of pickles.

Forskolin (7 $\beta$ -acetoxy-8, 13-epoxy-1 $\alpha$ , 6 $\beta$ , 9 $\alpha$ -trihydroxy-14-ene-11-one), a diterpene compound is the active principle (Shah et al., 1980). Minor diterpenoids, deacetylforskolin, 9-deoxyforskolin, 1, 9-deoxyforskolin, 1, 9-dideoxy-7-deacetylforskolin, and four other diterpenoids, have been reported to be present in the roots of *C. forskohlii* (Gabetta et al., 1989). Exports of 140 Metric ton *C. forskohlii* roots was recorded during 2005 to 2006 in the data relating to India's exports by commodities is compiled and published by the Directorate General of Commercial Intelligence and Statistics (DGCIS) of Government of India (DGCIS, 2006). It has been reported that the transverse section of the root of *C. forskohlii* shows the presence of forskolin and other terpenoids (Narayanan et al., 2002); this has been further confirmed by our present study. Considering the medicinal importance and economic benevolence of the *C. forskohlii*, the objective of our study has been to examine the distribution of the tannins, phlobatannins, saponins, flavonoids, terpenoids, cardiac glycosides and alkaloids of the roots and tubers of *C. forskohlii*. By studying the presence of phytochemicals in this plant, the uses of this plant in traditional treatment can be explained scientifically. It is reported on the growth and yield of *C. forskohlii* (Inamdar et al., 1984; Schaneberg and Khan, 2003; Saleem et al., 2006; Wu et al., 2007; Vennila and Jayanthi, 2008; Mastiholi and Hiremath, 2009). Another objective of our study has been to develop a histochemical test to locate the terpenoids and forskolin in the roots and tubers of *C. forskohlii*.

## MATERIALS AND METHODS

### Collection of plant samples

The roots and tubers of *C. forskohlii* were collected from the experimental medicinal garden of the Department of Botany, Burdwan University, Burdwan, India that were grown in field condition. The plant materials were processed and analyzed.

### Processing of plant samples

The roots and tubers of this plant were properly washed in tap water and then rinsed in distilled water. The rinsed roots and tubers were dried in an oven at a temperature of 35 to 40°C for 3 days. The dried roots and tubers of this plant were pulverized, using a sterile electric blender, to obtain a powdered form. The powdered form of roots and tubers of this plant were stored in airtight glass containers, protected from sunlight until required for analysis.

### Preparation of aqueous extract of plant samples

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

### Qualitative analysis on phytochemical constituents

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

### Histochemical localization of forskolin and other terpenoids

Hand-sections of fresh roots and tubers of *C. forskohlii* were cut and observed. Forskolin is reported to give violet colouration with vanillin in acetic acid and perchloric acid, which has been used as a spectrometric method for detection and quantification (Inamdar et al., 1984). This colour reaction was tried directly on transverse sections of the roots and tubers of *C. forskohlii*. Sections of the roots and tubers of *C. forskohlii* were first placed in 2 ml of 10% vanillin in acetic acid to which 2 to 3 drops of perchloric acid (70%) was added and placed on water bath (70°C) for 2 to 3 min (Abraham et al., 1988; Narayanan et al., 2002). 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 root and tuber 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 chloral hydrate. Thin layer chromatography (TLC) of chloral hydrate (75%) washings of these sections was done using standard forskolin (HiMedia Chemicals, Mumbai, India).

### Thin layer chromatography (TLC)

The TL separation was carried out using precoated TLC plastic sheets of 60F<sub>254</sub> silica gel (Merck Chemicals, Mumbai, India). TLC plate size 20 × 20 cm and toluene: ethyl acetate (80:20, v/v) solvent system. After developing, the plate was sprayed with anisaldehyde sulphuric acid reagent (1 ml concentrated H<sub>2</sub>SO<sub>4</sub> was added to 0.5 ml anisaldehyde in 50 ml acetic acid) and heated at 100 to 105 °C and the R<sub>f</sub> values were calculated.

## RESULTS AND DISCUSSION

### Qualitative analysis

Qualitative analysis showed (Table 1) that except the

**Table 1.** Qualitative analysis on phytochemical constituents of *C. forskohlii*.

Plant samples	Roots	Tubers
Tannins	+	+
Phlobatannins	+	+
Saponins	+	+
Flavonoids	+	+
Terpenoids	+	+
Cardiac glycosides	+	+
Alkaloids	-	-

+: Presence of phytochemical constituents. -: Absence of phytochemical constituents.

alkaloids; terpenoids, tannins, flavonoids, phlobatannins, saponins and cardiac glycosides were present in both the roots and tubers of *C. forskohlii*.

#### ***Coleus forskohlii*: Effects of terpenoids, tannins, flavonoids, phlobatannins, saponins and cardiac glycosides.**

*C. forskohlii* is an ancient root drug recorded in Ayurvedic medicine in India (Sastri, 1950). Terpenoids were found to be present in *C. forskohlii* (Table 1), forskolin, a labdane diterpene (7 $\beta$ - Acetoxy-8,13-epoxy-1 $\alpha$ , 6 $\beta$ , 9 $\alpha$ -trihydroxy-labd-14-ene-11-one) isolated from *C. forskohlii* (Bhat et al., 1977; Saleem et al., 2005), was observed to activate adenyl cyclases resulting in an increase in cAMP (Seamon and Daly, 1981; Rupp et al., 1986). The mechanisms of interaction of forskolin were studied in detail (Zhang et al., 1997; Tesmer et al., 1997; Tang and Gilman, 1995). Forskolin showed positive effects against a wide range of conditions such as asthma (Lichey et al., 1984), glaucoma (Caprioli and Sears, 1983), hypertension (Dubey et al., 1981), cancer (Agarwal and Parks, 1983), heart diseases (Kramer et al., 1987), diabetes (Ammon and Müller, 1984) and obesity (Allen et al., 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 getting a single class of voltage-dependent potassium channels from a clonal pheochromocytoma (PC12) cell line independent of adenylate cyclase activation (Hoshi et al., 1988).

Cardiac glycosides were found to be present in *C. forskohlii* (Table 1), a compound that has been shown to aid in treatment for congestive heart failure and cardiac arrhythmia. This is another reason why this plant is widely used in traditional medicine. Cardiac glycosides work by inhibiting the Na<sup>+</sup>/K<sup>+</sup> pump. This causes an increase in the level of sodium ions in the myocytes, which then leads to a rise in the level of calcium ions. This inhibition increases the amount of Ca<sup>2+</sup> ions

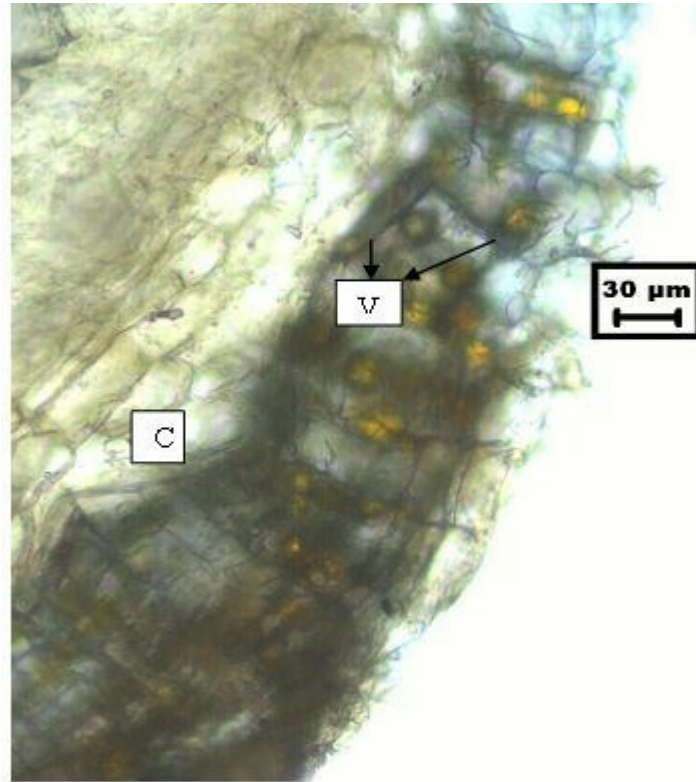
available for contraction of the heart muscle, improves cardiac output and reduces distention of the heart. Flavonoids were present in both the roots and tubers of *C. forskohlii* (Table 1), as flavonoids are known to act as antioxidant. Antioxidants, which could protect against oxidant and free radical injure, in addition to their medicinal properties. Oxidation from reactive oxygen species (ROS), radical-related damage of DNA and proteins has been proposed to play a key role in the development of age dependent diseases such as cancer, atherosclerosis, arthritis, Alzheimer's disease, other neurodegenerative disorder and other such conditions (Collins, 2005). *C. forskohlii* has been employed in the treatment of intestinal disorder such as dysentery and diarrhoea for its tannins (Table 1).

#### **Histochemical localization of forskolin and other terpenoids**

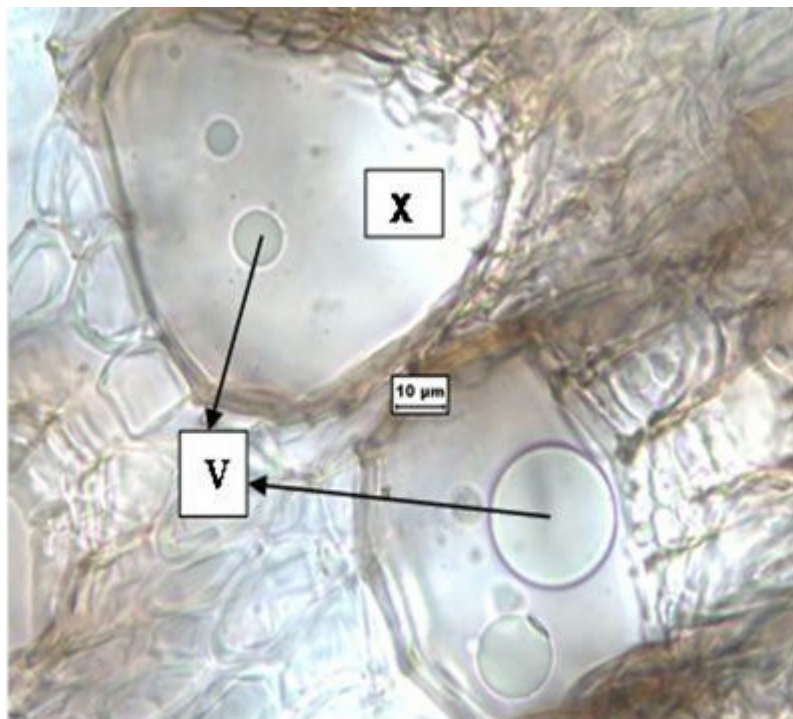
Histochemical analysis shows that forskolin was found in the cells of cork, cortex, medullary rays and xylem in roots and tuber of *C. forskohlii* (Figures 1 to 7). TLC of chloral hydrate washings showed presence of forskolin and other terpenoids in roots and tubers of *C. forskohlii*. Rf value of forskolin was 0.48. This confirms that the yellowish-red masses seen in the sections contain the terpenoids (Abraham et al., 1988).

#### **Conclusion**

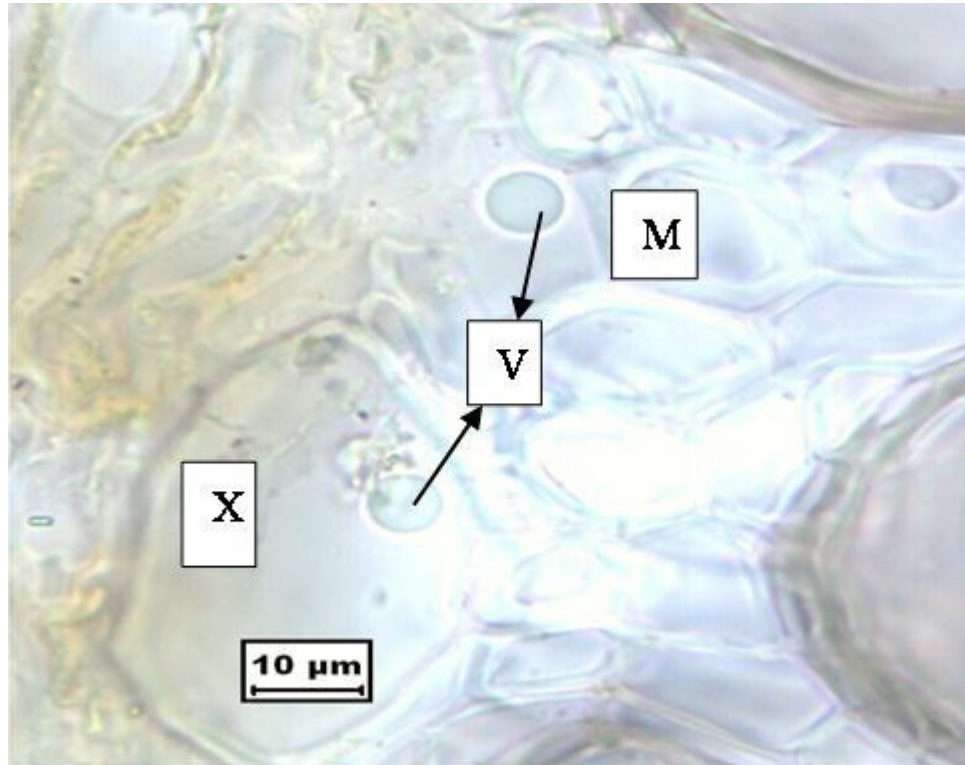
This study therefore has provided some biochemical basis for ethno pharmacological uses of this plant in the treatment and prevention of various diseases and disorders. The phytochemical screening on qualitative analysis shows that the roots and tubers of the *C. forskohlii* are rich in terpenoids, tannins, saponins, and flavonoids, cardiac glycosides and phlobatannins which are popular phytochemical constituents. Histochemical analysis shows that forskolin is found in the roots and tubers of *C. forskohlii*. These yellowish and reddish-brown masses are of diagnostic importance for this drug



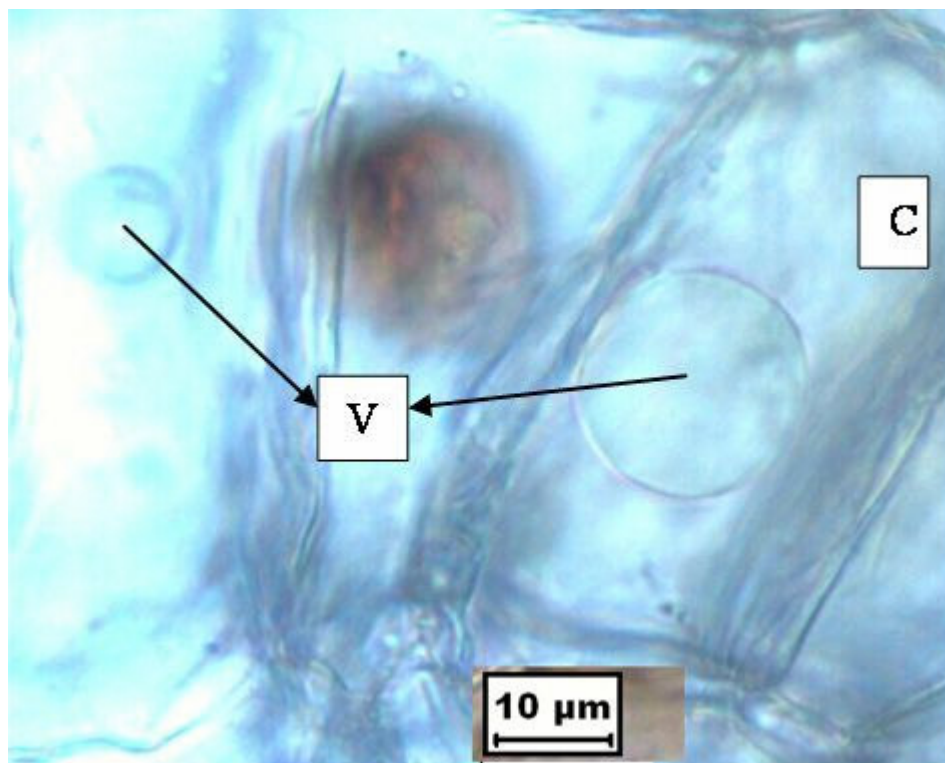
**Figure 1.** Transverse section of root of *C. forskohlii*. Violet stained vesicles (V) were observed within the cork cells (C) of root of *C. forskohlii*. Bar = 30 μm.



**Figure 2.** Transverse section of root of *C. forskohlii*. Violet stained vesicles (V) were observed within the xylem cells (X) of root of *C. forskohlii*. Bar = 10 μm.

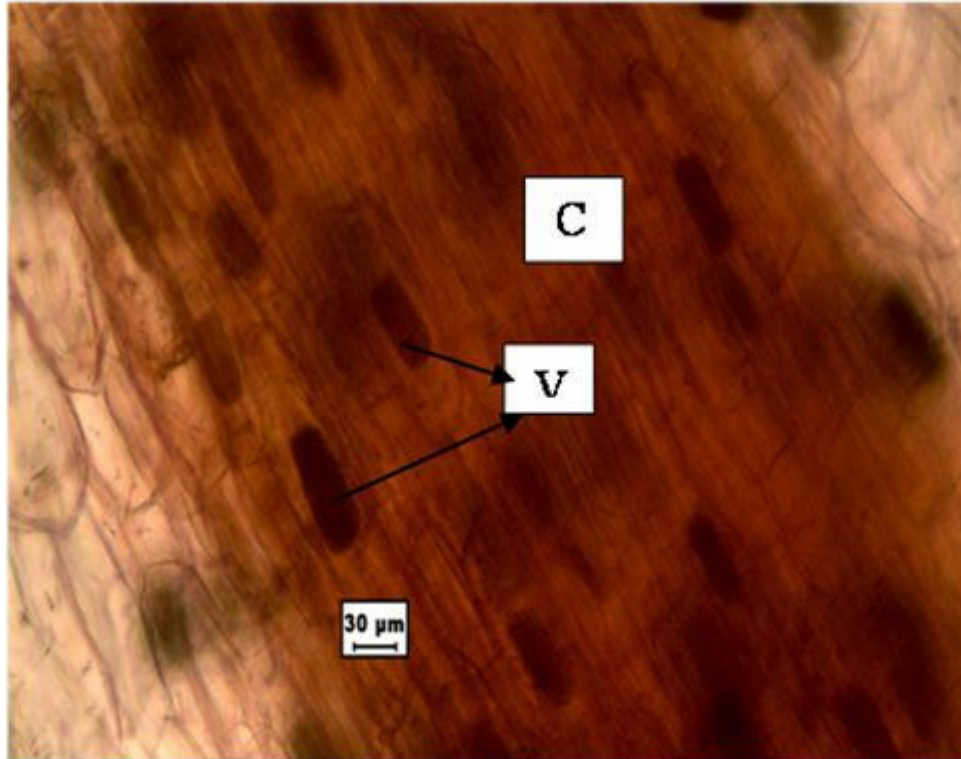


**Figure 3.** Transverse section of root of *C. forskohlii*. Violet stained vesicles (V) were observed within the medullary rays (M) and xylem cells (X) of root of *C. forskohlii*. Bar = 10 µm.

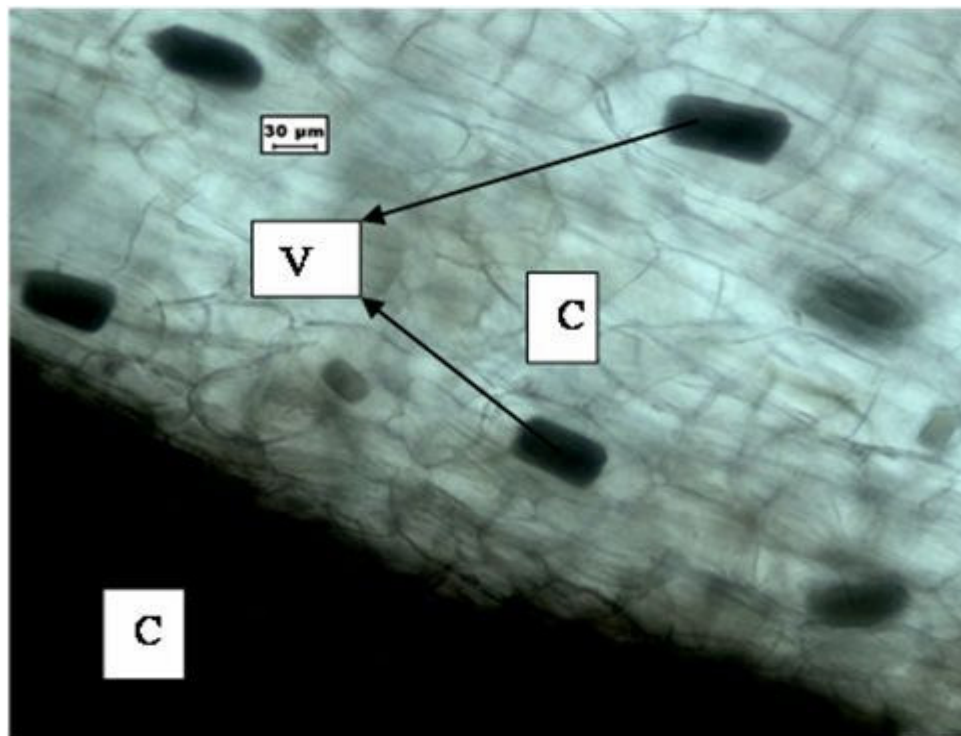


**Figure 4.** Transverse section of root of *C. forskohlii*. Violet stained vesicles (V) were observed within the cortex cells (C) of root of *C. forskohlii*. Bar = 10 µm.

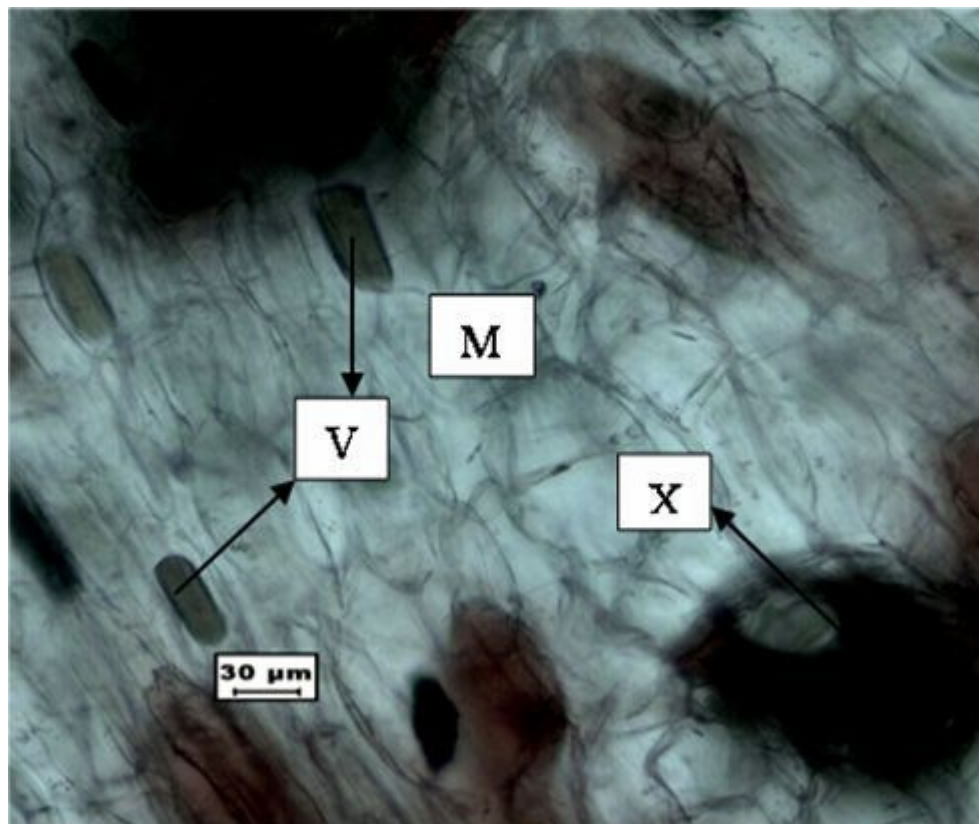




**Figure 5.** Transverse section of tuber of *C. forskohlii*. Violet stained vesicles (V) were observed within the cortex cells (C) of tuber of *C. forskohlii*. Bar = 30 µm.



**Figure 6.** Transverse section of tuber of *C. forskohlii*. Violet stained vesicles were observed within the cork and cortex cells of tuber of *C. forskohlii*. Bar = 30 µm.



**Figure 7.** Transverse section of tuber of *C. forskohlii*. Violet stained vesicles (V) were observed within the medullary rays (M) and xylem cells (X) of tuber of *C. forskohlii*. Bar = 30  $\mu$ m.

plant and can be used for its characterization.

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