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Biochemical profiling of *Trichodesmaindicum*

Shameema Yousuf[1]

School of Life & Allied Health Sciences, Glocal University, Mirzapur Pole, Saharanpur, Uttar Pradesh

Santosh Kumar Singh[2]

Associate Professor & Head, Research Division, Beehive P.G. College of Advance Studies, Selaqui, Dehradun, Uttarakhand

ABSTRACT

Trichodesmaindicum is one of the most significant medicinal herbs and is part from traditional to the modern therapeutic system. It is used to treat gastrointestinal disorders, cancer, aging and has antispasmodic and insecticidal potential. It is a herbaceous plant found in barren dry lands especially near to plains of hilly areas. In current study, *Trichodesma* accessions were analyzed for its photosynthetic pigment profiles, growth in terms of protein and carbohydrate contents and non-enzymatic antioxidants in leaf samples.Despite of growing in dry habitats, most of the accessions of *Trichodesmaindicum* were observed to contain very high phenolic and flavonoid contents. High protein content and accumulated ascorbate and proline in the leaf samples may be attributed to the high tolerance capacity and adaptive ability of this palnt in stressed environments.

Keywords: Protein, Ascorbate, Proline, Phenolic, Flavonoid

INTRODUCTION

Trichodesmaindicum is commonly called as Adhapushpi and Indian Borage. It belongs to the family Boraginaceae. In Ayurveda it is also referred as Adhomukha, Andhaka, Andpushpi, Gandhapushpika. It is an erect, branched annual herb with moderate height. Stalks are absent in leaves which are opposite in orientation, lanceolate and pointed at the tip and heart-shaped at the base. Stiff hairs arising from circular tubercles give it a hairy appearance. Flowers occur singly in the axils of the leaves and may be violet, blue, pink and white colored. It is reported to be present in Iran, India, and Mauritius especially in dry and non fertile landscapes.

Many medicinal properties of the plant are reported by various authors. The anti-inflammatory, anticancer potential and treatment of joint disorders using the extract of this plant are very popular

ISSN:0971-1260

Vol-22-Issue-10-November-2019

(Ali M 2008,Azhaguraman*et al* 2016). Anti-diarrheal (Perianayagam*et al* 2005), antispasmodic/lipoxygenase inhibitory activity (Taous Khan *et al* 2009), insecticidal (Taous Khan *et al* 2008) antimicrobial (Saboo*et al* 2013) activities are proved by researchers and are being used in many medical formulations. Each part of the plant is useful and Ethan botanical studies consider it as one of the important medicinal plant.

Since there is increasing interest in industry, academia and the health sciences in medicinal and aromatic plants, *Trichodesma* can be selected not only for the yield of active principles, but for the plant's ability to overcome diseases, climatic stresses and the hazards caused to mankind. The authors have performed many assays depicting and establishing biochemical profile of different accessions of *Trichodesmaindicum* which might be useful in the characterization of this significant herb.

MATERIALS AND METHODS

Various accessions of *Trichodesmaindicum* were collected from wild locality near the plains of Shivalik Hills of West Uttar Pradesh, India. Voucher specimens of each accession were also collected, dried, and stored in replicates at Glocal University and the Botanical Survey of India, Dehradun circle. Taxonomic identification and characterization of accessions were conducted with the help of BSI, Dehradun, Uttarakhand, India.

Extraction and estimation of pigments

Fresh leaves (0.02 g) from different *Trichodesma accessions* were taken and cut into small pieces and photosynthetic pigments were extracted with 80% (v/v) acetone. The extracts were centrifuged and pellets were re-suspended in 80% acetone, till it becomes colourless (Arnon 1949). The absorbance of the resulting solution were recorded at 663, 646 and 470 nm spectro photo metrically, and amount of chlorophylls and carotenoids were calculated by using the equations given by Lichtenthaler and Welburn (1983).

Chlorophyll a (µg/ml) = 12.21 (A_{663}) – 2.81 (A_{646})

Chlorophyll b (µg/ml) = 20.13 (A_{646}) – 5.03 (A_{663})

Carotenoids (µg/ml) = [(1000 A_{470}- 3.27 (Chl a) – 104 (Chl b)] / 227

Extraction and estimation of Flavonoid

Flavonoids were extracted in leaf samples by using the method of Jordan *et al* 1994 and were estimated as described by Mirecki and Teramura (1984) . The reaction mixture consisted acidified methanol: HCl: water (78: 20: 2, v/v) and put for 24h at 4^0C. The filtered extract was then used for measuring the absorbance at 320 nm, which was considered as indicative of relative concentration of UV–B absorbing pigments. Flavonoid contents were expressed as absorbance g^{-1} fresh mass of tissue at 320 nm.

ISSN:0971-1260

Vol-22-Issue-10-November-2019

Protein extraction and measurement

Protein contents were determined using Folins-Lowry method with lysozyme as the standard (Lowry *et al* 1951).Folin's- Ciocalteau colorimetric method relies on the formation of a copper-protein complex. On the reduction of phosphmolybdate and phosphotungstate anions present in Folin-Ciocalteau phenol reagent by tyrosine and tryptophan residues of the proteins, to heteropolymolybdenum blue and tungsten blue respectively, this gives a blue colored complex with absorption maxima at 750 nm. Extraction of plant protein was carried by the standard method of Jervis L (1989).1.0 g leaf samples werehomogenized and extracted with 0.01 M phosphate buffer (pH, 6.7). Precipitated protein was used for quantification assay.

Determination of total proline content

Proline contents in leaf homogenate of plants were estimated according to the method of Bates *et al*(1973).Fresh leaves were crushed in 3% aqueous sulfosalicylic acid, centrifuged at 10,000 g and then reacted with 3% glacial acetic acid and acid ninhydrin. Samples were heated for 1h in a water bath at 95^0C, cooled and extracted with toluene by vortexing for 15 sec with a test tube mixer. The toluene layer was then aspired and the absorbance was read at 520 nm using toluene as blank. The proline content in each sample was calculated from the standard curve.

Determination of ascorbate contents

Ascorbic acid content in leaves homogenate was estimated using the method given by Oser (1979).Fresh leaves were ground in 5% sulfosalicylic acid, and then the homogenate was centrifuged at 10,000 g for 10 min. The reaction mixture consisted of 2% sodium molybdate, 0.15 N H_2SO_4, 1.5 mM K_2HPO_4 and tissue extract. The reaction mixture was incubated at 60^0C in water bath for 40 min, cooled, centrifuged at 3000 g for 10 min and absorbance was recorded at 660 nm. The amount of ascorbic acid was calculated by comparing with standard curve using L-Ascorbate as standard.

Extraction and analysis of Total phenolic contents

The amount of total phenolics in *Trichodesma*samples were determined with the Folins-ciocalteu reagent using the method of Spanos and Wrolstad (1990), as modified by Lister and Wilson (2001).Results were expressed in plant methanol extracts as milligrams of gallic acid equivalent per gram of dry wt (mg GAE/g DW).

Extraction and estimation of Carbohydrate contents

Anthrone method was used for the estimation of total carbohydrate content in leaf samples. The sugars, in presence of concentrated sulphuric acid got dehydrated and produced furfural form (from pentoses) or 5-Hydroxymethylfurfural (from hexoses). It reacted with anthrone to produce colored compound with absorption maxima at 625 nm (Joslyn 1970 & Hodge 1962).

ISSN:0971-1260

Vol-22-Issue-10-November-2019

RESULTS AND DISCUSSION

Photosynthetic Pigments & Phenolic contents

Plants harvest solar energy and assimilate it into carbon compounds which provide cellular energy and carbon skeleton for various metabolic processes. Plant pigments play vital role in this process. This is the reason why pigment profile of a plant is an indicator of its biochemical and assimilatory health. Amount of chlorophyll a was estimated in the range of 3.306 mg pigment g^{-1} FW to 2.660 mg pigment g^{-1} FW). Chlorophyll b was varying between 1.1-3.3 mg pigment g^{-1} FW.Average value of carotenoid was estimated in the range of 1.3-1.91 mg pigment g^{-1} FW(figure 1 a). Total amount of pigments wereobserved to be in the range of 6.1- 7.4 mg pigment g^{-1} FW in all the accessions of *Trichodesmaindicum*.

The amount of total phenolic contents ranged from 30- 68 mg GAE /g of dry material (Figure 1 b) with the mean value of 42.56 mg GAE /gDW. It was found that there was a linear relationship between the total phenolic contents present in a plant and its antioxidant capacity (Sundaram*et al* 2006). It is well known that the phenolic compounds itself act as natural antioxidants (Javanmardi*et al* 2003).The high content of photosynthetic pigments and phenolicsin most accessions might be indicative of luxuriant growth even in dry and unfertile soils.

Total Flavonoid

Flavonoids are reported to exhibit antioxidant activity (Ramanathan, *et al* 1989) and are effective scavengers of superoxide anions (Robak and Gryglewski 1988). They prevent the penetration of harmful UV-radiation to the leaf interior (Devi *et al* 1999), so their concentration in the epidermal cells may also be the deciding factor regarding susceptibility to UV-B radiations. These compounds act as a light screen against damaging UV radiation in young leaves, provide resistance to pathogens, and act as antioxidants, enzyme inhibitors and precursors of toxic substances. Figure 2 a depicted the varying concentration of flavonoids in different *Trichodesma* accessions. Many authors have shown the similar results and contents of flavonoids in plants.Flavonoid contents were found to be in the range of 0.240- 1.21 A g^{-1} FW). Higher contents of UV-B absorbing pigment, flavonoid signifies the high UV-stress tolerant nature of the concerned accessions. The chemical evolution of flavonoids and other UV-B absorbing compounds has been assumed to play an important role in the evolution from aquatic plants to land plants (Rozema*et al* 2002). Flavonoids also have the ability to alter the fluidity of membranes (Arora 2002).

Total Ascorbate and Proline

The contents of ascorbic acid were measured in different accessions (figure 2 b). The mean values of ascorbate contents were found to be in the range of 45.17- 79.40 µg g^{-1} FW. The ascorbic acid is one of the most studied and powerful antioxidant (Horemans*et al* 2000). It has been detected in majority

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Vol-22-Issue-10-November-2019

of the plant cell types, organelles and in the apoplast. Under physiological conditions, ascorbic acid exists mostly in the reduced form in leaves and chloroplasts (Smirnoff 2000).

Proline also serves as an important antioxidant metabolite with several other important cellular functions. Figure 3a shows the various concentrations of proline in leaves of different *Trichodesma* accessions. The proline contents ranged from 5.93-14.25 (µg g^{-1} FW), in 20 accessions. The mean value of proline contents was observed to be9.75 µg g^{-1} FW. The accumulation of compounds such as proline in higher plant cells in response to environmental stress is well documented before proving the present study (Hare and Cress 1997,Cechin*et al* 2006). The function of proline in stressed plants is often explained by its property as an osmolyte, able to balance water stress (Delauney and Verma 1993).

Total Carbohydrate and Protein contents

Total carbohydrate and protein contents were analyzed to diversify different accessions for overall growth pattern. It was found that the carbohydrate contents in 20 accessions ranged from 5.5-21%. With the mean value 17.7%as depicted by the figure 3b.The protein contents varied from 8.9 to 28.5% and the mean value for all 20 accessions were recorded to be 17.5%.

Medicinal and aromatic plants are of prime economic importance because of their potential of having ingredients for its possible use in treatment of diseases. *Trichodesma*isone of the most important plants inthis regard. The protein and carbohydrate contents of accessions might be very useful in food industry when used as diet supplements, balancing and making the diet healthy.The concentration of some of the chemical compounds varies from species to species. Flavonoids, tannins, saponins, ascorbate, prolineetc are such compounds. The aim of the present study was to find out some key biochemical characters which can be used as a framework for understanding relationship within the genus, *Trichodesma*.Presence of antioxidant compounds as Ascorbate, proline, phenolic acids, carotenoids and others makethe planta significant and usable source in medicinal sector.

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ISSN:0971-1260

Vol-22-Issue-10-November-2019

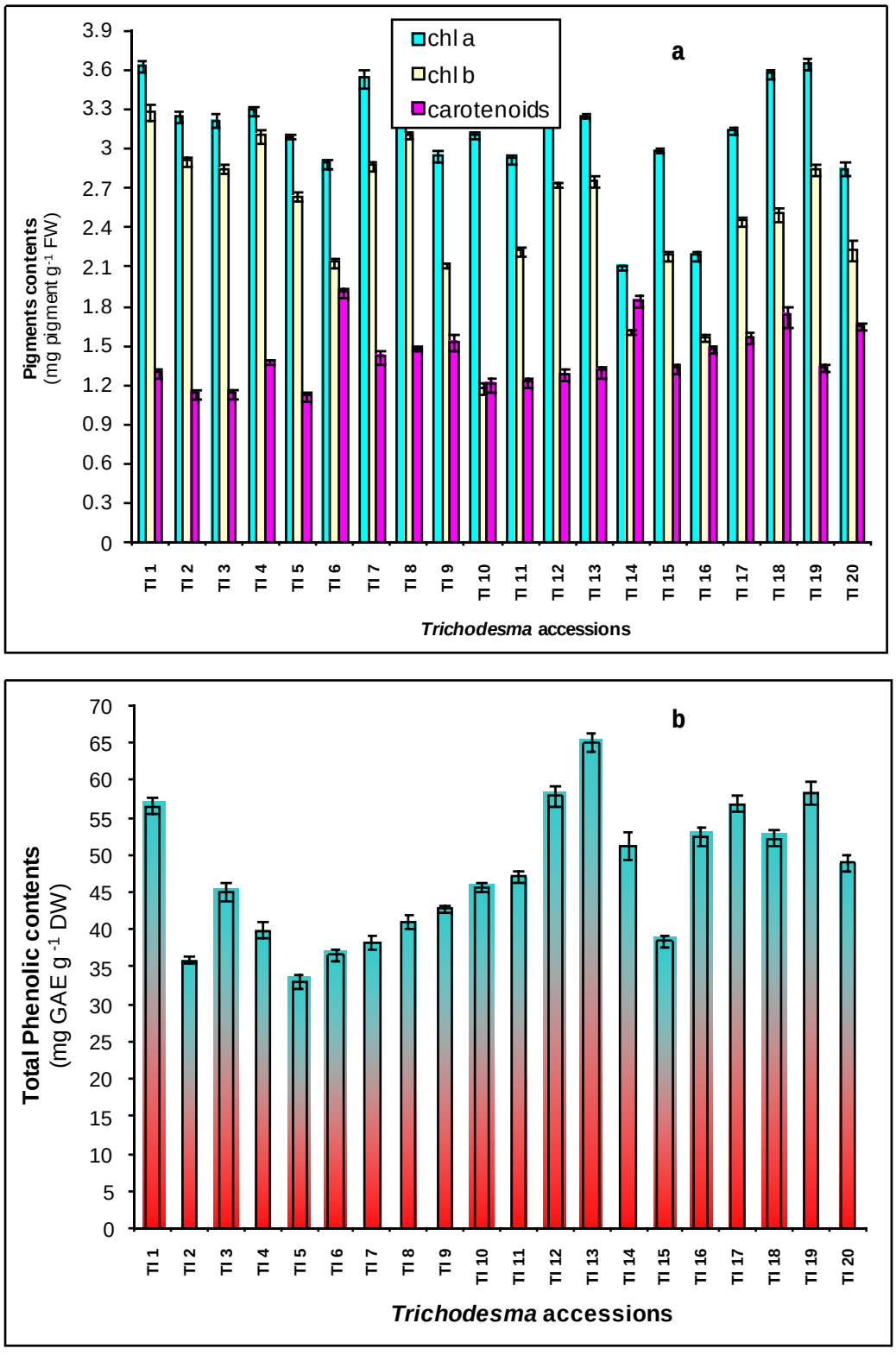

Figure 1 Evaluation of photosynthetic pigments (a) Chlorophyll a, b and carotenoids (b) Total phenolic contents in 20 accessions. The error bars represent the mean values of triplicates ± SE.

ISSN:0971-1260

Vol-22-Issue-10-November-2019

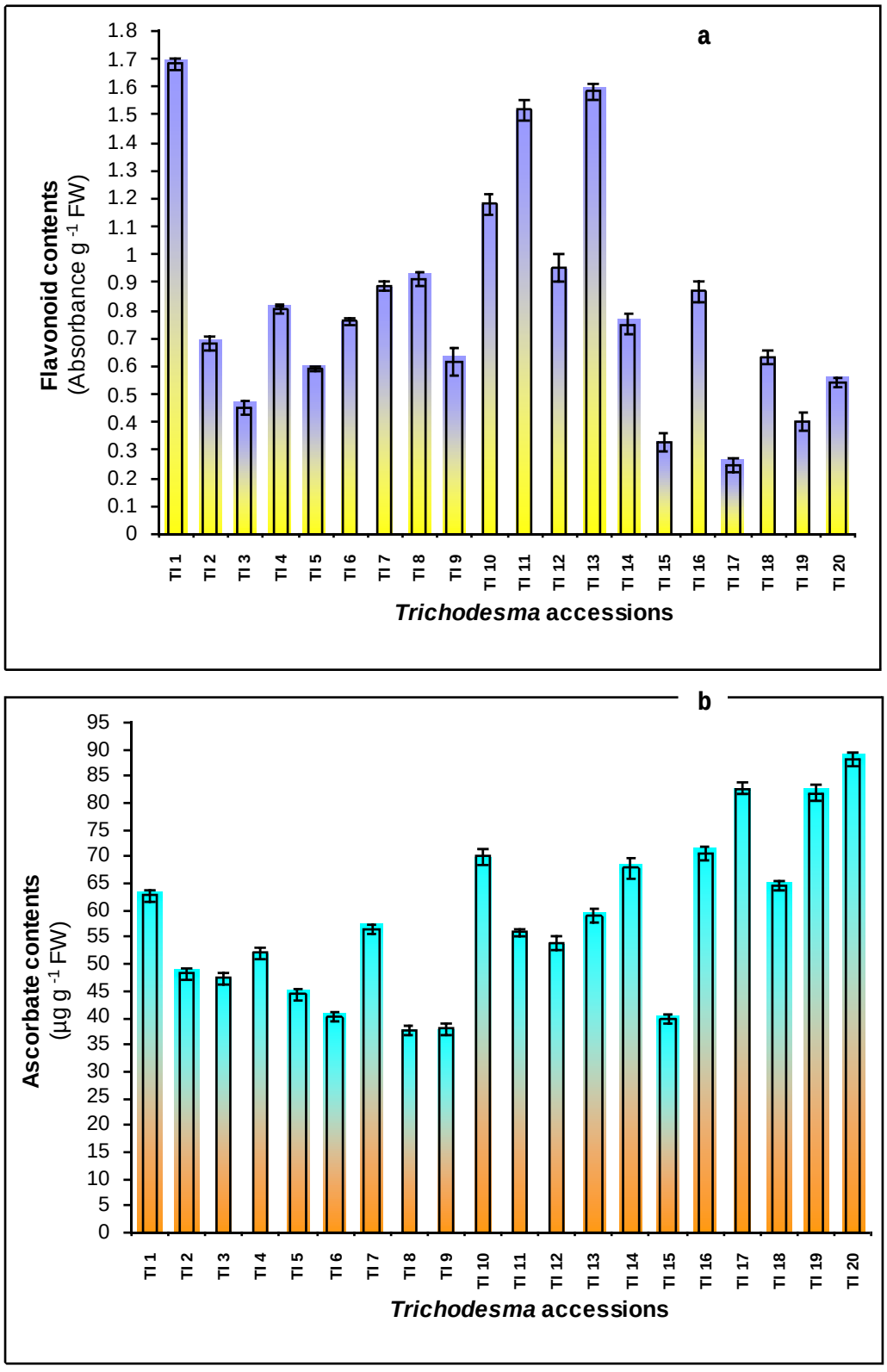

Figure 2 Evaluation of Flavonoid (a) and ascorbate contents(b) in*Trichodesma*. The error bars represent the mean values of triplicates ± SE.

ISSN:0971-1260

Vol-22-Issue-10-November-2019

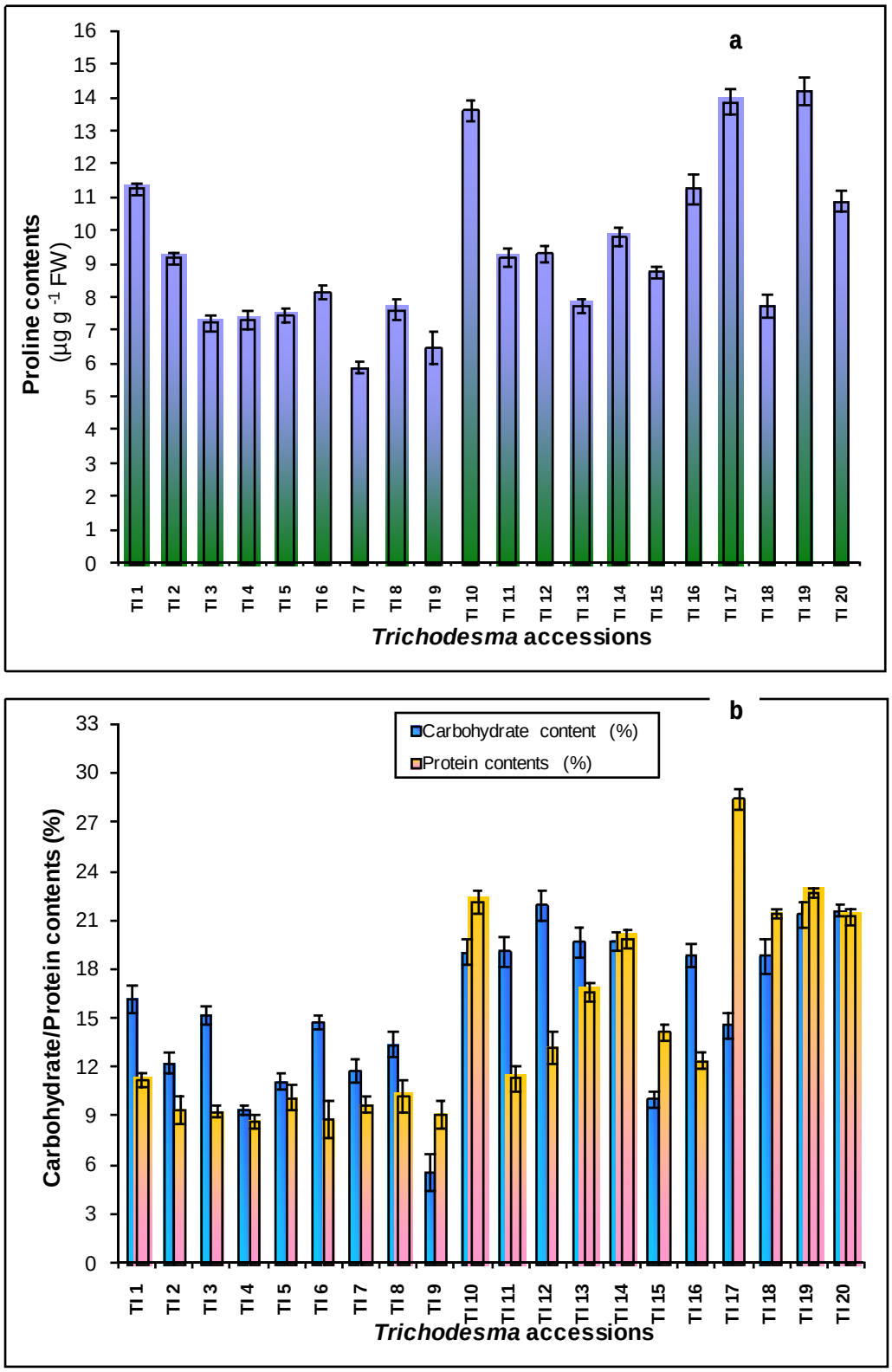

Figure 3 Poline (a) & carbohydrate and protein contents(b) in*Trichodesma*. The error bars represent the mean values of triplicates ± SE