

## HPLC ANALYSIS REVEALS PRESENCE OF ALKALOID IN LEAF AND SEED EXTRACTS OF *TRICHODESMA INDICUM* (L.) R. BR

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### ABSTRACT

Alkaloid contents of leaf, flower and seed samples of *Trichodesma indicum* were extracted using five different methods. Harborne's method produced maximum amount of alkaloid residues from leaf, flower and seed samples. The color test, TLC and HPLC confirmed the presence of alkaloid in leaf and seeds but not in flowers.

**Key words:** Alkaloid, *Trichodesma indicum*, HPLC, TLC.

### Introduction

*Trichodesma indicum* (family - Boraginaceae) is a medicinal herb distributed in tropical and sub tropical Asia, Africa and Australia (Samvatsar, 1996). In Chhattisgarh, it occurs in crop fields, bunds and wasteland during monsoon and winter season (Srivastava, 1989). Whole plant paste is useful for osteoarthritis, inflammation and conjunctivitis. Ash is used to clean wounds and scabies (Kulkarni and Ansari, 2004). The plant is used for expulsion of dead fetus (C.S.I.R., 1986) and prescribed for abortion (Tarafder, 1983) and as a brain tonic (Jain, 1991).

Alkaloids are a chemically heterogeneous group of natural substances and comprise more than 6000 basic nitrogen containing organic compounds, which occur in about 15% of all vascular plants and more than 150 different plant families (Kokate *et al.*,

2004). The plants containing Pyrrolizidine alkaloids (PAs) are probably the most common natural poisons affecting livestock, wildlife and human (Fu *et al.*, 2004). The edible sources, including milk, honey, wheat, herbal medicines, herbal tea, dietary supplements, and functional foods, have been found to be contaminated with PAs or originally contain PAs, which may potentially cause human health problems. Sixteen PAs and one pyrrolizidine alkaloid N-oxide (isatidine) from the three plant families Asteraceae, Boraginaceae and Fabaceae induce tumors in experimental animals (Fu *et al.*, 2002). Chronic ingestion of plants containing PAs has also led to cancer in experimental animals and metabolites of several PAs have shown to be mutagenic in the *Salmonella typhimurium*/mammalian microsome system (Prakash *et al.*, 1999). PAs serve as a part of the plant defense against

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predators (Hartmann and Witte, 1995). Being liver toxic for vertebrates (Mattocks, 1986) and mutagenic for insects (Frei *et al.*, 1992), they are strongly feeding deterrents for most herbivorous organisms. Its non-toxic form is N-oxides that convert into toxic tertiary amines (Hartmann and Toppel, 1987). PAs occur in *Trichodesma incanum* and *T. africana* (Dharmananda, 2002). However, confirmed reports are lacking on the presence of PA in *Trichodesma indicum*.

### Material and Methods

#### Collection and preparation of plant material

*Trichodesma indicum* plants are found on rice field bunds, grazing grounds and forests during rainy season. However, it is possible to grow plants and keep them in flowering state throughout the year under moist condition. The leaf, flower and seed samples were collected from plants of Raipur (Chhattisgarh). The plant materials were dried in shade and coarsely powdered before use.

#### Extraction of alkaloids

Five methods namely Harborne's (1998), Kokate *et al.* (2003), Evans (2002), Verma *et al.* (2007) and Djilani *et al.* (2006) were used to extract alkaloid content from leaf, flower and seed samples of *T. indicum*. Powdered samples were first treated with methanol for 1 - 4 days and filtered through filter paper. Filtrates were dried and acidified with 2 M sulphuric acid and extracted with chloroform in separating funnel. Aqueous acid layer was basified to 10 pH with  $\text{NH}_4\text{OH}$ , extracted with  $\text{CHCl}_3$ : MeOH (3:1). Then  $\text{CHCl}_3$ : MeOH layer was

filtered and evaporated. Basic extract contained most alkaloids.

#### Qualitative test and weighing of samples

The qualitative tests were performed with Dragendorff reagent, Ehrlich reagent and Mayer's reagent to screen the presence of alkaloid in small amount of samples. Weight of alkaloid content in dried extracts was determined by Higuchi and Brochmann-Hanssen (2004) method.

#### Thin Layer Chromatography (TLC)

TLC plates were made by mixing the silica gel with a small amount of water. The plate was dried and activated by heating in oven at 110 °C for 30 minutes. The extracts of leaf and seed samples were mixed separately in ammonia with a glass rod. The plate was then placed into chloroform: methanol (90:1) solvent system, in a sealed container. TLC plates were sprayed with Dragendorff reagent. The movement of samples was characterized by a retardation factor ( $R_f$ ).

#### High Performance Liquid Chromatography (HPLC)

The alkaloids were resolved by HPLC method. Optimum resolution and peak shape were obtained using the Perkin Elmer Series- 200, RP- C- 18 column with an acetonitrile and water (80: 20) as solvent system. The pressure was maintained at 950- 960 PSI and wavelength at 275nmr. The detector was UV- 200. The ambient extraction and HPLC analysis method were applied to two lots of *T. indicum* powder (leaves and seed) to determine the alkaloid content.

## Results and Discussion

### Weight of extracts

The extracts of leaf, flower and seed samples obtained by different methods were dried and their weights were determined. The alkaloid extract of leaf sample was green whereas the extracts of seed and flower were of cream color. The amount of residue obtained by Harborne's method was maximum 100.0 mg g<sup>-1</sup> leaf sample, 20.0 mg g<sup>-1</sup> flower and 75.0 mg g<sup>-1</sup> seed. Pyrrolizidine alkaloids occur in several plant species and their varieties. Mroczek (2006) used various techniques for extraction of PAs from comfrey. Kurucu and Murat (2002) isolated PA Echimidine-*N*-oxide from *Symphytum sylvaticum* Boiss. subsp. *sepulcrale* Greuter and Burdet var. *sepulcrale*

and PA Echimidine from *S. aintabicum*. Datta *et al.* (2003) reported that *Heliotropium indicum* (Boraginaceae) contains the anticancer PA, indicine-*N*-oxide (INO). Ji *et al.* (2005) analyzed sun hemp (*Crotalaria juncea* L.) seeds of nine populations originated from different parts of the world and found variation in quantity and type of PAs in the seeds.

### Color test for presence of alkaloids

Three reagents namely Dragendorff reagent, Ehrlich Reagent and Mayer's Reagent were used to determine the presence of alkaloid(s) in the dried extracts of leaf, flower and seeds. Dragendorff reagent, Ehrlich Reagent and Mayer's Reagent produced colors with leaf and seed samples (Table 1). Dried extracts from flowers failed to give color

TABLE 1: QUALITATIVE TEST OF ALKALOID IN LEAF, SEED AND FLOWERS OF *T. INDICUM*

SN	Method of Extraction	Sample Reagent	Color of sample with different reagents			Dry weight of extract of sample (mg <sup>-1</sup> )
			Dragendorff Reagent	Ehrlich Reagent	Mayer's Reagent	
1	Djilani <i>et al.</i>	Leaf	Orange-red	Magenta	Cream	30.0
		Seed	Orange-red	Magenta	Cream	32.0
		Flower	-	-	-	3.0
2	Evans	Leaf	Orange-red	Magenta	Cream	60.0
		Seed	Orange-red	Magenta	Cream	40.0
		Flower	-	-	-	10.0
3	Harborne	Leaf	Orange-red	Magenta	Cream	100.0
		Seed	Orange-red	Magenta	Cream	75.0
		Flower	-	-	-	20.0
4	Kokate <i>et al.</i>	Leaf	Orange-red	Magenta	Cream	50.0
		Seed	Orange-red	Magenta	Cream	32.0
		Flower	-	-	-	3.0
5	Verma <i>et al.</i>	Leaf	Orange-red	Magenta	Cream	10.0
		Seed	Orange-red	Magenta	Cream	5.0
		Flower	-	-	-	0.50

with any of the three reagents. These tests suggest the presence of alkaloids in leaf and seed samples of *T. indicum*. However, the alkaloid(s) are either absent or not detectable in flower samples. The most sensitive methods for detecting PAs on TLC are those using Ehrlich reagent (4- dimethylaminobenzaldehyde) (Mattocks, 1967). First, spray of the plates with a solution of orthochloranil, then with Ehrlich reagent, heating after each spray, best visualizes the unsaturated alkaloids (Molyneux and Roitman, 1980).

#### Thin Layer Chromatography (TLC)

The dried extracts of leaf, flower and seeds obtained by Harborne's method were separated by thin layer chromatography. The separated samples from TLC were detected by spraying the plates with Dragendorff reagent that converted the

samples to an orange color product. The leaf and seed samples showed orange color (Fig. 1) but flower samples failed to give any color. The  $R_f$  value for leaf and seed samples were 0.83 and 0.75, respectively.

#### High Performance Liquid Chromatography (HPLC)

Each peak of HPLC is labeled with retention time. Retention time indicates how long it takes a compound to come out of the HPLC column. The first few small peaks in the chromatogram (around 2-3 minutes) are "noise" from the injection made. The larger peaks represent chemicals in the sample. The leaf samples in HPLC showed retention time 1.71, 2.09, 2.44, 2.59, and 2.72 (Fig. 2). The seed samples in HPLC showed retention time 1.67, 2.02, 2.44, 2.57 and 2.68 (Fig. 3). Segall (1979) and Dimenna

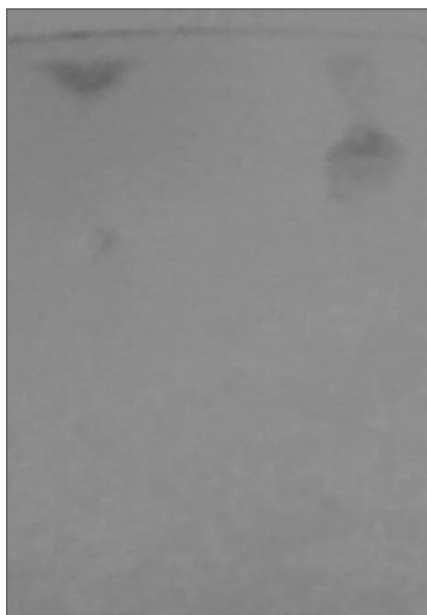


Fig. 1: Thin Layer Chromatography (TLC) of seed and leaf samples of extracted with Harborne's method. All plates sprayed with Dragendorff reagent to develop color.

Fig. 2: HPLC of leaf sample of *T. indicum*

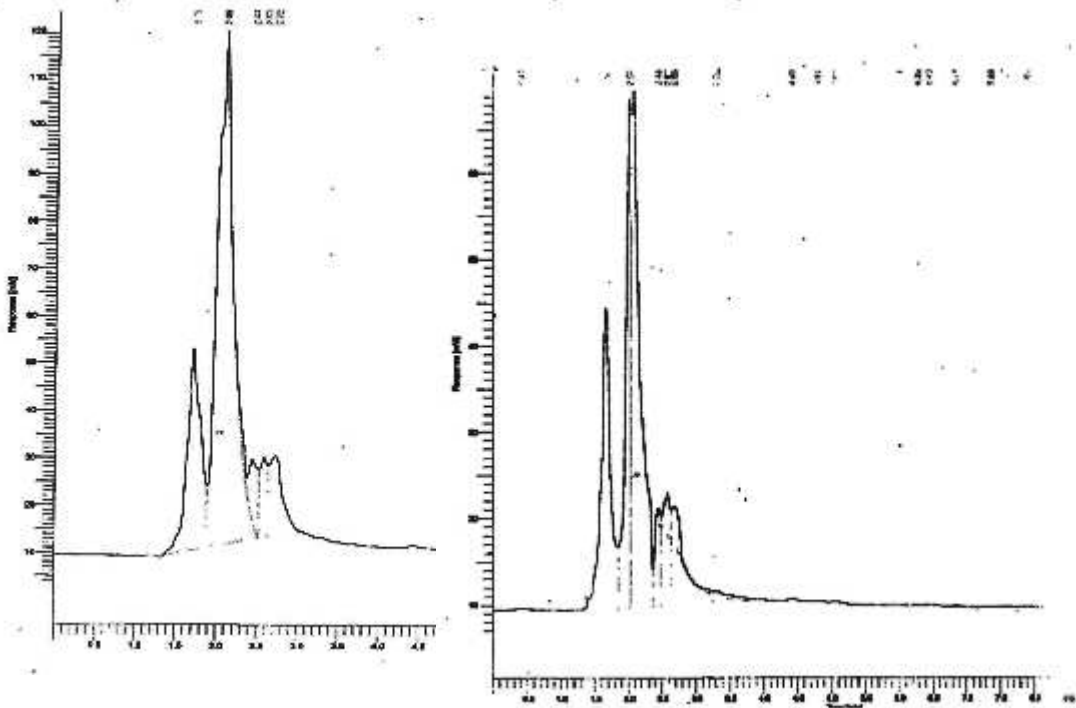


Fig. 3: HPLC of seed sample of *T. indicum*

*et al.* (1980) have described analytical or preparative scale HPLC separation of PAs, and Ramsdell and Buhler (1981) have reported an improved method. Souza *et al.* (2005) isolated PAs from the roots of *Heliotropium indicum* (Boraginaceae) and found Helindicine, a new PAs with unusual structural features, together with the known Lycopsamine. Zhang *et al.* (2008) developed a quantitative method using HPLC-MS2 for the determination of adonifoline, one of the retronecine-type hepatotoxic pyrrolizidine alkaloids in *Senecio scandens*. They determined quantities of adonifoline in extracts of 18 plant samples from different collection sources and from different parts of *S. scandens* by using HPLC/MS2 analysis. Li

*et al.* (2010) established high performance liquid chromatography with diode-array detector (HPLC-DAD) method for simultaneous determination of polyphenols including flavonoid glucosides and phenolic acids in *Ehretia thyrsoiflora* and several medicinal plants of the family Boraginaceae. This report suggests the presence of pyrrolizidine alkaloids in leaf and seed samples of *Trichodesma indicum*.

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