

## Phytochemical investigations of plant *Trichodesma indicum*

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### ABSTRACT

Various extracts of the plant *Trichodesma indicum* (Boraginaceae) were subjected to preliminary Phytochemical screening and it was shown that Flavanoids, Triterpenes, Tannins and Saponins were present. Flavanoids and Triterpenes were present in alcoholic extract, Tannins and saponins in aqueous extract, steroids and saponins in petroleum ether and chloroform extract. Further these various extracts were confirmed by thin layer chromatographic study.

**Key words:** Phytochemical screening, TLC, *Trichodesma indicum*.

### INTRODUCTION

#### Collection and extraction of plant

The plant was collected in Mysore and taxonomical identification of the plant specimen was authenticated by Prof. Shashikala, HOD of Botany, Sarada Vilas Science College, Mysore. The plant was removed and dried under shade and powdered in a mechanical grinder. The powder was extracted with various solvents. All chemicals and reagents used are of analytical grade.

#### Sample taken

*Trichodesma indicum* whole plant extract

#### Solvent

Petroleum ether, Benzene, Chloroform, Alcohol and Water.

#### Method used

Reflux method.

#### Apparatus

Heating mantle, beaker, round bottom

flask, measuring cylinder, double walled condenser, stand etc.

Proportion of the drug with the solvent - 1:4

Quantity of the drug taken 100g

Time-16hrs

Drying temperature - <50° C

#### Extraction procedure<sup>1</sup>

The preparation of various extracts was by successive solvent extraction. The material to be extracted was subjected to extraction with solvents in ascending order of polarity successively. The constituents which are soluble in both polar and non polar solvents can be extracted separately by adopting this approach. For each and every extraction the extract was evaporated under reduced pressure until the solvent had been removed to give an extract sample with a yield of w/w.

All the extracts were examined for their colour and consistency. Their percentage weights were calculated with reference to the dried sample. These data are compiled in table 1.

### Qualitative chemical tests for various chemical Phyto constituents

Qualitative tests were conducted for all the extracts to identify the various phyto constituents. The various tests for steroids, flavonoids and tannins were observed and the tests are recorded in table 2 which shows the qualitative chemical tests conducted for various extracts.

### Purification and separation of Flavanoidal compounds by column chromatography <sup>2</sup>

Attempts were made to purify and separate the Flavanoidal compound by taking alcoholic extracts (prepared by successive solvent extraction) and mixed with silica gel and kept overnight to get adsorbed on the silica gel. Then the column was packed with silica gel using petroleum ether for building the column. Then the silica gel along with alcoholic extract was packed at the top of the column and the column was developed using different solvents. The solvent mixtures were collected in the decreasing order of their polarity as shown in the table 3.

Different fractions were collected at the rate of 18 to 20 drops per minute and each fraction was subjected to TLC examination. The course of chromatogram is given below:

### Examination of the eluates

#### Fraction 1-10

All the fractions showed –ve test for Flavonoids. Hence further investigation was not carried out.

#### Fraction 15-54

All the fractions gave +ve test for Flavonoids and subjected to TLC examination. These fractions showed a single spot having same Rf value. Hence all the fractions were pooled

together and the solvents were removed under reduced pressure.

The residue was dissolved in alcohol and left overnight. The light brownish yellow compound was obtained which was soluble in EtOH, Acetone, MeOH, dil H<sub>2</sub>SO<sub>4</sub> and DMSO.

The isolated Flavanoidal compounds gave +ve test for the following color reactions:

1. FeCl<sub>3</sub> test: Dark green color
2. Magnesium ribbon HCl reduction test- Crimson red color (Shinoda test)
3. Zinc-HCl reduction test –yellow ppt
4. Ammonium Hydroxide solutes test-yellow fluorescence (UV)

### TLC study of isolated compounds<sup>3-6</sup>

Materials used- Glass plate 20x5cm size  
Adsorbent- Silica gel-G

Solvent system used

- a) Ethyl acetate: AA (9:1)
- b) Pet.Ether : ethanol (1:9)
- c) Toluene: Ethyl alcohol: acetone (7:2:2)

|                 |                      |
|-----------------|----------------------|
| Sample          | Isolated flavonoids  |
| Detecting agent | Antimony trichloride |
| Rf value        | 0.9                  |

Further it needs spectral studies to confirm the findings. In a similar fashion steroids (obtained from Pet.Ether- CHCl<sub>3</sub> extract) were subjected to column chromatography and fractions were tested for solvents collected at regular intervals of time and each fraction was subjected to TLC study which showed two different Rf values i.e, 0.9 & 0.75 with different solvent systems which are shown in the Table 4.

Table 1

| S.No | Name of the extract | Nature   | Colour          | Wt. of Extract |
|------|---------------------|----------|-----------------|----------------|
| 1.   | Pet.Ether           | Resinous | Yellowish green | 2g             |
| 2.   | Chloroform          | Resinous | Greenish        | 2.5g           |
| 3.   | Alcohol             | Resinous | Greenish brown  | 4g             |
| 4.   | Aqueous             | Resinous | Brownish yellow | 3.5g           |

Similarly triterpenoids were isolated by taking alcoholic extract with the help of column chromatography & fractions were collected, tested for triterpenoids and gave positive response and later it was subjected to TLC study. It has also shown

two different Rf values (0.9 & 0.72) with different nature of their residues. Further studies like UV, IR & Mass, NMR spectral studies are required to confirm the findings.

Table 2

| S.No. | Chemical test | Pet.Ether | CHCl <sub>3</sub> | Alcohol | Aqueous |
|-------|---------------|-----------|-------------------|---------|---------|
| 1.    | Steroids      | +ve       | +ve               | -ve     | -ve     |
| 2.    | Triterpenes   | -ve       | -ve               | +ve     | +ve     |
| 3.    | Saponins      | -ve       | -ve               | +ve     | +ve     |
| 4.    | Alkaloids     | -ve       | -ve               | -ve     | -ve     |
| 5.    | Flavonoids    | -ve       | -ve               | +ve     | +ve     |
| 6.    | Tannins       | -ve       | -ve               | +ve     | +ve     |

Table 3

| S.No | Fractions | Eluate                    | Nature                | Color |
|------|-----------|---------------------------|-----------------------|-------|
| 1.   | 1-4       | PetEther(100%)            | colorless             | -ve   |
| 2.   | 5-10      | Pet.Ether+Benzene (50:50) | V.light yellow        | -ve   |
| 3.   | 11-15     | Benzene+EtOH(75:25)       | light brownish yellow | +ve   |
| 4.   | 16-20     | Benzene+EtOH (50:50)      | yellowish brown       | +ve   |
| 5.   | 21-25     | Benzene+EtOH (25:75)      | Light orange yellow   | +ve   |
| 6.   | 26-30     | EtOH(100%)                | greenish yellow       | +ve   |
| 7.   | 31-37     | Acetone+MeOH(50:50)       | brownish yellow       | +ve   |
| 8.   | 38-42     | Acetone+MeOH(25:75)       | light yellow          | +ve   |
| 9.   | 43-48     | MeOH(100%)                | brownish yellow       | +ve   |
| 10.  | 49-54     | EtOH(100%)                | greenish yellow       | +ve   |

Table 4: TLC study of the various extracts of *Trichodesma indicum*

| Sl.No | Chemical group                | Solvent system                                   | Spray reagent                      | Rf value      |
|-------|-------------------------------|--|------------------------------------|---------------|
| 1     | Steroids<br>EtOH:diethylamine | a)CHCl <sub>3</sub> :MeOH (7:3)                  | Liebermann                         | 0.9           |
|       |                               | b)Toluene:<br>Burchard Reagent                   |                                    | 0.9&0.75      |
|       |                               | c)CHCl <sub>3</sub> :EtOH (7:3)                  |                                    | 0.9&0.72      |
| 2     | Triterpenoids                 | CHCl <sub>3</sub> : MEOH (7:3)                   | Antimony<br>Trichloride<br>Reagent | 0.87&0.72     |
|       |                               | a) EA:HAc (9:1)                                  |                                    | 0.9,0.81&0.54 |
| 3     | Flavonoids                    | b)CHCl <sub>3</sub> : MeOH (1:1)                 |                                    | 0.92 & 0.75   |
|       |                               | c)Toluene:<br>EtOH:acetone (2:4:4)               | 0.93 & 0.86)                       | 0.92 & 0.85   |
|       |                               | d)EtOH: formic acid: HAc:water<br>(100:11:11:26) |                                    |               |

## RESULTS AND DISCUSSION

*Trichodesma indicum* is a plant containing steroids, triterpenoidal saponins & Flavanoids. The qualitative tests & chromatographic studies substantiate the presence of Steroids, triterpenoidal saponins & flavanoids. The presence of these phytochemical constituents may have different biological activities. The Pharmacological screening of various extracts that are taken up is under progress in our laboratories.

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