



## Phytochemical Analysis and *In-vitro* Antioxidant Activity of Aerial Parts of *Trichodesma indicum*

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### ABSTRACT

This study was conducted to assess the physicochemical analysis, qualitative and quantitative phytochemical analysis and antioxidant activity of various solvent (*n*-hexane, ethyl acetate, ethanol, and water) extract obtained from *Trichodesma indicum* aerial parts. The physicochemical analysis was carried out using standard reported methods. Successive solvent extraction was carried out using *n*-hexane, ethyl acetate, and ethanol by the Soxhlet method, followed by maceration with water. Qualitative phytochemical screening was done using standard procedures. The extracts were analyzed for its total phenolic and total flavonoid contents by Folin - ciocalteu and Aluminium chloride colorimetric methods. For the extracts, antioxidant activity was screened using three different methods, 1, 1-diphenyl-2-picryl-hydrazyl assay, Nitric oxide assay, and Superoxide dismutase assay. Ascorbic acid has been used for antioxidant activity as a positive control. The percentage of inhibition was measured at various levels of concentration. The total ash, water-soluble ash, acid insoluble ash, sulfated ash, and the moisture content of the powdered drug was found to be 15.30, 07.50, 03.99, 21.00, 05.00%w/w. The qualitative phytochemicals found in the extracts are carbohydrates, proteins, and amino acids, flavonoids, phenols, phytosterols, saponins, tannins, and glycosides. The total phenolic and total flavonoid content of the extracts was found in the sequence of ethyl acetate > *n*-hexane > ethanol > aqueous. All the extracts demonstrated significant concentration-dependent antioxidant activity in DPPH assay, Nitric oxide assay, and Superoxide dismutase assay. The findings indicated that all solvent extracts of *Trichodesma indicum* have promising antioxidant activity and that could be the presence of phytochemicals present in the extract.

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### INTRODUCTION

Nature has provided the vast diversity of medicinal plants and potent bioactive constituents for humankind as long many years; however, plants are the treasures for the source of medicines for the primary health care system (Khyade *et al.*, 2017). The medicinal plants and its parts have been used to eradicate the diseases since the olden days. Some of the naturally available phytoconstituents are used for the management of various diseases because these are accepted to be safe with fewer side effects. Secondary metabolites such as polyphenols,

cannabinoids, alkaloids, and isothiocyanates are an important class of plant constituents reported to have antioxidant, anti-inflammatory, (Borneo *et al.*, 2009; Katalinic *et al.*, 2006) hypoglycemic, anti-allergic, antibiotic, anti-carcinogenic and neuroprotective effects (Kumar *et al.*, 2018; Vanisree Mulabagal and Tsay, 2004).

Some of the disorders are such as Cancer, Multiple sclerosis, Parkinson's, Alzheimer's, Arthritis and Atherosclerosis are may be due to the effect of elevated levels of free radicals in the body. Reactive nitrogen species (RNS) and Reactive oxygen species (ROS) are more abundantly produced pro-oxidants produced through normal metabolism or exposure to UV radiation or pollutants (Ghosh *et al.*, 2008; Ognjanović *et al.*, 2008). Reactive oxygen species are continuously produced inside the living organisms for specific purposes and for various reasons. To prevent the damage caused by ROS on living system specifically DNA, proteins and lipids, the organisms are well-composited antioxidant systems, which comprises enzymes like glutathione peroxidase, catalase, superoxide dismutase and macromolecules like ferritin, ceruloplasmin, albumin and also small molecules such as  $\alpha$ -carotene, reduced glutathione, R-tocopherol, and ascorbic acid. Plant and animals are composed of antioxidant systems and it is difficult to measure individually. Hence several methods are being adopted to estimate the total antioxidant activity of biological samples (Blois, 1958).

Plant extracts provide biologically active compounds and source of potent antioxidants for the treatment of oxidative stress-related disorders. Plant-based medicines have the potential to cure the diseases because of their multiple bioactive constituents and comparatively less toxicity for the treatment of diverse medical conditions, including oxidative stress-related disorders. A huge number of plants have been evaluated for their antioxidant properties (Krishnaswamy, 2008; Zengin *et al.*, 2011).

*Trichodesma indicum* is an herbaceous plant commonly known as Adhapushpi from the family Boraginaceae. It is indigenous to India, Africa and this species were considered to be newly naturalized (Wang and Chang, 2014). *Trichodesma indicum* is a small erect, spreading, branched, rugose annual herb; it grows about 50 -75cm tall. The flowers are solitary, axillary and blue in color. The flowering starts from September to November and January to March. The fruits consist of four nutlets, smooth on the outer surface and wrinkled on the inner surface. The fruiting will be throughout the year. The leaves

are opposite- alternate, sessile, hirsute-pubescent and acute auriculate at the base. The apex of the corolla is long-tailed and curved. These leaves are simple, oblong-lanceolate, acute apex and having an entire margin.

*Trichodesma indicum* are more abundantly present in various parts of India and has been used in the Indian system of medicine. Despite its medicinal value, it remains unrecognized in herbal medicine. It is a well-known medicinal plant and has high ethnopharmacological value for treating skin diseases, allergy, arthritis, wound healing, dysentery, and arthralgia. Each part of the plant is useful and was reported for its antioxidant (Kothapalli *et al.*, 2014), anti-inflammatory (Perianayagam *et al.*, 2006), analgesic and antipyretic (Perianayagam *et al.*, 2011), antimicrobial (Perianayagam *et al.*, 2012) and antidiabetic activity (Narendra *et al.*, 2016).

## MATERIALS AND METHODS

### Reagents

All the chemicals used in the experiment were in analytical grade. Rutin, Gallic acid, Ascorbic acid are purchased from Merck, Germany. Nitroblue tetrazolium, Sulphanilamide, orthophosphoric acid, Phenazodinium methosulphate, Nicotinamide adenine dinucleotide, 1, Naphthyl ethylene diamine are obtained from Sigma Aldrich, India. 1-diphenyl-2-picrylhydrazyl (DPPH) and Sodium nitroprusside was obtained from Loba Chemie, India.

### Plant material collection

The fresh and matured aerial parts of the plant used for the study was collected from local region Mysuru, Karnataka, India, during August – September, identified and authentication was done by Dr. Jayaraman, Plant Anatomy Research Centre, Chennai with the voucher specimen number PARC/2018/3741. The collected plant material was washed, cleaned and dried under the shade. The dried material was powdered using an electrical blender and the uniform size was achieved using 40 $\mu$ m mesh size. The plant powder was preserved in an airtight container to avoid the absorption of atmospheric moisture and microbial attack.

### Pharmacognostical study

The pharmacognostic parameters, such as macroscopic and microscopic parameters were screened and reported in our earlier study. The proximate values such as total ash, water-soluble ash, acid insoluble ash, sulfated ash, ethanol-soluble extractive, water-soluble extractive and moisture content was assessed as per the standard methods referred

from Ayurvedic pharmacopeia of India (Pharmacopoeia, 2001; World Health Organization, 2002).

### Preparation of extracts (Successive solvent extraction)

The shade dried plant powder was taken and successively extracted with soxhlation method using the solvents such as *n*-hexane 50-60°C, Ethyl acetate 60-70°C, Ethanol 78°C. The extraction was carried out successively using solvents from non-polar to polar. The powdered plant drug (aerial parts of *Trichodesma indicum*) was packed into the soxhlet apparatus. The solvent was taken in a round bottom flask and hot extraction was carried out for 48 hours. Each time before extracting with next higher polar solvent the marc was dried and extracted finally, the marc was macerated with water. The obtained crude extracts were filtered, concentrated under a rotary evaporator, recovered the solvent under reduced pressure and dried under the vacuum. Each extract was weighed to obtain the percentage yield of extract. Qualitative and quantitative phytochemical analysis of the extracts was performed and the antioxidant activity of all the extracts were assessed.

### Qualitative phytochemical analysis

To investigate the primary and secondary metabolites present in the extract, different qualitative chemical tests were performed (Mohammed, 1998). Each extract (*n*-hexane, ethylacetate, ethanol and aqueous extract) was tested for the presence or absence of metabolites such as alkaloids, carbohydrates, tannins, glycosides, terpenoids, saponins, flavonoids, proteins and amino acids, steroids, phenolics, etc. using reported methods (Brain and Brain, 1975; Harborne, 1973).

### Quantitative phytochemical analysis

#### Total phenolic content determination (TPC)

Using Folin ciocalteu process, all the extracts were screened for TPC. The dried *n*-hexane, ethyl acetate, ethanol and aqueous extract are dissolved in small quantities of Dimethyl Sulphoxide (DMSO) and diluted at a concentration of 1mg/ml with distilled water. For the reference curve, the standard Gallic acid (0-60( $\mu$ g/ml) was taken. The extract of 0.5ml was added to 2.5ml of folin reagent (10%) and mixed for 3minutes. 2ml of sodium carbonate solution (7.5% w/v) was added to the above mixture and made up to 10ml with distilled water. The solution was allowed to stand for 30minutes at room temperature. The solution absorbance was measured at 760nm using a UV-VIS spectrophotometer. The TPC was expressed in  $\mu$ g of Gallic acid equivalent (GAE)/mg of the extract (Singleton et al., 1999).

#### Total flavonoid content determination (TFC)

All the extracts have been screened for their TFC using Aluminum chloride colorimetric method. All the dried extracts were dissolved in a small quantity of Dimethyl Sulphoxide (DMSO) and diluted at a concentration of 1mg/ml with distilled water. The reference curve was prepared at a concentration of 0-100 $\mu$ g/ml using rutin standard drug dissolved in methanol. The diluted sample (0.5ml) or rutin (2ml) was combined with 0.1ml of 10% w/v aluminum chloride solution and 0.1ml of 0.1mM potassium acetate solution. The volume was made with distilled water up to 5ml. The solution has been kept for 30minutes at room temperature. The mixture absorbance was measured at 415nm using a UV-VIS spectrophotometer. The TFC was expressed in  $\mu$ g of rutin equivalent (RUE)/mg of the extract (Chang et al., 2002).

### In vitro antioxidant activity

#### 1, 1-diphenyl-2-picrylhydrazyl (DPPH) Assay

The reported method was used to assess the DPPH radical scavenging activity of *n*-hexane, ethyl acetate, ethanol and aqueous extracts of *Trichodesma indicum* (Shen et al., 2010). The standard ascorbic acid and sample was dissolved in methanol and prepared at 1mg/ml concentrations. Concentrations of 200, 100, 50, 25, 12.5, 6.25, 3.12, 1.07 $\mu$ g/ml dilutions were made using methanol. Freshly prepared 3ml of 0.1mM solution of DPPH in methanol was mixed 1ml of the sample solution. The solutions were kept for 30minutes at room temperature and the absorbance was determined at 517nm. The solutions were prepared in triplicates for the analysis and calculated the mean values of absorbance. The percentage of DPPH radical scavenging activity was determined using the following formula-

$$(\%) \text{ DPPH radical scavenging inhibition} = [(A_0 - A_t/A_0)] \times 100$$

Where  $A_0$  was control absorbance and  $A_t$  was sample absorbance. All the experiments were performed in triplicate and the graph was plotted with the mean  $\pm$  SD values.

#### Nitric oxide assay (NO)

Nitric oxide assay was performed for *n*-hexane, ethyl acetate, ethanol and aqueous extracts using the process (Singh et al., 2012). 2.5 ml of 5 mM sodium nitroprusside solution was prepared with phosphate buffer saline (pH 7.4) and combined with 1.0 ml of extracts at different concentrations (100-500  $\mu$ g/ml). The mixture was incubated for 3hrs at 30°C and to the mixture, 2.5 ml of freshly prepared Griess reagent (1%) was added (which was prepared by dissolving 1gm of Sulphanilamide in few ml of water

and 2ml of orthophosphoric acid was added. Finally, added 100mg of Naphthyl ethylene diamine (0.1% w/v) and made up to 100ml with water. The control solution was prepared without adding the sample solution but the equal amount of buffer was added and conducted in the same manner. The absorbance of the formed color during diazotization of nitrite with sulphanilamide and its successive coupling with naphthyl ethylenediamine hydrochloride reaction mixture was determined within 15-30 seconds at 546 nm and the calibration curve was plotted by concentration against absorbance. The same procedure was followed for standard ascorbic acid and which was compared with samples. The nitric oxide radicals scavenging effect was calculated using the following formula.

$$(\%) \text{ Nitric oxide radical scavenging inhibition} = [(A_0 - A_t/A_0)] \times 100$$

Where  $A_0$  was control absorbance and  $A_t$  was absorbance of sample or extract. All the experiments were determined in triplicate and the graph was plotted with the mean  $\pm$  SD values (Singh *et al.*, 2012).

#### Superoxide dismutase assay (SOD)

Inhibition of nitroblue tetrazolium (NBT) is the basic principle involved in the SOD assay. The reduction of NBT by superoxide radicals is due to the development of the blue-colored compound and it is measured at 560nm under UV spectrophotometer. The procedure followed according to the method stated by (Rukmini *et al.*, 2004). The reaction mixture was prepared by mixing 0.3ml of extracts with 25  $\mu$ l of 186 $\mu$ M Phenazodim methosulphate (PMS), 75  $\mu$ l of 300  $\mu$ M Nitro blue tetrachloride (NBT), 75  $\mu$ l of 780 $\mu$ M Nicotinamide adenine dinucleotide (NADH) and the mixture was allowed for reaction for few minutes at 30°C and the reaction was stopped by the addition of 250  $\mu$ l of glacial acetic acid vigorously. The mixture was agitated with 1.5ml of butanol and placed at room temperature for 10minutes. Further, the mixture was centrifuged at 4°C for 10minutes at 4000rpm. The obtained supernatant was collected and the absorbance was measured at 560nm. The enzyme source was not added to the control group. The percentage inhibition was calculated using the below formula.

$$\% \text{ Superoxide radical scavenging inhibition} = [(A_0 - A_t/A_0)] \times 100$$

Where  $A_0$  was control absorbance and  $A_t$  was sample absorbance. All the tests were carried out in triplicate and the graph was plotted with the mean  $\pm$  SD values.

## RESULTS AND DISCUSSION

The physicochemical constants such as extractive values, ash values and loss on drying were found to validate and standardize the herbal drugs. Any variation in any of the reported parameters in the present work indicates the adulteration of the drug.

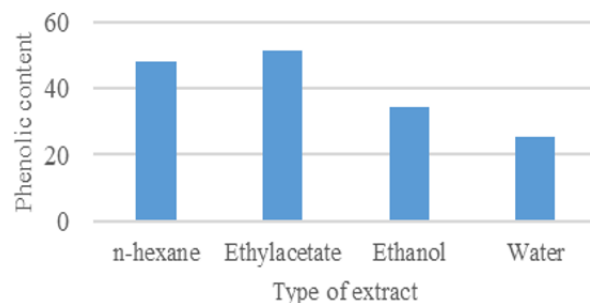


Figure 1: Total phenolic content of extracts

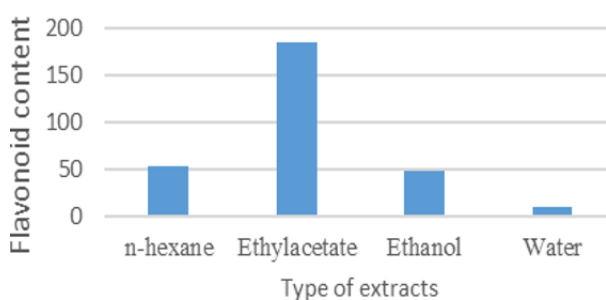


Figure 2: Total flavonoid content of extracts

The physicochemical constants such as total ash, water-soluble ash, acid insoluble ash, sulfated ash and the moisture content of the powdered drug were observed as per the WHO guidelines are given for quality control techniques for medicinal plant materials and the results are tabulated in Table 1.

The plant powder was made and the obtained powdered drug was extracted using different solvents of increasing polarity. The percentage yield of *n*-hexane extract was 03.10%w/w, ethyl acetate 02.45%w/w, Ethanol 03.95%w/w. The aqueous extract has the highest extractive value of 14.04 %w/w as compared to other extracts. The values are reported in Table 2.

To find out the chemical composition of all extracts, different qualitative chemical tests were carried out. All the secondary metabolites such as alkaloids, tannins, glycosides, essential oils are essential for physiological activity and are synthesized in the plants. In addition to that, primary metabolites also important and utilized by man as food articles.

*n*-hexane extract found to contain only phytosterols. Ethyl acetate extract contains diterpenes and triter-



**Table 1: Physicochemical Values of *Trichodesma indicum***

| Parameters                 | Values (%w/w) |
|----------------------------|---------------|
| Total ash                  | 15.30         |
| Acid-insoluble ash         | 07.50         |
| Water-soluble ash          | 03.99         |
| Sulfated ash               | 21.00         |
| Alcohol soluble extractive | 02.70         |
| Water-soluble extractive   | 14.04         |
| Moisture content           | 05.00         |

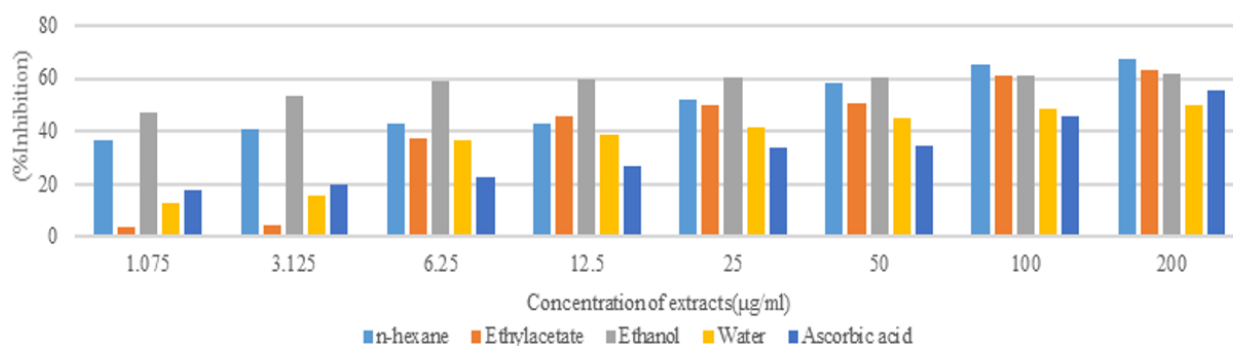
**Table 2: Extractive Values of *Trichodesma indicum***

| Solvent          | Yield (% w/w) | Color of extract |
|------------------|---------------|------------------|
| <i>n</i> -hexane | 3.10          | Yellowish green  |
| Ethyl acetate    | 2.45          | Dark green       |
| Ethanol          | 3.95          | Dark green       |
| Distilled water  | 14.04         | Dark brown       |

**Table 3: Qualitative Analysis of Phytochemicals Present in *Trichodesma indicum* Plant**

| Constituents               | <i>n</i> -hexane extract | Ethyl acetate extract | Ethanol extract | Aqueous extract |
|----------------------------|--------------------------|-----------------------|-----------------|-----------------|
| Alkaloids                  | -                        | -                     | -               | -               |
| Flavonoids                 | -                        | +                     | +               | +               |
| Tannins                    | -                        | -                     | +               | +               |
| Diterpenes and triterpenes | -                        | +                     | +               | +               |
| Saponins                   | -                        | -                     | +               | +               |
| Glycosides                 | -                        | +                     | +               | +               |
| Proteins and amino acids   | -                        | -                     | +               | -               |
| Carbohydrates              | -                        | +                     | +               | +               |
| Phenols                    | -                        | +                     | +               | +               |
| Phytosterols               | +                        | +                     | -               | +               |

'+' indicates presence and '-' indicates the absence of phytoconstituents

**Figure 3: Determination of DPPH Assay**

penes, flavonoids, glycosides, carbohydrates, phenols, and phytosterols. Ethanol and aqueous extract found to have flavonoids, diterpenes and triterpenes, tannins, protein, and amino acids, saponins, glycosides, and phenols but proteins and amino acids are not found in the aqueous extract. The contents are tabulated in Table 3.

The extracts (*n*-hexane, ethyl acetate, ethanol and aqueous) were standardized for total flavonoids and phenolics. The extracts showed a notable amount of flavonoids and phenolics. The total phenolic content was in the descending order from ethylacetate > *n*-hexane > ethanol > aqueous extract. The highest amount of total phenolics was found in ethylacetate extract 52.48  $\mu\text{g}$  of GAE/mg of the extract while least in aqueous extract 24.58  $\mu\text{g}$  of GAE/mg of the extract. The highest flavonoid content was recorded in the descending order ethylacetate > *n*-hexane > ethanol > aqueous extract. The highest amount of total flavonoids was found in ethylacetate extract 52.48  $\mu\text{g}$  of RUE/mg of extract whereas least in aqueous extract 24.58  $\mu\text{g}$  of RUE/mg of extract as shown in Figure 1 and Figure 2.

Exposure to UV radiation, stress, alcohol consumption, high cholesterol consumption, and smoking can elevate cell oxidation (Kohen and Nyska, 2002). The natural polyphenolic compounds act as reducing agents, singlet oxygen quenchers and hydrogen donors due to their presence of hydroxyl groups in their structures and produces antioxidant activity (Javanmardi, 2003). Hence the polyphenolic compounds directly contribute antioxidant action and have hindrance effect on free radical-related disorders (Duh et al., 1999; Tanaka et al., 1988).

The DPPH radical scavenging activity was observed for *n*-hexane, ethyl acetate, ethanol and aqueous extracts at the concentrations of 200, 100, 50, 25, 12.50, 6.25, 3.12, 1.07  $\mu\text{g}/\text{ml}$ . The percentage of inhibition was shown in (Figure 3). The *n*-hexane extract has the highest inhibition followed by ethyl acetate and ethanol when compared to ascorbic acid, which was a standard control and the aqueous extract showed less inhibition as compared to all above.

The nitric oxide assay was performed for *n*-hexane, ethyl acetate, ethanol and aqueous extracts at the concentrations of 100-500  $\mu\text{g}/\text{ml}$  and the observed results are presented in (Figure 4).

Superoxide radicals scavenging activity was observed at the concentration of 100-500  $\mu\text{g}/\text{ml}$  and the observed percentage inhibition was expressed in (Figure 5). The observed results showed the antioxidant activity of all the extracts and it was compared with Ascorbic acid.

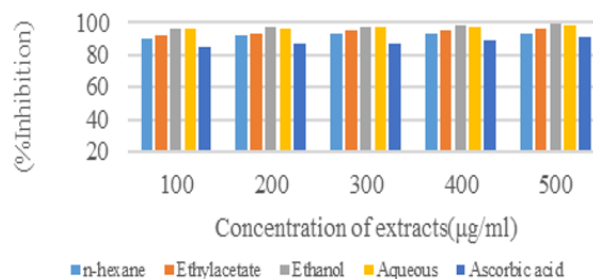


Figure 4: Determination of Nitric oxide assay

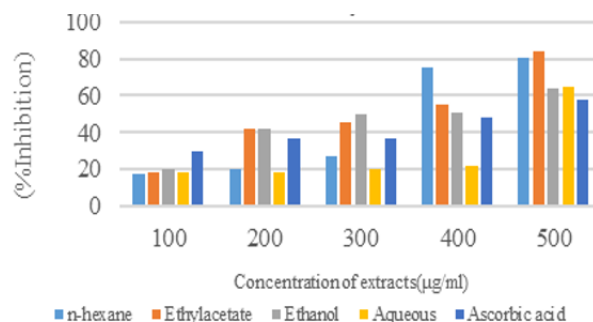


Figure 5: Determination of Superoxide dismutase assay

Identification of plants with potent phytoconstituents for the treatment of ailments becomes much significant and it is necessary to examine and identify the various pharmacognostical parameters to produce the standard medicine.

## CONCLUSIONS

The observed results ensures the plant identity and gives us an idea for the identification of adulterants present in the drug and it improves the quality of the drug. Further, it helps us to standardize the drug using the above mentioned all pharmacognostic parameters. The study also demonstrated that the extracts of *Trichodesma indicum* have multiple bioactive compounds which showed the antioxidant activity. The plant extracts might be beneficial for halting and declining the progress of various oxidative stress-induced diseases. It can also be utilized for the preparation of potent natural antioxidants for the treatment of oxidative stress induced neurodegenerative disease.

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