QUANTITATIVE ANALYSIS OF TOTAL PHENOLIC AND FLAVONOID CONTENTS IN ETHANOLIC LEAF EXTRACT OF TRICHODESMA ZEYLANICUM (BURM. F.) R. BR.

Jayesh Ramdas Pande
Arts, Commerce and Science College
Arvi, District- Wardha
Email- jayeshpande54@gmail.com

Abstract :

Trichodesma zeylanicum (Burm. f.) R. Br. is an ethnoveterinary medicinal plant traditionally used for gastrointestinal disorders in livestock. The present study evaluated the total phenolic and flavonoid contents of its ethanolic leaf extract to validate its traditional use. Total phenolic content, estimated by the Folin–Ciocalteu method, was found to be 82.98 ± 1.45 mg gallic acid equivalents (GAE)/g extract, while total flavonoid content determined by the aluminium chloride method was 8.30 ± 1.00 mg quercetin equivalents (QE)/g extract. The high phenolic and appreciable flavonoid content supporting the ethnoveterinary use of T. zeylanicum in the management of gastrointestinal ailments in livestock.

Keywords: *Trichodesma zeylanicum*, Ethnoveterinary medicine, Folin–Ciocalteu, Gallic acid, Aluminium chloride, Quercetin, Total phenolic content, Total flavonoid content

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Introduction :

Trichodesma zeylanicum (Burm. f.) R. Br., a member of the family **Boraginaceae** is an ethnomedicinally important annual herb widely distributed throughout **peninsular India** extending into **West Bengal** (Hooker, 1883; Cooke, 1958; Banerjee & Pramanik, 1978). The species is commonly known as *Jalshirasi* and is referred to as *Adah Pushpi* and *Jhingi* in Ayurvedic literature, while various indigenous communities recognize it by vernacular names such as *Hetenuriyaa* and *Jinghini*. Morphologically the plant is characterized by an erect growth habit reaching up to 0.5 m in height, softly hairy stems and branches and oblong-lanceolate leaves bearing hispid hairs on both surfaces. The light blue flowers arranged in cymes, campanulate corolla lobes and ovoid nutlets serve as key diagnostic features. (Hooker, 1883; Cooke, 1958). *Trichodesma zeylanicum* is well known in traditional and folk medicine for its wide range of therapeutic applications. In ethnoveterinary practices particularly in rural regions a fermented decoction prepared from leaves soaked in buttermilk is administered orally to livestock to treat **gastritis, stomach ulcers, bloody stools and other gastrointestinal disorders**. Ethnomedicinal reports further indicate that decoctions of leaves and roots are used in the treatment of **diarrhea, dysentery, intestinal worms, cough, itching and abdominal pain** while root scrapings are applied externally for **wound dressing, poisoning and as an antimicrobial agent** (Bosch, 2006; Ngonda, 2013). Phenolic compounds and flavonoids constitute two major classes of bioactive secondary metabolites that significantly contribute to

the therapeutic potential of medicinal plants. These compounds are widely recognized for their antioxidant, antimicrobial and gastroprotective properties, which play an important role in the prevention and management of gastrointestinal disorders (Harborne, 1998; Kumar & Pandey, 2013). In ethnoveterinary medicine plants rich in phenols and flavonoids are frequently utilized for the treatment of digestive ailments in livestock due to their ability to neutralize free radicals and inhibit pathogenic microorganisms (Cushnie & Lamb, 2005). Therefore the present study emphasizes the estimation of total phenolic content and total flavonoid content of the selected plant to scientifically validate its traditional use and to support its relevance in the management of gastrointestinal ailments in livestock.

Materials and Methods :

Sample collection and extraction :

Fresh leaves of *Trichodesma zeylanicum* (Burm. f.) R. Br. were collected from Gonapur village, Tehsil Prabhat Pattan, which forms part of the Satpura range, following standard ethnobotanical collection practices (Jain, 1991; Martin, 1995). The plant material was initially identified with the assistance of a local ethnoveterinary practitioner as recommended for documentation of traditional knowledge (Jain & Rao, 1977). The taxonomic identity of the collected specimen was subsequently authenticated and verified by Dr. N. M. Dongarwar through critical comparison with standard regional floras (Hooker, 1872–1897; Cooke, 1903). A voucher specimen was prepared and deposited with the accession number 2025/211 for future reference in accordance with herbarium preservation guidelines (Bridson & Forman, 1998). The collected leaf samples were thoroughly washed under a hydro-alcoholic solution to remove adhering dirt and contaminants (Harborne, 1998). The cleaned leaves were then shade-dried in a closed room under ambient conditions to prevent contamination by dust particles and degradation of bioactive compounds (Saxena et al., 2013). After complete drying the plant material was coarsely powdered using an electric grinder. Approximately 25 g of the dried leaf powder was subjected to extraction using ethanol as solvent by the Soxhlet extraction method, a widely employed technique for phytochemical studies (Soxhlet, 1879; Kokate, Purohit, & Gokhale, 2014). The resulting extracts were allowed to dry at room temperature under sterile conditions until complete solvent evaporation (Harborne, 1998). The dried extracts were then collected and preserved in airtight containers and stored under refrigerated conditions until further use.

Determination of total phenolic content :

The total phenolic content of the plant extract was determined using the Folin–Ciocalteu method with slight modifications (Ainsworth & Gillespie, 2007). Briefly, 0.5 mL of the extract was mixed with 0.5 mL of Folin–Ciocalteu reagent. The mixture was allowed to stand at 25 °C for 5–8 minutes, after which 2 mL of 7.5% (w/v) sodium carbonate solution was added. The final volume was adjusted to 8 mL with distilled water and the reaction mixture was incubated at room temperature for 2 hours. The absorbance was then measured at 725 nm using a UV–Visible spectrophotometer. Gallic acid was used as the standard for calibration, and the total phenolic content was expressed as milligrams of Gallic acid equivalents per gram

of plant extract (mg GAE/g).

Determination of total flavonoids content :

The total flavonoid content (TFC) was determined using a colorimetric assay as described by Joshi et al. (2024) and John et al. (2014) with minor modifications. Briefly, 100 µL of the plant extract was mixed with 4 mL of distilled water. To this, 0.3 mL of 5% sodium nitrite solution was added and the mixture was allowed to stand for 5 min. Subsequently, 0.3 mL of 10% Aluminium chloride solution was added and incubated for 6 min. Thereafter, 2 mL of 1 M sodium hydroxide was added to the reaction mixture. The final volume was immediately adjusted by adding 3.3 mL of distilled water, followed by thorough mixing. The absorbance of the resulting solution was measured at 510 nm against a reagent blank using a UV–Visible spectrophotometer. Quercetin was used as the reference standard for constructing the calibration curve. The total flavonoid content of the extract was expressed as milligrams of Quercetin equivalents per gram of sample (mg QE/g)

Result and discussion :

Determination of total phenolic content :

The total phenolic content of the *Trichodesma zeylanicum* R. Br. [Leaf] was determined using the Folin-Ciocalteu method at 750 nm of absorbance in terms of the Gallic acid equivalent in mg/g of the extract. The total phenolic content was calculated with the help of the graph shown in Figure: 1 and the standard curve equation was $y = 0.0021x + 0.1142$, where $R^2 = 0.9935$, absorbance of blank is 0.027. The total phenolic contents [Gallic acid equivalents, mg/g] in the ethanolic dry extract were calculated to be 82.98±1.45 mg/g.

Table No.1: Total phenolic content extract of Trichodesma zeylanicum R. Br. [Leaf]

Test-tube No.	Absorbance	Mean of Absorbance
1	0.201	0.201
2	0.203	
3	0.200	

x=[y-c]/m	C= x/1000 [mg/ml]	A= cxv/m [TPC Mg GAE/g extract]	Mean	Standard Deviation	Mean ± SD [mg GAE/g crude extract]
41.33	0.0413	82.66	82.98	1.45	82.98±1.45
42.28	0.0422	84.57			
40.85	0.0408	81.71			

The values are the mean of three experiments ± SD. Statistical data shows significant difference at #P<0.001. SD: Standard deviation

Determination of total flavonoid content :

The total flavonoid content of the *Trichodesma zeylanicum* R. Br. [Leaf] was determined using the Aluminum chloride colorimetric assay at 510 nm of absorbance in terms of the Quercetin equivalent in mg/g of the extract. The total flavonoid content was calculated with the help of the graph shown in Figure: 2 and the standard curve equation was y = 0.002x + 0.3607, where R^2 = 0.992, absorbance of blank is 0.083. The total flavonoid content [Quercetin equivalents, mg/g] in the ethanolic dry extract was calculated to be 8.30±1.00mg/g.

Table No.2: Total flavonoid content of extract of Trichodesma zeylanicum R. Br. [Leaf]

Test-tube No.	Absorbance	Mean of Absorbance
1	0.369	0.3690
2	0.370	
3	0.368	

x=[y-c]/m	C= x/1000 [mg/ml]	A= cxv/m [TPC Mg GAE/g extract]	Mean	Standard Deviation	Mean ± SD [mg GAE/g crude extract]
4.15	0.00415	8.3	8.30	1.00	8.30±1.00
4.65	0.00465	9.3			
3.65	0.00365	7.3			

The values are the mean of three experiments ± SD. Statistical data shows significant difference at #P<0.001. SD: Standard deviation

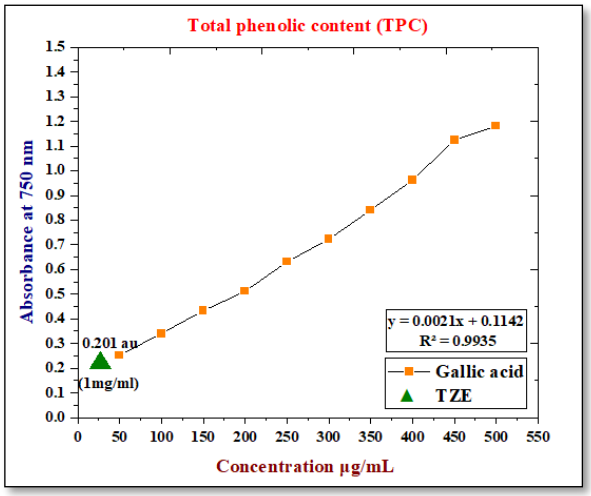

Figure 1: Total Phenol content of ethanolic leaf extract of *Trichodesma zeylanicum*

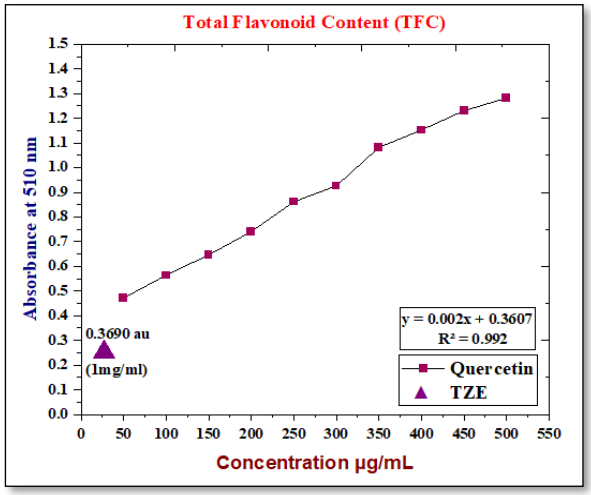

Figure 2: Total flavonoid content of ethanolic leaf extract of *Trichodesma zeylanicum*

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The quantitative estimation of total phenolic and flavonoid contents in the ethanolic leaf extract of *Trichodesma zeylanicum* R. Br. revealed a substantial presence of bioactive secondary metabolites. The total phenolic content was found to be 82.98 ± 1.45 mg gallic acid equivalents (GAE)/g extract indicating a high phenolic concentration. Phenolic compounds are well recognized for their strong antioxidant potential due to their ability to donate hydrogen atoms or electrons and to chelate metal ions thereby neutralizing free radicals and reducing oxidative stress (Ainsworth & Gillespie, 2007; Pandey & Rizvi, 2009). The high phenolic content observed in the present study suggests that *T. zeylanicum* leaves may significantly contribute to antioxidant-mediated therapeutic effects. This is particularly relevant in ethnoveterinary medicine where oxidative stress is often associated with gastrointestinal disorders, infections and inflammatory conditions in livestock. Previous studies have demonstrated a strong correlation between phenolic content, antioxidant, antimicrobial and anti-inflammatory activities of medicinal plants (Dai & Mumper, 2010; Siddiqui et al., 2017). Therefore, the elevated phenolic content supports the traditional use of *T. zeylanicum* in treating gastrointestinal ailments. The total flavonoid content of the ethanolic leaf extract was determined to be 8.30 ± 1.00 mg quercetin equivalents (QE)/g extract which although lower than the phenolic content, still represents a biologically significant level. Flavonoids are an important class of polyphenols known for their antioxidant, antimicrobial, antidiarrheal and gastroprotective properties (Panche et al., 2016). Their ability to inhibit lipid peroxidation, modulate enzyme activity and protect intestinal mucosa may explain their role in alleviating gastrointestinal disorders. The comparatively lower flavonoid content may be attributed to solvent selectivity, plant maturity, environmental factors or differential biosynthesis of phenolic subclasses in *T. zeylanicum.* Similar variations in flavonoid concentration have been reported in several ethnomedicinal plants when extracted using ethanol (Rashid et al., 2018; Joshi et al., 2024). Nevertheless, even moderate flavonoid levels can synergistically enhance the biological activity of phenolic-rich extracts. Overall, the substantial phenolic content coupled with appreciable flavonoid levels suggests that the ethanolic leaf extract of *Trichodesma zeylanicum* possesses strong antioxidant potential. These findings scientifically validate the traditional ethnoveterinary applications of the plant and indicate its potential as a natural source of antioxidant compounds for managing gastrointestinal disorders in livestock.

Conclusion :

The ethanolic leaf extract of *Trichodesma zeylanicum* R. Br. showed a high total phenolic content (82.98 ± 1.45 mg GAE/g) and a moderate total flavonoid content (8.30 ± 1.00 mg QE/g). These results indicate a strong antioxidant potential of the plant mainly attributed to its phenolic constituents. The findings support the traditional ethnoveterinary use of *T. zeylanicum* in the management of gastrointestinal disorders in livestock and highlight its potential for further pharmacological studies.

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