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Phytochemical profiling of *Terminalia chebula* Retz: A comparative study of fruit and seed accessions from North-East and South IndiaShobith Murthy Mahadeva*, Umesh Kanna Subramani**, Parthiban Kalappan Thangamuthu*, Senthilraja Kandasamy**, Venkatesan Subramanian** and Devanand Pachanoor Subbian*[◆]

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Abstract

The current research was carried out to delineate the phytochemical profiles of fruits and seeds of 30 candidate plus trees (CPTs) of *Terminalia chebula* Retz. collected from diverse geographical locations in northeastern and southern parts of India. The crude samples were analyzed for total tannin content (TTC), total phenol content (TPC), total carbohydrate content, and total protein content using standard biochemical assays. Significant variations ($p < 0.01$) were observed among the genotypes for all parameters. The TTC ranged from 222.45-423.82 mg/100 g, TPC from 465.77-625.49 mg/100 g, carbohydrates from 603.71-786.73 mg/100 g and proteins from 211.51-357.97 mg/100 g in fruits whereas, the seeds showed lower concentrations (TTC: 43.87-262.34 mg/100 g, TPC: 369.97-563.33 mg/100 g, carbohydrates: 295.03-491.28 mg/100 g and proteins: 87.67-259.25 mg/100 g). Fruits generally contained higher concentrations of bioactive compounds compared to seeds. Based on the results, the genotypes TNTC 01 (for tannin and phenol), TNTC 05 (for tannin), TNTC 03 (for phenol), APTC 02 and ASTC 02 (for carbohydrates), MHTC 05 and APTC 04 (for proteins) showed consistently higher levels of phytochemicals and could be recommended for further research and cultivation. The wide variation observed among the genotypes highlights the importance of careful selection and standardization when utilizing *T. chebula* for medicinal purposes. The finding supports traditional medicinal uses of *T. chebula* and demonstrates the need to consider genetic and environmental factors in determining the phytochemical profiles of medicinal plants.

1. Introduction

The deciduous tree *Terminalia chebula* Retz. a member of the Combretaceae family, is known by various names including Harar, Chebulic myrobalan, Haritaki, and Black myrobalan. This species thrives predominantly across the Indian subcontinent, with significant populations extending into China, Southeast Asian countries like Myanmar and Vietnam, and the island nation of Sri Lanka (Kumar *et al.*, 2020). In India, the tree's distribution is particularly extensive and flourishes in the sub-Himalayan belt, spanning from the Ravi River in the west to the states of West Bengal and Assam in the east. The species can be found at elevations reaching up to 1500 m (4900 ft) in the Himalayan region. Wild populations of *T. chebula* are abundant in northern India's forests, central provinces, and Bengal. The tree's distribution also encompasses southern regions, including Madras, Mysore, and the lower areas of the former Bombay Presidency (Mahadeva *et al.*, 2024).

The pharmaceutical industry has taken a keen interest in *T. chebula*, particularly its fruits and seeds. These plant parts are valued for their rich phytochemical content, making them valuable resources in

the development of various medicinal products (Kailash *et al.*, 2022). Phytochemicals are naturally occurring substances found in the majority of plants. They serve as primary and secondary plant metabolites with anti-inflammatory, antimicrobial, antidiabetic, and antihyperglycaemic effects, and are widely distributed in medicinal plants and herbs (Yazan and Armania, 2014). The use of herbal remedies has grown recently because of their low adverse effect rate on a global scale (Gunasekar *et al.*, 2019). The yearly value of plants with remarkable therapeutic qualities exported worldwide was estimated to be at 2.2 billion USD with several hundred billion dollars invested in the herbal medicine sector (Ahn, 2017; Awuchi, 2019). Researchers from all around the world studied these plants to find their safe dosages due to a lack of scientific and evidence based understanding about them (Sultan *et al.*, 2023).

One such plant that was used in herbal concoctions to treat a variety of health issues is myrobalan (*T. chebula*), known as the "king of medicinal plants" in Ayurveda (Cock *et al.*, 2015). The unripe fruit is highly regarded for its aperient and astringent qualities, which makes it beneficial for treating diarrhea and dysentery. It is also utilized to enhance gastrointestinal health because of the matured fruit's adsorption capacity and purgative properties (Chellammal, 2022). It is regarded as a brain, memory, and eye tonic by Unani physicians. In addition, it is used to clear yellow bile, and cure epilepsy, piles, diarrhea, and headaches (Akbar, 2020). Because of its wide range of tannins and flavonoids, it has the potential to be an effective medicinal agent against several illnesses (Meher *et al.*, 2018; Zhao *et al.*, 2021).

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The ancient ayurvedic formulation known as “Triphala” prominently features these fruits as a key component. This blend is renowned for its purported ability to invigorate the body with youthful vitality while enhancing mental acuity and sensory perception. Beyond its medicinal applications, the tree from which these fruits are harvested proves to be a versatile resource. The wood finds use in crafting furniture, while its bark contributes to the tanning process. Additionally, various parts of the tree serve purposes in clinical settings (Wadkar and Pinjari, 2023). In recent decades, there has been a notable resurgence of interest in herbal remedies. This trend spans across both developed and developing nations, reflecting a global shift towards natural healing modalities (Saxena *et al.*, 2013).

Natural materials have a far broader variety of medicinal uses than synthetic organic compounds manufactured in a lab. These natural products have been used for treating pathological conditions in both contemporary as well as conventional medicine (Chen *et al.*, 2021). Natural products are now frequently employed as a basis for optimizing drug discovery leads, with synthetic alteration added to aid decrease adverse effects and post-bio availability. About two-third of the pharmaceuticals approved by authorities are inspired by natural products (Atanasov *et al.*, 2015). Apart from being utilized in medicines, natural products, and their derivatives have also been added to food as flavors and herbs, antimicrobial and antioxidants to protect food freshness (Mishra and Tiwari, 2011). A few of the bioactive components of these plants include steroids, terpenoids, flavonoids, carotenoids, alkaloids, tannins, and phenols (Nigam *et al.*, 2020). Despite the numerous medicinal benefits that *T. chebula* offers, less research has been done on the phytoconstituents of this plant. Hence, the phytochemical profile of *T. chebula* was emphasized in the current study. The study’s main focus is on the conceptual basis for myrobalan’s efficacy as a natural substitute for synthetic

medicines. Against this back drop, the current study was subjected to preliminary screening of phytochemical constituents of *T. chebula* fruits and seeds accessions from north-eastern and southern parts of India.

2. Materials and Methods

2.1 Experimental site

The study was carried out at the Forest College and Research Institute, Mettupalayam, Tamilnadu, India (11°192 N, 76°562 E). The experiment was laid out in Completely Randomized Design (CRD). Analysis of Variance (ANOVA) was carried out using SPSS software to test the significance among the genotypes.

2.2 Plant materials

The matured fruits were collected from a total of 30 genotypes in different *T. chebula* dominant geographical locations of India *viz.* Tamil Nadu, Maharashtra, Kerala, Karnataka, Assam, and Andhra Pradesh during January-March, 2024 (Table 1) (Figure 1). The fruits were collected from superior trees in each location chosen using the comparison check tree method based on several growth parameters. The trees were identified with the aid of standard monographs (Khare, 2007) Plant Identification No.: 506159.

2.3 Preparation of plant extract

The fruits were washed, sun-dried, cut open by hammer, and the outer pericarp and inner endocarp were separated manually (Singh *et al.*, 2020). The seeds were extracted from each fruit. The dried fruits and seeds were ground to fine powder by grinder separately and were kept in zip-locked bags and stored. The powdered samples were used for further biochemical studies.



Figure 1: (a) Fruit and (b) Seed of *T. chebula*.

Table 1: Geomorphological details of 30 CPTs of *T. chebula*

State	Accessions	District	Location	Longitude	Latitude
Tamil Nadu	TNTC 01	Dharmapuri	Chiteri hills	78.3008	11.5313
	TNTC 02	kallakuruchi	Kalvarayan hills	78.7114	11.8166
	TNTC 03	Tiruvannamalai	Jamunamarthur	78.9457	12.6545
	TNTC 04	Tiruchirappalli	Pacchamalai hills	78.6252	11.2874
	TNTC 05	Salem	Shervarayan hills	78.1328	11.5037
Maharashtra	MHTC 05	Jalgaon	Jalgaon	75.3316	21.0048
	MHTC 04	Gadchiroli	Gyarapatti	80.0142	20.0957
	MHTC 03	Ratnagiri	Dapoli	73.1785	17.7491
	MHTC 02	Gadchiroli	Bhimankhoji	80.0104	20.1036
	MHTC 01	Akola	Patur	76.5545	20.2333
Kerala	KETC 05	Palakkad	Attapadi	76.3424	11.0851
	KETC 04	Wayanad	Tholpatty	76.0354	11.5639
	KETC 03	Wayanad	Wayanad	76.0231	11.5245
	KETC 02	Idduki	Chinnar	77.1222	10.1824
	KETC 01	Idduki	Marayur	77.0941	10.1658
Karnataka	KATC 05	Uttara Kannada	Jagalabetta	74.5099	15.3274
	KATC 04	Kodagu	Ponnampet	77.7491	13.2146
	KATC 03	Uttara Kannada	Siddapura	74.5306	14.2723
	KATC 02	Bangalore rural	Chikkabalapur	77.7491	13.2146
	KATC 01	Chitradurga	Hemanthgiri	76.4013	14.1868
Assam	ASTC 05	Jorhat	Guhainkur	94.5364	26.9148
	ASTC 04	Jorhat	Gyanpith	94.1953	26.7638
	ASTC 03	Jorhat	Baligaon	94.4128	26.6672
	ASTC 02	Jorhat	Alengigaon	94.4129	26.6671
	ASTC 01	Kokrajhar	Habrubil	89.9801	26.4486
Andhra Pradesh	APTC 05	Tirupati	Talakona	79.1872	13.8118
	APTC 04	Anantapur	Muntimandagu	79.3583	13.7995
	APTC 03	Eluru	Duripodalu	79.3832	13.7902
	APTC 02	Sri Sathya Sai	Kadri	78.0837	14.0930
	APTC 01	Cudappah	Puttampalli	78.8749	14.4234

The present study was carried out based on the objective of biochemical characterization of fruits and seeds of *T. chebula*. The collected fruits and seeds were subjected to a variety of standard qualitative assays to estimate the total tannin content, total phenol content, total carbohydrate content, and total protein content. The standard chemicals used for analysis were purchased from Otto Chemical Pvt. Ltd., Mumbai, Maharashtra.

2.4 Phytochemical screening

To estimate primary and secondary metabolites, phytochemical examination of the samples was carried out. Carbohydrates and proteins were screened for primary metabolites, whereas phenols and tannins were screened for secondary metabolites.

2.4.1 Total tannin content (Folin-Denis method)

The idea underlying tannin estimates is that tannin-like substances in alkaline solutions decrease phosphotungstomolybdic acid to generate a strongly colored blue solution, the intensity of which is correlated with the amount of tannins present. In triplicate, the total tannin content (TTC) was determined using a spectrophotometer set at 700 nm. Following the protocol of Qahtan and Alatar (2024), the Folin-Denis reagent, sodium carbonate solution, standard tannic acid solution, and working standard solutions were prepared.

To calculate TTC, 0.5 g of the powdered material was dissolved in 75 ml of water to extract the tannin, and the flask was gently heated for 30 min. After centrifuging the dissolved material for 20 min at 2000 rpm, the supernatant was collected in a 100 ml volumetric

flask, and the volume was adjusted. 1 ml of the sample extract was put into a volumetric flask of 100 ml. 10 ml of sodium carbonate solution was added to the flask along with 5 ml of Folin-Denis reagent and diluted to 100 ml. After 30 min, the observation was read at 700 nm. In parallel, a blank was made using water in place of the sample. A standard was prepared with tannic acid ranging from 0-100 µg. The TTC of samples was calculated as tannic acid equivalence from the standard graph and expressed as mg/100 g.

2.4.2 Total phenol content (Folin-Ciocalteu method)

Based on the principle that phenols react with phosphomolybdic acid in the Folin-Ciocalteu reagent in an alkaline medium to form a blue colored complex (molybdenum blue), the Folin-Ciocalteu technique was used to quantify the amount of phenol. Total phenol content (TPC) was estimated using 80% ethanol, 20% Na₂CO₃, the Folin-Ciocalteu reagent, and standard catechol (Srihanam *et al.*, 2021).

0.5 g of powdered sample was used to extract the sample, and 10 ml of 80% ethanol was used to ground it in a pestle and mortar. After centrifuging for 20 min at 10000 rpm, the supernatant was collected and dried completely by evaporating it. A known volume of distilled water (5 ml) was used to dissolve the residue. 1 ml of the dissolved residue was pipetted into test tubes and the volume was made up to 3 ml with water. 0.5 ml of Folin-Ciocalteu reagent was added to the test tube, and 2 ml of Na₂CO₃ solution was added after 3 min. After carefully mixing, the test tube was submerged in boiling water for 1 min. Using a UV-Spectrophotometer, the absorbance was measured at 650 nm after cooling and compared with a blank. A standard curve was prepared using different concentrations of catechol and was used to find the concentration of TPC in the test sample expressed as mg/100 g.

2.4.3 Total carbohydrate content (Anthrone method)

In the Anthrone technique of carbohydrate measurement, carbohydrates are first hydrolyzed into simple sugars using diluted hydrochloric acid (HCl). Glucose is dehydrated to hydroxymethyl furfural in a heated acidic media. When combined with anthrone, this chemical generates a green product whose absorbance maxima occurs at 630 nm (Udeozo *et al.*, 2018).

The sample was extracted using 2.5 N HCl, and standard glucose and anthrone reagents were used for analysis (Udeozo *et al.*, 2018). 5 ml of 2.5 N HCl was added to 100 mg of the sample, which was placed in a boiling water bath for 3 h to hydrolyze it. Solid Na₂CO₃ was added to the tube after cooling to neutralize the effervescence. The volume was made up to 100 ml and centrifuged. After collecting the supernatant, 0.5 ml aliquot was utilized for analysis. Both the blank and the standards were prepared at the same time. Distilled water was added to all the tubes, including the sample tubes, to make up the volume to 1 ml. 4 ml of anthrone was added to each tube and was heated in a boiling water bath for 8 min. The tubes were cooled rapidly and absorbance was read at 630 nm using UV-Spectrophotometer. A standard graph was prepared and was used to estimate the amount of carbohydrates present in the sample expressed as mg/100 g.

2.4.4 Total protein content (Lowry's method)

The total protein content is estimated using the principle that the blue color is produced as a result of the protein's tryptophan and tyrosine, which reduce the phosphomolybdic phosphor components in the Folin-Ciocalteu reagent; the blue color is also the result of the protein's biuret reaction with the alkaline cupric tartrate.

reagent A (2% Na₂CO₃ in 0.1 N NaOH), reagent B (0.5% CuSO₄ in 1% potassium sodium tartrate), reagent C (alkaline copper solution- mix 50 ml reagent A and 1ml reagent B before use), Reagent D (Folin-Ciocalteu reagent), phosphate buffer (KH₂PO₄ + K₂HPO₄), albumin bovine fraction V solution and working standard were prepared for the estimation of total protein in the test samples (Saqib *et al.*, 2024).

A phosphate buffer was used to extract the protein from the amole. 500 mg of the material was ground in 10 ml of buffer using a pestle and mortar before being centrifuged. To estimate the amount of proteins, the supernatant was collected. A series of test tubes were pipetted with 0.1 ml of the sample extract along with various concentrations of the working standard. Volume was made up to 1 ml in each test tube, and a blank tube containing 1 ml of water was used. 5 ml of reagent C was added to each tube, including blank. They were mixed and left to stand for 10 min. 0.5 ml of reagent D was added and mixed thoroughly. The mixture was left to incubate at room temperature in the dark for 30 min, which led to the development of blue color. The readings were taken at 660 nm using a UV-Spectrophotometer. A standard graph was drawn and was used to estimate the amount of protein in the sample expressed as mg/100 g.

3. Results

The results revealed that the estimated amounts of several phytochemicals, *viz.*, total tannin content, total phenol content, total carbohydrate content, and total protein content were significantly higher in fruits compared to seeds.

3.1 Total tannin content of fruits and seeds

The Folin-Denis reagent technique was used to determine the total tannin content in comparison with the standard tannic acid curve ($y = 0.109x - 0.0933$, $R^2 = 0.9967$) (Figure 2a). Significant variations in total tannin content ($p < 0.01$) were observed among the fruits from the thirty candidate plus trees of *T. chebula* and it ranged from 423.82 mg/100 g (TNTC 05) to 222.45 mg/100 g (MHTC 05) (Table 2). The highest total tannin content was observed in TNTC 05 accession (423.82 mg/100 g), followed by TNTC 01 (418.13 mg/100 g) against the general mean of 300.46 mg/100 g. The lowest tannin content was recorded by MHTC 05 (222.45 mg/100 g), followed by KATC 02 (222.63 mg/100 g).

In case of seeds from 30 genetic resources of *T. chebula* the total tannin content ranged from 43.87 mg/100 g (KATC 05) to 262.34 mg/100 g (KATC 04) (Table 3). The highest tannin content was noticed in KATC 04 (262.34 mg/100 g), followed by TNTC 04 (231.39 mg/100 g) against the general mean of 127.89 mg/100 g. The lowest was recorded in KATC 05 (43.87 mg/100 g), followed by (58.43 mg/100 g) (Table 3).

3.2 Total phenol content of fruits and seeds

The Folin-Ciocalteu technique was used to calculate the total phenol content in comparison with the standard catechol curve ($y = 0.3078x - 0.2587$, $R^2 = 0.9969$) (Figure 2b). The accession TNTC 01 (625.49 mg/100 g) exhibited significantly highest total phenol content ($p < 0.01$), followed by TNTC 03 (595.22 mg/100 g) compared with the general mean of 552.98 mg/100 g. The lowest phenol content was registered by KETC 01 (465.77 mg/100 g), followed by KATC 04 (487.70 mg/100 g). Holistically, the phenol content ranged between 465.77 mg/100 g and 625.49 mg/100 g as illustrated in Table 2.

Concerning seeds, the total phenol content varied from 369.97 mg/100 g (ASTC 05) to 563.33 mg/100 g (KETC 05) among the 30 candidate plus trees of *T. chebula*. Significantly highest total phenol content was observed in KETC 05 (563.33 mg/100 g), followed by TNTC 05 (558.13 mg/100 g) and the lowest was recorded by ASTC 05 (369.97 mg/100 g), followed by MHTC 01 (375.17 mg/100 g) (Table 3).

3.3 Total carbohydrate content of fruits and seeds

The Anthrone technique was used to estimate the total amount of carbohydrates against the standard D (+) Glucose curve ($y = 0.3391x -$

0.3897 , $R^2 = 0.9956$) (Figure 2c). The significantly highest total carbohydrate content ($p < 0.01$) was exhibited by APTC 02 (786.73 mg/100 g), followed by ASTC 02 (785.97 mg/100 g) when compared with the general mean of 707.65 mg/100 g. The lowest carbohydrate content was registered by KATC 02 (603.71 mg/100 g), followed by MHTC 04 (609.94 mg/100 g) (Table 2).

In case of total carbohydrate content of seeds among the 30 genetic resources, the highest carbohydrate content was recorded in KATC 02 (491.28 mg/100 g), followed by KATC 01 (483.45 mg/100 g). The lowest carbohydrate content was observed in APTC 01 (295.03 mg/100 g), followed by KETC 03 (295.91 mg/100 g) (Table 3).

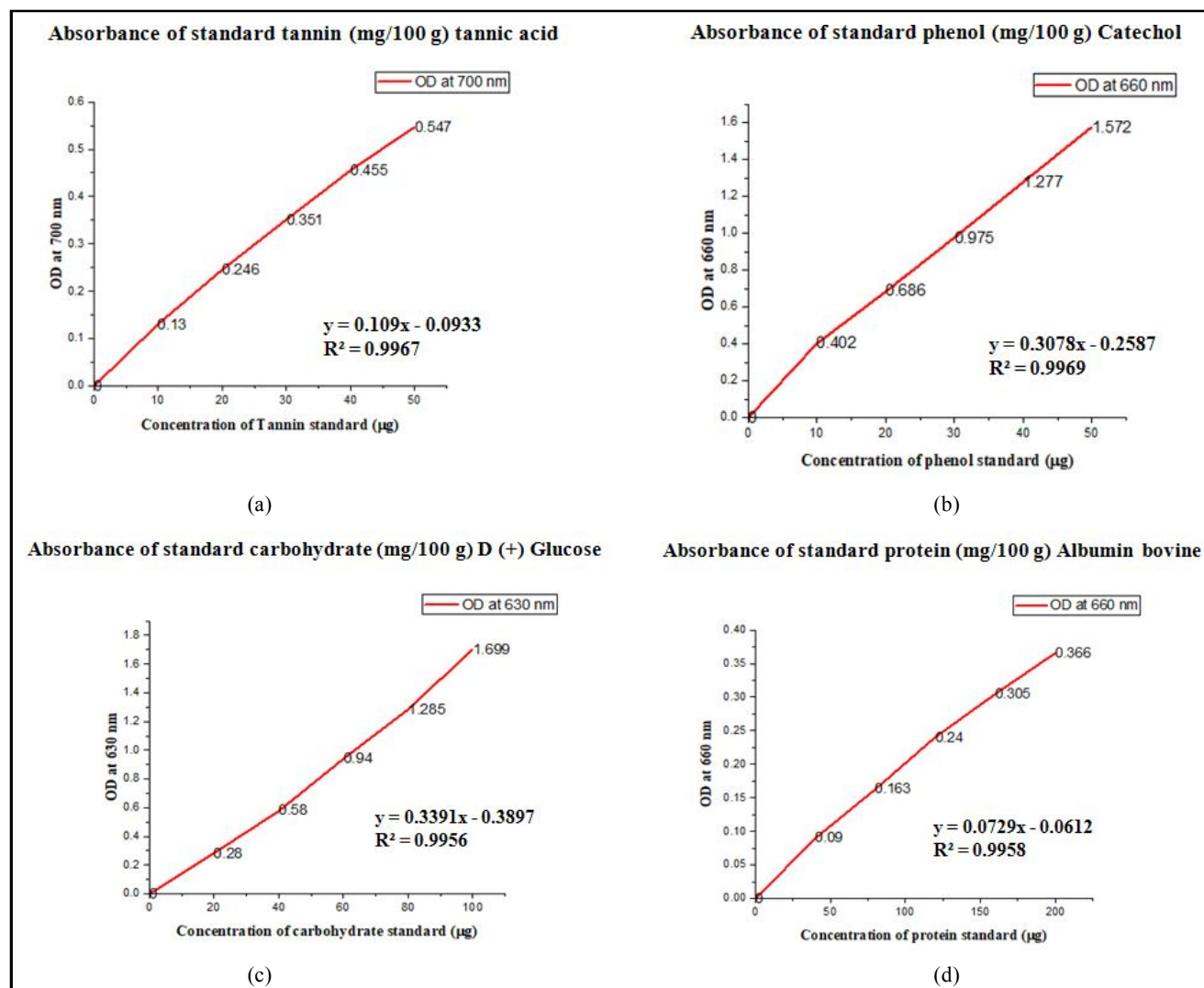


Figure 2: Standard curves of (a) tannin, (b) phenol, (c) carbohydrate and (d) protein.

3.4 Total protein content of fruits and seeds

The Lowry's technique was used to determine the total protein content against the standard albumin bovine curve ($y = 0.0729x - 0.0612$, $R^2 = 0.9958$) (Figure 2d). Significant variations in total protein content ($p < 0.01$) were observed among the fruits collected from the 30 CPTs of *T. chebula* and it ranged from 211.51 mg/100 g (TNTC 03) to 357.97 mg/100 g (MHTC 05) (Table 2). The highest protein

content was observed in MHTC 05 (357.97 mg/100 g), followed by APTC 04 (342.21 mg/100 g) against the general mean of 276.72 mg/100 g. The lowest protein content was recorded by TNTC 03 (211.51 mg/100 g), followed by ASTC 02 (216.20 mg/100 g) (Table 2). In case of seeds, the highest total protein content was noticed in TNTC 04 (259.25 mg/100 g), followed by TNTC 02 (238.40 mg/100 g) whereas the lowest protein content was observed in KATC 02 (87.67 mg/100 g), followed by ASTC 01 (89.86 mg/100 g).

Table 2: Variations in the amount of phytochemicals in fruits of selected CPTs of *T. chebula*

S. No.	Accession No.	Tannin (mg/100 g)	Phenol (mg/100 g)	Carbohydrate (mg/100 g)	Protein (mg/100 g)
1	APTC 01	408.32**	562.86	690.30	274.52
2	APTC 02	407.95**	574.3	786.73**	249.41
3	APTC 03	394.19**	526.32	656.70	270.79
4	APTC 04	396.94**	582.69**	739.97*	342.21**
5	APTC 05	347.40*	499.18	701.10	266.87
6	ASTC 01	234.56	507.53	776.20**	217.74
7	ASTC 02	226.30	558.68	785.97**	216.20
8	ASTC 03	252.90	533.63	713.31	256.60
9	ASTC 04	225.38	587.91**	771.82**	230.84
10	ASTC 05	291.44	564.95	664.79	278.88
11	KATC 01	228.13	562.86	720.51	246.13
12	KATC 02	222.63	561.81	603.71	255.59
13	KATC 03	228.13	560.77	746.97**	308.36**
14	KATC 04	371.25**	487.70	782.38**	299.18*
15	KATC 05	351.07**	557.64	732.00*	335.34**
16	KETC 01	247.01	465.77	653.33	276.16
17	KETC 02	341.90*	555.55	713.56	317.80**
18	KETC 03	314.37	584.78**	682.60	268.49
19	KETC 04	301.80	572.25*	743.60*	282.74
20	KETC 05	269.20	585.82**	622.75	275.07
21	MHTC 01	269.42	583.74**	732.00*	326.03**
22	MHTC 02	263.00	516.93	767.35**	256.44
23	MHTC 03	271.25	558.68	666.34	233.42
24	MHTC 04	237.31	546.16	609.94	281.64
25	MHTC 05	222.45	491.88	738.21*	357.97**
26	TNTC 01	418.13**	625.49**	701.94	324.93*
27	TNTC 02	343.73*	549.29	708.93	264.38
28	TNTC 03	259.33	595.22**	728.60*	211.51
29	TNTC 04	244.65	546.16	659.48	300.27*
30	TNTC 05	423.82**	582.69**	628.56	276.16
	Mean	300.46	552.98	707.65	276.72
	SEd	12.69	6.57	9.78	6.91
	CD = 0.05	26.66	13.79	20.54	14.52
	CD = 0.01	48.24	24.95	37.17	26.27

*Significant at 5%

**Significant at 1%

Table 3: Variations in the amount of phytochemical in seeds of selected CPTs of *T. chebula*

S. No.	Accession No.	Tannin (mg/100 g)	Phenol (mg/100 g)	Carbohydrate (mg/100 g)	Protein (mg/100 g)
1	APTC 01	171.31**	377.25	295.03	241.10**
2	APTC 02	229.57**	379.33	300.34	121.64
3	APTC 03	143.87	465.61	377.30	94.25
4	APTC 04	145.82	408.44	337.49	189.59**
5	APTC 05	118.51	457.30	421.52*	116.16
6	ASTC 01	167.67*	430.27	421.40*	89.86
7	ASTC 02	67.54	390.76	351.56	180.82**
8	ASTC 03	65.72	470.81	372.83	165.48*
9	ASTC 04	103.95	508.23**	331.21	93.15
10	ASTC 05	93.02	369.97	460.45**	96.44
11	KATC 01	92.05	415.72	483.45**	94.58
12	KATC 02	86.59	376.21	491.28**	87.67
13	KATC 03	58.43	533.18**	297.68	124.93
14	KATC 04	262.34**	416.76	433.02**	145.75
15	KATC 05	43.87	481.21*	302.99	123.84
16	KETC 01	118.51	515.51**	469.17**	123.84
17	KETC 02	104.82	485.36*	479.02**	146.85
18	KETC 03	123.84	494.72**	295.91	93.15
19	KETC 04	125.66	376.21	298.57	119.45
20	KETC 05	109.41	563.33**	379.06	121.64
21	MHTC 01	80.28	375.17	386.14	95.34
22	MHTC 02	94.84	403.24	303.87	103.01
23	MHTC 03	88.46	467.69	395.75	160.00*
24	MHTC 04	80.28	446.90	426.79**	93.57
25	MHTC 05	76.64	401.16	345.45	146.24
26	TNTC 01	152.97*	479.13*	420.56*	197.81**
27	TNTC 02	213.18	519.67**	348.02	238.40**
28	TNTC 03	165.72*	502.81**	395.87	88.09
29	TNTC 04	231.39**	414.68	366.68	259.25**
30	TNTC 05	220.60**	558.13**	401.18	228.53**
	Mean	127.89	449.49	379.65	139.35
	SEd	10.51	10.71	11.33	9.40
	CD = 0.05	22.08	22.49	23.78	19.74
	CD = 0.01	39.95	40.70	43.04	35.73

*Significant at 5%

**Significant at 1%

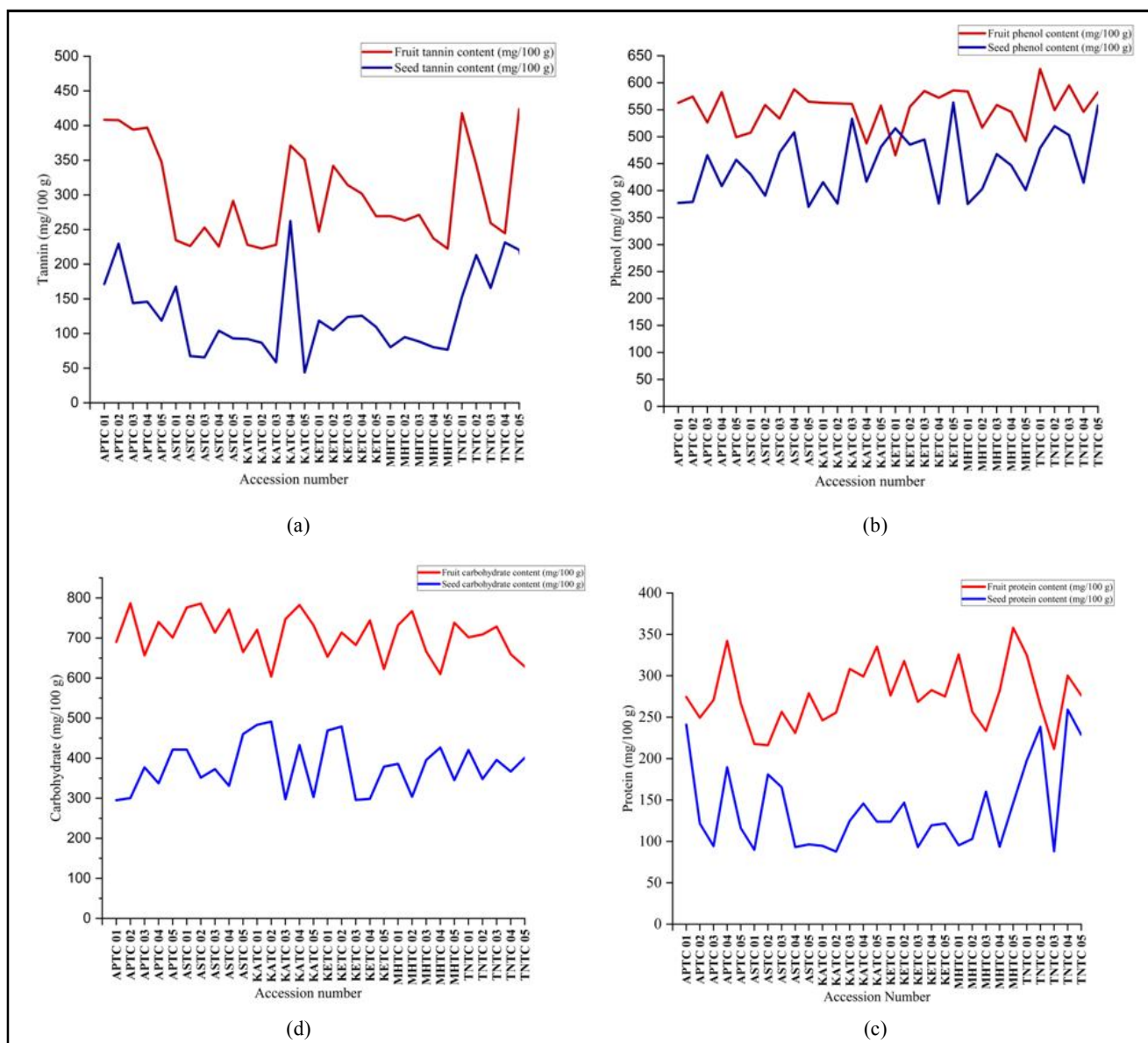


Figure 3: (a) Variation in total tannin content, (b) Variation in total phenol content, (c) Variation in total protein content and (d) Variation in total carbohydrate content.

4. Discussion

The presence of various phytochemicals in the current study proves the varied pharmaceutical applications of the species. The total tannin, phenol, carbohydrates, and protein content provide baseline data for various bioactivities.

4.1 Total tannin content

Tannins have been linked to several health benefits, such as anti-mutagenic, antibacterial, and antioxidant qualities (Mahendran and Rahman, 2020). The total tannin content was registered highest in fruits compared to seeds (Figure 3a). *T. chebula* fruits serve as the primary source of tannin. The tannin concentration of the fruit is larger than that of the other parts due to the presence of higher levels of cellulose, hemicellulose, lignin, and extractive components in fruits (Sharma, 2019). Additionally, tannins are present in regions where

trees develop, particularly in the phloem and xylem layers that lie between the cortex and the epidermis. This suggests that tannins have an impact on tissue growth in these locations (Das *et al.*, 2020). The presence of a high concentration of tannin lies on par with the findings of Nawwal *et al.* (2021) who recorded much higher concentrations of tannins, carbohydrates, phenols, and glycosides in *Terminalia arjuna*. Similar studies were carried out by Shahzad *et al.* (2022) who recorded the highest total tannin content in butanol aerial fraction (BUAE) (143.36 ± 4.32 mg. TA. Eq. g^{-1} DE), followed by chloroform aerial fraction (CFAE) (104.32 ± 4.22 mg. TA. Eq. g^{-1} DE) in *Terminalia neotaliala*.

Significant variations in the phytochemical composition were observed among different genotypes. Geographical location, maturation stage, growing environment, and raw material processing conditions all affect the diversity in chemical composition (Nisha *et*

al., 2023). The results lie on par with the findings of Suraweera *et al.* (2020) who analyzed the samples of *T. chebula* fruits to study the effect of climatic zone variation on chemical profile. Significant differences in total tannin content (% w/w) were recorded in samples collected from five different geographical locations of Sri Lanka, *i.e.*, Padiyathalawa: 33.40 ± 0.17 , Buththala: 43.39 ± 0.41 , Gampaha: 41.13 ± 0.61 , Bibila: 42.31 ± 0.23 and Colombo: 34.12 ± 0.01 .

4.2 Total phenol content

Several research investigations (Mahendran and Rahman, 2020; Begum *et al.*, 2020) suggest that phenolic compounds are a crucial constituent for the antioxidant action of medicinal plants. The results showed that the total phenol content ranged from 465.77 mg/100 g and 625.49 mg/100 g in fruits, whereas from 369.97 mg/100 g to 563.33 mg/100 g in seeds. The total phenol content in seeds was significantly lower compared to fruits (Figure 3b).

In a previous study conducted by Guleria *et al.* (2020) on *T. chebula*, the total phenolic content was found to be much higher in ethanolic extracts of fruits (359 ± 12.73 mg/g gallic acid equivalents, GAE) and leaves (54.77 ± 1.12 mg/g GAE). These results affirm the dominance of phenols in fruit extracts, similar to the current assay. Similar studies were conducted by Singh and Kumar (2020) who found higher total phenolic content in *T. chebula* (43.90 mg/ml GAE per 100 mg fruit extract) compared to *Embllica officinalis* (39.87 mg/ml GAE per 100 mg fruit extract). The findings indicated that *T. chebula* had significantly increased levels of phenolic and flavonoid content, with unit values of 3 mg/ml compared to *E. officinalis*. As a result, the high phenolic content of *T. chebula* fruits may directly enhance antioxidant activity by scavenging free radicals, which may have useful healthcare applications (Rai *et al.*, 2023).

4.3 Total carbohydrate content

The total carbohydrate content varied from 786.73 mg/100 g to 603.71 mg/100 g in fruits whereas in seeds it ranged from 491.28 mg/100 g to 295.03 mg/100 g. From the study, it was evident that the fruits of *T. chebula* registered the highest carbohydrate content in comparison to seeds (Figure 3c).

A study by Asati *et al.* (2020) indicated the presence of carbohydrates in the fruit extract powder of *T. chebula*. Previous investigation by Ananta *et al.* (2019) and Tewari *et al.* (2020) in *T. chebula* recorded total carbohydrate content of 7.45 ± 0.28 to 17.37 ± 0.32 $\mu\text{g}/\text{mg}$ plant extract and $38.1 \pm 0.52\%$ which confirm the results of the current study. The total carbohydrate content was estimated for a range of species, *viz.*, *Eugenia roxburghii* (Giri *et al.*, 2022), *Spondia pinnata*, *Castanopsis hystrix* (Angami *et al.*, 2024), *Terminalia catappa* (Ogbu *et al.*, 2024) and *Phyllanthus emblica* (Saini *et al.*, 2024). The presence of good amount of carbohydrates in the fruits of *T. chebula* indicates that the fruits possess nutritional and anti-nutritional properties and could be considered promising for the nutraceutical and pharmaceutical industries (Samreen and Ahmad, 2022).

4.4 Total protein content

Determination of protein content is essential for evaluating the nutritional worth and possible medicinal uses of the species (Santhosha and Mohan, 2023). It offers insights into the fruit's bioactive components, which may contribute to its medical properties, and helps with quality control of herbal formulations

(Rizvi *et al.*, 2022). In the current study, protein was found in both fruits and seeds. The total protein content was significantly higher in fruits (ranging from 211.51 mg/100 g to 357.97 mg/100 g) as compared to seeds (ranging from 87.67 mg/100 g to 259.25 mg/100 g) (Figure 3d).

The current study lies on par with the findings of a study conducted by Sakthi and Rajeev (2020) who concluded the presence of proteins in both fruits and seeds thus emphasizing the species' potential as a food supplement in the nutraceutical sector. Similar studies were carried out in *Terminalia catappa* wherein the highest protein content was found in the exocarp and mesocarp, fruits, bark, and leaves (Saqib *et al.*, 2024). A plethora of scientists estimated the total protein content in different species, *viz.*, *Pinus roxburghii* (Khan *et al.*, 2024), *Eucalyptus microcorys* (Faria *et al.*, 2023), *Juglans regia* (Turfan *et al.*, 2020), *Syzygium caryophyllatum* (Chandra *et al.*, 2023), *Ficus carica* (Sedaghat *et al.*, 2022) and *Tilia miqueiana* (Wu *et al.*, 2023).

5. Conclusion

The study revealed significant variations in the phytochemicals (tannin, phenols, carbohydrates, and proteins) in fruits and seeds across the thirty genotypes studied. Certain genotypes consistently showed high levels of phytochemicals across multiple parameters, suggesting their potential for further research and cultivation. The study confirms the presence of important bioactive compounds in *T. chebula*, supporting its traditional medicinal uses. The wide variation in phytochemical content among genotypes highlights the importance of careful selection and standardization when using *T. chebula* for medicinal purposes. Based on the results, the genotypes TNTC 01 (tannin and phenol), TNTC 05 (tannin), TNTC 03 (phenol), APTC 02 and ASTC 02 (carbohydrates), MHTC 05 and APTC 04 (proteins) showed consistently higher levels of phytochemicals and could be recommended for further research and cultivation. This research provides valuable data on the biochemical composition of *T. chebula* fruits and seeds from diverse geographic sources. The findings can guide future research on optimizing cultivation practices, selecting high-yielding varieties, and standardizing herbal preparations. Additionally, this information may be useful for quality control in the herbal medicinal industry and further exploration of the plant's therapeutic potential. The study also demonstrates the importance of considering both genetic and environmental factors in determining the phytochemical profile of medicinal plants. Further research could explore the specific environmental conditions or genetic factors contributing to higher phytochemical content in certain genotypes.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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