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RESEARCH ARTICLE

Production of secondary metabolites by *in-vitro* callus culture of *Terminalia bellirica* (Gaertn.) Roxb.

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Abstract

Terminalia bellirica (Gaertn.) Roxb. that widely grows in Indian subcontinent is known as 'Behada' in Marathi and 'Bibhitak' in Sanskrit. The plant is known for its several medicinal uses in Ayurveda and other traditional medicine systems. *T. bellirica* is reported to contain a diverse array of secondary metabolites, the main class being polyphenols. In the present study, callus culture of *T. bellirica* was established and the ability of the cultured cells to produce secondary metabolites was tested and compared with the fruit rind extract and the leaf extract of the plant. The qualitative analyses revealed that the cultured cells are able to synthesize all the secondary metabolites present in the fruit rind extract. The quantitative analysis of tannins by Folin-Ciocalteu reagent revealed a tannin content of 11.55 mg/g dry weight of the cultured cell samples, whereas estimation by reverse phase HPLC revealed presence of 0.0202 mg/g dry weight of gallic acid equivalents in the callus extract. This study confirms the production of secondary metabolites by callus culture system of *T. bellirica*.

Keywords: Callus culture; Phytochemical analysis; Tannins; *Terminalia bellirica* (Gaertn.) Roxb.

1. Introduction

Plants, being sessile organisms, produce an array of secondary metabolites to defend themselves against biotic or abiotic constraints. The secondary metabolites can have antimicrobial activity, act as deterrents for herbivorous animals, attractants for pollinators, or establish symbiotic associations. The number of secondary metabolites produced by plants is estimated to be over 200,000 (Wu et al., 2021). Plant secondary metabolites can be classified into four major groups: terpenoids, phenolic compounds, N-containing compounds (alkaloids) and S-containing compounds (glucosinolates, thiosulfinates, defensins and thiosins).

The genus *Terminalia,* which includes about 200 species, belongs to the family Combretaceae. The genus name is derived from the Latin word "*terminus*" since the leaves are arranged at the tip of the stem (Saxena et al., 2013). *T. bellirica* is one of the components of the popular Ayurvedic herbal formulation "*Triphala*", along with *Terminalia chebula* and *Phyllanthus emblica*. *Triphala* promotes digestion and detoxification, and, as an antioxidant, provides overall wellness. *T. bellirica* is known for its high tannin content. Tannins are a complex group of polyphenolic compounds and are distinguished from other polyphenolic compounds by their ability to bind proteins. Tannins are classified into two groups: condensed tannins (proanthocyanidins) and hydrolysable tannins (polyesters of gallic acid and its dimer ellagic acid ester, bound usually to a glucose). Condensed and hydrolysable tannins have a large number of free phenolic groups that form strong hydrogen bonds at multiple sites with proteins and carbohydrates. Tannins may also complex with proteins through hydrophobic binding (Sieniawska, 2015). Structural analysis of the polyphenolic compounds found in the leaf extracts of *T. bellirica* by HPLC-PDA-MS/MS showed the presence of nearly 50 compounds, mainly ellagitannins and proanthocyanidins. *In-silico* docking experiments revealed that the compounds abundant in the extracts were able to bind to the Bcl-2: Bim (BH3) interaction surface, which might be responsible for the anti-apoptotic effect. In the same study, the leaf extract was found to exhibit antioxidant properties and a hepatoprotective role (Sobeh et al., 2019). *T. bellirica* exhibited high antimicrobial activity against enteric pathogens like *V. cholerae, Salmonella, Shigella, E. coli* and *Staphylococcus aureus*. The ethanol extract of the fruit rind was successfully employed to reduce the microbial load in the tap water (Pujari and Pethkar, 2024). The ethanolic fruit extract of *T. bellirica* showed significant anticryptococcal activity against clinical isolates. *T. bellirica* extract has immunosuppressant effects at low concentrations, while it has stimulatory activity at high concentrations. This suggests a potential therapeutic application of this plant in the treatment of diseases associated with the functions of phagocytes and lymphocytes (Valli and Shankar, 2013). Other important medicinal activities reported are anticancer activity, angiogenesis activity, antidepressant activity, anti-urolithiatic effect, and effect on LDL oxidation and macrophage inflammation (Kumar and Khurana, 2018). Aqueous extract of fruits of *T. bellirica* were shown to possess antidiabetic activity by stimulating the secretion and action of insulin as well as by inhibiting starch digestion and protein glycation (Kasabri et al., 2010). Diverse bioactivities of *T. bellirica* have been ascribed to the presence of many bioactive phytochemicals, such as glucoside, tannins, corilagin, gallic acid, ellagic acid, ethyl gallate, galloyl glucose, chebulagic acid, and arjunolic acid. Polyphenols have been proven to have anti-oxidant and anti-inflammatory properties (Gupta et al., 2020).

Despite the extensive research into secondary metabolites over such a long period of time, very few species of higher plants have been systematically studied for their pharmacological potential. Plant secondary metabolites are complex chemical structures with multiple chiral centres and labile bonds; the chemical synthesis of these molecules is a very challenging task. Moreover, most of the source plants are wild, which presents a risk of overexploitation of their natural habitats. The slow growth rate of plants, the need for biotic and abiotic factors for the induction of synthesis of the

Figure 1. *T. bellirica* plant grown in a pot

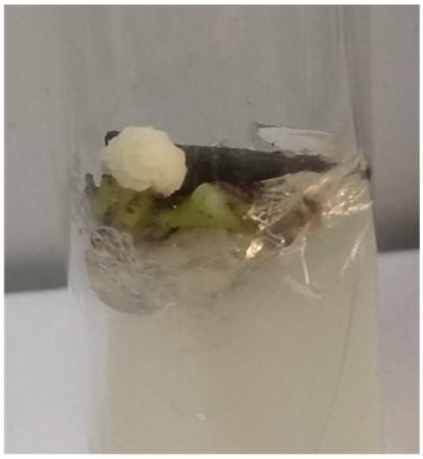


Figure 2. Initiation of callus from the leaf explant of *T. bellirica*

bioactive molecule, makes the extraction more difficult (Pickens et al., 2011). Therefore, there is a need for a method that enables the continuous production of an identified and useful bioactive compound. The *in-vitro* culture of plant cells and tissues under controlled conditions has emerged as a novel approach for the production of plant natural products. The *in-vitro* micropropagation of plants or the *in-vitro* culture of plant organs (usually roots) or callus culture can provide plant material capable of producing secondary metabolites (Espinosa-Leal et al., 2018). The technique of producing bioactive metabolites using plant cell culture technology has received more attention in recent years. The undifferentiated *in-vitro* cultured cells are capable of producing a variety of secondary metabolites under laboratory conditions. Plant cell cultures have advantages like consistency in yield irrespective of season, shorter production cycle compared to the plant, and improved biosafety due to aseptic conditions. Several commercial plant cell culture systems have been developed for the production of valuable plant secondary metabolites for example, paclitaxel from *Taxus sp*., rosamarinic acid from *Salvia offensinalis* (L). and resveratrol from *Vitis labrusca* (L.) (Marchev et al., 2020). To establish a plant cell culture system for the production of secondary metabolites, the first step is the successful establishment of a friable callus from the explant. Developed friable callus can be scaled up further for the production of biomass capable of producing bioactive metabolites. For this purpose, various approaches like elicitation, metabolic engineering, and genetic modification can be applied. Elicitation with methyl jasmonate was successfully used to enhance production of total phenolics and flavonoids in the cell suspension cultures of *Sageretia thea* (Osbeck) M.C. Johnst, which were raised from friable callus, in a balloon-type bubble bioreactor (Kim et al., 2023).

In the present study, a callus culture of *T. bellirica* was established, and the ability of the cultured cells to produce a variety of secondary metabolites was compared with that of the fruit rind extract and leaf extracts. Qualitative detection of different phytochemicals and quantitative estimation of tannins was carried out in the callus cells and was compared with that of the fruit rind extract.

2. Material and method

2.1. Collection and authentication of the plant parts

Terminalia bellirica (Gaertn) Roxb. plant located in the botanical garden of Dr. Babasaheb Ambedkar Marathwada University, Chhatrapati Sambhajinagar, India, was identified (19°54'10.4 N 75°18'45.0 E) and plant parts were collected for experimental work. The plant was authenticated at the Department of Botany, Government Institute of Science, Chhatrapati Sambhaji Nagar, India. The voucher sample was prepared and deposited at the same department, with code GISA/BOT/AP-2024-1.

2.2. Germination of seeds

Mature and dry nuts of the plant were collected in the month of April. The pericarp of the fruits was removed, and the inside hard seeds were soaked in water for 5 days, changing water every day till all the tannins from the seeds were washed out. The seeds (10 no.) were then planted in soil and cocopeat mixture (1:1) with regular watering.

Figure 3. Fully developed callus of *T. bellirica* after 6 weeks of incubation in the dark at 30±2°C

Table 1. Fresh weight and morphology of callus on different media

SN	Medium code	2,4-d Mg/l	6-bap Mg/l	Fresh wt. of callus in g	Morphology of callus
1	MS-1	0.5	0.0	0.5 (±0.1)	White, friable
2	MS-2	0.4	0.1	1.8 (±0.16)	White, friable
3	MS-3	0.3	0.2	1.9 (±0.13)	White, friable
4	MS-4	0.25	0.25	3.1 (±0.21)	White brown friable
5	MS-5	0.1	0.4	0.9 (±0.61)	White, friable

Table 2. Phytochemical analyses of leaf extract, fruit rind extract, and callus extract

SN	Name of the secondary metabolite tested	Name of the test	Leaf Extract	Fruit rind Extract	Callus Extract
		Ether extract			
1	Essential oils	Pleasant smell	ND	ND	ND
2	Lipids & Fats	Spot test	ND	ND	ND
3	Steroids & Triterpenes	Salkowski reaction	ND	ND	+
4	Carotenoids	Sulfuric acid test	ND	+	+
		Alcoholic extract			
5	Cardiac glycosides	Trichloroacetic acid test	ND	ND	ND
		Keller- Killiani reaction	ND	ND	ND
6	Saponins	Foam formation	ND	+	+
		Lead acetate test	ND	+	+
7	Phenolic Glycosides	Potassium ferrocyanide test	+	+	+
		Ferric sulphate test	+	+	+
8	Phloroglucides	Potassium ferrocyanide test	+	+	+
		Conc. nitric acid test	+	+	+
9	Anthrocenocides	Sodium hydroxide test	+	+	+
10	Flavonoids	Alkaline reagent test	+	+	+
		Lead acetate test	+	+	+
11	Coumarins	Ammonia solution test	+	+	ND
12	Quinones	Alcoholic KOH test	+	+	+
13	[1]Tannins	Ferric chloride test	+	+	+
14	Alkaloids	Mayer's reagent test	ND	ND	ND
		Aqueous extract			
15	Glucides	Molisch's reagent test	+	+	+
16	Polyphenols	Potassium ferrocyanide test	+	+	+
17	[1]Tannins	Ferric chloride test	+	+	+
		Catechol tannins	ND	ND	ND
18	Polyuronides (Pectin, gum mucilage)	Alcohol test	ND	+	ND

+ Present, ND- Not detected, [1] Gallic tannins

Table 3. Estimation of total phenols and Tannins by the Folin-Ciocalteu method

Sample. No.	Medium code	Total phenols µg/100µL sample	Total phenols mg/g dry wt.	Tannins µg/100µL sample	Tannins mg/g dry weight
TB1	Callus extract	27.75±0.1	13.87±0.08	23.11±1.0	11.55±0.5
TB2	Fruit rind extract	364.9±0.0	182.45±0.0	231.01±1.5	115.45±0.8

Data are given as Mean ± Standard error, N=3

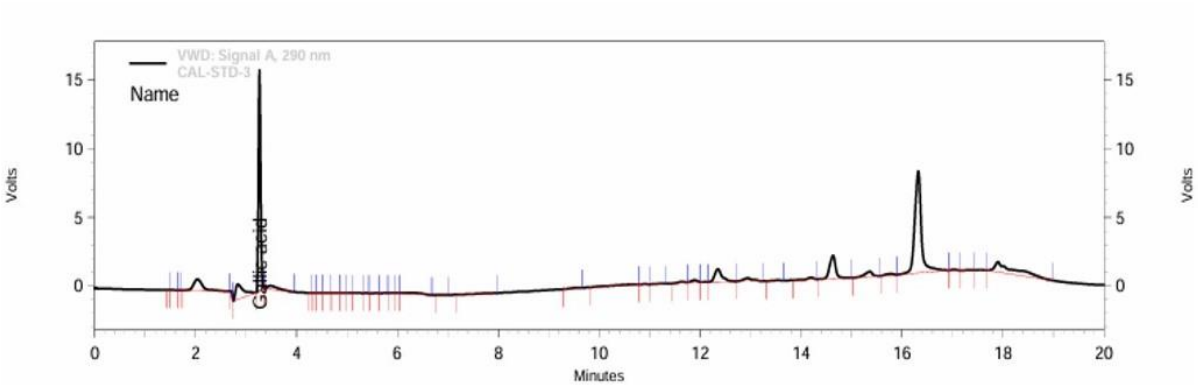
Table 4. Estimation of tannins as gallic acid equivalent by HPLC in callus extract of *T. bellirica*.

Sample code	Sample	Amount of Gallic acid equivalent in the injected 5 µl sample in ng	Amount of Gallic acid equivalent mg/gram dry weight	Projected figures for 1 L culture medium	
				Biomass dry weight g/L	Amount of Gallic acid equivalent mg /L of medium
TB1	Callus culture extract	1.012	0.0202	205	4.141
TB2	Fruit Rind extract	170.058	3.401	-	-

2.3. Induction of callus from juvenile leaves

Murashige and Skoog medium (HiMedia Laboratories Pvt. Ltd., India) was used as the basal medium for all the tissue culture experiments. For induction of callus, media containing auxin, 2,4-dichlorophenoxy acetic acid (2,4-D, HiMedia Laboratories Pvt. Ltd., India), and cytokinin,6- benzyl adenine (BAP, HiMedia Laboratories Pvt. Ltd., India) were prepared, with ratios (0.5:0, 0.4:0.1, 0.3:0.2, 0.25:0.25, 0.1:0.4 mg/L). To all the combinations, refined table sugar (3%) and agar-agar (0.6%) were added. Medium (20 ml) was poured into a 100 ml capacity bottle, labelled properly and sterilized at 110°C for 40min. New juvenile leaves of the plants (4 to 6 cm in length) were collected fresh. The leaves were washed with running tap water, followed by 10 min in 2% Bavistin solution. The leaves were then surface sterilized with ethanol (30s), followed by sodium hypochlorite (5%), with 2-3 drops of Tween-20 (10min). Finally, the explants were washed three times with sterile distilled water. The leaves were cut into 1 cm square explants along the central vein and inoculated on the medium so that the anterior side faced upward with cut edges touching the medium. For each medium combination, 5 culture bottles were inoculated. The culture bottles were incubated in the dark at 30±2°C and observed for callus initiation.

Sample: CAL-STD-3
Injection Volumn: 5 ul
Injection Amount: 4 ng



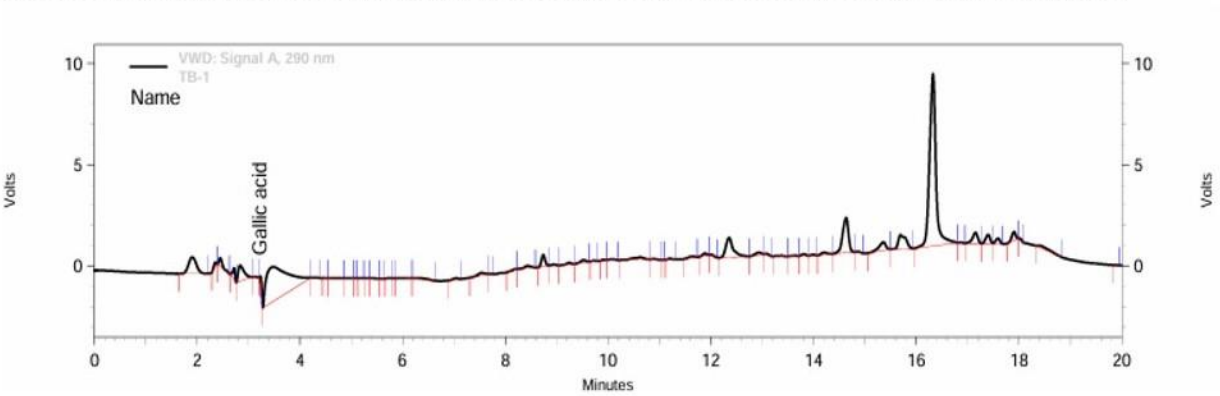
VWD: Signal A, 290 nm Results

Pk #	*Name*	*Retention Time*	*Area*	*Amount (ng)*
7	Gallic acid	3.267	797606	**4.000 CAL**

Totals		797606	**4.000 CAL**

Figure 4. Chromatogram for standard gallic acid

Sample: TB-1
Sample Preparation: 1. Centrifuged @ 10,000 rpm, 10 Degree Celcius
2. Clear supernatent 100 ul + 900 ul mixture of MeOH & Water (80:20 v/v)
3. Filtered through 0.22um syringe filter
Injection Volumn: 5 ul
Injection Amount:



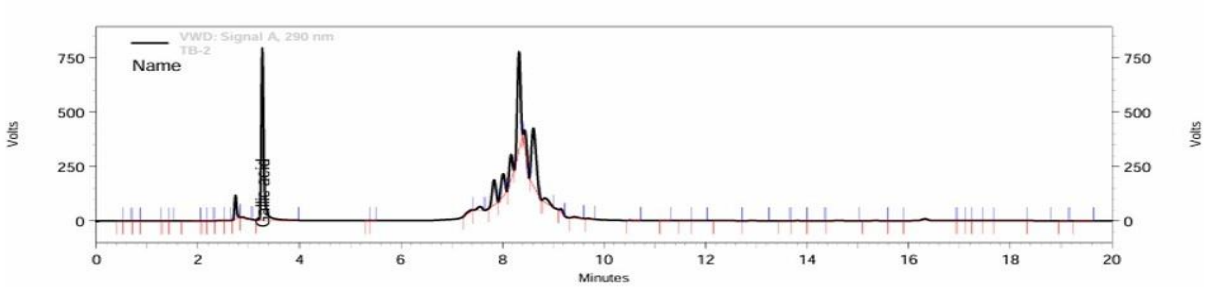
VWD: Signal A, 290 nm Results

Pk #	*Name*	*Retention Time*	*Area*	*Amount (ng)*
7	Gallic acid	3.233	27673	**1.012**

Totals		27673	**1.012**

Figure 5. Chromatogram of sample TB1 callus extract of *T. bellirica*

Sample: TB-2
Sample Preparation: 1. Centrifuged @ 10,000 rpm, 10 Degree Celcius
2. Clear supernatent 100 ul + 900 ul mixture of MeOH & Water (80:20 v/v)
3. Filtered through 0.22um syringe filter
Injection Volumn: 5 ul
Injection Amount:



VWD: Signal A, 290 nm Results

Pk #	Name	Retention Time	Area	Amount (ng)
14	Gallic acid	3.270	46533154	**170.058**
Totals			46533154	**170.058**

Figure 6. Chromatogram of sample TB2, fruit rind extract of *T. bellirica*

2.4. Qualitative phytochemical analyses of leaf extract, fruit rind extract and callus extract

Comparative qualitative phytochemical analyses of the leaf extract, fruit rind extract and callus extract were carried out to study and compare the diversity of secondary metabolites produced. Shade-dried fine powder of fruit rind, leaves and dried callus were subjected to sequential extraction, first with lipophilic (non-polar) solvent, diethyl ether (Loba Chemie Pvt. Ltd. India.), second with intermediate polar solvent methanol (Fisher Scientific, India), followed by water, a strong polar solvent. Fine powders of the plant materials and dried callus (1 g) were mixed with diethyl ether (10 ml). The mixture was shaken for 24 h on a rotary shaker. The extracts were filtered and concentrated by evaporating at room temperature for 1 h. The concentrated extracts were used for the identification of lipophilic constituents. The same dried fruit rind, leaves and dried callus powder, after extraction with ether, were extracted with methanol (80%, 10 ml) for 24 h. The filtered extracts were concentrated to a minimum volume by evaporating excess solvent in oven at 45°C for 4 h and used to identify alcohol-soluble constituents. After extraction with diethyl ether and alcohol, the fruit rind powder, leaf powder, and callus powder were dried and extracted again with deionized distilled water for 24 h. The extract was filtered and water-soluble constituents were identified from the extract. Various analytical tests were performed to identify the phytoconstituents, as reported earlier (Tailang and Sharma, 2008; Pujari and Pethkar, 2024).

2.5. Quantitative estimation of total phenolics and tannins using the Folin-Ciocalteu method (Makkar, 2013)

(i) Extract Preparation- Fine powder of fruit rind and the dried callus samples were soaked (1 g) in methanol (10 mL, 80%) for 24 h, then sonicated for 20 min in an ultrasonication bath (Kumar Sales Corporation, Mumbai) and kept for the next 24 h. The extracts were filtered first through ordinary filter paper and then through Whatman filter paper No.1, and were collected.
(ii) Reagents- Folin-Ciocalteu reagent (SISCO Research Lab. Pvt. Ltd., India) 1N was prepared by diluting the commercially available 2N reagent with an equal amount of distilled water and stored in a brown bottle at 4°C (FC reagent). Standard Tannic acid solution (0.1 mg/ml) was prepared by dissolving Tannic acid (HiMedia Laboratories Pvt. Ltd., India) in deionized distilled water (25mg in 25 mL) and then diluting to 1:10 with deionized distilled water. Sodium carbonate (20%) was prepared by dissolving Sodium carbonate x10 H_2O (40 g) in deionized distilled water (200 ml). Insoluble polyvinyl polypyrrolidone (HiMedia Laboratories Pvt. Ltd., India, PCT1003) was used to remove tannins from the mixture in the second step.
(iii) Preparation of standard graph- A calibration curve was prepared by taking standard tannin solution (0.1mg/ml) in a series of test tubes viz. 0.0, 0.02, 0.04, 0.06, 0.08 and 0.1 ml. In each test tube, the volume was made up to 0.5 ml by adding the appropriate amount of water, followed by FC reagent (0.25 ml) and sodium carbonate (20%, 1.25 ml). After 40 min the absorbance was measured at 725 nm. A standard graph was plotted for absorbance verses concentration (Figure 4).
(iv) Analysis of total phenols- For the estimation of total phenols, a sample (0.1 ml) was mixed with distilled water (0.4 ml). To this FC reagent (0.25 ml) and sodium carbonate (20%, 1.25 ml) were added. The tubes were vortexed, and after 40 min absorbance was recorded at 725 nm. The concentration of total phenols as tannic acid equivalents were calculated from the above calibration curve, and total phenolic content was recorded as 'A'µg.
(v) Removal of tannin from the tannin-containing extract- PVPP (0.1g) was added to a test tube containing distilled water (1.0 ml) and a tannin-containing extract sample (1.0 ml). The mixture was vortexed and kept at 4°C for 15 min. The tubes were vortexed again and were centrifuged at 3000g for 10 min. The supernatant contains phenolics other than tannins, as tannins precipitate with PVPP. The phenolic content of the supernatant (0.1 ml) was measured as mentioned above. The non-tannin phenolic content was calculated using a standard curve. The non-tannin phenol content was expressed as 'B' µg. (A-B). The amount of tannins as tannic acid equivalent (µg/100 µl sample) was calculated by substracting value of B from A.

2.6. Quantitative estimation of tannins as gallic acid equivalent by HPLC

Quantitative estimation of tannins in the fruit rind extract and callus extract was carried out using High Performance Liquid Chromatography system (Agilent Technologies, USA, Model 1260 infinity). Gallic acid monohydrate (TCI (India) Pvt. Ltd.) was used as standard. Shade-dried samples were crushed to fine powder in a mortar and pestle and were added to a measured amount of methanol (0.1g/1 ml 80%). The methanol extracts were sonicated in an ultrasonication bath for 20 minutes and centrifuged at 10,000 rpm at 10°C. Clear supernatant (100 µl) was mixed with methanol (900 µl, 80%) and filtered through a 0.22 µm syringe filter. A calibration graph for Gallic acid was obtained with concentrations of 1, 2, 4, 6, 8, and 10 ng (Figure 5). Water containing 0.1% Formic acid [A]: Acetonitrile [B] was used as

mobile phase by a gradient run of [A] %: 0 to 2 min: 95%, 2 to 7 min: 50%, 7 to 12 min: 25%, 12 to 15 min: 5%, 15 to 20 min: 95%, with a flow rate of 1 ml/min, at an oven temperature of 40°C, in a Kromasil column (C-18, 250 x 4.6 mm, 5 µm). The injection volume was 5 µl and the wavelength of detection was 290 nm. From the chromatograms obtained the estimated concentration of gallic acid equivalents in the sample were calculated in mg/g dry weight of sample.

3. Result

31. Germination of the seeds

After a month, 9 seeds out of 10 germinated. The plantlets were then transferred to large pots from bags. These plants were used as mother plants for the collection of explants.

3.2. Induction of callus from juvenile leaves

After 10 to 12 days of inoculation, callus formation started from the cut ends of the veins. After 8 weeks of incubation, the calluses were harvested, and fresh weight was recorded. From Table 1, it can be observed that the MS-4 combination with equal concentrations of auxin and cytokinin (0.25 mg/L 2,4-D & 0.25mg/L BAP) supported maximum growth of callus.

3.3. Qualitative phytochemical analyses of leaf extract, fruit rind extract and callus extract

The qualitative analysis of the extracts showed that callus cells were able to biosynthesize almost all the metabolites, including phenolic glycosides, flavonoids, quinones, hydrolysable tannins and polyphenols present in the leaf and fruit rind extract (Table 2). Carotenoids and saponins, which could not be detected in the leaf extract, were detected in the callus extract.

3.4. Quantitative estimation of total phenolics and tannins using the Folin-Ciocalteu method

The Folin-Ciocalteu method quantifies the phenolic content by measuring the reduction of a mixture of phosphotungstic and phosphomolybdic acid (Folin-Ciocalteu reagent) by the phenolic compounds, resulting in a blue-coloured complex. The intensity of the blue colour is directly proportional to the amount of phenolic compounds present in the sample. The absorbance of the blue complex was measured at 725 nm. This method was coupled with the use of insoluble matrix, polyvinyl polypyrrolidone (PVPP), which precipitates tannin phenols. The concentration of tannins was calculated by subtracting the concentration of non-tannin phenols from the concentration of total phenols.
The results are expressed as tannic acid equivalents (Table 3). The amount of tannins estimated in the callus sample was 11.55 mg/g dry weight, and the fruit rind extract was 115.45 mg/g dry weight.

3.5. Quantitative estimation of tannins as gallic acid equivalent by HPLC

In the RP-HPLC analyses the retention time for standard Gallic acid was found to be 3.267 min. at 290 nm and at an injection volume of 5 µl. The concentration of Gallic acid equivalents estimated in the callus extract was 0.02 mg/g dry weight, and in the fruit rind extract, 3.4 mg/g dry weight (Table 4).

4. Discussion

It was observed that the germination frequency and survival rate of *T. bellirica* seeds was satisfactory. Woody plants are recalcitrant to grow and differentiate *in-vitro*. During the juvenile phase, starting from the embryo and perhaps lasting up to a decade, tissues from woody plants are responsive to in vitro conditions. As maturation sets in, tissues from mature trees become less responsive to tissue culture (Ahuja, 1993). To get the juvenile explants the plants were raised in a pot. Other than the age of a tree, the response of an explant/tissue is also determined by the genotype, physiological state of the tissue, time of the year when the explant is cultured, and the composition of the medium. It was found that all the combinations of 2,4-D: BAP were able to induce callus formation, but the one with equal ratio induced a soft, friable and fast-growing callus. Friable soft callus contains fast proliferating undifferentiated cells, and is the prerequisite for the establishment of cell suspension culture on a large scale in a bioreactor.

In the present study the phytochemical studies proved the ability of the callus cells to produce all the metabolites which were present in the fruit rind extract. In a recent metabolome profiling of the plant using LCMS/MS analysis, 74 nonredundant metabolites were reported in *T. bellirica*, with polyphenols as the major group of metabolites. The polyphenols mainly found to be present were ethyl gallate, pyrogallol, gallic acid, ferulaldehyde, and sinapaldehyde. The most abundant polyphenols in *T. bellirica* are ellagic acid, quercetin, chebulanin, methyl gallate, and especially ferulaldehyde, which play an important role in activating Nrf2 transcription factor, which consequently enhances the expression of antioxidant response elements, contributing overall cellular antioxidant defense (Hegde et al., 2024). The quantitative estimation by the Folin-Ciocalteu reagent also shows the presence of marked tannins in the callus culture. Earlier, Sun et al (2016) reported maximum resveratrol accumulation (0.15 mg/g dry weight), and biomass accumulation (329.45 g/L) of cultured cells of *Vitis amurensis*; in an air lift bioreactor. In another study production of resveratrol by *Vitis vinifera* cv. cell suspension cultures with the highest level of resveratrol (1.40 mg/g dry wt.) have been reported (Donnez et al., 2011). In view of these reports, there is further scope for the optimisation of culture conditions for the development of callus culture of *T. bellirica* into a large-scale cell culture system for the production of bioactive metabolites.

5. Conclusion

In the present study callus culture of *T. bellirica* was successfully established from juvenile leaves. The qualitative phytochemical analyses confirmed the capability of callus cells to synthesise all the secondary metabolites under laboratory conditions. Moreover, carotenoids, triterpenes and saponins, which could not be detected in the leaf extract, were detected in the callus cells. Quantitative analyses confirmed a notable total phenols and tannins concentration in the cultured cells. This study pointed to the possibility of scaling up the cell culture system of *T. bellirica* to enhance the production of secondary metabolites.

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Conflict of interest

The authors declare that there is no conflict of interest.

Contribution of the authors

AAP carried out experimental work and prepared the manuscript, AVP supervised the work and carried out proofreading of the manuscript.

References

Ahuja MR.1993. *Micropropagation of Woody Plants*. Kluwer Academic Publishers. Pp 3-9.

Donnez D, Kim KH, Antoine S, Conreux A, De Luca V, Jeandet P, Clement C, and Courot E. 2011. Bioproduction of resveratrol and viniferins by an elicited grapevine cell culture in a 2 L stirred bioreactor. Process Biochemistry 46 (5): 1056-1062. https://doi.org/10.1016/j.procbio.2011.01.019

Espinosa-Leal CA, Puente-Garza CA, and García-Lara S. 2018. In vitro plant tissue culture: means for production of biological active compounds. Planta 248: 1-18. https://doi.org/10.1007/s00425-018-2910-1

Gupta A, Kumar R, Bhattacharyya P, Bishayee A, and Pandey AK. 2020. *Terminalia bellirica* (Gaertn.) roxb. (Bahera) in health and disease: A systematic and comprehensive review. Phytomedicine 77: 153278. https://doi.org/10.1016/j.phymed.2020.153278

Hegde SN, Devi KL, Choudhary M, Menon N, and Singh G. 2024. A comprehensive metabolome profiling of *Terminalia chebula*, *Terminalia bellerica*, and *Phyllanthus emblica* to explore the medicinal potential of Triphala. Scientific Reports 14 (1): 31635. ps://doi.org/10.1038/s41598-024-80544-6

Kasabri V, Flatt PR, and Abdel-Wahab YH. 2010. *Terminalia bellirica* stimulates the secretion and action of insulin and inhibits starch digestion and protein glycation in vitro. British journal of nutrition 103 (2): 212-217. doi:10.1017/S0007114509991577

Kim J, Han J, Murthy HN, Kim J, Kim M, Jeong T, and Park S. 2023. Production of Secondary Metabolites from Cell Cultures of *Sageretia thea* (Osbeck) M.C. Johnst. Using Balloon-Type Bubble Bioreactors. Plants 12 (6): 1390. https://doi.org/10.3390/plants12061390

Kumar N, and Khurana SM. 2018. Phytochemistry and medicinal potential of the *Terminalia bellirica* Roxb. (Bahera). Indian Journal of Natural Products and Resources (IJNPR) 9 (2): 97-107.

Makkar HP. 2013. *Quantification of tannins in tree and shrub foliage: a laboratory manual*. Springer Science and Business Media.

Marchev AS, Yordanova ZP and Georgiev MI. 2020. Green (cell) factories for advanced production of plant secondary metabolites. Critical reviews in biotechnology 40(4): 443-458. https://doi.org/10.1080/07388551.2020.1731414

Pickens LB, Tang Y, Chooi YH. 2011. Metabolic engineering for the production of natural products. Annul Review of Chemical and Biomolecular Engineering 2:211–236. https ://doi.org/10.1146/annur ev-chembioeng - 061010-114209

Pujari AA, and Pethkar AV. 2024. Harvesting the potential of medicinal plant extracts for anti-diarrhoeal treatment and water purification. Journal of Bioresources 12 (2) :107. https://doi.org/10.5281/zenodo.14892397

Saxena V, Mishra G, Saxena A, and Vishwakarma K. 2013. A comparative study on quantitative estimation of tannins in *Terminalia chebula, Terminalia bellirica, Terminalia arjuna* and *Saraca indica* using spectrophotometer. Asian Journal of Pharmaceutical and Clinical Research 6 (3):148-149.

Sieniawska E. 2015. Activities of tannins–from in vitro studies to clinical trials. Natural Product Communications 10(11): 1934578X1501001118.

Sobeh M, Mahmoud MF, Hasan RA, Abdelfattah MAO, Osman S, Rashid H, El-Shazly AM, and Wink M. 2019. Chemical composition, antioxidant and hepatoprotective activities of methanol extracts from leaves of *Terminalia bellirica* and *Terminalia sericea* (Combretaceae). PeerJ 7: e6322 DOI 10.7717/peerj.6322

Sun D, Li C, Qin H, Zhang Q, Yang Y, and Ai J. 2016. Somatic embryos cultures of *Vitis amurensis* Rupr. in air-lift bioreactors for the production of biomass and resveratrol. Journal of Plant Biology 59: 427-434. https://doi.org/10.1007/s12374-016-0022-7.

Tailang M, and Sharma A. 2008. *Phytochemistry (Theory and Practical)*. Birla Publishing House, New Delhi.

Valli S, and Shankar SG. 2013. *Terminalia bellerica*: A promising challenge to cryptococcosis. International Journal of Pharmaceutical Research and Bio-Science 2 (5): 154-169

Wu T, Kerbler SM, Fernie AR, and Zhang Y. 2021. Plant cell cultures as heterologous bio-factories for secondary metabolite production. Plant Communications 2 (5):100235. DOI: 10.1016/j.xplc.2021.100235. PMID: 34746764; PMCID: PMC8554037