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Isolation and characterization of β-sitosterol from *Terminalia arjuna* fruit and its growth-promoting effect on beneficial fungi

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Abstract

Terminalia arjuna, an important medicinal plant in traditional Indian systems, has been extensively studied for its cardioprotective bark. However, limited attention has been given to its fruit, which contains several biologically active phytochemicals with potential antioxidant, anti-inflammatory, and immunomodulatory properties. This study aimed to isolate and partially purify phytoactive compounds from the fruit of *T. arjuna* using chromatographic techniques, characterize them, and assess their effect on the growth of industrially important fungal strains. Dried and powdered *T. arjuna* fruit was extracted using 70% methanol. The crude extract was subjected to sequential solvent partitioning followed by column chromatography. The resulting fractions were evaluated using TLC, High Performance Liquid Chromatography (HPLC), and LC-MS for partial purification and confirmation of the phytochemicals. The growth-promoting effect of the purified compound was tested on *Trichoderma harzianum* by measuring optical density after 48 hours of incubation in the presence of the compound. Methanolic extraction of *T.arjuna* fruit yielded approximately 1652 mg of dried extract. Column chromatography followed by TLC profiling led to the identification of three pooled fractions (A, B, and C), each with distinct banding patterns and Rf values. Among them, Fraction B exhibited a prominent TLC band at Rf 0.133 and was the most abundant (421 mg). HPLC analysis confirmed the high purity of Fraction B, showing a single dominant peak at retention time 3.113 minutes with an area percentage of 97.8%. In growth promotion assays using *Trichoderma harzianum*, Fraction B significantly enhanced fungal biomass, with a 32.54% increase in OD600 compared to control. LC-MS analysis in positive ion mode identified a molecular ion at m/z 415 [M+H]+ and a key fragment at m/z 256, consistent with β-sitosterol. The presence of β-sitosterol in fruit of *T. arjuna* reflects the plant's long-recognized therapeutic significance and is associated with various beneficial pharmacological effects. This study established that the fruit of *T. arjuna* can serve as a viable source of β-sitosterol, which may have potential as a natural growth enhancer for industrial fungi.

Keywords: *Terminalia arjuna*, Phytochemicals, β-sitosterol, TLC, LC-MS, *Trichoderma harzianum*, Fungal growth.

1. Introduction

Terminalia arjuna (Roxb.) Wight & Arn., a prominent member of the family Combretaceae, holds a revered place in traditional Indian medicine, particularly in Ayurveda and Unani systems [1,2]. While the bark of this tree is widely documented for its cardioprotective and antiviral properties[3,4], recent studies have turned attention to the fruit of *T. arjuna*, which has shown promise due to its rich phytochemical profile [5].

Several studies have explored the potential of *T. arjuna* extracts in influencing the growth of industrially significant microorganisms, owing to its diverse phytochemical constituents such as tannins, flavonoids, and saponins [6,7]. Methanolic and aqueous extracts of *T. arjuna* have demonstrated antimicrobial activity against a range of bacterial species, including *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*, many of which are of industrial relevance in fermentation and biotechnology processes [8,9]. In particular, the ethanolic fruit extract has shown inhibitory effects against pathogenic and spoilage bacteria, indicating its potential as a natural preservative or biocontrol agent in industrial settings [5]. Similarly, industrially important fungal species such as *Aspergillus niger*, *Trichoderma harzianum*, and *Penicillium chrysogenum* have exhibited varied responses to *T. arjuna* extracts, suggesting the extract's modulatory role in fungal growth and metabolism [10]. The fruit is known to contain several biologically active compounds, including polyphenols, flavonoids, saponins, and phytosterols [11,12], which have industrial importance.

Accurate identification and structural characterization of plant-derived compounds necessitate the use of ad-

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vanced analytical methods. Thin Layer Chromatography (TLC) remains a fundamental technique for preliminary screening, offering a rapid and cost-effective approach to assess compound purity and profile phytochemical constituents. For more precise and confirmatory analysis, Liquid Chromatography–Mass Spectrometry (LC-MS) plays a crucial role by combining chromatographic separation with mass-based detection, enabling both the identification and partial structural elucidation of bioactive compounds such as β-sitosterol. The complementary use of TLC for profiling and LC-MS for compound validation provides a reliable analytical framework for the study of plant metabolites [13,14].

In our previous study, we evaluated the effect of extracts from ten medicinal plants on the growth of these industrially important fungi [10]. Among them, *T. arjuna* extract demonstrated the most significant enhancement in fungal growth and sporulation, indicating the presence of one or more bioactive compounds with growth-promoting properties. These findings highlighted the potential of *T. arjuna* as a natural stimulant for fungi widely used in biotechnology and pharmaceutical industries, for instance, in enzyme production, biocontrol, and antibiotic synthesis. Based on these observations, the present study was carried out to isolate and characterize the active compound(s) from the fruit of *T. arjuna* responsible for this biological activity through the application of advanced analytical techniques, with a focus on establishing a cost-effective, plant-based medium for enhanced fungal cultivation.

2. Materials and methods

2.1. Collection and preparation of plant material

Unprocessed fruits of *T. arjuna* were purchased from a local supplier (Yeshwanthpur market, Bangalore, India) and the plant material was authenticated by in-house botanist of Skanda Life Sciences, Bangalore, India. The plant specimens were further authenticated as per tests given in the Indian Pharmacopeia 2010 edition [15]. The fruits were washed thoroughly with distilled water, shade-dried at room temperature for 10 days, and ground into a fine powder using a mechanical grinder. The powder was stored in airtight containers at 4°C until extraction.

2.2. Extraction of phytochemicals

Dried fruit of *T. arjuna* was ground to a fine powder and sieved through mesh No. 80 for uniform particle size. The powder was extracted with 70% methanol-water (100 mL) at 50°C for 15 minutes under continuous stirring. The process was repeated four times, and the supernatants were pooled, filtered through Whatman No. 1 filter paper, and concentrated using a rotary evaporator to obtain the crude methanolic extract. The extract was stored at 4°C for further analysis.

Two grams of *T. arjuna* extract were dissolved in 10 mL of water with continuous stirring for 30 minutes. The aqueous solution was then sequentially extracted with equal volumes of methanol, chloroform, and hexane by vigorous mixing, followed by phase separation. An additional 10 mL of each solvent was added gently along the container wall to enhance separation, and the mixture was allowed to stand for 10 minutes. Each solvent layer was collected, evaporated, and weighed. The fractions were labeled as methanol, chloroform, and hexane extracts, respectively and referred to as liquid-liquid fractions (LLF) hereafter. The process was repeated to obtain sufficient yields, which were analyzed by TLC to assess purity.

2.3. Preliminary screening by TLC

To perform a preliminary screening of the extract and assess the presence of bioactive compounds, TLC was carried out using standard procedures. Sample solutions were prepared at a concentration of 10 mg/mL in methanol. From this, 2.5 µL of each sample was carefully spotted onto pre-coated TLC plates (silica gel 60 F254, 0.25 mm thickness), ensuring consistent application for accurate comparative analysis. The TLC plates were developed in a saturated chamber containing a solvent system of chloroform: methanol in a 9.5:0.5 ratio, selected based on its polarity and effectiveness in separating phytosterol compounds. Care was taken to ensure that the spotted samples remained above the solvent level to prevent sample dissolution.

Once the solvent front reached an appropriate distance (~3/4 of the plate), the plates were removed from the chamber and air-dried at room temperature. The separated bands were visualized under ultraviolet (UV) light at 254 nm to detect fluorescent compounds. Each visible spot was analyzed, and the retention factor (Rf) was calculated. Distinct spots with characteristic Rf values suggested the presence of different phytochemical constituents, providing initial evidence for compound separation.

2.4. Separation of phytochemicals by column chromatography and TLC

The methanol LLF was subjected to column chromatography to further purify the bioactive constituents. A total of 500 mg of the methanol LLF was first dissolved in 3 mL of 5% methanol to ensure complete solubilization of the extract. The stationary phase used was silica gel (60–120 mesh), which was packed into a glass column using chloroform as the packing solvent to maintain appropriate polarity and uniform flow. The sample was carefully loaded onto the column, and elution was carried out using a stepwise gradient of methanol in chloroform, ranging from 5% to 100% methanol.

Fractions were collected sequentially in appropriately labeled tubes based on observed phase separations and elution volumes. Each fraction was then monitored using TLC, following the previously described method, to determine the composition and purity of eluted components. The retention factor (Rf) values were compared and visual assessment of compound separation of compounds was visually assessed under UV light, followed by pooling of fractions with similar profiles. After confirming the presence of the target compound in selected fractions, the solvents were removed using a rotary evaporator under reduced pressure to yield semi-purified residues. These residues were subjected to HPLC for further analysis.

2.5. Partial purification and characterization of isolated compounds

2.5.1. HPLC analysis

The partially purified fractions were subjected to HPLC analysis using a Shimadzu LC-Prominence system equipped with a C4 column (150 mm × 4.6 mm, 3 µm particle size). Separation was carried out under a low-pressure gradient using a methanol: water (50:50 v/v) mobile phase at a flow rate of 0.5 mL/min. A 5 µL injection volume was

used, and detection was performed at 254 nm. The purity of the isolated compound was assessed based on the percentage peak area in the chromatogram in comparison with known standards.

2.5.2. LC-MS analysis of partially purified extract

LC-MS was performed using an API Sciex 4000 Mass Spectrometer coupled with an HPLC system. The analysis was conducted in both positive and negative ion modes, with a precursor ion scan range of 50 Da to 100 Da. Instrument parameters were optimized for sensitivity and specificity, with curtain gas set at 25, gas1 at 40, gas2 at 60, needle voltage at 5000 V, and a delustering potential of 70 V.

2.6. Growth promotion test using turbidometry

The partially purified fractions obtained through HPLC were evaluated for their growth-promoting activity by measuring turbidity as an indicator of fungal biomass development. A suspension of *Trichoderma harzianum* was grown in Potato Dextrose Broth (PDB) and incubated at 27 ± 2°C for 24h to 48h. The culture was standardized to 1×10^8 cells/mL using 0.5 McFarland standards. Extract fractions from column chromatography were prepared at 20% concentration in sterile water. A 1:1 dilution of 2X sterile PDB and extract was made to achieve 1X broth containing 10% extract and fungal suspension. A control without extract was also prepared. All tubes were incubated for 48 h at 27 ± 2°C. Fungal growth was assessed by measuring optical density (OD) at 600 nm. Growth inhibition (%) was calculated by comparing OD values of treated samples with the control using the following formula:

% Growth inhibition = ([{OD sample-OD cntrol}]/OD control) x100

2.7. Statistical analysis

All experiments were conducted in triplicate, and results are presented as mean ± standard deviation (SD). Statistical analysis was performed using GraphPad Prism version 8.0 (GraphPad Software, San Diego, CA, USA). Differences between treated and control groups in the growth promotion assay were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons. A p-value of less than 0.05 was considered statistically significant.

3. Results

3.1. Extraction of solvent fractions from *T. arjuna*

Table 1 shows the yields of LLFs of *T. arjuna* fruit. Among the three solvents used, the methanol fraction yielded 1652 mg of extractable material, indicating a high content of polar phytoconstituents. The chloroform fraction yielded 400 mg, representing the intermediate polarity components, while the hexane extract showed yield of 32 mg, reflecting the limited presence of non-polar compounds in the fruit matrix.

3.2 TLC profiling of solvent extracts from *T. arjuna*

TLC analysis was carried out for separation and profiling of *Terminalia arjuna* fruit extract. The methanol, chloroform, and hexane fractions of *Terminalia arjuna* fruit revealed distinct phytochemical profiles in TLC analysis, with varying Rf values (Table 2) and band characteristics under UV and visible light (Figure 1), indicating solvent-dependent compound distribution. The methanol extract showed four prominent bands with Rf values of 0.21, 0.28, 0.69, and 1.0 (Table 2). These bands appeared green under UV 254 nm and light blue to grey-green under visible light, suggesting the presence of conjugated compounds and sterol-like constituents. The chloroform extract exhibited the highest number of bands (seven in total), with Rf values ranging from 0.16 to 1.0. Bands were predominantly green to light green at 254 nm, with some showing light blue fluorescence under UV 366 nm (Figure 1). The consistent brown coloration under visible light suggested the presence of polyphenolic or steroidal compounds. The hexane extract showed only two detectable bands at Rf values 0.23 and 1.0 (Table 2) with ligh-

Table1. Summary of yield of phytochemicals after liquid-liquid fractionation.

Sample	Solvent	Yield
T. arjuna fruit extract	Methanol	1652mg
	Chloroform	400 mg
	Hexane	32mg

Table 2. TLC characteristics of solvent extracts from *T. arjuna* fruit.

Solvent extract	TLC band# at 254 nm	Retention factor	TLC band characteristics		
			254 nm	366 nm	Visible light
Methanol	4	0.21	Green	-	Brown
		0.28	Green	Light Blue	Brown
		0.69	Green	Light Blue	Brown
		1	Green	Light Blue	Grey Green
Chloroform	6	0.16	Green	-	Brown
		0.22	Green	-	Brown
		0.28	Green	-	Brown
		0.33	Light Green	-	Brown
		0.34	Light Green	Light Blue	Brown
		0.68	Light Green	Light Blue	Brown
		1	Light Green	Light Blue	Grey Green
Hexane	1	0.23	Light Green	-	Brown
		1	Light Green	Light Blue	Grey Green

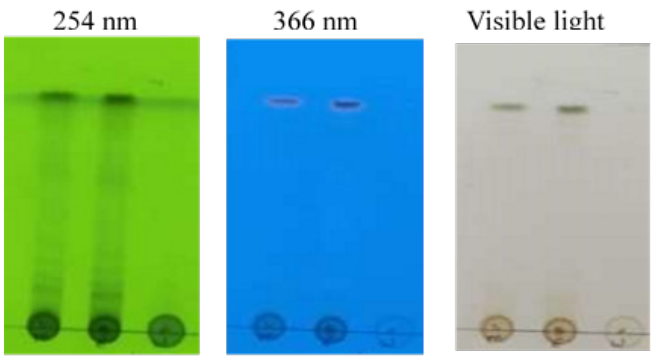

Fig. 1. TLC chromatogram of *T. arjuna* solvent extracts (lane 1-Methanol extract, lane 2-Chloroform extract, lane 3-Hexane extract).

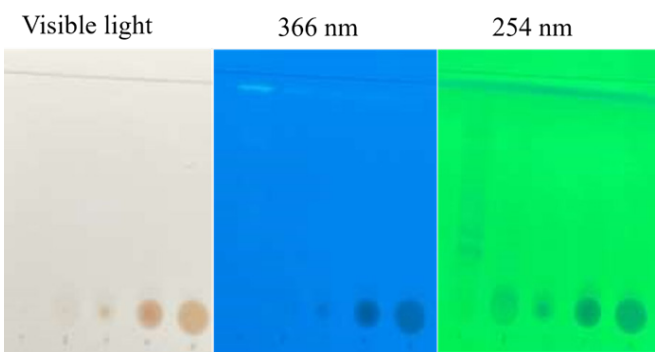

Fig. 2. TLC chromatogram of *T. arjuna* column fraction prior to pooling.

ter coloration compared to the other extracts. These bands appeared light green under 254 nm and developed light blue fluorescence at 366 nm, indicating limited but specific non-polar constituents (Figure 1).

3.3. Separation, partial purification and profiling of column fractions from *T.arjuna* methanol LLF

Column chromatography of the methanol LLF of *Terminalia arjuna* fruit yielded five primary fractions, which were subjected to TLC analysis prior to pooling. The TLC characteristics, including band positions (Rf values) and their visual profiles under 254 nm, 366 nm, and visible light, are summarized in Table 3. Fraction 1 exhibited three distinct bands with Rf values of 0.28, 0.33, and 0.34, all showing consistent light green fluorescence under 366 nm and corresponding brown coloration under visible light. Fractions 2 to 5 each showed a single band in the lower Rf range (~0.11–0.13), with blue or pink hues under 366 nm and brown coloration under visible light (Figure 2).

Based on the TLC profiles, fractions with similar Rf values and band characteristics were pooled, resulting in three major pooled samples: A (fraction 1), B (fractions 2–3), and C (fractions 4–5). Pooled fraction A retained the three bands seen in its parent fraction, whereas pooled fractions B and C showed a single dominant band with slightly differing Rf values (0.133 and 0.145, respectively), indicating minor differences in compound mobility (Table 4 and Figure 3). The respective yields of pooled fractions A, B, and C were 201 mg, 421 mg, and 523 mg, suggesting substantial recovery of compounds, particularly in the lower Rf ranges, which may correspond to more polar constituents.

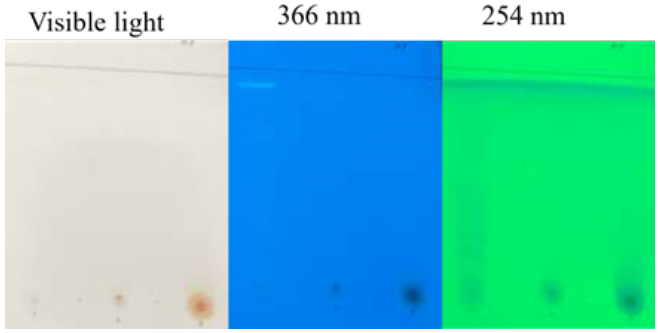

Fig. 3. TLC chromatogram of pooled *T. arjuna* column fraction.

Table 3. TLC characteristics of column fractions of *T. arjuna* methanol extract prior to pooling.

Column fraction#	TLC band # 254 nm	Retention factor	TLC band characteristics		
			366 nm	254nm	Visible light
		0.28	Light Green	Light Blue	Brown
1	3	0.33	Light Green	Light Blue	Brown
		0.34	Light Green	Light Blue	Brown
2	1	0.123	Blue	Light Pink	Brown
3	1	0.113	Blue	Light Pink	Brown
4	1	0.121	Blue	-	Brown
5	1	0.131	Blue	Light Pink	Brown

Abbreviations: TLC, thin-layer chromatography.

Table 4. TLC characteristics of pooled column fractions of *T. arjuna* extract.

Column Fraction	TLC band# 254 nm	Retention Factor	TLC Profile characteristics			Yield (mg)
			366 nm	254nm	Visible light	
		0.28	light green	light blue	light pink	
1 (A)	**3**	0.33	light green	light blue	light pink	201
		0.34	light green	light blue	light pink	
2-3 (B)	1	0.133	blue	light pink	light pink	421
4-5 (C)	1	0.145	blue	light pink	light pink	523

Abbreviations: TLC, thin-layer chromatography.

3.4. HPLC analysis of pooled fractions from *T. arjuna* fruit extract

HPLC analysis of the pooled fractions from methanol extract revealed varying compound profiles. Sample A showed three peaks at 3.091, 3.473, and 4.131 min with area percentages of 45.79%, 15.39%, and 29.79%, indicating a mix of compounds (Figure 4A). Sample B exhibited a single major peak at 3.113 min with 97.797% area, suggesting high purity (Figure 4B). Sample C displayed two peaks at 3.100 and 3.466 min, accounting for 84.64% and 15.36% of the area, respectively (Figure 4C).

3.5. Growth promotion assay of *T. harzianum* using extract fractions

The growth-promoting effect of partially purified fractions of *Terminalia arjuna* was assessed by measuring the OD at 600 nm after 48 hours of incubation in Potato Dextrose Broth (PDB). The control sample (PDB with inoculum only) showed an OD of 2.148. Sample B (PDB + inoculum + 10% extract) exhibited the highest growth promotion with an OD of 2.847, corresponding to a 32.54% increase in fungal growth relative to control and was highly significant. Sample C (PDB + inoculum + 10% extract) also demonstrated enhanced growth with an OD of 2.585, reflecting a 20.32% increase (Table 5).

Sample B exhibited a single prominent peak at a retention time of 3.113 with an area percentage of 97.8%, indicating high purity. Due to its chemical consistency and purity, Sample B was selected for further structural elucidation by LC-MS. Also, in the growth promotion assay, Sample B demonstrated a significant increase in fungal biomass, further supporting its selection for further characterisation.

3.6. LC-MS analysis and tentative identification of β-Sitosterol in partially purified fraction of *T. arjuna*

Sample B was scanned in mass spectrometer in both positive and negative ionization modes. Total Ion Chromatogram (TIC) and Extracted Ion Chromatogram (EIC) is shown in Figure 5A and Figure 5B, respectively. Abundant mass obtained in positive mode and negative mode has been summarized in Table 6.

LC-MS analysis of the partially purified fraction (Sample B) revealed key signals supporting the presence of β-sitosterol. In positive ionization mode, a major molecular ion peak was observed at *m/z* 415 $[M+H]^+$, corresponding to the protonated molecular ion of β-sitosterol,

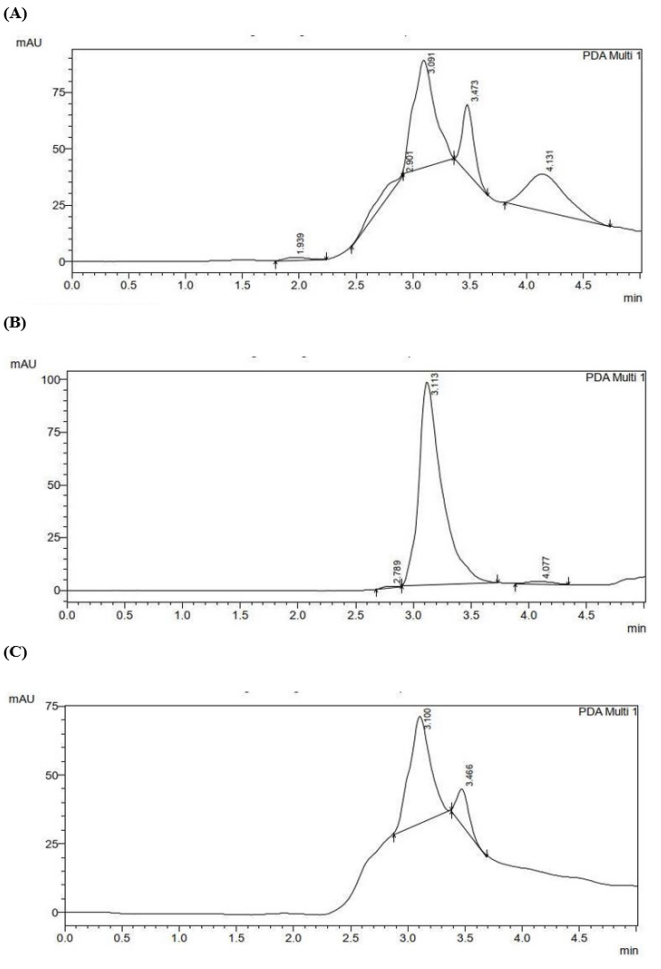

Fig. 4. HPLC analysis of pooled fractions from *T. arjuna* fruit extract (A) sample A, (B) sample B and (C) sample C.

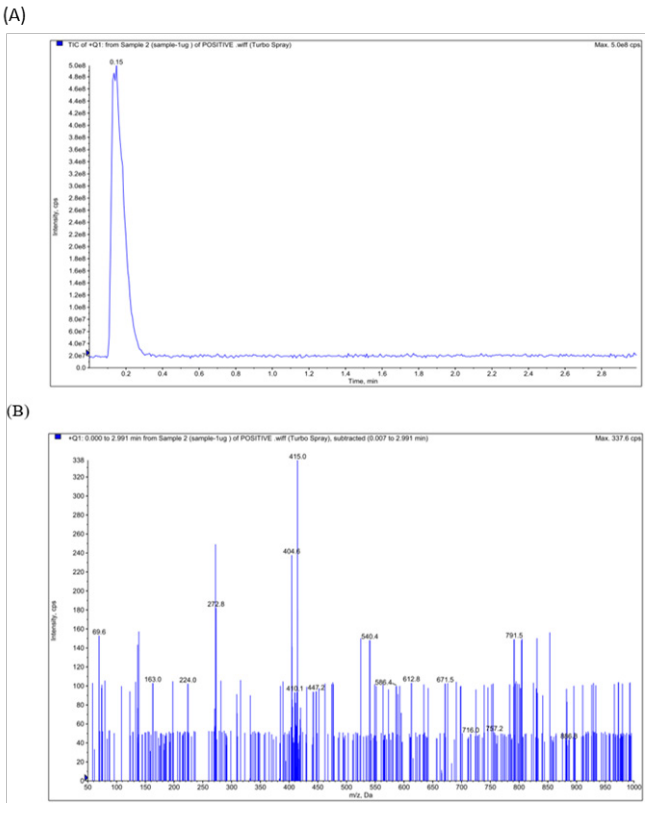

Fig. 5. (A) Total Ion Chromatogram (TIC) and **(B)** Ion Chromatogram (EIC) of partially purified *T. arjuna* extract in positive mode.

Table 5. Assessment of fungal (*T .harzianum*) growth promotion by partially purified extract of *T. arjuna* fruit.

Sample	OD @ 600nm	% Growth
Control (PDB + inoculum)	2.148	0.0
Sample B (PDB + inoculum + 10% extract)	2.847	32.5
Sample C (PDB + inoculum + 10% extract)	2.585	20.3

Abbreviation: OD, optical density.

Table 6. Summary of LC-MS analysis of *T. arjuna* extract sample B.

Mode	Retention Time (min)	Mass Spectrum
		Abundant mass recorded $(M + H)^{+1}$
Positive	0.0 to 2.991	415 404.6 272.8
Negative	0.007 to 0.270	256.1 284.1 265.3

which has a molecular weight of 414.7 g/mol. Additionally, a characteristic fragment ion at *m/z* 256 was detected, which is typically generated by the cleavage of the side chain along with part of the steroidal nucleus. These mass spectral features closely match previously reported profiles for β-sitosterol, allowing for its tentative identification in the *T. arjuna* fruit extract.

4. Discussion

The current study was undertaken to isolate, partially purify, and characterize the key phytosterol from the fruit of *T. arjuna*, and to evaluate its potential as a growth enhancer for industrially relevant fungal species. Recent investigations suggest that the fruit is also a rich reservoir of bioactive phytochemicals, including flavonoids, saponins, and phytosterols [16]. In this study, the successful identification of β-sitosterol through a combination of chromatographic and spectroscopic techniques highlights the phytochemical richness of *T. arjuna* fruit, a plant part not as extensively studied as the bark, which is rich in arjunic acid and arjunolic acid [3].

β-sitosterol, a plant sterol structurally similar to cholesterol, was confirmed as the major bioactive constituent in the methanol extract of *T. arjuna* fruit. Its identification was validated using TLC and LC-MS analyses. The LC-MS data, showing a molecular ion peak at m/z 414 $[M+H]^+$, further corroborated the presence of β-sitosterol [17–19]. These findings demonstrate the effectiveness of the adopted purification protocol and affirm *T. arjuna* fruit as a promising source of β-sitosterol. β-sitosterol has attracted significant scientific interest for its potential to modulate lipid metabolism, reduce inflammation, and exert antioxidant, anticancer, and immunomodulatory effects [20,21]. Industrially, β-sitosterol is incorporated into dietary supplements, functional foods, and cosmetic formulations for its bioactive and skin-protective effects. Characterizing β-sitosterol from *T. arjuna* fruit reveals it as a promising natural source and supports its use in cost-effective, plant-based nutraceutical and biotechnological applications.

In this study, we have demonstrated that β-sitosterol positively influences the growth of selected fungal strains widely used in biotechnology. Treatment with partially purified β-sitosterol fractions significantly increased the biomass of *Trichoderma harzianum* under laboratory conditions. *T. harzianum* serves as a potent biocontrol agent in agriculture [22,23]. The enhanced growth observed in the presence of β-sitosterol suggests a potential avenue for improving the efficacy and scalability of fungal-based biocontrol formulations. These findings open new possibilities for integrating naturally derived phytosterols in microbial cultivation strategies to augment bio-efficacy and yield in agricultural biotechnology.

The precise mechanism through which β-sitosterol enhances fungal growth is yet to be elucidated. One hypothesis is that phytosterols integrate into fungal cell membranes, modulating membrane permeability and fluidity, thereby facilitating improved nutrient uptake or triggering growth-related signalling pathways [24]. Another possibility is that β-sitosterol serves as a structural analog to ergosterol, a critical fungal membrane component, allowing it to substitute in membrane biosynthesis under certain conditions. Although exogenous sterol uptake in fungi is typically limited, certain conditions, such as sterol-auxotrophic mutants or low ergosterol biosynthesis environments, may permit assimilation [25].

The current findings are in agreement with our earlier study, in which crude methanolic extracts of *T. arjuna* enhanced fungal growth and sporulation, particularly for *T. harzianum* and *P. chrysogenum* [10]. The present study builds on earlier findings by identifying β-sitosterol as a key bioactive compound contributing to the growth-promoting effect of the plant extract. Other researchers have also noted that similar growth-promoting or modulating effects of phytosterols on microorganisms have been reported in previous studies. Enhanced mycelial growth in fungal cultures exposed to plant sterols under nutrient-limited conditions [26]. Supplementation with phytosterol-rich plant extracts significantly improved sporulation and biomass production in *Trichoderma viride* cultures [27].

The industrial implications of these findings are significant. The supplementation of microbial culture media with phytosterols or phytosterol-rich plant extracts could serve as a cost-effective strategy to boost fungal biomass or metabolite production. This may be particularly valuable in fermentation-based industries where rapid fungal growth is desirable. Moreover, since β-sitosterol is derived from plant sources, it supports the current trends toward sustainable, animal-free bioprocessing methods. Given the increasing cost and environmental concerns of synthetic or animal-derived media additives, plant-based enhancers could provide an ecologically and economically viable alternative [28].

Future studies should also explore the synergistic effects of β-sitosterol with other phytochemicals present in *T. arjuna*. It is likely that the effect of the partially purified plant extract is the result of additive or synergistic interactions among multiple constituents, including flavonoids and saponins, which are also known to affect microbial physiology. Furthermore, investigating the effects of β-sitosterol on gene expression and enzymatic activity in fungi could provide deeper insights into its biological mode of action. New approaches, including transcriptomic and proteomic profiling, may be valuable in understanding

how β-sitosterol influences fungal metabolism, sporulation, and secondary metabolite production.

This study tentatively confirms the presence of β-sitosterol in *T. arjuna* collected from Bangalore, India. However, phytochemical profiles in medicinal plants can differ widely depending on the location and time of collection. Environmental conditions such as temperature, soil composition, and precipitation play a significant role in influencing the type and concentration of active compounds. As a result, the findings presented here may not fully represent *T. arjuna* grown in other regions or under different seasonal conditions, and broader generalizations should be made with caution.

This study confirms the presence of β-sitosterol in the fruit of *T. arjuna*, a phytosterol widely recognized for its medicinal properties. The identification and partial purification of this bioactive compound provide valuable contributions to natural product research, highlighting the therapeutic potential of plant-derived sterols. Findings of this study demonstrate that *T. arjuna* fruit serves as a promising natural source of β-sitosterol and that this compound enhances the growth of key industrial fungi. These findings not only expand the pharmacological relevance of *T. arjuna* but also provide insight into a sustainable, plant-based approach to boosting fungal biomass production in commercial fermentation processes. Further work is needed to achieve complete purification and in-depth structural elucidation of β-sitosterol to better understand its mechanisms and explore its full range of applications.

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