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Original Article

Integrated Phytochemical and Chromatographic Analysis for Detection of Adulteration in *Terminalia chebula* Fruit Rind Powder

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ABSTRACT

Background: *Terminalia chebula* Retz. is a well-recognized medicinal plant widely used in the Ayurveda and Siddha systems of medicine, wherein the fruit rind is designated as the official therapeutic component. Admixture of the nut (kernel) portion with fruit rind powder has become a growing concern due to increased demand, potentially compromising quality, safety and therapeutic efficacy. **Objectives:** This study was designed to develop useful analytical approach for the identification and detection of nut admixture in *Terminalia chebula* fruit rind powder. **Materials and Methods:** Comparative evaluation of fruit rind and nut samples was performed using physicochemical analysis, total fat estimation by gravimetric (Soxhlet) extraction, fatty acid profiling through Fatty Acid Methyl Ester (FAME) derivatization followed by gas chromatography, phenolic marker assessment using HPLC-PDA and triglyceride detection using HPLC-ELSD. Mixture and spiking studies at different fruit rind-nut ratios were conducted to evaluate chromatographic variations. **Results:** The nut and kernel portions showed significantly higher total fat and triglyceride content than the fruit rind, whereas the fruit rind showed elevated concentrations of key phenolic markers, including gallic acid, chebulinic acid, chebulagic acid and ellagic acid. Chromatographic profiling showed distinct and reproducible differences between samples, with proportional enhancement of triglyceride peaks observed as nut content increased. **Conclusion:** The integrated phenolic and lipid profiling approach established in this study provides a sensitive, reproducible and analytical strategy for detecting nut admixture in *Terminalia chebula* fruit rind powder, thereby ensuring authenticity, quality control and compliance with pharmacopeial standards.

Keywords: Adulteration detection, Fatty Acid Methyl Ester (FAME) Analysis, HPLC-ELSD, HPLC-PDA, Lipid Profiling, *Terminalia chebula*.

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INTRODUCTION

Terminalia chebula Retz. (Combretaceae) is an important medicinal plant with notable pharmaceutical relevance, extensively used in the Ayurveda and Siddha medicine systems of medicine (Chatoopadhyay *et al.*, 2007; Bulbul *et al.*, 2022; Wang *et al.*, 2024; Kunle *et al.*, 2012). Adulteration of herbal raw materials is a major concern that adversely affects the quality, safety, and therapeutic efficacy of herbal medicines. Common forms of adulteration include substitution with morphologically similar species, admixture of inferior-quality plant parts, incorporation of exhausted drug material after extraction of active constituents, and intentional addition of foreign matter such as starch, sand, or soil to increase bulk. For example, medicinal plant materials are often substituted with closely related species or mixed with non-medicinal plant parts that resemble the authentic drug. *Terminalia chebula* Retz. commonly known as Haritaki, is an important medicinal plant extensively used in traditional medicine. The dried mature fruit pericarp (fruit rind) constitutes the official drug and is incorporated in numerous classical formulations owing to its documented antioxidant, laxative, antimicrobial, anti-inflammatory and rejuvenative properties (Bag *et al.*, 2013; Kannan *et al.*, 2015). The pharmacological activity of *Terminalia chebula* is primarily attributed to its hydrolysable tannins and phenolic compounds, such as gallic acid, chebulinic acid, chebulagic acid and ellagic acid, which are considered important bioactive markers for quality assessment. Classical pharmacopeial texts clearly state that only the fruit rind should be used for medicinal preparations, whereas the nut (seed/kernel) portion is excluded due to its limited therapeutic

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relevance (Government of India, 2001; Singh *et al*, 2017). However, the growing global demand for herbal raw materials and large-scale commercial processing have increased the risk of unintentional or deliberate admixture of the nut portion with fruit rind powder (Mishra *et al*., 2011; Karunakaran *et al*., 2020). Since morphological and organoleptic differentiation becomes impractical after pulverization, conventional quality control measures may fail to detect such adulteration. This raises significant concerns regarding product consistency, efficacy and regulatory compliance (Liang *et al*., 2004; Kunle *et al*., 2012). Such adulteration can compromise the quality, efficacy and standardization of the crude drug. Therefore, reliable phytochemical and chromatographic techniques are required to detect adulteration and ensure the authenticity of *T. chebula* fruit rind powder used in herbal formulations.

Previous reports indicate that the nut and kernel portions contain comparatively higher lipid and triglyceride content, whereas the fruit rind is rich in phenolic compounds (Rathinamoorthy *et al*., 2014). Therefore, differential phenolic and lipid profiling may provide a robust analytical strategy for detecting nut admixture. It was hypothesized that an integrated chromatographic approach combining phenolic marker quantification and triglyceride profiling would enable sensitive and reproducible identification of nut adulteration in *T. chebula* fruit rind powder (Reich *et al*., 2007).

Although *Terminalia chebula* fruit rind is widely used as a raw material in herbal formulations, reports on analytical methods specifically designed to detect adulteration of fruit rind powder with the seed (nut) portion are limited. Accordingly, the present study was designed to establish and optimize a multi-technique analytical framework incorporating physicochemical evaluation, total fat estimation, Fatty Acid Methyl Ester (FAME) profiling, High-Performance Liquid Chromatography - Photodiode Array. Phenolic analysis, and High-Performance Liquid Chromatography - Evaporative Light Scattering Detector triglyceride detection to ensure authenticity and pharmacopeial compliance of *T. chebula*. Such an approach may serve as a useful tool for the authentication and quality assessment of *T. chebula* raw materials

MATERIALS AND METHODS

Chemicals and reagents

All chemicals and reagents used in the study were of HPLC grade to ensure accurate and reproducible results. HPLC-grade water, acetonitrile, methanol, and ethanol were obtained from well-established suppliers, including Merck, Sigma-Aldrich, Fisher Scientific, and Loba Chemie. Reagents including potassium dihydrogen phosphate, orthophosphoric acid, hydrochloric acid, acetone, potassium ferricyanide, ferric chloride, sodium carbonate, Folin Ciocalteu reagent, formic acid, toluene, ethyl acetate, methanolic sodium hydroxide (2%), boron trifluoride, hexane, and glacial acetic acid were obtained from certified analytical-grade sources.

Instruments used

The instruments used in the present study included a NABERTHERM muffle furnace, Servewell Instruments Pvt. Ltd., hot air oven, ultrasonic cleaner and PURELAB Classic ELGA water purification system. Sample processing and temperature-controlled procedures were carried out using Servewell ring water bath and REMI water bath shaker. Weighing and moisture analysis were performed using a Contech Precisa XB-220A analytical balance (CA-234) and an OHAUS MB45 halogen moisture analyzer, respectively. Chromatographic analysis were conducted using a CAMAG LINOMAT V automated thin-layer chromatography system, a Shimadzu high-performance liquid chromatography system (UV Prominence SPD-M20A), and a Shimadzu gas chromatograph (AOC-5000).

Collection and authentication of plant material

The whole fruit, fruit rind and nut samples of *Terminalia chebula* were collected from Kodaikanal, Tamil Nadu, on 18 November 2018 and authenticated by Dr. R. Kannan, Botanist, Department of Pharmacognosy, R&D Division, The Himalaya Wellness Company, Bengaluru. Voucher specimens (NPD/180/17/F, NPD/176/17, and NPD/119/19) have been deposited for future reference and verification. Samples were subjected to the following analysis.

Physical and Phytochemical Analysis

The samples fruit rind and nut of *T. chebula* were evaluated for physicochemical parameters using standard methods, including Loss on Drying, total ash, acid-insoluble ash, water-soluble extractive and alcohol-soluble extractive values, in accordance with the Indian Pharmacopoeia 2022. Furthermore, phytochemical analysis was conducted to quantify Total Tannins and Total Polyphenols.

Samples of fruit rind and nut, were subjected to physical analysis mainly Loss on Drying, Total Ash value, Acid insoluble ash, Water soluble extractive value, Alcohol soluble extractive value as per Indian Pharmacopoeia.

Phytochemical analysis (Harborne, 1998) was carried out to determine. i) Total Tannins (Singletom *et al*., 1965). ii) Total Polyphenols (Sanmuga *et al*., 2018) by UV spectrophotometry. iii) HPLC-PDA analysis was also carried out to determine phenols (Chebulagic acid, Chebulinic acid, Ellagic acid and Gallic acid). iv) TLC fingerprinting was carried out to determine the differences in fruit rind and nut pattern.

Estimation of Total Tannin by UV spectrophotometry

Total tannin content was estimated using UV-visible spectrophotometry with tannic acid as the reference standard. 1% w/v solutions of potassium ferricyanide and ferric chloride were prepared separately in purified water. A standard stock solution of concentration 10 µg/mL was prepared by dissolving tannic acid in purified water.

For sample preparation, 100 mg of the sample was refluxed with purified water at 100 ± 2°C for 1 hr, then cooled and transferred to a 500 mL volumetric flask, and the volume was adjusted with purified water.

Aliquots of 0.2 mL of the sample solution and 1 mL of the standard solution were taken separately into 10 mL volumetric flasks.

To each standard and sample flask, 1 mL of 1% potassium ferricyanide and 1 mL of 1% ferric chloride solutions were added, and the volume was made up to 10 mL with purified water. A reagent blank was prepared in the same manner without the addition of sample or standard.

After standing for 30 min, the absorbance was recorded at 720 nm against the blank, and the total tannin content was calculated and expressed as % w/w of tannic acid equivalents.

Calculation

$$\%\ \text{w/w of Total Tannins} = \frac{A_1}{A_2} \times \frac{W_1}{100} \times \frac{1}{100} \times \frac{V_1}{V_2} \times \frac{V_3}{W_2} \times \%\ \text{Purity of Standard}$$

where:

A_1 = Absorbance of sample.

A_2 = Absorbance of standard.

W_1 = Weight of standard (mg).

W_2 = Weight of sample (mg).

V_1 = Volume of standard taken for reaction (mL).

V_2 = Volume of sample taken for reaction (mL).

V_3 = Total volume of sample extract (mL).

Estimation of Total Polyphenol by UV spectrophotometry

Total polyphenol content was estimated using Folin-Ciocalteu reagent by spectrophotometric method with pyrogallol as the reference standard. A sodium carbonate solution (290 g in 1000 mL purified water) and Folin-Ciocalteu reagent diluted in a 1:1 ratio with purified water were used for the analysis. A standard stock solution of pyrogallol (500 µg/mL) was prepared and further diluted to obtain a working standard solution of 25 µg/mL.

For sample preparation, approximately 1 g of the powdered sample was refluxed with purified water at 97 ± 2°C for 30 min, then cooled, filtered, and the extract volume was adjusted to 250 mL. An aliquot of this extract was further diluted as required for analysis.

For the assay, 2 mL each of the standard and sample solutions were mixed with Folin-Ciocalteu reagent and sodium carbonate solution, and the mixtures were allowed to stand for 30 min. The absorbance was measured at 760 nm against a reagent blank.

The total polyphenol content was calculated using the appropriate formula and expressed as pyrogallol or tannic acid equivalents.

The percentage content of total polyphenols, expressed as pyrogallol or tannic acid equivalents (depending on the standard used), was calculated using the following formula:

$$\%\text{Total Polyphenols} = \frac{A_1}{A_2} \times \frac{W_1}{V_1} \times \frac{5}{100} \times \frac{2}{25} \times \frac{V_2}{W_2} \times \frac{50}{1} \times \frac{25}{2} \times \%\ \text{Purity of Standard}$$

where:

A_1 = Absorbance of sample.

A_2 = Absorbance of standard.

W_1 = Weight of standard (mg).

W_2 = Weight of sample (mg).

V_1 = Volume of standard solution prepared (100 mL).

V_2 = Volume of sample solution prepared (250 mL).

High-Performance Liquid Chromatography-Photo Diode Array detector (HPLC-PDA) to analyse phenolic compounds

Stock solution of Chebulagic acid, Chebulinic acid, Ellagic acid and Gallic acid were prepared by dissolving 10 mg in 10 mL of methanol, respectively with sonication and suitably diluted. The solution was filtered through a 0.45 µm syringe filter. Sample *Terminalia chebula* fruit rind and nut extract (300 mg) was prepared separately by refluxing with 70 mL of methanol at 80°C for 30 min, followed by cooling, addition of about 25 mL of purified water, sonication for 5 min, dilution to 100 mL with purified water and filtration through a 0.45 µm syringe filter. Chromatographic analysis was carried out using a Phenomenex Luna C18 column (250 × 4.6 mm, 5 µm) at 254 nm, employing a gradient elution of potassium dihydrogen orthophosphate buffer (adjusted with orthophosphoric acid) and acetonitrile at a flow rate of 1.5 mL/min. The column temperature was maintained at 45°C, with a total run time of 32 min. A volume of 20 µL of both standard and sample solutions was injected, and the active constituents were quantified based on peak area. The gradient programme was initiated at 0% acetonitrile (0.01 min), increased to 8% at 7 min, 15% at 12 min, 20% at 22 min, and 50% at 25 min, followed by re-equilibration to 0% at 28 min within a total run time of 32 min.

Thin Layer Chromatography (TLC)

To know the differences in phytoconstituents pattern in fruit rind and nut of *T. chebula*, the TLC method was optimized by evaluating four different mobile phase compositions, among which ethyl acetate: toluene: formic acid: water (30:1.5:4:3) was selected as the optimized system as it provided clear and well-defined separation of the compounds. Chromatographic separation was performed on pre-coated silica gel 60 F_{254} TLC plates (20 × 10 cm). Fruit rind and Nut samples (500 mg) were individually refluxed with methanol at 80°C for 30 min, filtered and suitably diluted. Samples were applied as 12 mm bands with a spotting volume of 2 µL. Development was carried out in a CAMAG twin-trough chamber (20 × 10cm) pre-saturated for 30 min. Plates were developed up to 8.5 cm from the point of application, removed and air-dried. Visualization was performed under UV light at 254 nm and 366 nm before and after derivatization. The derivatizing reagents included diphenyl boric acid aminoethyl ester (10 g/L in methanol) and macrogol 400 (PEG, 50 g/L in methanol). The developed plates were dipped sequentially in the derivatizing solutions, dried and documented under UV light at 366 nm.

Extraction of total fat by gravimetric method

5 g of the sample was accurately weighed and transferred into tarred cellulose thimbles, which were then dried at 102 ± 2°C for 2 hrs. Three to four glass boiling beads (5 mm) were placed into each extraction flask and the flasks were dried at 100 ± 2°C for at least 30 min, cooled and weighed. Subsequently, 120-150 mL of light petroleum ether (40-60°C) was added to each extraction flask to ensure complete coverage of the test portion when the thimbles were in the boiling position.

The extraction units were assembled over an electrical heating mantle or a water bath. The solvent in the extraction flask was heated until boiling and the heat source was adjusted so that the solvent dripped from the condenser into the sample chamber at a rate of approximately 3-5 drops per second using condenser cooling water. The thimble containing the sample was placed inside the Soxhlet extraction unit, which was connected to the extraction flask, and the condensing unit was attached to the top of the Soxhlet apparatus. The entire assembly was maintained on a water bath.

Extraction of fat from the sample was carried out for 4 hrs. After completion of extraction, the extraction flask was removed from the Soxhlet unit and placed on an evaporation water bath to remove the solvent. The flask was then heated in an oven at 102 ± 2°C for 30 min to dry the residue. Finally, the flask was cooled in a desiccator to room temperature and weighed to a constant weight.

$$\%\text{Total Fat} = \frac{W_2 - W_1}{W_3} \times 100$$

where W_1 is the weight of the empty flask (g), W_2 is the weight of the flask with extracted fat (g), and W_3 is the weight of the sample (g).

Fatty acid Methyl ester derivative - Gas chromatography

Triglycerides present in the sample (washed nut, unwashed nut, crushed sample, whole fruit rind and nut) were converted into Fatty Acid Methyl Esters (FAME) by alkali hydrolysis followed by trans-esterification using boron trifluoride. The formed FAMEs were separated by liquid-liquid extraction and used for chromatographic analysis (Christe, 2003).

A quantity of 500 mg of reference standard containing saturated and unsaturated fatty acids was accurately weighed and transferred into a 250 mL round-bottom flask. To this, 5 mL of 14% methanolic boron trifluoride solution was added, and the mixture was refluxed at 80°C for 30 min. After cooling, the solution was transferred to a 250 mL separating funnel and extracted with 15 mL of hexane by shaking for 1 min.

Then, 5 mL of saturated sodium chloride solution was added and gently shaken for 30 sec. The organic layer was collected and washed three times with 10 mL of water. Finally, the organic layer was dried by filtration through anhydrous sodium sulphate and used for analysis.

The total fat extract obtained from the previous step was treated with 2% w/v methanolic sodium hydroxide and refluxed at 80°C for 15 min. Subsequently, 5 mL of 14% methanolic boron trifluoride solution was added, and the mixture was further refluxed at 80°C for 30 min.

After cooling, the solution was transferred to a separating funnel and extracted with 15 mL of hexane. Then, 5 mL of saturated sodium chloride solution was added and gently shaken. The organic layer was collected and washed three times with 10 mL of water. The final organic layer was dried by filtration through anhydrous sodium sulphate bed and subjected to Gas chromatography. The sample was also prepared separately in the above manner.

Gas Chromatographic Analysis

Gas chromatographic analysis was performed using BPX-70 capillary column (30 m × 0.25 mm, 0.25 µm film thickness) with Flame Ionization Detector (FID). Nitrogen was used as the carrier gas at a flow rate of 1 mL/min. The injection volume was 1 µL with split ratio of 1:20.

The oven temperature was maintained at 120°C for 4 min, increased at a rate of 5°C per min to 250°C and held for 5 min. The injector and detector temperatures were maintained at 250°C and 275°C, respectively. Fatty acids were identified by comparing the retention times of sample FAMEs with those of the standard FAME mixture.

HPLC - ELSD Analysis of Triglycerides

Samples (Unwashed nut kernel, Crushed sample kernel, Whole fruit kernel, fruit rind and nut) of 100 mg was individually weighed accurately and transferred to a 25 mL volumetric flask, to which 15 mL of 100% acetone was added and sonicated for 15 min . The volume was then made up to the mark with acetone and the solution was filtered through a 0.45 µm syringe filter. High-Performance Liquid Chromatography with Evaporative Light Scattering Detection (HPLC-ELSD) was carried out using a Phenomenex Luna C18 (250×4.6 mm, 5 µm) column at a wavelength of 254 nm, with a flow rate of 1.5 mL/min and an injection volume of 20 µL. The mobile phase consisted of 100% acetone (phase A) and 100% acetonitrile (phase B), following a gradient program: at 0.01 min - 50% B, 10.00 min - 0% B, 25.00 min - 0% B, 28.00 min - 50% B, 32.00 min - 50% B, and 32.01 min - stop, for a total run time of 32 min. The column temperature was maintained at 30.6°C. The ELSD detector operated at a total flow of 1.5 mL/min with a B concentration of 50%, a detector temperature of 40°C, gain set to 1 and gas flow at 1.5. A 20 µL sample was injected and the chromatogram was recorded; the triglycerides pattern was then compared with the chromatogram of standard glycerol mono stearate (Hokapek ***et al.***, 1999).

Ethical Statement

This study did not involve human participants or animal subjects. Ethical approval was not required for this botanical research.

Statistical Analysis

All experiments were performed in triplicate and data expressed as mean values where applicable.

RESULTS

All the analysis was carried out as per the method described in Methodology section to identify the differences in fruit rind and nut samples of *T. chebula* which would be helpful to detect the adulteration further during routine analysis.

Physical and Phytochemical Analysis

The fruit rind and nut were subjected to Physical and Phytochemical Analysis to know the differences in the fruit rind and nut.

Physical Analysis

Loss on drying, Total ash, Acid insoluble ash, Water soluble extractive value and alcohol soluble extractive values was carried out as per standard procedure and the results obtained are as below in Table 1.

Phytochemical Analysis

Fruit rind and nut sample of *T. chebula* were subjected to phytochemical analysis to analyse - Total tannins and Total polyphenols by UV spectrophotometry. Chebulagic acid, Chebulinic acid, Ellagic acid and Gallic acid was analysed by HPLC. Results are depicted in Table 2.

High-Performance Liquid Chromatography with PDA Detection to analyse phenolic compounds

The HPLC-PDA chromatographic analysis of *Terminalia chebula* fruit rind and nut samples showed the presence of major phenolic markers, namely gallic acid, chebulinic acid, chebulagic acid, and ellagic acid (Figure 1). Gallic acid was detected at a retention time of 6.983 min, while chebulinic acid was observed at 16.378 min. Chebulagic acid eluted at 18.256 min, followed by ellagic acid at 19.242 min in Fruit rind. The chromatogram of the fruit rind showed prominent and well-resolved peaks corresponding to these compounds, whereas the nut sample also showed peaks at similar retention times with variations in peak intensity and area, indicating compositional differences between the fruit rind and nut portions.

Overlay Chromatogram of HPLC - Fruit Rind and Nut Samples of *T. chebula*

The overlay HPLC-PDA chromatograms of *Terminalia chebula* fruit rind and nut samples showed distinct differences in their phenolic profiles. The fruit rind exhibited prominent and well-resolved peaks corresponding to gallic acid at approximately 6.98 min, chebulinic acid at around 16.44 min, and ellagic acid at approximately 19.24 min, followed by chebulagic acid eluting at about 19.55 min.

Mixture of fruit rind and nut spiked to different ratios by HPLC-PDA technique

Different proportions of standard markers and samples were spiked to observe variations in fruit rind and nut. The spiking study confirmed the identity of the major phenolic markers present in the sample, and all solvent ratios showed consistent results. After spiking with reference standards, a significant increase in peak intensity was observed at the specific retention times without any shift in retention time across all ratios. Gallic acid was detected at a retention time of 6.98 min, chebulinic acid was observed at 16.44 min, ellagic acid eluted at 19.24 min and chebulagic acid appeared at 21.92 min.

TLC: The fruit rind and nut were subjected to TLC. Images obtained are as below.

Thin layer chromatographic profiling revealed distinct banding patterns for the fruit rind and nut samples both at 254 nm and at 366 nm. Fruit rind showed many spots at various R_f (0.3, 0.42, 0.51, 0.67, 0.72). Whereas there were no distinct spot observed in nut sample (Table 3 and Figure 2).

Estimation of total fat by gravimetric method

The total fat content of different samples was determined by the gravimetric method and expressed as percentage w/w.

Fatty acid Methyl ester derivative - Gas chromatography

Figure 3 depicts the Fatty acid Methyl ester derivative analysed by Gas chromatography. Washed nut, Unwashed nut, Crushed sample, Whole fruit and fruit rind spectra is overlayed and the present study confirms that fatty acids are distributed in all parts of the fruit.

Table 1: Physical parameters of *Terminalia chebula* Fruit rind and Nut: A) LOD; B) Total ash value; C) Acid insoluble ash value; D) Water soluble extractive value; E) Alcohol soluble extractive value.

Physical Parameters	Fruit Rind (Mean + SD)	Nut (Mean + SD)
LoD %	7.97± 0.32	8.03± 0.21
Total ash value %	2.88± 0.18	1.89± 0.15
Acid insoluble ash value %	0.79± 0.08	0.41± 0.03
Water soluble extractive value % w/w	33± 0.06	12.51± 0.02
Alcohol soluble extractive value % w/w	9.93± 0.02	12.32± 0.01

Triglycerides in *Terminalia chebula* by HPLC-ELSD

The chromatographic analysis of triglycerides in *Terminalia chebula* samples (whole fruit, fruit rind, nut) showed a consistent triglyceride pattern. Figure 4 gives the overlay of Triglyceride pattern of washed nut, unwashed nut, crushed sample, whole fruit and fruit rind. Triglycerides pattern were observed in all samples of the kernel part.

Comparative Study of Triglyceride Patterns in Mixtures of Fruit Rind and Nut Powder at Different Ratios Using HPLC-ELSD

Triglyceride patterns in mixtures of Fruit rind and Nut powder were analyzed at different ratios such as. A) 90:10; B) 70:30; C) 50:50; D) 30:70; E) 10:90. The sample mixture containing fruit rind and nut in a 10:90 ratio showed a notable triglyceride response, indicating a higher contribution of triglycerides from the nut portion.

Qualitative Analysis of Triglycerides by HPLC-ELSD and Fat Extraction Using Petroleum Ether from Different Parts of *Terminalia chebula* Fruit

The qualitative analysis of triglycerides by HPLC-ELSD following petroleum ether extraction from different parts of *Terminalia chebula* fruit revealed distinct distribution patterns.

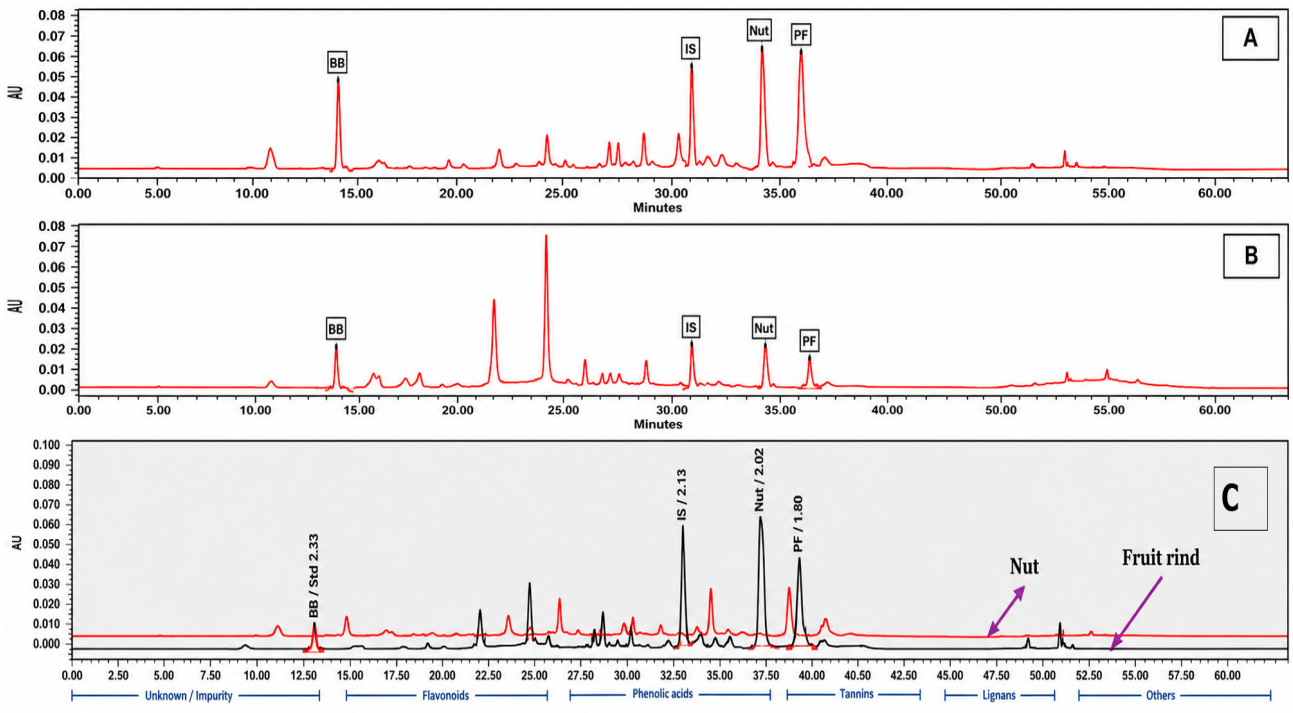

Figure 1: HPLC Chromatogram of phenols in *Terminalia chebula* fruit rind and nut. A) Chromatogram of Fruit rind; B) Chromatogram of Nut; C) Overlay of fruit rind and nut.

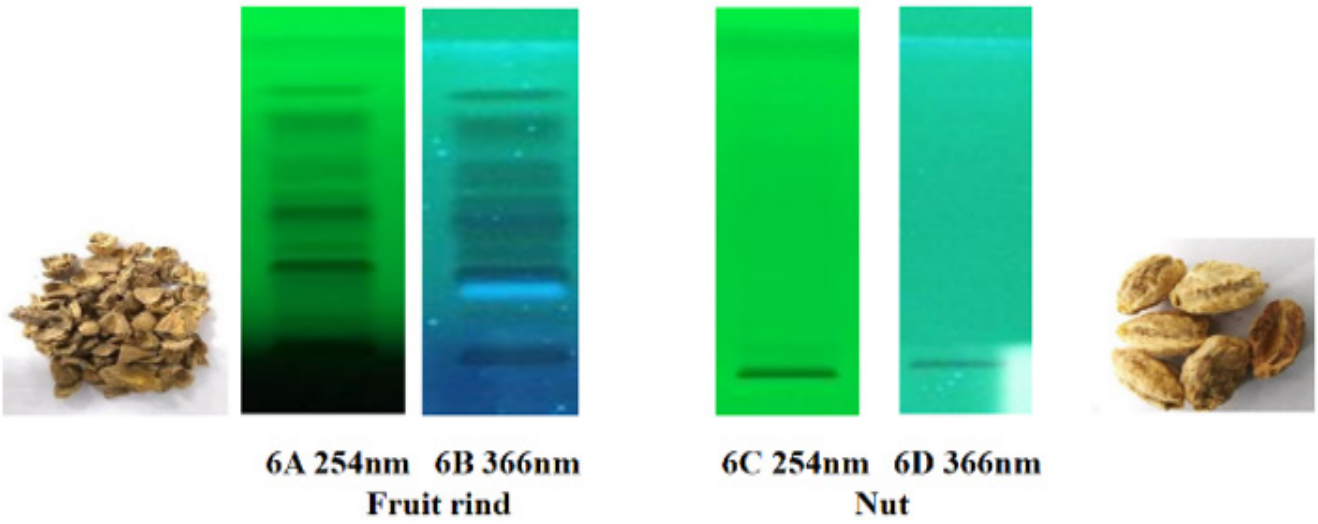

Figure 2: TLC pattern of fruit rind and nut at 254 nm and 366 nm.

Table 2: Chemical Parameters of *Terminalia chebula* Fruit rind and Nut: A) Total tannins; B) Total polyphenols; C) Total chebulagic acid; D) Total chebulinic acid; E) Total Ellagic acid; F) Total Gallic acid.

Chemical Parameter %	Fruit Rind	Nut
Total tannins	41.04	19.9
Total polyphenols	20.25	18.01
Chebulagic acid	7.91	0.68
Chebulinic acid	4.28	0.71
Ellagic acid	0.78	0.16
Gallic acid	1.05	0.26

Overlay Chromatogram of Different Parts of *Terminalia chebula* Fruit showing Triglyceride Response by HPLC-ELSD Method

The overlay chromatogram of different parts of *Terminalia chebula* fruit was repeatedly analyzed by HPLC-ELSD and it clearly demonstrates variation in triglyceride response among the samples. The whole fruit sample exhibited a distinct triglyceride peak cluster in the highlighted region (approximately 8-14 min), indicating the presence of lipid constituents.

HPLC-ELSD Analysis of Triglycerides, Total Fat Extraction of Fruit Rind-Nut Mixtures at Different Ratios

Total fat extracted from fruit rind and nut mixtures prepared in different ratios was dissolved in acetone to obtain suitable concentrations for HPLC-ELSD analysis. For the 50:50 fruit rind-nut mixture, 0.135 g of the sample was dissolved in 10 mL of acetone, yielding a concentration of 13.5 mg/mL. In the 70:30 ratio, 0.168 g of the extract was dissolved in 5 mL of acetone to obtain a concentration of 33.6 mg/mL. Similarly, for the 90:10 fruit rind-nut mixture, 0.207 g of the extract was dissolved in 5 mL of acetone, resulting in a concentration of 41.4 mg/mL. These prepared solutions were subsequently subjected to HPLC-ELSD analysis for qualitative evaluation of triglyceride patterns. All three ratios exhibited a triglyceride pattern in the HPLC-ELSD analysis suggesting the suitability of the method in identifying the adulterant from the genuine drug.

DISCUSSION

Physical and Phytochemical Analysis

The fruit rind and nut were subjected to Physical and Phytochemical Analysis.

The assessment of physical parameters demonstrated distinct variations between the fruit rind and nut samples. The fruit rind exhibited higher water-soluble extractive values, whereas the nut showed relatively higher alcohol-soluble extractive values, reflecting differences in the composition of extractable constituents. The Loss on Drying (LOD) was found to be higher in the nut, indicating greater moisture content, while the total ash and acid-insoluble ash values were comparatively higher in the fruit rind, suggesting a greater presence of inorganic and siliceous matter. Most of the parameters complied with pharmacopeial standards. These findings emphasize the significance of using the fruit rind alone in medicinal formulations to ensure quality, uniformity and adherence to established standards.

The phytochemical analysis demonstrated a markedly higher concentration of bioactive constituents in the fruit rind compared to the nut. The fruit rind showed substantially greater levels of total tannins, total polyphenols and key marker compounds such as chebulagic acid, chebulinic acid, ellagic acid and gallic acid. In contrast, the nut contained considerably lower amounts of these constituents. These findings clearly indicate that the fruit rind is the principal reservoir of therapeutically important phytochemicals, thereby justifying its exclusive use in traditional medicinal preparations and supporting the detection of nut admixture through chemical profiling.

High-Performance Liquid Chromatography with PDA Detection to analyse phenolic compounds

The HPLC-PDA chromatographic analysis of *Terminalia chebula* fruit rind and nut samples showed the presence of major phenolic markers, namely gallic acid, chebulinic acid, chebulagic acid, and ellagic acid. The chromatogram of the fruit rind showed prominent and well-resolved peaks corresponding to these compounds, whereas the nut sample also showed peaks at similar retention times with variations in peak intensity and area, indicating compositional differences between the fruit rind and nut portions.

Overlay Chromatogram of HPLC - Fruit Rind and Nut Samples of *T. chebula*

The overlay HPLC-PDA chromatograms of *Terminalia chebula* fruit rind and nut samples showed distinct differences in their phenolic profiles (Reich *et al.*, 2007). The fruit rind exhibited prominent and well-resolved peaks corresponding to gallic acid at approximately 6.98 min, chebulinic acid at around 16.44 min, and ellagic acid at approximately 19.24 min, followed by chebulagic acid eluting at about 19.55 min. These marker compounds appeared with higher peak intensity and area in the fruit rind sample. In contrast, the nut sample showed peaks at similar retention times but with comparatively reduced intensity, indicating lower concentrations of these phenolic constituents. The chromatographic overlay clearly demonstrates quantitative variation between the fruit rind and nut portions.

The spiking study confirmed the identity of the major phenolic markers present in the sample and all solvent ratios showed consistent results. After spiking with reference standards, a significant increase in peak intensity was observed at the specific retention times without any shift in retention time across all ratios. Gallic acid was detected at a retention time of 6.98 min, chebulinic acid was observed at 16.44 min, ellagic acid eluted at 19.24 min and chebulagic acid appeared at 21.92 min.

Thin layer chromatographic profiling revealed distinct banding patterns for the fruit rind and nut samples both at 254nm and at 366nm. The fruit rind exhibited multiple well-resolved spots corresponding to tannins, polyphenols and flavonoids, indicating a richer phytochemical composition. In contrast, the nut showed fewer and less intense spots, reflecting a lower concentration of these constituents. The clear difference in TLC profiles confirms the suitability of TLC as a reliable tool for differentiating the fruit rind from nut admixture in *Terminalia chebula*.

Table 3: Total fat estimation by gravimetric method - whole fruit, kernel, crushed fruit, fruit rind and nut.

Samples	Sample wt. (g)	% w/w total fat
Whole fruit	5.0455	2.10
Kernel	5.0105	3.36
Crushed	5.0035	2.27
Fruit rind	5.0229	1.32
Nut	5.0021	2.83

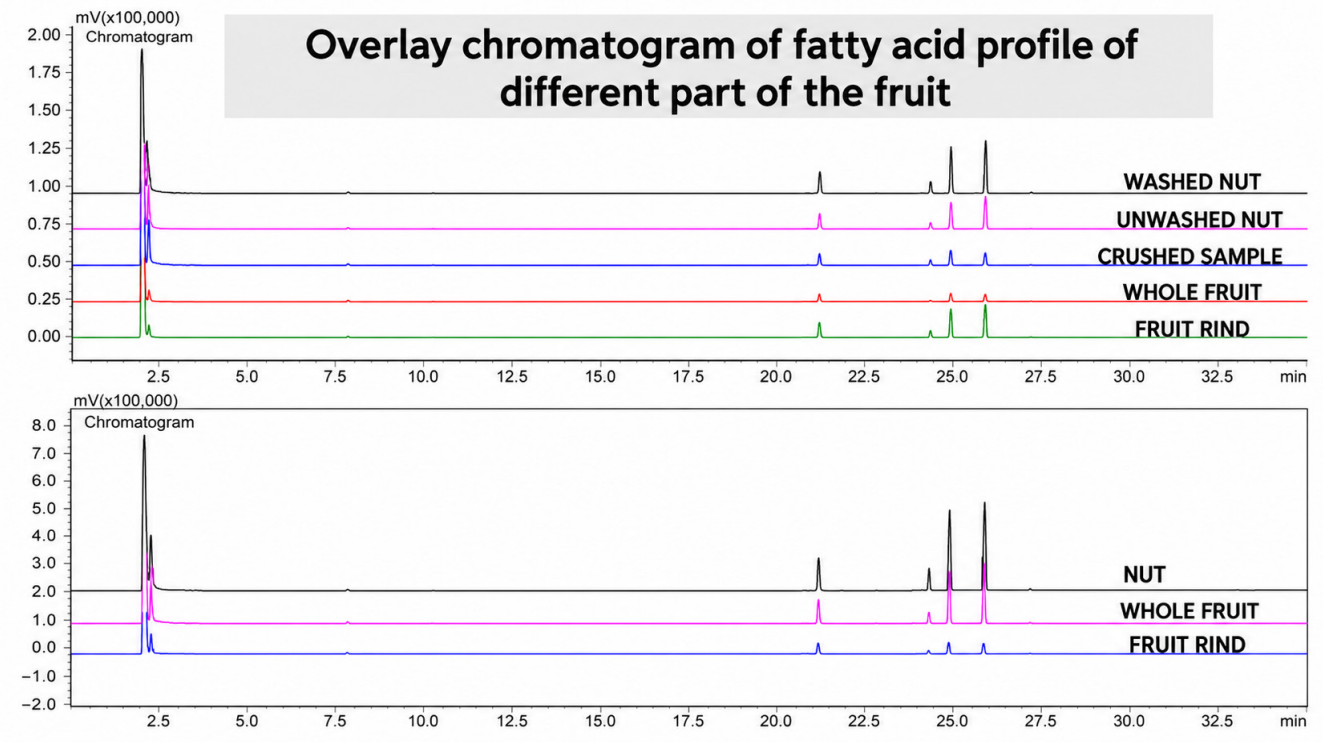

Figure 3: Overlay chromatogram of fatty acid profile of different part of the fruit. Chromatogram of Washed nut, Unwashed nut, Crushed sample, Whole fruit, Fruit rind; Chromatogram of Nut, Whole fruit and Fruit rind.

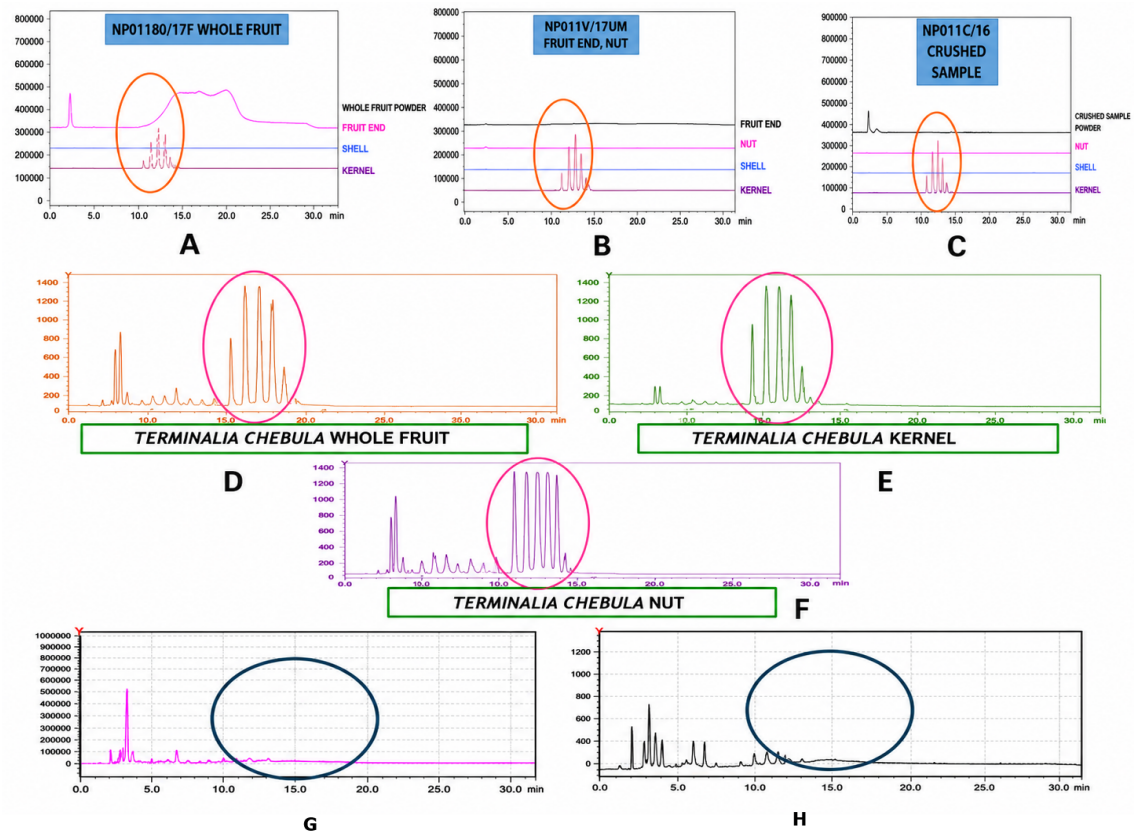

Figure 4: HPLC-ELSD Overlay chromatogram of various parts of fruit showing triglyceride pattern of *Terminalia chebula* -Whole fruit, Fruit rind and Nut, Crushed sample. A) Chromatogram of Whole fruit, Fruit rind, Shell and Kernel; B) Chromatogram of Fruit rind, Nut, Shell and Kernel; C) Chromatogram of Crushed sample powder, Nut, Shell and Kernel; D) Whole fruit; E) Kernel; F) Nut; G) Fruit rind; H) Shell.

The total fat content of different samples was determined and the results revealed that the kernel sample showed the highest total fat content of 3.36%, followed by the nut sample with 2.83%. The crushed sample exhibited 2.27% total fat, while the whole fruit sample showed 2.10%. Among all the samples analyzed, the fruit rind demonstrated the lowest fat content of 1.32%. These findings indicate that the fat content is comparatively higher in the kernel and nut portions than in the fruit rind of *Terminalia chebula*.

Fatty acid Methyl ester derivative was analysed by Gas chromatography. The nut portion exhibited a comparatively higher fatty acid content than the fruit rind. The fatty acid profiling revealed the presence of capric acid, palmitic acid, stearic acid, oleic acid, linoleic acid, and linolenic acid, indicating a diverse composition of both saturated and unsaturated fatty acids within the fruit.

The chromatographic analysis of triglycerides in *Terminalia chebula* samples (whole fruit, fruit rind, nut) showed a consistent triglyceride pattern. The characteristic triglyceride pattern was predominantly present in the kernel fraction and was minimal in the fruit rind. Similarly, in crushed sample, the triglyceride peaks matched the kernel pattern, indicating the presence of kernel-derived lipid components. Overall, the triglyceride profile was consistently prominent in the kernel part across all batches, confirming that triglycerides are primarily localized in the kernel portion of *Terminalia chebula*. The developed method was suitable to detect the adulterant in *T. chebula* fruit rind powder.

The method developed could detect the triglycerides in rind and nut and it was observed that the nut which is often an adulterant, had a higher contribution of triglycerides from the nut portion.

The qualitative analysis of triglycerides by HPLC-ELSD following petroleum ether extraction from different parts of *Terminalia chebula* fruit revealed distinct distribution patterns. The whole fruit extract exhibited a clear triglyceride peak pattern in the highlighted region, indicating the presence of lipid constituents. The kernel sample showed prominent and well-defined triglyceride peaks with higher intensity, confirming that triglycerides are predominantly concentrated in the kernel portion (Rathinamoorthy *et al.*, 2014). Similarly, the nut sample displayed a comparable triglyceride pattern, supporting its lipid-rich nature. In contrast, the fruit rind and shell samples showed either minimal or negligible triglyceride peaks in the corresponding region. The comparative chromatograms clearly demonstrate that triglycerides are mainly localized in the kernel and nut portions, while the fruit rind and shell contain very low levels of these lipid components.

The overlay chromatogram of different parts of *Terminalia chebula* fruit sample exhibited a distinct triglyceride peak cluster in the highlighted region indicating the presence of lipid constituents. The nut sample and crushed sample nut showed prominent and well-defined triglyceride peaks with higher intensity, closely matching the kernel sample, confirming that triglycerides are predominantly associated with the kernel portion. In contrast, the fruit rind samples displayed minimal or negligible triglyceride response in the same retention time region.

The overlay comparison clearly indicates that triglyceride content is significantly higher in the kernel and nut portions, while the fruit rind contains very low levels of triglycerides.

Total fat extracted from fruit rind and nut mixtures in all three ratios exhibited a triglyceride pattern in the HPLC-ELSD analysis suggesting the suitability of the method in identifying the adulterant from the genuine drug.

The results obtained from phytochemical screening and chromatographic profiling demonstrated distinct differences between authentic fruit rind powder and adulterated samples containing the nut portion. The integrated analytical approach employed in this study enabled reliable detection of adulteration through complementary phytochemical and chromatographic markers. These findings highlight the prospective utility of the proposed methodology as a quality control tool for authentication and standardization of *Terminalia chebula* fruit rind powder in herbal industries.

CONCLUSION

This study systematically examined the chemical differences between the fruit rind and nut (kernel) of *Terminalia chebula* using complementary chromatographic techniques to address concerns related to raw material authenticity. Traditional literature consistently identifies the fruit rind as the therapeutically active portion due to its richness in hydrolysable tannins and phenolic constituents, while the nut is recognized as comparatively lipid-rich and not intended for medicinal use. Despite this distinction, analytical differentiation between these parts in powdered form has remained limited. The present findings clearly demonstrate substantial compositional variation between the fruit rind and nut. The nut and kernel portions showed markedly higher total lipid and triglyceride content, along with significant levels of fatty acids such as capric, palmitic, stearic, oleic, linoleic, and linolenic acids. In contrast, the fruit rind exhibited higher concentrations of key phenolic markers, including gallic acid, chebulinic acid, chebulagic acid, and ellagic acid. Controlled mixture and spiking experiments revealed progressive increases in triglyceride peak intensity corresponding to higher proportions of nut material, indicating that even partial inclusion of the nut noticeably modifies the chemical profile. These observations support the hypothesis that integrated phenolic and lipid profiling can effectively distinguish fruit rind from nut material. Collectively, these findings deepen the scientific insight into the part-specific chemical composition of *Terminalia chebula* and reaffirm the traditional practice of exclusively utilizing the fruit rind for therapeutic purposes. Further studies may focus on evaluating variability across wider commercial sources and defining practical compositional benchmarks for routine quality assessment.

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ABBREVIATIONS

FAME: Fatty Acid Methyl Ester; **HPLC-PDA:** High-Performance Liquid Chromatography - Photodiode Array; **HPLC-ELSD:** High-Performance Liquid Chromatography - Evaporative Light Scattering Detector; **LOD:** Loss on Drying; **TLC:** Thin Layer Chromatography; **UV:** Ultraviolet; **PEG:** Macrogol 400; **FID:** Flame ionization detector.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

This study establishes a dual-profiling method to identify the adulteration of *T. chebula* fruit rind with its nut kernel. While the rind is rich in therapeutic phenolics, the nut contains significant triglycerides and fatty acids. The integrated use of HPLC-PDA and HPLC-ELSD allows for sensitive detection of nut admixture in powdered samples.

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