Wiley
Journal of Food Biochemistry
Volume 2025, Article ID 8590029, 9 pages
https://doi.org/10.1155/jfbc/8590029

WILEY

Research Article

Bioactivity Studies of Different Solvent Extracts of Defatted Residues From *Terminalia catappa* L. Seed Kernels

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Received 24 January 2025; Accepted 25 April 2025

Academic Editor: Lin Han

Terminalia catappa Linn., also referred to as tropical almond or Indian almond, can play a significant role in improving food and nutritional security. The objective of this research was to assess the antioxidant, antihyperglycemic, and antiobesity potentials of the defatted residues from seed kernels of purple and yellow cultivars. The defatted residues obtained using a micro–screw-press oil extractor were subjected to sequential extraction using n-hexane, dichloromethane (DCM), and methanol (MeOH) as solvents. The crude extracts of both cultivars were subjected to the evaluation of total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activities, namely, DPPH, $ABTS^{+}$, and ferric reducing antioxidant power (FRAP). They were also subjected to enzyme inhibitory activities against α-amylase and lipase. Among the extracts, the MeOH extract of the yellow cultivar showed the highest TPC, superior antioxidant activities (DPPH and $ABTS^{+}$), and strongest enzyme inhibitory activities. In contrast, the purple cultivar exhibited the highest FRAP activity. Gallic acid was the major phenolic constituent occurring in high concentrations in the defatted residues. These findings enlighten the potential uses of defatted residues of the *T. catappa* seed kernels, particularly those from the yellow cultivar as an ingredient for nutraceutical and functional food applications.

Keywords: antihyperglycemic activity; antiobesity activity; antioxidant capacity; *Terminalia catappa* Linn.; underutilized seeds

1. Introduction

Food and nutritional security are major global concerns, especially in the context of world population growth and depletion of natural resources. To address these challenges, exploring unconventional food sources is a proactive approach. Sadia et al. [1] highlighted the use of plants such as *Fragaria nubicola, Rosa moschata, Rosa webbiana,* and *Rubus anatolicus* as potential sources to enhance food security in various regions. Similarly, *Terminalia catappa* L., widely known as tropical or Indian almond, can also play a significant role in improving food and nutritional security. *T. catappa* is a versatile tree species of the Combretaceae family [2] native to tropical regions are attributed to its

bioactive compounds. This tree can be identified as an underutilized or underexploited species for food use in Sri Lanka as well as the rest of the world. Therefore, it is crucial to study the nutritional composition and medicinal values of the seed kernel to utilize them to their maximum potential. A preliminary study by Wijesekera et al. [3] highlighted the nutritional composition and functional properties of the whole seed kernel of *T. catappa*, particularly having potent antioxidative and antihyperglycemic effects. Harnessing the potential of underutilized fruits might offer solutions not only for food shortages during climate change but also provide alternative remedies for disease conditions such as diabetes as well as contribute to sustainable, nutritious solutions for food security.

T. catappa seed kernel is a high source of edible oil as per the preliminary nutritional assessment which suggested its suitability for oil extraction by commercial screw press-machine [3]. Despite the known benefits of *T. catappa* kernels, the defatted residue, which is a by-product of oil extraction is supposed to be discarded as a waste. However, recent studies suggest that its residues may be a good source of protein and minerals. The protein content of defatted residues of *T. catappa* was found to be more than twice that of the whole kernel [4]. While the bioactivities of the whole kernels and its oil have been already documented [3, 5, 6], research strictly focusing on the functional food properties of defatted residue remains limited. Although some studies have begun to investigate the properties of defatted residues in other plants, research specific to defatted residues of *T. catappa* is scarce, and its bioactive constituents remain underexplored. In this study, we attempted to explore the bioactivity of different solvent extracts of the defatted residues from *T. catappa* kernels, focusing on their antioxidant, antihyperglycemic, and antiobesity potentials.

2. Materials and Methods

2.1. Sampling. The purple and yellow cultivars of *T. catappa* seeds were collected from the Central Province of Sri Lanka from February to May 2023. The initial processing involved with drying the seeds at 55°C for 8 h using a blower-assisted drying oven (Biobase, model-BOV-V230F, China). After drying, the seeds were carefully opened to extract the kernels, which were maintained under refrigeration for future analysis.

2.2. Reagents and Instruments. Enzymes porcine pancreatic α-amylase and porcine pancreatic lipase were obtained from Sigma-Aldrich, while the rest of the chemicals and reagents employed in the assays were of analytical grade, unless stated otherwise. UV absorbance measurements were carried out using a microplate reader (Synergy HTX BioTek Multimode Reader, BioTek Instruments, USA).

2.3. Preparation of Defatted Residues. Separated kernels were dried in the oven at 55°C for 8 h using a blower-assisted drying oven (Biobase, model-BOV-V230F, China). After drying the kernels, size reduction was performed using a mortar and pestle. To separate the oil, the kernel pieces were then fed into a micro–oil expeller (Komet DD85, Germany). After the oil extraction, the defatted residue was recovered and ground into a powder and then kept in a refrigerator at 4°C until additional analyses were performed.

2.4. Preparation of Defatted Residue Extracts. Two hundred grams of defatted residues from each cultivar were subjected to sequential extraction with n-hexane, dichloromethane (DCM), and methanol (MeOH) using an ultrasound-assisted extraction method (Rocker ultrasonic cleaner, model-Soner 206H) for a 30-min duration. For each solvent type, the extraction procedure was repeated three times. The resulting extracts underwent concentration via a rotary evaporator (Heidolph, Laborota 4000) under reduced pressure. This was followed by vacuum drying (vacuum oven, Heraeus instrument, Germany) for a duration of 3-4 h. The dried extracts were then preserved at −18°C.

2.5. Assessment of Total Phenolic Content (TPC). The TPC of defatted residues from *T. catappa* extracts was quantified using the Folin–Ciocalteu method, outlined by Gunarathne et al. [7] with minor adjustments. Briefly, 2 mg of each extract was dissolved in 0.2 mL of 4% dimethyl sulfoxide (DMSO) and 4.8 mL of distilled water. Then, in a 96-well microplate, 50 μL aliquot of this solution was mixed with 15 μL of distilled water and 105 μL of 10% Folin–Ciocalteu reagent. After 3 min of incubation, 80 μL of 7.5% Na_2CO_3 was added, then the mixture was incubated in the dark at room temperature (RT) for 30 min. Absorbance was recorded at 765 nm. TPC of crude extracts were represented as mg of gallic acid equivalent (GAE) per g of crude extract.

2.6. Assessment of Total Flavonoid Content (TFC). The TFC of defatted residues of *T. catappa* was determined by aluminum chloride colorimetric method outlined by Gunarathne et al. [7] with minor adjustments. Briefly, 2 mg of each extract was dissolved in 0.2 mL of 4% DMSO and 4.8 mL of distilled water. A 50-μL aliquot of this solution was mixed with 20 μL of 5% $NaNO_2$ in a 96-well microplate. After 6 min of incubation at RT, 20 μL of $AlCl_3$ was added. A 200-μL 4% NaOH solution was added after another 6 min of incubation, allowed to stand for 15 min, and the absorbance was recorded at 510 nm. TFC in the crude extracts were denoted as mg of catechin equivalent (CE) per g of crude extract.

2.7. Evaluation of Antioxidant Activity

2.7.1. 2, 2-Diphenylpicrylhydrazyl (DPPH) Radical Scavenging Assay. As outlined by Gunarathne et al. [7], the antioxidant capacity from crude extracts of defatted residues of *T. catappa* was measured following the DPPH radical scavenging method. In brief, a series of concentrations ranging from 7.123 ppm to 2000 ppm of crude extracts were prepared by dissolving the samples in MeOH. In a 96-well microplate, 150 μL of each concentration was mixed with

60 μL of 0.3 mM DPPH. The mixture was then incubated for 30 min at RT. Absorbance was recorded at 517 nm against the control, with ascorbic acid serving as the positive control. Finally, the percentage radical scavenging activity (% RSA) was calculated accordingly, and plotting % RSA against the sample concentration of each extract allowed for the graphic determination of the IC_{50} values.

$$\text{RSA \%} = \frac{\delta A_{\text{control}} - \delta A_{\text{sample}}}{\delta A_{\text{control}}} \times 100, \quad (1)$$

where $\delta A_{\text{control}} = \text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{control blank}}$, $\delta A_{\text{sample}} = \text{Absorbance}_{\text{sample}} - \text{Absorbance}_{\text{sample blank}}$, and RSA % is the percentage of radical scavenging activity.

2.7.2. Ferric Reducing Antioxidant Power (FRAP) Assay. The experiment was carried out as previously outlined by Gunarathne et al. [7] with minor adjustments. Briefly, 8 mg of the sample was dissolved in 0.2 mL of 4% DMSO and 4.8 mL of distilled water. A 96-well microplate was then filled with 50 μL of the sample and 200 μL of FRAP solution. The mixture was then incubated for 4 min at RT, and absorbance was recorded at 593 nm. The results were reported as mmol $FeSO_4$ per gram of crude extract. The positive control in this experiment was ascorbic acid. The FRAP solution was prepared by combining 10 mM of TPTZ solution (in 40 mM of HCl), 10 mM of $FeCl_3.6H_2O$ solution, and 40 mL of 300 mM acetic buffer (pH 3.6) in a 1:1:10 ratio. It was then heated to 37°C just before use.

2.7.3. $ABTS^+$ Radical Scavenging Assay. The assay was conducted following the method outlined by Adekola et al. [8] with minor adjustments. Equal volumes of ABTS (7.8 mM) and potassium persulfate (2.45 mM) were combined to create the ABTS stock solution, which was then left at RT for 16 h in darkness. Subsequently, a working ABTS solution was obtained by diluting the stock with PBS (pH 7.4) to achieve an absorbance of 0.70 ± 0.02 at 734 nm. For sample preparation, crude extracts were obtained by dissolving 6 mg of the sample in 0.09 mL of 3% DMSO and 2.91 mL of distilled water. Then, a 96-well microplate was filled with 50-μL aliquot of this solution and 150 μL of the ABTS working solution. The mixture was then allowed to incubate for 30 min at RT, after which the absorbance was recorded at 734 nm. Ascorbic acid served as the positive control, and the results were reported as millimoles of Trolox per gram of crude extract.

2.8. Evaluation of Enzyme Inhibitory Activity

2.8.1. α-Amylase Inhibition Assay. The crude extracts from defatted residues of *T. catappa* were evaluated for their capacity to inhibit α-amylase activity using the protocols outlined by Nickavar et al. [9] and Ulpathakumbura et al. [10] with minor adjustments. Initially, concentration series ranging from 6000 to 36,000 ppm of crude extracts were prepared by dissolving them in distilled water containing 5% DMSO. In a semicentrifuge tube, 50 μL of each sample solution was mixed with 50 μL of a 20-mg/mL α-amylase enzyme solution. After a 30-min incubation at RT, 100 μL of 1% starch solution was added, followed by a further 10-min incubation. The DNSA reagent (100 μL) was then added, and the mixture was heated in a water bath at 85°C for 15 min. The mixture was cooled, diluted with distilled water, and 200 μL was moved to a 96-well microplate for absorbance measurement at 540 nm. Acarbose served as the positive control. The percentage of α-amylase inhibition was calculated according to the following equation, and IC_{50} values were determined by plotting the percentage of inhibition against the sample concentration.

$$\text{Percentage } \alpha - \text{amylase inhibition} = \frac{\delta A_{\text{control}} - \delta A_{\text{sample}}}{\delta A_{\text{control}}} \times 100, \quad (2)$$

where $\delta A_{\text{control}} = \text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{control blank}}$ and $\delta A_{\text{sample}} = \text{Absorbance}_{\text{sample}} - \text{Absorbance}_{\text{sample blank}}$

2.8.2. Lipase Inhibition Assay. The assay was performed following the methodology outlined by Ulpathakumbura et al. [10] with slight modifications. A 100-mM phosphate buffer (pH 7.4) was prepared using sodium chloride, potassium chloride, potassium dihydrogen phosphate, disodium hydrogen phosphate, and triton-X-100 as the primary reagents. Additionally, a working solution of p-NPB was made by combining 10 μL of p-NPB with 10 mL of acetonitrile. Crude extract concentrations ranging from 19.53 to 5000 ppm were prepared by dissolving the samples in distilled water. In a 96-well microplate, 100 μL of phosphate buffer (pH 7.4) was mixed with 25 μL of the sample solution, followed by the addition of 50 μL of the enzyme solution. After the incubation of 15 min, at 37°C, 25 μL of p-NPB working solution was added, and the mixture was incubated for another 30 min at 37°C. Absorbance was recorded at 400 nm. Orlistat (Orslim tablet) was served as the positive control. The percentage of lipase inhibition was determined according to the equation, and IC_{50} values were determined by plotting of inhibition percentage against sample concentration.

$$\text{Percentage lipase inhibition} = \frac{\delta A_{\text{control}} - \delta A_{\text{sample}}}{\delta A_{\text{control}}} \times 100, \quad (3)$$

where $\delta A_{\text{control}} = \text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{control blank}}$ and $\delta A_{\text{sample}} = \text{Absorbance}_{\text{sample}} - \text{Absorbance}_{\text{sample blank}}$.

2.9. Phytochemical Profiling by LC-MS. The phytochemical profiling was performed using the LC-MS analysis, as outlined by Ulpathakumbura et al. [10] with minor modifications. For the extraction, 50 mg of each *T. catappa* powder sample was treated with 2.5 mL of 70% aqueous methanol (HPLC grade methanol, > 99.99% in ultrapure water) and subjected to ultrasonication for 15 min. The resulting extract was filtered using a syringe filter (25 mm, 0.45 μm) before proceeding to the LC-MS analysis. The system used in the LC-MS analysis was an ultrahigh pressure liquid chromatograph (UPLC) (UltiMate 3000, Thermo Scientific, Germany) equipped with components, including autosampler (ACC-3000), quaternary pump (LPG-3400SD), and diode array detector (DAD-3000) capable of detecting signals at wavelengths of 224 nm, 254 nm, 280 nm, and 360 nm. Fragment ion analysis m/z was conducted using an iron trap mass spectrometer (LCQ FLEET, Thermo Scientific, USA) with an electrospray ionization (ESI) source in full scan and auto MS^n mode. An Ascentis RP-Amide column (5 μm, 15 cm × 4.6 mm, Supelco Analytical, Merck, Germany) was filled with a 10-μL aliquot of the sample. MeOH (A) and acidic water containing 0.01% formic acid (B) were combined to generate the mobile phase, which flowed at a rate of 0.400 mL/min under gradient elution. The gradient program was as follows: 90% of Solvent B from 0 to 5 min, 90%–2% of Solvent B from 5 to 65 min, 2% of Solvent B from 65 to 70 min, 2%–90% of Solvent B from 70 to 75 min, 90% of Solvent B from 75 to 80 min. The mass spectra were acquired in negative ion mode under the following operating conditions: sheath N_2 gas flow rate of 36 arbitrary units; aux N_2 gas flow rate of nine arbitrary units; heat temperature, 350°C; spray voltage, 4.50 kV; capillary voltage, −40.00 V; capillary temperature, 320°C; and tube lens, −95.00 V. Mass chromatograms were recorded in the range of 110–1500 m/*z*. To calibrate the system for qualitative and quantitative analysis, nine phenolic compounds were employed as authentic standards.

2.10. Statistical Analysis. Data from triplicate analyses ($n = 3$) were presented as mean ± standard deviation (SD). Statistical evaluation was conducted using the Minitab 17 software package and one-way ANOVA. When significant differences were observed, Tukey's test was applied at a 5% significance level to determine mean differences. Pearson's linear correlation was used to assess the relationship between bioactivities at a 5% level.

3. Results

Table 1 presents the TPC and TFC of n-hexane, DCM, and methanol (MeOH) extracts from the defatted residues of *T. catappa* cultivars. The results of the antioxidant activities, including DPPH, FRAP, and ABTS assays, are summarized in Table 2, while Table 3 highlights their enzyme inhibitory activities. Table 4 details the distribution of selected phenolic constituents in MeOH extracts of the whole kernel and defatted residues of *T. catappa*. Finally, Table 5 provides Pearson's linear correlation coefficients (r) among TPC, TFC, DPPH, FRAP, ABTS, α-amylase inhibition, and lipase inhibition of the MeOH extract.

4. Discussion

4.1. TPC of Defatted Residues of T. catappa Kernels. Phenolic compounds, synthesized through secondary metabolic pathways of plants [11], were recognized as crucial agents in adaptive protection. According to the findings in Table 1, the highest TPC was exhibited by MeOH extracts, while the lowest TPC was shown by the DCM extracts, and no TPC was detected in the n-hexane extracts of either cultivar. Between the MeOH extracts, the yellow cultivar showed the highest TPC content. Likewise, the highest TPC content between the DCM extracts was shown by the yellow cultivar. Nevertheless, there was no remarkable difference between the DCM extracts of cultivars, while a remarkable difference ($p < 0.05$) was detected between the MeOH extracts of *T. catappa* cultivars. Although some data on the TPC of *T. catappa* whole kernel were reported [3], literature availability is scarce regarding the intervarietal TPC comparison of their defatted residue. Based on the analysis of the whole kernels, Wijesekera et al. [3] reported that the purple cultivar showed the highest TPC in the MeOH extract. The phenolic content of crude plant extracts varied generally depending on factors such as cultivar types and solvent choices for extraction. MeOH being a polar solvent was favored for its efficiency in extracting phenolic compounds and yielded higher TPC in extracts of *T. catappa* due to its ability to effectively extract polar phenolic compounds. n-hexane and DCM extracts were found to show lower TPC because n-hexane being a nonpolar solvent was not efficient at extracting polar phenolic compounds, while DCM, a mid-polar solvent, was less effective than highly polar solvents such as MeOH in extracting more polar phenolic compounds in plants. These findings emphasized the importance of solvent selection in ensuring accurate phenolic content from plant sources.

4.2. TFC of Defatted Residues of T. catappa Kernels. Flavonoids, a group of phenolic compounds with low molecular weight, are abundant and widely distributed among plants. TFC values obtained for different crude extracts of *T. catappa* are displayed in Table 1. The data showed that the n-hexane extracts had the greatest TFC values, followed by the DCM extracts, with the lowest values being shown by the MeOH extracts. Among these extracts, a higher TFC content was exhibited by the yellow cultivar compared to the purple cultivar. Statistical analysis showed a remarkable ($p < 0.05$) difference among these extracts of *T. catappa* cultivars. To compare the findings of this study, the availability of the literature data is scarce on the TFC of defatted residues obtained from purple and yellow cultivars. Notably, TFC values were detected not only in MeOH extracts but also in nonpolar (n-hexane) and mid-polar (DCM) extracts. Hence, it was suggested that nonpolar and mid-polar flavonoids might have been present in *T. catappa,* with higher levels being in the yellow cultivar compared to the purple cultivar. This observation is aligned with the solvent preferences of flavonoids as described earlier by Pinho and Ferreira [12], where polar flavonoids favor aqueous solutions and pure alcohols, while isoflavones and flavanones are generally soluble in nonpolar solvents, but not all flavones and flavonols show this behavior due to

Table 1: Total phenolic and flavonoid contents of n-hexane, dichloromethane (DCM), and methanol (MeOH) extracts obtained from defatted residues of *Terminalia catappa* cultivars.

Assay	Cultivar	Type of extract		
		n-hexane	DCM	MeOH
Total phenolic content (mg gallic acid equivalent (GAE)/g of crude extract)	Purple	ND	$2.04^{a} \pm 0.10$	$2.17^{a} \pm 0.18$
	Yellow	ND	$2.31^{a} \pm 0.00$	$9.52^{b} \pm 0.18$
Total flavonoid content (mg catechin equivalent (CE)/g of crude extract)	Purple	$12.65^{a} \pm 0.04$	$10.02^{a} \pm 0.06$	$3.29^{a} \pm 0.08$
	Yellow	$49.06^{b} \pm 2.64$	$26.61^{b} \pm 1.94$	$12.02^{b} \pm 0.70$

Note: Each value in the table represents the mean of three replicates. Means within each column bearing different superscripts are significantly ($p < 0.05$) different.
Abbreviation: ND, not detected.

Table 2: Antioxidant activities of n-hexane, DCM, and MeOH extracts obtained from defatted residues of *Terminalia catappa* cultivars.

Assay	Cultivar	Type of extract		
		n-hexane	DCM	MeOH
DPPH radical scavenging activity (IC_{50} value/ppm)	Purple	> 2000	1422.64 ± 79.59	$178.20^{a} \pm 3.25$
	Yellow	> 2000	> 2000	$226.01^{b} \pm 4.10$
IC_{50} value of ascorbic acid (ppm) 2.11 ± 0.02				
Ferric reducing antioxidant power (mmol $FeSO_4$/g of crude extract)	Purple	ND	ND	$0.40^{b} \pm 0.02$
	Yellow	ND	ND	$0.34^{a} \pm 0.01$
$ABTS^{+}$ radical scavenging activity (mmole of trolox/g of crude extract)	Purple	ND	ND	$38.50^{a} \pm 0.00$
	Yellow	ND	ND	$91.66^{b} \pm 0.00$

Note: Each value in the table represents the mean of three replicates. Means within each column bearing different superscripts are significantly ($p < 0.05$) different.
Abbreviations: IC_{50}, half maximal inhibitory concentration; ND, not detected.

Table 3: Enzyme inhibitory activities of different solvent extracts obtained from defatted residues of *Terminalia catappa* cultivars.

Assay	Cultivar	Type of extract		
		n-hexane	DCM	MeOH
α-Amylase inhibitory activity (IC_{50} value/ppm)	Purple	> 6000	$3404.82^{a} \pm 5.63$	$3294.99^{b} \pm 17.13$
	Yellow	ND	$4545.97^{b} \pm 66.78$	$960.28^{a} \pm 47.81$
IC_{50} value of acarbose (ppm) 2.07 ± 0.11				
Lipase inhibitory activity (IC_{50} value/ppm)	Purple	> 1000	> 1000	$444.42^{b} \pm 25.70$
	Yellow	> 1000	> 1000	$249.56^{a} \pm 1.30$
IC_{50} value of Orlistat (ppm) 27.06 ± 0.70				

Note: Each value in the table represents the mean of three replicates. Means within each column bearing different superscripts are significantly ($p < 0.05$) different.
Abbreviations: IC_{50}, half maximal inhibitory concentration; ND, not detected.

structural differences. This distinction in solvent affinity has been a reflection of the diverse chemical nature of flavonoids.

4.3. Evaluation of Antioxidant Properties

4.3.1. DPPH Radical Scavenging Assay. The DPPH radical scavenging activity is employed widely to assess the antioxidant potential of plant substances in vitro. The percentage radical scavenging activities and the IC_{50} values for the two cultivars of *T. catappa* are given in Table 2. According to Marques et al. [13], the color changes observed in the DPPH radical were closely tied to the quantity of total extractable phenols. Consequently, a lower IC_{50} value was interpreted as an indication of higher levels of antioxidant activity in the extract. MeOH extracts had the most effective and highest radical scavenging activity for both cultivars of *T. catappa*. More specifically, the MeOH extract of *T. catappa* purple exhibited an IC_{50} value of 178.20 ± 3.25 ppm, while *T. catappa* yellow showed a slightly higher IC_{50} value of 226.01 ± 4.10 ppm. This difference suggested that *T. catappa* purple possessed greater radical scavenging activity when compared to *T. catappa* yellow in MeOH extracts.

In DCM extracts, *T. catappa* purple demonstrated a notably lower IC_{50} value of 1422.64 ± 79.59 ppm compared to *T. catappa* yellow, where the value exceeded 2000 ppm. In fact, both cultivars were found to exhibit weak antioxidant activity in n-hexane extracts, as indicated by IC_{50} values exceeding 2000 ppm. This weak activity was related to the presence of nonphenolic antioxidants in the nonpolar solvent fractions as suggested by Ojha et al. [14]. These results are favorably aligned with the findings of Wijesekera et al.

Table 4: Distribution of selected phenolic compounds present in MeOH extracts from whole and defatted residues of *Terminalia catappa* kernels.

Phenolic compound	Content (mg/100 g of defatted residues)			
	DP	DY	WP	WY
Gallic acid	$2.95^{c} \pm 0.19$	$3.46^{d} \pm 0.28$	$2.87^{b} \pm 0.11$	$2.04^{a} \pm 0.19$
Rutin acid	$2.57^{d} \pm 0.23$	$0.18^{c} \pm 0.01$	$0.12^{a} \pm 0.01$	$0.16^{b} \pm 0.01$
Vanillin acid	$2.35^{c} \pm 0.20$	$0.02^{b} \pm 0.00$	$0.01^{a} \pm 0.00$	$0.01^{a} \pm 0.00$
Ferulic acid	$0.94^{c} \pm 0.08$	$0.68^{b} \pm 0.05$	$0.03^{a} \pm 0.00$	ND
Catechin acid	$0.43^{d} \pm 0.02$	$0.11^{c} \pm 0.00$	$0.01^{a} \pm 0.00$	$0.04^{b} \pm 0.00$
Sinapic acid	$0.18^{c} \pm 0.01$	$0.09^{b} \pm 0.00$	$0.05^{a} \pm 0.00$	$0.09^{b} \pm 0.00$
Chlorogenic acid	$0.01^{a} \pm 0.00$	$3.01^{d} \pm 0.23$	$0.22^{c} \pm 0.01$	$0.03^{b} \pm 0.00$
p-coumaric acid	$0.01^{a} \pm 0.00$	$0.01^{a} \pm 0.00$	$0.93^{b} \pm 0.08$	$0.98^{c} \pm 0.07$
Caffeic acid	$0.01^{a} \pm 0.00$	$0.02^{b} \pm 0.00$	$0.01^{a} \pm 0.00$	$0.01^{a} \pm 0.00$

Note: Each value in the table represents the mean ± SD of three replicates. The means that do not share a similar superscription letter within the rows are significantly different at 95% confidence ($\alpha = 0.05$).
Abbreviations: DP, defatted purple; DY, defatted yellow; ND, not detected; WP, whole purple; WY, whole yellow.

Table 5: Pearson's linear correlation coefficients (r) within TPC, TFC, DPPH, FRAP, ABTS, inhibition of α-amylase, and inhibition of lipase of MeOH extract.

	TPC	TFC	IC_{50} of DPPH	FRAP value	$ABTS^{+}$	IC_{50} of α-amylase	IC_{50} of lipase
TPC	—	—	—	—	—	—	—
TFC	0.994	—	—	—	—	—	—
IC_{50} of DPPH	0.997	0.999	—	—	—	—	—
FRAP	−0.971	−0.991	−0.982	—	—	—	—
ABTS	0.999	0.990	0.994	−0.963	—	—	—
IC_{50} of α-amylase	−0.994	−0.977	−0.986	0.940*	−0.997	—	—
IC_{50} of lipase	−0.999	−0.987	−0.993	0.958	−1.000	0.998	—

Note: DPPH, DPPH radical scavenging activity; ABTS, $ABTS^{+}$ radical scavenging activity.
Abbreviations: FRAP, ferric reducing antioxidant power; TFC, total flavonoid content; TPC, total phenolic content.
*No significant correlation ($p > 0.05$).

[3], who also observed variations in antioxidant activity based on extracting solvents and cultivar types. Similar to this study, polar solvents such as methanol proved to be more effective than nonpolar solvents such as n-hexane. While Wijesekera et al. [3] focused on the whole kernel extracts, this study examined the antioxidant potential of the extracts from defatted residues. This was done so because there is hardly any literature data on the antioxidant activity of defatted residues of *T. catappa kernels*.

4.3.2. FRAP Assay. The assay was employed to evaluate the conversion of ferric ions (Fe^{3+}) into ferrous ions (Fe^{2+}) by antioxidants under acidic pH conditions [15]. The mean FRAP values obtained for different crude extracts using different solvents are shown in Table 2. FRAP values were not detected for both n-hexane and DCM extracts of *T. catappa*. Nevertheless, the MeOH extracts of *T. catappa* purple exhibited a FRAP value of 0.40 ± 0.02 mmol $FeSO_4$/g of extract, while *T. catappa* yellow showed a slightly lower FRAP value (0.34 ± 0.01 mmol $FeSO_4$/g of extract). The FRAP value of *T. catappa* purple was found to be remarkably higher ($p < 0.05$) than *T. catappa* yellow, indicating a variation in FRAP between the two cultivars. When compared to the positive control (ascorbic acid, 41.23 ± 0 mmol of $FeSO_4$/g of extract), the reducing power of the MeOH extract of the individual *T. catappa* cultivar was remarkably ($p < 0.05$) lower. The findings of this research indicated that the antioxidant capacity, as reflected by FRAP values, varies with cultivar type and extracting solvent.

4.3.3. $ABTS^{+}$ Radical Scavenging Activity. The antioxidant activities of the defatted residues from the kernels of *T. catappa* cultivars were also evaluated by measuring their $ABTS^{+}$ radical scavenging ability using a trolox equivalent antioxidant capacity (TEAC) assay, as outlined by Annegowda et al. [16]. According to Table 2, radical scavenging activities were not detected in both n-hexane and DCM extracts of the defatted residues of *T. catappa*. Nevertheless, the MeOH extracts of *T. catappa* purple exhibited a radical scavenging activity of 38.50 ± 0.00 mmol of trolox/g of crude extract, while *T. catappa* yellow showed a slightly higher radical scavenging activity (91.66 ± 0.00 mmol of trolox/g of crude extract). Based on the statistical analysis, the radical

scavenging activity of *T. catappa* yellow was remarkably higher ($p < 0.05$) than *T. catappa* purple, indicating a variation in radical scavenging activity between them. When compared to the positive control (ascorbic acid, 4.12 ± 0.01 mM of trolox/g), the radical scavenging activity of the MeOH extract of each cultivar was remarkably ($p < 0.05$) lower. For comparison purpose, there was hardly any literature data on the radical scavenging activity of defatted residues from *T. catappa* cultivars.

4.4. Enzyme Inhibitory Activity

4.4.1. α-Amylase Inhibitory Activity. One of the primary enzymes in the human body responsible for breaking down starch into simpler sugars is α-amylase. The process of carbohydrate digestion can be slowed down by inhibiting this enzyme, leading to a reduction in the rate at which glucose is absorbed. As a result, the postmeal spike in blood glucose levels can be minimized. This mechanism suggested that α-amylase inhibitors might offer potential benefits to diabetic patients in improving glucose control [17]. According to the results presented in Table 3, only MeOH extracts exhibited potent inhibitory effects against α-amylase. Aside from this, weaker activities were observed for DCM extracts of *T. catappa* cultivars. The n-hexane extract of *T. catappa* yellow did not demonstrate any inhibitory activity, whereas *T. catappa* purple exhibited $46.62 \pm 0.40\%$ of inhibition at the concentration of 6000 ppm, suggesting a probable IC_{50} value higher than 6000 ppm for this extract. In MeOH extracts, higher inhibitory activity was observed for *T. catappa* yellow cultivar (960.28 ± 47.81 ppm) when compared to *T. catappa* purple cultivar (3294.99 ± 17.13 ppm). There was a remarkable ($p < 0.05$) difference between them. Similarly, among the DCM extracts, *T. catappa* purple exhibited stronger inhibition ($IC_{50} = 3404.82 \pm 5.63$ ppm) when compared to *T. catappa* yellow ($IC_{50} = 4545.97 \pm 66.78$ ppm), with a remarkable difference ($p < 0.05$) between them. The earlier findings of Wijesekera et al. [3] was also aligned with the observation that the polar solvents such as MeOH are more effective in extracting compounds that are potent to inhibit α-amylase. Similar to the present study, the previous study also reported higher inhibitory activity for the yellow cultivar when compared to the purple cultivar, emphasizing the influence of the cultivar differences on enzyme inhibition. The positive control, acarbose demonstrated the highest α-amylase inhibitory activity among all crude extracts with an IC_{50} value of 2.07 ± 0.11 ppm.

4.4.2. Lipase Inhibitory Activity. The lipase inhibitory activities of *T. catappa* crude extracts are presented as IC_{50} values in Table 3. Significant lipase inhibitory activities were observed only in the MeOH extracts of both cultivars, while weak activities were observed for n-hexane and DCM extracts of both cultivars. Particularly, MeOH extract of *T. catappa* purple had an IC_{50} value of 444.42 ± 25.70 ppm, while *T. catappa* yellow had a slightly lower IC_{50} value of 249.56 ± 1.30 ppm. This indicated that the MeOH extract of *T. catappa* yellow cultivar had stronger inhibitory activity than *T. catappa* purple cultivar. For the DCM extracts at 1000 ppm, percentage inhibitions of $34.50 \pm 0.45\%$ and $31.04 \pm 0.54\%$ were exhibited by *T. catappa* yellow and *T. catappa* purple, respectively, suggesting that the IC_{50} values for these extracts might have exceeded 1000 ppm. For n-hexane extracts at 1000 ppm, percentage inhibitions of $23.65 \pm 0.26\%$ and $22.24 \pm 0.42\%$ were shown by *T. catappa* yellow and *T. catappa* purple, respectively, indicating that the IC_{50} values for these extracts could have been higher than 1000 ppm. Thus, among all crude extracts, the MeOH extract of *T. catappa* yellow was found to exhibit the highest antiobesity potential. In this experiment, Orlistat used as the control demonstrated remarkably ($p < 0.05$) higher lipase inhibition with an IC_{50} value of 27.06 ± 0.70 ppm.

4.5. Phenolic Profiles of Whole and Defatted Residues of T. catappa Kernels. The distribution of various phenolic constituents in the methanol extracts of defatted residues and whole kernels of *T. catappa* are shown in Table 4. Except for ferulic acid, all nine phenolic constituents were detected in all sample extracts. Ferulic acid was not detected in the whole kernel of the yellow cultivar most probably due to low abundance. Among the phenolic constituents, gallic acid was found to be most abundant in both defatted residues and whole kernels. The defatted yellow (DY) exhibited the highest gallic acid content (3.46 ± 0.28 mg/100 g), while the whole yellow (WY) had the lowest content (2.04 ± 0.19 mg/100 g). The gallic acid content of the crude extracts increased in the ascending order of WY < WP < DP < DY, with remarkable ($p < 0.05$) differences. The next most abundant phenolic constituent was rutin, with the highest content (2.57 ± 0.23 mg/100 g) found in defatted purple (DP), while the lowest content (0.12 ± 0.01 mg/100 g) found in the whole purple (WP). The proportion of vanillin acid ranged from 2.35 ± 0.20 mg/100 g to 0.01 ± 0.00 mg/100 g, DP showing the highest content (2.35 ± 0.20 mg/100g), while WP and WY showing the lowest content (0.01 ± 0.00 mg/100 g). The amount of vanillin present in DY was 0.02 ± 0.00 (mg/100 g). With regard to the proportion of vanillin, no remarkable difference was observed between WP and WY, but remarkable ($p < 0.05$) differences were detected between other samples. Ferulic acid was not detected in WY, but it was present in the other samples (ranging from 0.94 ± 0.08 mg/100 g to 0.03 ± 0.00 mg/100 g). In fact, remarkable ($p < 0.05$) differences were observed among them. Regarding the catechin content, the DP had the highest amount (0.43 ± 0.02 mg/100 g), while WP had the lowest (0.01 ± 0.00 mg/100 g). Catechin content also showed remarkable ($p < 0.05$) differences among all samples. According to Table 04, the values were found to align in the order of DP > DY > WY > WP. When coming to sinapic acid, DP had the highest content (0.18 ± 0.01 mg/100 g), followed by DY and WY (0.09 ± 0.00 mg/100 g). It should be noted that WP contained the lowest content of 0.05 ± 0.00 mg/100 g. With regards to sinapic acid content, remarkable differences ($p > 0.05$) were not noticed between DY and WY, but remarkable ($p < 0.05$) differences noted between other samples. The highest chlorogenic content was

detected for DY (3.01 ± 0.23 mg/100 g), while the lowest content was displayed by DP (0.01 ± 0.00 mg/100 g). Chlorogenic acid content differed remarkably ($p < 0.05$) among the samples, increasing in the order of DP < WY < WP < DY. With regard to p-coumaric acid, both DP and DY recorded equal amounts of 0.01 ± 0.00 mg/100 g, whereas WP and WY presented higher contents at 0.93 ± 0.08 mg/100 g and 0.98 ± 0.07 mg/100 g, respectively. Regarding caffeic acid, the highest content was observed in DY (0.02 ± 0.00 mg/100 g), the caffeic acid amounts found in DP, WP, and WY were in equal amounts (0.01 ± 0.00 mg/100 g). There were no remarkable changes in caffeic acid content between DP, WP, and WY.

4.6. Analysis of Correlation Between Bioactivities. Pearson's correlation coefficients (r) for different bioactivities of the MeOH extracts of defatted residues are presented in Table 5. According to the results, the biological activities of the MeOH extracts exhibited correlations with both TPC and TFC of MeOH extracts. Particularly, TPC and TFC of MeOH extracts demonstrated strong positive correlations with IC_{50} values of DPPH and $ABTS^+$ radical scavenging while exhibiting strong negative correlations with the FRAP value as well as IC_{50} values of α-amylase and lipase inhibition. Additionally, the IC_{50} of DPPH showed strong negative correlations with the FRAP value and IC_{50} values of α-amylase and lipase inhibition but high positive correlations with ABTS. Furthermore, the IC_{50} of DPPH had high negative relationships with FRAP values and IC_{50} of α-amylase and lipase inhibitions. There were no remarkable correlations between the FRAP values and the IC_{50} of lipase inhibition. Finally, ABTS had the highest negative relationships with the IC_{50} of α-amylase and lipase inhibition, whereas the IC_{50} of α-amylase had the highest positive relationships with the IC_{50} of lipase.

5. Conclusion

This study highlighted the bioactive potential of defatted residues from purple and yellow cultivars of *T. catappa* seed kernels with regard to their antioxidant, antihyperglycemic, and antiobesity properties. The bioactivities of crude extracts from both cultivars varied based on the solvent polarity differences. Among the three solvent extracts, MeOH extracts showed the highest TPC content for both cultivars, with the yellow cultivar containing a significantly higher amount. Meanwhile, the n-hexane extract of *T. catappa* yellow cultivar showed the highest TFC value. Based on these findings, the defatted residues of both cultivars could be rich sources of flavonoid and phenolic compounds. In terms of antioxidant activity, the MeOH extracts of the yellow cultivar demonstrated the highest DPPH and $ABTS^+$ radical scavenging activity, while the MeOH extracts of the purple cultivar exhibited the greatest FRAP value. The MeOH extract of the yellow cultivar showed the highest antihyperglycemic potential through α-amylase inhibition and exhibited the greatest antilipase activity, suggesting notable antiobesity potential in the residues. Gallic acid was identified as the most dominant phenolic compound, with higher concentrations occurring in the defatted residues than the whole seed extracts. Based on the overall observation, the yellow cultivar consistently outperformed the purple cultivar in all bioactivities, highlighting its potential uses in nutraceutical and functional food applications.

Data Availability Statement

The data supporting this study's findings are available from the corresponding author upon request.

Conflicts of Interest

The authors declare no conflicts of interest.

Author Contributions

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Funding

This study was partly funded by the National Institute of Fundamental Studies, Hanthana Road, Kandy, Sri Lanka. The authors extend their sincere appreciation to the Researchers Supporting Project number: RSPD2024R729 and King Saud University, Riyadh, Saudi Arabia.

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