

## Nutritional Composition, Anti-oxidative and Anti-hyperglycemic Potential of the Kernels of Two Varieties of *Terminalia catappa* L.

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### ABSTRACT

**Purpose:** An attempt was made in this study to compare the differences in nutritional composition, antioxidant capacity, and anti-hyperglycemic effect of the kernel of purple and yellow varieties of *Terminalia catappa* L (*T. catappa* L).

**Research Method:** Sampling of ripe fallen seeds of yellow and purple varieties of *T. catappa* L. was done in the Central Province of Sri Lanka from October to December 2022. Moisture, crude fat, ash, and crude protein contents of seed kernels of the two cultivars were determined using AOAC methods. The differences in fatty acid composition and micro-mineral distributions were also determined using the relevant methods. Sequential extraction of the powdered samples of individual cultivars was performed with hexane, ethyl acetate, and methanol as solvents. The total phenolic content, DPPH radical scavenging activity, alpha-amylase, and alpha-glucosidase inhibitory activities were determined in vitro using relevant assays.

**Findings:** Regardless of the cultivar, the main constituents of the seed kernels were fat ( $63.30 \pm 4.16\%$ ) followed by protein ( $25.58 \pm 0.51\%$ ). Oleic, linoleic, and palmitic were detected as the major fatty acids of the oil fraction. Among the solvent extracts, the methanol extract of the purple variety had the highest total phenolic content ( $17.77 \pm 0.15$  mg of GAE/g). Interestingly, ethyl acetate extracts of both cultivars showed significantly ( $p < 0.05$ ) higher inhibition against alpha-glucosidase ( $IC_{50}$ :  $104.13a \pm 1.22$  mg/dm<sup>3</sup>) while the methanol extract of the yellow variety showed the strongest alpha-amylase inhibitory activity ( $IC_{50}$ :  $2137.95 \pm 118.54$  mg/dm<sup>3</sup>).

**Value:** The seed kernel of *T. catappa* L is a rich source of nutrients and can be used as a raw material for the development of foods meant for diabetic patients.

**Keywords:** Antidiabetic, Antioxidant activity, *Terminalia catappa* L seed, Nutritional kernel.

### INTRODUCTION

*Terminalia catappa* L., also known as ‘kottamba’, is one of the tree species belonging to the family, Combretaceae. Being a large woody tree, it is generally grown as an ornamental plant in home gardening. The botanical attributes of *T. catappa* in its leaf, fruits, and seeds are described in several previous communications. Since time immemorial, *T. catappa* has been recognized as an important plant in the traditional and alternative medical scriptures. The scientific findings about the bioactivities and medicinal properties of the fruits, leaves, roots, and stem bark of

this tree have been well-documented through several previous studies (Khan *et al.* 2014; Yeh *et al.*, 2012; Kinoshita *et al.*, 2007; Liu *et al.*, 1996; Fan *et al.*, 2004; Lin *et al.*, 1997; Nagappa *et al.*, 2003). Similar to many forest species, *T. catappa* is a perennial crop that exists in different varietal forms.

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The two main varieties bearing yellow and purple fruits are spread across the different agroecological regions of Sri Lanka. Though *T. catappa* is not yet grown on plantation scales, tens of thousands of trees are found along riverbanks and water logging areas of both dry and wet zones. The fruit of *T. catappa* is sessile, laterally compressed, smooth-skinned, and had an ovoid to ovate-shaped drupe (Anand *et al.*, 2015). Several hundred metric tons of fruits of this tree are shed on the ground annually during the major flowering seasons and wasted without any proper use. The kernel of the seeds is made up of two delicate cotyledons, which are strongly intertwined and protected by a seed coat which is usually pale brown in color. They are usually similar in shape and size to those of almond seeds in the commercial world. Although these seeds have edible kernels that are underutilized, investigations related to their potential use as a source of nutrients are useful, especially during times of food insecurity and economic hardships. Despite several studies carried out in other countries, a comparison of the varietal differences of *T. catappa* of Sri Lankan varieties on the nutritional composition of the kernel is essential probably due to influences by soil type, climate, and environmental factors. Apart from this, investigation on the anti-hyperglycemic potential of *T. catappa* in vitro is rare along with other bioactivities to assess its suitability as a dietary supplement for diabetic patients. The findings of this study would provide background information required for its economic exploitation in the future.

## MATERIALS AND METHODS

### Materials

Sampling of ripe fallen seeds of yellow and purple varieties of *T. catappa* was done in the Central Province of Sri Lanka from October to December 2022. Initially, seeds were dried at 55 °C for 8 h in a blower-assisted drying oven (Biobase, BOV-V230F, China). The dried seeds were then split and opened to recover the kernels, which were ground into powder form by using an electric grinder. The powdered kernel samples were then kept under refrigerated conditions until further analysis. The chemicals and reagents of analytical grade were employed in the assays unless otherwise specified.

### Proximate Composition Analysis

Determinations of the nutritional parameters related to proximate composition were performed in accordance with the procedures described in the AOAC International (2019) manual. After determining moisture

content (AOAC Official Method 934.06), oil content (AOAC Official Method 948.22 method), ash content (AOAC Official Method 942.05), and crude protein content (Kjeldahl method AOAC Official Method 970.02), the total carbohydrate content was estimated according to the following equation:

$$\text{Total carbohydrate content (\%)} = 100 - (\text{Moisture\%} + \text{ash\%} + \text{protein\%} + \text{fat\%}) \quad (1)$$

### Mineral Analysis

Macro-and-micro-mineral distribution was analyzed by following the procedure previously used by Marasinghe *et al.* (2019). Digestion of kernel powder samples was carried out in a microwave digester (CEM MARS 6, USA) with the addition of 3 mL of 65% nitric acid to 0.25 g of flour. The digested mixture was filtered into a 100 mL volumetric flask and diluted with distilled water to fill up to the mark. The resulting solution was used for the analysis of macro-and-micro minerals using an ICP spectrophotometer (Thermo Scientific, iCAP 7000 series, USA).

### Fatty Acid Analysis

The fatty acid distribution of the oil component of the seed kernel was determined by following the procedure previously used by Marasinghe *et al.* (2019). Before analysis, each individual oil sample was transformed into fatty acid methyl esters (FAME) as per the method described by Gunarathne *et al.* (2022a). The system used to inject the FAME sample was a gas-liquid chromatograph (GC-2010, Shimadzu Corporation, Japan) fitted with a polar capillary column RTX-5 (0.32 mm internal diameter, 30 m length, and 0.25 μm film thickness; Restex Corp., Bellefonte, PA) and a flame ionization detector (FID). The oven temperature was programmed as follows: the initial temperature was 130 °C (held for 1 min), then increased from 130 to 170 °C at a rate of 6.5 °C/min, followed by a rise from 170 to 215 °C at 2.75 °C/min, and maintained at 215 °C for 12 min. Then, the temperature was increased from 215 to 230 °C at 4 °C/min and held for 3 min. The injector and detector temperatures were maintained at 270 °C and 280 °C, respectively. Hydrogen was used as the carrier gas at a constant pressure mode of 43 cm/s. The split ratio of the injector was set to 50:1. Retention times of each peak were compared with those of standard fatty acid methyl esters to identify individual fatty acids. The percentage of each fatty acid was calculated by dividing the individual fatty acid peak area by the total peak area for all fatty acids.

### Crude Kernel Extract Preparation

Powdered samples of the kernels (200 g) of each variety were extracted with hexane, ethyl acetate (EtOAc), and methanol (MeOH) in a sequential manner using an ultra-sonication bath (Rocker ultrasonic cleaner, model Soner 206H), in accordance with the procedure previously described by Gunarathne *et al.* (2022b). The crude extracts were stored at -18 °C until further analysis.

### Determination of Total Phenolic Content (TPC)

TPC was assayed by following the Folin-Ciocalteu (FC) method previously elaborated by Adekola *et al.* (2017) with slight modifications. TPC of the crude extracts was expressed as mg gallic acid equivalent (GAE) per g of crude extract.

### Anti-oxidative Property Analysis

**DPPH radical scavenging activity:** The assay was performed according to the procedure previously used by Adekola *et al.* (2017). A concentration series of each individual crude extract was prepared by reconstituting the crude extract in methanol (MeOH). Then, 150  $\mu$ L aliquots of each concentration solution were added to 60  $\mu$ L of 0.3 mM DPPH solution in a 96-well microplate, and allowed to stand for 30 minutes in the dark at room temperature. The absorbance values were recorded against the control at 517 nm. Ascorbic acid was employed as the positive control. The percent inhibition (%) was calculated using the following equation, and IC<sub>50</sub> values were obtained from the graphical plot:

$$\text{RSA (\%)} = \left[ \frac{\delta A_{\text{control}} - \delta A_{\text{sample}}}{\delta A_{\text{control}}} \right] \times 100$$

Where:

$$\delta A_{\text{control}} = \text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{control blank}},$$

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

RSA (%), Percentage of radical scavenging activity.

**Anti-hyperglycemic effect analysis:** Alpha amylase inhibition: Alpha-amylase inhibitory activity of the crude extracts of *T. catappa* seed kernel was done by following the procedure used by Gunarathne *et al.* (2022b) with minor adjustments. Crude extract in a concentration series was prepared in deionized water. In this experiment, acarbose was used as the positive control. After plotting the Alpha-amylase (%) inhibition against sample concentration, the IC<sub>50</sub> values were obtained graphically.

$$\text{Alpha-amylase (\%)} = \left[ \frac{\delta A_{\text{control}} - \delta A_{\text{sample}}}{\delta A_{\text{control}}} \right] \times 100$$

Where:

$$\delta A_{\text{control}} = \text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{control blank}},$$

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

**Alpha-glucosidase inhibition:** Alpha-glucosidase inhibitory activity of crude extracts of the seed kernel was carried out by following a procedure used by Gunarathne *et al.* (2022b) with minor adjustments. The assay was performed in a 96-well microplate. Crude extract in a concentration series was prepared in deionized water. Acarbose was used as the positive control in this experiment. Calculation of the % inhibition of Alpha-glucosidase was based on the following equation. After plotting % inhibition vs. the sample concentration, the IC<sub>50</sub> value was calculated graphically.

$$\text{Alpha-glucosidase (\%)} = \left[ \frac{\delta A_{\text{control}} - \delta A_{\text{sample}}}{\delta A_{\text{control}}} \right] \times 100$$

Where:

$$\delta A_{\text{control}} = \text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{control blank}},$$

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

### Statistical Analysis

In all experiments, measurements were taken in triplicate (n=3) and the data were presented as the mean value  $\pm$  standard deviation. One-way analysis of variance (ANOVA) was performed on all data using Tukey's test of the MINITAB (version 14) statistical package. When the F values were significant, mean differences were compared using Tukey's test at a 5% significance level.

## RESULTS AND DISCUSSION

### Proximate Composition

A comparison of the proximate compositions of *T. catappa* kernels of yellow and purple varieties is presented in Table 01. As the kernel of *T. catappa* is eaten in raw and sun-dried or roasted forms, the proximate compositional comparison would provide valuable information for its future applications (Agatemor and Mark, 2006). The sun-dried seed kernels particularly bear good potential for marketing as a dietary supplement in the health sector. The overall proximate data suggest that all components except protein content were significantly ( $p < 0.05$ ) different. Edible seed kernels with high protein content are highly regarded as supplements for those suffering from diabetes. Based on a previous research study in Ghana, hardly any significant ( $p > 0.05$ ) difference

in individual parameters of proximate composition was noticed between the two varieties of *T. catappa* (Oduro *et al.*, 2009). Among the macro-nutrients of the kernels, the fat component was the most dominant; its value for the yellow variety ( $66.25 \pm 0.28$  %) was significantly ( $p < 0.05$ ) higher than that of the purple variety ( $60.36 \pm 0.77$  %). A previous study conducted in Ghana showed that the fat contents of the kernel on a wet basis of the yellow and purple cultivars were 35.69 % and 36.9 %, respectively (Oduro *et al.*, 2009). According to another study from Benin, the fat content of the kernels of *T. catappa* was 61.76 % on a dry weight basis (Ladele *et al.*, 2016), which is roughly similar to the fat content found in our study. In another study by Santos *et al.* (2022), the lipid yields of purple and yellow varieties of *T. catappa* were found as 57 % and 54 %, respectively. However, hardly any literature data is available on the inter-varietal differences in the fat contents of Sri Lankan varieties of *T. catappa*. The fat content reported in the present study was higher when compared to those of other edible nuts such as cashews (48.27 %) (Rico *et al.*, 2016) and pistachios (45.4 %) (Bulló *et al.*, 2015).

Protein was the second largest component of the kernels of *T. catappa* used in this study (Table 01). The protein contents of the purple and yellow varieties were  $25.95 \pm 0.09$  % and  $25.22 \pm 0.92$  %, respectively, but the difference noticed between these two values was insignificant ( $p > 0.05$ ). According to Oduro *et al.* (2009), the protein contents of *T. catappa* kernels of yellow and purple varieties were 23.05 % and 22.19 %, respectively. Similar to the results obtained in the present study, the difference between the protein contents of yellow and red varieties of *T. catappa* was insignificant ( $p > 0.05$ ). Ladele *et al.* (2016) previously found that the protein content of the *T. catappa* kernels of Benin was  $20.14 \pm 0.95$  % on a dry basis. A separate study by Akpakpan and Akpabio (2012) also showed that the protein content of the wet seed kernels of *T. catappa* from Nigeria was about 33.69 %. Based on the findings of the present study, the protein content of *T. catappa* was slightly higher than that of the other edible seed nuts such as cashews (21.3 %) (Rico *et al.*, 2016) and pistachios (20%) (Bulló *et al.*, 2015). This is a salient feature of *T. catappa* when considering protein malnutrition prevalent among children living in the developing world.

The moisture content of edible seeds would play a crucial role in their shelf life stability during storage. Although the low moisture content of the kernels not only improves the texture and eating quality, it might also have an impact on storage stability. In this study, the moisture contents of kernels of purple and yellow

varieties were  $2.44 \pm 0.14$  % and  $2.88 \pm 0.20$  %, respectively (Table 01). The moisture values recorded for the kernels of both varieties are well below the limit to prevent any microbial contamination during storage. Though these values would look roughly similar, the difference between them was significant ( $p < 0.05$ ). According to Oduro *et al.* (2009), the moisture contents of the wet seed kernels of yellow and purple varieties of *T. catappa* were 32.06 % and 31.05 %, respectively. In this case, the values recorded for the moisture content are high enough to induce fungal growth in these seeds during storage.

When considering the ash contents of kernels, micromineral distribution is a vitally important parameter as many of them act as co-factors of metabolic processes. As shown in Table 01, the ash contents of purple and yellow varieties of *T. catappa* kernels were  $4.30 \pm 0.05$  % and  $4.03 \pm 0.28$  %, respectively. Although these values look roughly similar, they are significantly ( $p < 0.05$ ) different from each other. As reported previously by Oduro *et al.* (2009), the ash contents of the yellow and purple varieties of *T. catappa* kernels in Ghana were  $2.68 \pm 0.02$  % and  $2.76 \pm 0.11$  %, respectively. In a separate study from Benin, Ladele *et al.* (2016) reported that the ash content of *T. catappa* kernel was  $3.98 \pm 0.43$  %, which was relatively lower than the values reported in Table 01. The differences noticed in the values of the ash content from different reports were within a narrow range.

The carbohydrate component of kernels generally consists of multiple nutrients including simple sugars, starches, crude fiber, etc. The data from Table 01 show that the total carbohydrate contents of *T. catappa* kernels of purple and yellow varieties were  $6.95 \pm 0.62$  % and  $1.62 \pm 1.26$  %, respectively. The low values of total carbohydrate contents were recorded because the seed kernels of *T. catappa* were found to possess high amounts of fat and proteins. The total carbohydrate content of the purple variety was remarkably ( $p < 0.05$ ) higher than that of the yellow variety. The occurrence of low amounts of total carbohydrates in these kernels is a distinct advantage when considering dietary supplements meant for diabetic patients. Based on the previous findings of Oduro *et al.* (2009), the carbohydrate contents of the yellow and purple varieties of *T. catappa* kernels from Ghana were 4.92 % and 5.24 %, respectively. These results are in agreement with the values reported in Table 01. Moreover, the carbohydrate contents of *T. catappa* found in this study were remarkably lower than those found in some other popular edible nuts such as cashews (20.5 %) (Rico *et al.*, 2016), pistachios

**Table 1: Varietal differences in proximate composition of the kernel of *T. catappa***

Variety	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Total carbohydrate by difference (%)
Purple	2.44a± 0.14	25.95a±0.09	60.36a±0.77	4.30b±0.05	6.95b ± 0.62
Yellow	2.88b± 0.20	25.22a± 0.92	66.25b± 0.28	4.03a ± 0.28	1.62a ± 1.26

Each value in the table represents the mean of three replicates. This means that each row bearing different superscripts is significantly ( $p < 0.05$ ) different.

(27.5 %) (Bulló *et al.*, 2015), etc. According to Gray and Threlkeld (2019), edible seeds which are low in starches, but high in proteins are potentially useful as supplements for those who follow dieting as per medical advice.

**Fatty Acid Distribution**

The fatty acid data of the oil component of the kernels are given in Table 02. Palmitic (16:0), oleic (C18:1), and linoleic (C18:2) acids were the major fatty acids of *T. catappa* kernels. Statistical analysis showed that the differences between the two varieties of different fatty acids were insignificant ( $p>0.05$ ). Based on the classification of fatty acids, the unsaturated fatty acids (USFA) were predominant in the oils of these kernels. This is in agreement with the previous findings reported by Santos *et al.* (2022). The total proportion of USFA in *T. catappa* seed kernels of purple and yellow varieties was 58.76% and 59.17 %, respectively. Only marginal differences were noticed between the two varieties about the proportions of USFA. Oleic (C18:1) and linoleic acids (C18:2) were the most predominant USFA of the two varieties; oleic acid content (C18:1) of purple and yellow varieties were  $29.19 \pm 1.90$  % and  $28.86 \pm 0.71$  %, respectively.

Likewise, the saturated fatty acid contents (SFA) of purple and yellow varieties were  $41.33 \pm 3.59$  % and  $40.83 \pm 0.84$  %, respectively. Palmitic acid (16:0) was the most predominant SFA in *T. catappa* kernel and its proportions in purple and yellow varieties were  $35.72 \pm 3.87$  % and  $35.07 \pm 1.08$  %, respectively. Among the other identified SFA, lauric (C12:0), myristic (C14:0), heptadecenoic (C17:0), and stearic (C18:0) were present in low amounts. Investigations carried out in other parts of the world already indicated that the USFA contents of *T. catappa* kernels were remarkably higher than the SFA contents. For instance, Santos *et al.* (2022) previously found that the USFA contents of the purple and yellow varieties were 62.90 % and 60.8 %, respectively. Generally, the occurrence of high amounts of poly-unsaturated-fatty acids (PUFA) such as  $\alpha$ -Linolenic (C18:3n3) and  $\omega$ -Linolenic (C18:3n6) acids is nutritionally advantageous, but they would promote auto-oxidation in oil seeds during storage or

in industrial frying.

When comparing *T. catappa* kernels with other edible kernels, noteworthy differences existed in the distribution of fatty acids. According to Rico *et al.* (2016), the most dominant SFA in cashew was palmitic acid while the most dominant USFA was oleic acid. Although a high amount of linoleic acid was present in the cashew kernel, its content is relatively lower when compared to the kernels of *T. catappa* of the two varieties (Table 02). According to Shokraii (1977), the proportions of oleic and linoleic acids in pistachio nuts were 49.5 % and 31.8 %, respectively. Nevertheless, the relative proportions of these two fatty acids were higher than those of the same found in *T. catappa* kernels. On the other hand, the palmitic acid content of pistachio nuts was lower (13.4 %) when compared to that of *T. catappa* kernels. Dietary fatty acids generally play a crucial role in the etiology of many diseases as mentioned in several previous scientific studies. For instance, increased intake of PUFAs might reduce the risk factors leading to congenital heart diseases. Further to this, consumption of edible kernels composed of linoleic (C18:2) and oleic acid (C18:1) is encouraged as they are known to help manage chronic ailments common among elderly patients (Gray and Threlkeld, 2019). Research evidence has already indicated that a high intake of SFA is associated with the reduction of insulin sensitivity but the contrary is true for USFA such as linoleic acid (Buist, 2010). A recent systematic review and meta-analysis also highlighted the benefits of oils composed of alpha-linolenic acid on glycemic control among individuals suffering from type 2 diabetes.

**Mineral Distributions**

A comparison of mineral compositions of the *T. catappa* kernels of the two varieties is shown in Table 03. Minerals are one of the most essential nutrient components as they perform a range of functions that include aiding the synthesis of enzymes, hormones, and other substances required for the normal physiological functions of the human body (Dobrowolska-Iwanek *et al.*, 2022). As diet is the main supply line of minerals, investigation of foodstuffs rich in minerals is important.

**Table 2: Varietal differences in fatty acid (FA) compositions of the kernel of *T. catappa***

Component FA	Notation	Purple	Yellow
Lauric acid	C12:0	ND	0.07 ± 0.06
Myristic acid	C14 :0	0.07a ± 0.02	0.14a ± 0.04
Palmitic acid	C16 :0	35.72a ± 3.87	35.07a ± 1.08
Heptadecenoic acid	C17 :0	0.40a ± 0.00	0.49a ± 0.05
Stearic acid	C18 :0	5.14a ± 0.30	5.06a ± 0.19
Oleic acid	C18 :1	29.19a ± 1.90	28.86a ± 0.71
Linoleic acid	C18 :2	28.89a ± 1.64	29.79a ± 1.53
Linolenic acid	C18:3	0.69a ± 0.07	0.53a ± 0.02
Saturated Fatty Acids	∑ SFA	41.33a ± 3.59	40.83a ± 0.84
Unsaturated Fatty acids	∑ USFA	58.76a ± 3.47	59.17a ± 0.84

Each value in the table represents the mean of three replicates. Means within each row bearing different superscripts are significantly ( $p < 0.05$ ) different.

As shown in Table 03, potassium (K), calcium (Ca), and magnesium (Mg) were the most abundant macro-minerals present in both varieties of *T. catappa*. For instance, the K content of purple and yellow varieties was  $7575 \pm 292.82$  mg/kg and  $6861.41 \pm 0.75$  mg/kg, respectively. Likewise, the Ca contents of purple and yellow varieties were  $2294 \pm 98.24$  mg/kg and  $2687.52 \pm 9.50$  mg/kg, respectively. The Mg content of the purple variety ( $2273.92 \pm 91.50$  mg/kg) was significantly ( $p < 0.05$ ) lower than that of the yellow variety ( $2529.37 \pm 5.42$  mg/kg). Ladele *et al.* (2016) previously stated that the K content of the *T. catappa* kernel was 1718.12 mg/100 g (dry basis), which might contribute to more than 36 % of the daily requirement of the K intake. Zn, Cu, Mn, Ba, Sr, B, Co, and Ag were the micro-minerals present in *T. catappa* kernels (Table 03). Among these, Zn was the most abundant trace mineral present in *T. catappa* kernels and the content of Zn in the yellow variety ( $42.57 \pm 0.68$  mg/kg) was significantly ( $p < 0.05$ ) higher than that of the purple variety ( $32.72 \pm 2.84$  mg/kg).

Availability of Zn in the diet is essential as it is the cofactor of many enzymes affecting body growth and food digestion. On the other hand, deficiency of Zn might lead to retardation of sexual immaturity and impaired immune responses. Regarding Cu content, the difference noticed was insignificant ( $p > 0.05$ ) between yellow ( $22.09 \pm 0.22$  mg/kg) and purple ( $22.31 \pm 1.58$  mg/kg) cultivars of *T. catappa*. Deficiency of Cu in the body might cause Menkes disease. Generally, legumes, whole grains, nuts, and seed kernels are known to be rich sources of Cu. The Mn content of *T. catappa* seed kernels in purple and yellow cultivars was 13.91 mg/kg and 20.95 mg/kg, respectively. Notably, the Mn content of the yellow cultivar was remarkably ( $p < 0.05$ ) higher than that of the purple variety. Mn is a cofactor of many enzymes that metabolize carbohydrates, lipids, and amino acids. Seed nuts, whole grains, and leafy

vegetables are said to be rich sources of Mn. According to Table 03, Ba contents of the *T. catappa* seed kernels of purple and yellow varieties were 10.97 mg/kg and 10.28 mg/kg, respectively. As seen from Table 03, the occurrence of heavy metals in the kernels of *T. catappa* is rarely detected.

### TPC and Anti-Oxidative Properties

A comparison of the TPC of the kernel extracts of the two varieties of *T. catappa* is presented in Table 04. Recent reports have highlighted the role of polyphenols in free radical scavenging, which is attributed to their antioxidative capacity (Marques *et al.*, 2012). In a sequential extraction process with three solvents of increasing polarity, the TPC was recorded only for MeOH extracts while it was non-detectable in the extracts of hexane and EtOAc (Table 04). In comparison to non-polar or low-polar solvents like hexane and ethyl acetate, MeOH would have displayed its enhanced power to extract phenolic compounds in the kernels of *T. catappa*. After the removal of the lipid components by hexane and EtOAc, most of the polar phenolic constituents of the kernels would remain in the MeOH extract. Based on the varietal differences, the highest TPC content of MeOH extracts was detected in the purple variety.

The varietal difference in the  $IC_{50}$  values of the antioxidant power of different crude extracts of *T. catappa* is shown in Table 04. As ascorbic acid is one of the potent antioxidants, it was used as the positive control in this study. It displayed strong radical scavenging activity ( $IC_{50}$  value =  $8.52 \pm 0.11$  mg/dm<sup>3</sup>) when compared to the crude extracts of the two seed kernels. Generally, the lower the  $IC_{50}$  value, the greater the antioxidant power of the plant extract. The radical scavenging activity of hexane and MeOH extracts was relatively higher than

**Table 3: Varietal differences in mineral composition of the kernel of *T. catappa***

Minerals	Purple (mg/kg)	Yellow (mg/kg)
Potassium (K)	7575 b $\pm$ 292.82	6861.41 a $\pm$ 0.75
Calcium (Ca)	2294 a $\pm$ 98.24	2687.52 b $\pm$ 9.50
Magnesium (Mg)	2273.92 a $\pm$ 91.50	2529.37 b $\pm$ 5.42
Zinc (Zn)	32.72 a $\pm$ 2.84	42.57 b $\pm$ 0.68
Copper (Cu)	22.31 a $\pm$ 1.58	22.09 a $\pm$ 0.22
Manganese (Mn)	13.91 a $\pm$ 1.38	20.95 b $\pm$ 0.15
Barium (Ba)	10.97 a $\pm$ 1.01	10.28 a $\pm$ 0.14
Strontium (Sr)	4.30 a $\pm$ 0.35	7.26 b $\pm$ 0.13
Boron (B)	2.95 a $\pm$ 0.23	3.72 b $\pm$ 0.11
Cobalt (Co)	1.08 a $\pm$ 0.54	0.34 a $\pm$ 0.04
Silver (Ag)	0.04 b $\pm$ 0.0	0.02 a $\pm$ 0.0

Each value in the table represents the mean of three replicates. Means within each row bearing different superscripts is significantly ( $p < 0.05$ ) different.

**Table 4: Total phenolic contents (TPC) and IC<sub>50</sub> values of DPPH radical scavenging activity of different solvent extracts of *T. catappa* seed**

Variety	TPC (mg of GAE/g of extract)		
	Hexane extract	EtOAc extract	MeOH extract
Purple	ND	ND	17.77b $\pm$ 0.15
Yellow	ND	ND	9.63a $\pm$ 0.15
	IC 50 value/mg/dm <sup>3</sup> of DPPH		
	Hexane extract	EtOAc extract	MeOH extract
Purple	477.08b $\pm$ 29.32	1845.95b $\pm$ 56.96	533.56b $\pm$ 30.16
Yellow	280.79a $\pm$ 10.11	786.62a $\pm$ 73.99	409.84a $\pm$ 9.82

Each value in the table represents the mean of three replicates  $\pm$  standard deviation. Means within each row bearing different superscripts is significantly ( $p < 0.05$ ) different. Abbreviations: ND, not detected.

those of EtOAc extracts of both varieties (Table 04). Among these, the lowest IC<sub>50</sub> was recorded for the hexane extract of the yellow variety (280.79  $\pm$  10.11 mg/dm<sup>3</sup>). The antioxidant capacity in terms of DPPH radical scavenging of plant extracts was dependent on factors like plant type, cultivar differences, as well as the extracting medium (Gunaratne *et al.*, 2022b). Previously, Krishnaveni (2014) stated that the antioxidant activities of plants might be influenced by varietal differences, geographical location, and growth conditions. To compare the findings of the present study, there was hardly any data from the literature about the antioxidant power of the kernel of *T. catappa*.

### Anti-hyperglycemic Properties

A comparison of the IC<sub>50</sub> values of Alpha-glucosidase inhibitory activity of different extracts of the two varieties is presented in Table 05. Adekola *et al.* (2017) previously stated that the inhibition of carbohydrate-hydrolyzing enzymes in the digestive tract is one of the most effective ways to reduce the rate of absorption of glucose into the bloodstream of diabetic patients. A dose-dependent increase in inhibition against Alpha-glucosidase activity has been displayed

by various plant extracts. The overall results of this study suggested that the kernels of both varieties of *T. catappa* could have the potential to act as anti-hyperglycemic agents since they exerted inhibitory activity against Alpha-glucosidase. According to Table 05, higher inhibitory potentials were observed for the EtOAc extracts of both varieties since they had lower IC<sub>50</sub> values. Additionally, a significant ( $p < 0.05$ ) difference was noticed between the EtOAc extracts of the two varieties regarding IC<sub>50</sub> values. In this case, it is believed that the omega-3-rich lipid components present in EtOAc extracts could form amylose-lipid complexes, which might induce resistance to the hydrolysis of starch in rice, corn, etc. (Tamura *et al.*, 2022). This phenomenon has already demonstrated the benefits of oils with alpha-linolenic acid on glycemic control in individuals suffering from type 2 diabetes.

As shown in Table 05, the inhibitory activity against the Alpha-glucosidase enzyme displayed by the purple variety was higher (IC<sub>50</sub> = 104.13  $\pm$  1.22 mg/dm<sup>3</sup>) when compared to that of the yellow variety (IC<sub>50</sub> = 197.46  $\pm$  16.07 mg/dm<sup>3</sup>). All three extracts of the purple variety were found to display higher inhibitory

**Table 5: IC<sub>50</sub> values for  $\alpha$ -glucosidase and  $\alpha$ -amylase inhibitory activities of different solvent extracts of *T. catappa* seeds**

Assay	Cultivar	IC <sub>50</sub> value/mg/dm <sup>3</sup>		
		Hexane extract	EtOAc extract	MeOH extract
$\alpha$ -glucosidase inhibitory activity	Purple	1367.80±117.89	104.13a±1.22	312.52a±0.54
	Yellow	<2000	197.46b±16.07	>2000
$\alpha$ -amylase inhibitory activity	Purple	>7000	2436.23±189.76	3006.08b±231.87
	Yellow	ND	ND	2137.95a± 118.54

activities when compared to those of the yellow variety. Nevertheless, information is scarce in the literature to compare the results of Alpha-glucosidase inhibitory activity displayed by the kernel extracts of the two varieties. The data presented in Table 05 further provide details of the IC<sub>50</sub> values corresponding to Alpha-amylase inhibitory activities of the extracts of the two varieties. According to Table 05, higher inhibitory potentials (lower IC<sub>50</sub> values) were displayed by MeOH extracts of both varieties, but this particular activity for the EtOAc extract was detected only in the purple variety. Noteworthy ( $p<0.05$ ) differences were found between the MeOH extracts of the two varieties regarding their IC<sub>50</sub> values of inhibitory activity against the Alpha-amylase enzyme. While the highest activity was exhibited by the yellow variety (IC<sub>50</sub> = 2137.95 ± 118.54 mg/dm<sup>3</sup>), the lowest was found with the purple variety (IC<sub>50</sub> = 3006.08 ± 231.87 mg/dm<sup>3</sup>).

## CONCLUSION

This study compared the differences in the nutritional composition, TPC, anti-oxidative, and anti-hyperglycemic potentials of yellow and purple varieties of *T. catappa* distributed in the central province of Sri Lanka. The kernels of the two varieties of *T. catappa* were high in both fat and protein, but low in total

carbohydrate contents. Except for protein, all macro-nutrient components displayed significant differences in their proportions. Zn, Cu, Mn, Ba, Sr, B, Co, and Ag were detected as trace minerals in both varieties, but Zn was the most abundant trace mineral present. The oil components of both varieties were found to possess different fatty acids; palmitic, oleic, and linoleic were present as predominant in both varieties. The oils of both varieties having high amounts of essential fatty acids, especially linoleic acid, was an attractive merit. As the kernels of both varieties had positive indications of anti-oxidative and anti-hyperglycemic activity, consumption of them could be beneficial for diabetic patients. Hence, they can be suitably incorporated into foods that are meant for special diets.

## Conflict of Interest

The authors declare that there is no conflict of interest.

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