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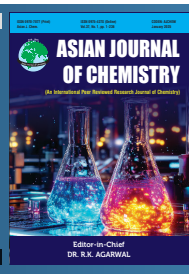

Ethanolic Extract of *Terminalia catappa* Kernel: A Natural Remedy with Promising Pharmacological Potential

H.A.L.D. JAYAWARDHANA, D.H.D. DEWAGEDONA and C.S.K. RAJAPAKSE*

Department of Chemistry, University of Kelaniya, Kelaniya, Sri Lanka

*Corresponding author: Fax: +94 112911916; Tel: +94 112903275; E-mail: shashikala@kln.ac.lk

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A relationship between humans and nature began with our ancestral reliance on natural resources for survival and health. This relationship has profoundly influenced the field of pharmacology through the discovery and utilization of natural product drugs, demonstrating the enduring importance of nature in medicine and the ongoing exploration of its therapeutic potential. This study investigates the pharmacological potential of the ethanolic extract of Ceylon almond kernel (*Terminalia catappa*), highlighting its antioxidant (IC_{50} = 21.68 ± 1.97 µg/mL), anti-inflammatory (IC_{50} = 180.14 ± 3.99 µg/mL) and α-amylase inhibitory (IC_{50} = 147.77 ± 4.74 µg/mL) activities. It also showed antibacterial activity against various pathogens and minimal UV protection (SPF = 5.27 ± 0.63). These results suggest that the Ceylon almond kernel could be a promising natural source for pharmaceutical applications and drug development.

Keywords: Antibacterial activity, Anti-inflammatory activity, α-Amylase inhibitory, Sun protection factor, *Terminalia catappa*.

INTRODUCTION

Homo sapiens relied on natural ecosystems for existence and health, forming a close relationship with nature. As hunter-gatherers, early humans knew their environment and used medicinal plants for food, shelter and healing [1]. This deep interaction with nature laid the groundwork for pharmacological discoveries [2]. In the context of pharmacology, using natural products as medicines has ancient roots across various cultures. Traditional medication, such as traditional Chinese medicine [3], Ayurveda [4] and indigenous healing practices [5], have long utilized natural substances derived from plants, animals and minerals for therapeutic purposes. These early practices represent humanity's early attempts to understand and harness the healing properties of the natural world.

Historically, natural product drugs have been pivotal in the pharmacological advancements. Examples include the discovery of morphine from opium poppies for pain relief [6], quinine from cinchona bark for treating malaria [7] and aspirin from willow bark for pain and fever relief [8]. These natural products served as the basis for modern pharmaceuticals and inspired the development of synthetic analogs with enhanced efficacy and reduced side effects. Even today, natural product drugs play a significant role in drug discovery and development. Natural products offer a vast chemical diversity and complex biological activities that make them valuable sources of new medicines [9]. Inspiring by their ecological interactions and evolutionary adaptations, pharmaceutical research is exploring natural chemicals for new therapeutic prospects [10].

Terminalia catappa, generally known as tropical almond, Indian almond, Malabar almond, sea almond, country almond and beach almond are 30-45 feet large, branched and spread like a crown, tropical tree native to the Asian and Australian continents [11]. This plant belongs to the Combretaceae family and commonly found in coastal areas around the Indian and Pacific oceans [12]. In most urban areas, this plant is grown for several reasons for example, the deep shade provided by its large leaves, the edible kernels that can be consumed as nuts and its role in protecting the soil from the heavy fall of its leaves [13,14].

The parts of *Terminalia catappa* plant, including leaves, bark and fruit, encompass numerous phytochemicals [15] that provide significant biological properties such as antioxidant [16,17], antimicrobial [16-18], antidiabetic [19], anthelmintic [20], anti-tumor [21], anti-aging [22] and anti-inflammatory [23] activities, in addition to possessing an exceptional

nutritional profile [24], which remains largely unknown to the common people. Moreover, *T. catappa* has an edible kernel consumed as a nut and tastes like almond [25]. Most researchers have revealed the health-promoting bioactivities of *T. catappa* leaves, bark and fruit. However, not much scientific data is available on the pharmacological potential of its kernel [26]. Therefore, the main goal of this study was to determine the antioxidant, antibacterial, anti-inflammatory, *in vitro* α-amylase inhibitory activities, total phenolic and flavonoid contents and sun protec-tion factor (SPF) of the ethanolic extract of the kernel of ripened fruits of *T. catappa*.

EXPERIMENTAL

Sample preparation: *Terminalia catappa* ripened fruits were collected from home gardens in the Gampaha district, Sri Lanka. Plant material was authenticated by Department of Plant and Molecular Biology, University of Kelaniya, Sri Lanka. Those were allowed to dry at room temperature and fruits were cracked to obtain the kernel. The kernels were washed with distilled water and air-dried for 7 days. Next, they were ground into a powder using a mechanical grinder (Innovex IBL002/400W), packed in an airtight bag and stored at 4 ºC until further use.

Extraction: The extraction of chemical constituents in the kernels was done using maceration [27]. Dried *T. catappa* kernel powder (10.00 g) was added to 99% ethanol (100 mL) in a volumetric flask. It was kept for 7 days at room temperature while shaking occasionally. It was filtered under gravity using a Whatman filter paper. The solvent of the filtrate was evaporated using a rotary evaporator (IKA®RV 10 digital, Germany) under given conditions (25 ºC, 30 rpm). The extract was stored at 4 ºC until further use.

Antioxidant activity: 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay was performed with few modifications according to Dewage *et al.* [28] to determine the antioxidant activity of sample. A concentration series of the extract was prepared using methanol as solvent (250, 125, 62.5, 31.25, 15.63, 7.82, 3.91, 1.95 µg/mL). DPPH in methanol (40 µL, 0.25 mg/mL) was added to sample (160 µL) in a 96-well plate. The absorbance was measured at 517 nm using a microplate reader (Thermo-Fisher Scientific 1510-01360C) after incubating the samples in dark for 15 min. A concentration series of the standard, Butylated hydroxytoluene (BHT) was prepared using methanol as solvent (250, 125, 62.5, 31.25, 15.63, 7.82, 3.91, 1.95 µg/mL). The above-mentioned procedure was followed using BHT. DPPH (40 µL) in methanol (160 µL) was used as negative control and methanol (200 µL) was used as blank. The experiment was triplicated (n = 3). The percentage inhibition (%) was calculated using eqn. 1:

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

where A_{sample} = absorbance of sample/standard; $A_{control}$ = absorbance of the control which replaced the sample with distilled water. The IC_{50}, the concentration of antioxidant required to reduce the DPPH radical concentration by 50% was determined by the plot of percent scavenging activity against concentrations of the samples/standard.

Anti-inflammatory activity: The anti-inflammatory activity of the extract was determined by a spectrophotometric method [29]. A concentration series of the ethanolic extract of kernel (1000, 500, 250, 125, 62.5, 31.25, 15.625 µg/mL) was prepared using phosphate buffer (pH 6.9) as solvent. The sample (1000 µL) was mixed with egg albumin from a fresh hen's egg (1000 µL). Then the sample was incubated for 15 min at room temperature and then the reaction was halted by heating the mixture at 70 ºC for 5 min. The mixture was allowed to cool to room temperature and its absorbance was measured at 660 nm using a microplate reader (Thermo-Fisher Scientific 1510-01360C). A concentration series of standard sodium diclofenac (1000, 500, 250, 125, 62.5, 31.25, 15.625 µg/mL) was prepared using phosphate buffer (pH 6.9) as solvent. The same experimental procedure was repeated with the reference (standard sodium diclofenac) solution. Phosphate buffer (pH 6.9, 500 µL) was used as the negative control instead of sample/reference solution. The experiment was carried out in triplicates (n = 3).

The percent inhibition was determined from eqn. 2 and the IC_{50} value was calculated by the plot of percent inhibition *vs*. concentrations of the samples/reference.

$$\text{Inhibition } (\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100 \quad (2)$$

A_{sample} = absorbance of the sample/reference; $A_{control}$ = absorbance of the control which replaced the sample with phosphate buffer. The IC_{50} defined as the concentration with 50% inhibition, which was determined by the plot of percent inhibition *vs*. concentrations of the samples/reference.

Antibacterial activity: The agar well diffusion method [18] was used to evaluate the antibacterial activity of the extract of kernel. *Escherichia coli* (ATCC 25922), *Salmonella enteric* (ATCC 14028), *Staphylococcus aureus* (ATCC 25923), *Bacillus cerus* (ATCC 11778), *Pseudomonas aeruginosa* (ATCC 9027), *Citrobacter freundii* (ATCC 8090), *Enterobacter aerogenes* (ATCC 13048), *Enterococcus faecalis* (ATCC 29212) were obtained from the American Type Culture Collection (ATCC). The ATCC bacterial cultures were grown on nutrient agar slants (Oxoid, UK) and incubated at 37 ºC in an incubator (Memmert, Germany) for 24 h. A bacterial suspension of each bacterial strain was prepared by using pure ATCC cultures. The separated bacterial colony was inoculated into the nutrient broth (5 mL) and incubated at 37 ºC for 24 h to prepare a suspension from a culture that represents 10^8 CFU/mL (approximately 0.5 McFarland standard). Sterilized Muller Hinton agar (20 mL) was poured into a sterilized and labeled petri dish, allowed to solidify and dried for 10 min at 55 ºC in an incubator to evaporate the excess moisture on the surface. Bacterial culture (10^8 CFU/mL) was swabbed with a sterile cotton swab on the agar plate. After drying, a well was prepared with a diameter of 10 mm by aseptically punching it with a sterile cork borer. The samples (100 µL) with three different concentrations (2000, 1000 and 500 µg/mL) were introduced into the wells separately and agar plates were incubated at 37 ºC for 24 h in an incubator. Amoxicillin (2000 µg/mL, 100 µL) and distilled water were

used as the positive and negative controls, respectively. All the tests were triplicated (n = 3). The resulting inhibition zone around the well was measured to the nearest millimeter along two axes and the mean of two measurements was calculated and reported.

Total phenolic content: The total phenolic content (TPC) of the ethanolic extract of Ceylon almond kernel was analyzed using a modified Folin-Ciocalteu method with gallic acid as the standard reference [30]. Stock solutions of both gallic acid and the extract were prepared at 1000 µg/mL in distilled water. A series of gallic acid dilutions (100 to 6.25 µg/mL) was used to construct a standard calibration curve. For assay, in a 96-well plate, 50 µL of either the standard or the extract solution was mixed with 50 µL of distilled water, 50 µL of 10% Folin-Ciocalteu reagent and, after a 5 min reaction period, 50 µL of 1 M sodium carbonate solution was added. The mixture was left to incubate in dark at room temperature for 1 h. Absorbance was then recorded at 765 nm using a microplate reader (Thermo-Fisher Scientific 1510-01360C). The TPC of the extract was calculated from the standard curve and expressed as milligrams of gallic acid equivalents (GAE) per gram of extract. Each measurement was conducted in triplicate (n = 3).

Total flavonoid content: The ethanolic extract of Ceylon almond kernel was assessed using a modified $AlCl_3$ colorimetric method [31], with quercetin as standard. Stock solutions of quercetin and extract (1000 µg/mL) were prepared in distilled water and a quercetin dilution series (100-6.25 µg/mL) was prepared for calibration. In a 96-well plate, 25 µL of sample or standard was mixed with 100 µL distilled water and 10 µL of 50 g/L NaNO, followed by 5 min of incubation. Subsequently, 15 µL of 100 g/L $AlCl_3$ in methanol was added and incubated for 6 min. Afterward, 50 µL of 1 M NaOH and 50 µL distilled water were added and stirred the mixture for 30 sec. Absorbance was measured at 510 nm using a microplate reader (Thermo-Fisher Scientific 1510-01360C). The TFC was calculated using the standard curve and expressed as mg quercetin equivalents (QE) per gram of extract and all experiments were conducted in triplicate.

α-Amylase inhibitory activity: The *in vitro* α-amylase inhibitory activity of the ethanolic extract of Ceylon almond kernel was evaluated using 3,5-dinitrosalicylic acid (DNS) reagent, following a modified method [32]. Phosphate buffer (pH 6.9) was prepared, along with solutions of human pancreatic amylase (0.5 mg/mL), 1% wheat starch and DNS reagent. Acarbose served as reference compound, with stock and serial dilutions (1000-15.625 µg/mL) prepared for both acarbose and extract. For assay, 500 µL of sample or reference solution was mixed with 500 µL of enzyme solution and incubated for 10 min at room temperature. Starch solution (500 µL) was added and the mixture was incubated for an additional 10 min. DNS reagent (500 µL) was then added and the mixture was heated at 95 ºC for 10 min. Absorbance was measured at 540 nm using a microplate reader (Thermo-Fisher Scientific 1510-01360C). Phosphate buffer was used as the negative control. The experiment was conducted in triplicate (n = 3). The percent inhibition (%) was determined using eqn. 3:

$$\text{Inhibition } (\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100 \quad (3)$$

where A_{sample} = Absorbance of sample; $A_{control}$ = Absorbance of control, which replaced the sample with phosphate buffer. IC_{50}, is defined as the concentration with 50% inhibition which was determined by the plot of percent inhibition *vs*. concentrations of the samples/standard.

Sun protection factor (SPF): The *in vitro* sun protection factor (SPF) of ethanolic extract of Ceylon almond kernel was assessed using a spectrophotometric method [33]. A reference sunscreen and the extract were prepared as 2 mg/mL solutions in ethanol. Absorbance was measured in triplicate (n = 3) using a UV-vis spectrophotometer (Orion Aqua Mate 8000, Thermo-Scientific) over 290-320 nm at 5 nm intervals, with ethanol as blank. SPF values for both the extract and reference sunscreen were calculated using the Mansur equation (eqn. 4) [34]:

$$SPF = CF \times \sum_{320}^{290} EE \times (\lambda) \times I(\lambda) \times abs(\lambda) \quad (4)$$

where CF = Correction factor (=10), EE = erythemal effect spectrum, I = Solar intensity spectrum, abs = absorption of sample/reference solution, λ = wavelength.

The EE × I values are constants, which are predetermined [35] and are listed in Table-1.

TABLE-1
NORMALIZED PRODUCT FUNCTION USED IN THE CALCULATION OF SPF

Wavelength (nm)	EE × I (normalized)	Wavelength (nm)	EE × I (normalized)
290	0.0150	310	0.1864
295	0.0817	315	0.0839
300	0.2874	320	0.0180
305	0.3278	Total	1.0000

Statistical analysis: All experiments were conducted in triplicate and the data are presented as mean ± standard deviation. The results were statistically analyzed using GraphPad Prism 7.00 software. Tukey's pairwise test was performed to determine significant differences between the means, with a significance level set at $p \leq 0.05$.

RESULTS AND DISCUSSION

Antioxidant activity: In this study, the antioxidant activity of ethanolic extract of *T. catappa* kernel was evaluated by DPPH free radical scavenging assay [28]. Fig. 1 shows the DPPH free radical scavenging activity of ethanolic extract of *T. catappa* kernel alongside the antioxidant activity of the reference, BHT. The ethanolic extract of *T. catappa* kernel and standard BHT exhibited DPPH free radical scavenging activity with IC_{50} values of 21.68 ± 1.97µg/mL and 12.30 ± 0.79 µg/mL, respectively. The results revealed that the ethanolic extract of *T. catappa* kernel has a strong antioxidant potential, even though it is slightly less than that of BHT.

Krishnaveni *et al*. [26] reported that the reducing power activities of aqueous extract of *T. catappa* kernel grown in Namakkal District, India is found to be 142.66 ± 6.11 ascorbic

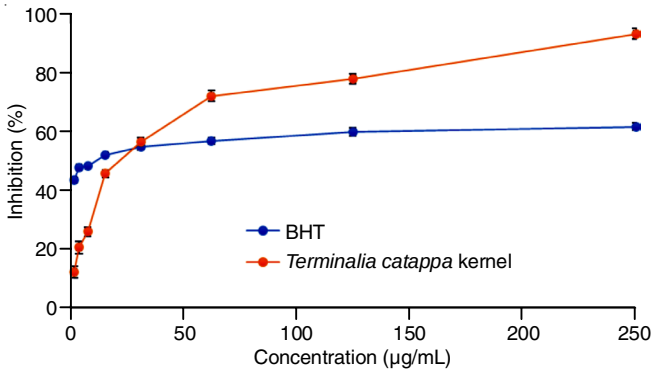

Fig. 1. DPPH radical scavenging activity (inhibition %) of BHT and ethanolic extract of *Terminalia catappa* kernel. BHT was used as the reference compound. Data are expressed in mean ± SD (n = 3)

acid equiv. mg/g dry material and showed that phenols and flavonoids in *T. catappa* kernel induces the potential of antioxidants. Similarly, Chanda *et al*. [36] investigated the IC_{50} value for the acetone leaf extract of *T. catappa* and found to be 28.5 µg/mL. In contrast, the methanolic leaf extract exhibited the highest scavenging activity with an IC_{50} value of 23 µg/mL, which is comparable to the IC_{50} value of standard ascorbic acid (11.4 µg/mL). Mwangi *et al*. [17] reported the IC_{50} values were 8.723 µg/mL for L-ascorbic acid, 13.42 µg/mL for the methanol extract and 13.04 µg/mL for aqueous extract of *T. catappa* plant materials. Both L-ascorbic acid and *T. catappa* extracts showed increased scavenging activity with increasing concentrations. In another study, the radical scavenging activity of *T. catappa* seed oil was also assessed by DPPH assay which yielded IC_{50} values of 950 µg/mL for cold-pressed oil and 2529 µg/mL for hot-pressed oil [16].

Anti-inflammatory activity: Fig. 2 shows the *in vitro* anti-inflammatory activity of ethanolic extract of *T. catappa* kernel alongside the anti-inflammatory activity of the reference sodium diclofenac. The ethanolic extract of *T. catappa* kernel and reference sodium diclofenac exhibited anti-inflammatory activity with IC_{50} values of 180.14 ± 3.99 µg/mL and 67.62 ± 5.67 µg/mL, respectively. Thus, this activity is less compared to that of standard, sodium diclofenac and the result suggests that the ethanolic extract of *T. catappa* kernel could be used as an additive in natural anti-inflammatory formulations.

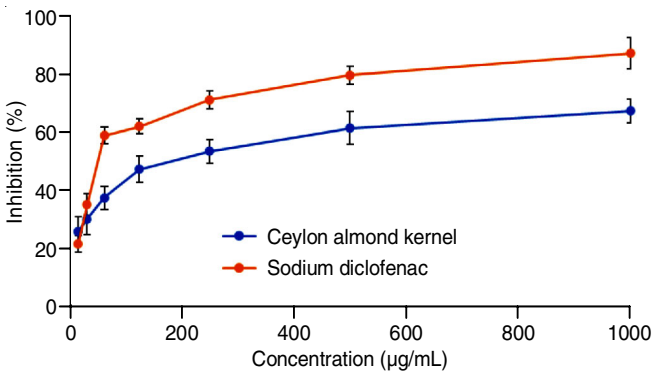

Fig. 2. Anti-inflammatory activity (inhibition %) of sodium diclofenac and ethanolic extract of *Terminalia catappa* kernel. Sodium diclofenac was used as the reference compound. Data are expressed in mean ± SD (n = 3)

Fan *et al*. [37] stated that the chloroform fraction of the ethanolic leaf extract of *T. catappa* was effective in mitigating inflammation in chronic and acute 12-O-tetradecanoylphorbol-13-acetate (TPA) models. Abiodun *et al*. [38] investigated the impact of ethanolic extract of the bark of *T. catappa* L. on the release of NO and IL-1β from cultured RAW 264.7 cells. Their findings showed that pre-treating these cells with ethanolic extract of the bark of *T. catappa* L. significantly diminished the LPS-induced secretion of both NO and IL-1β. This indicates that the ethanolic extract of the bark of *T. catappa* L. possesses anti-inflammatory and immunomodulatory properties, effectively countering the effects of pro-inflammatory mediators in RAW 264.7 cells.

Antibacterial activity: The antibacterial activity of the ethanolic extract of *T. catappa* kernel against *E. coli* (ATCC 25922), *S. enteric* (ATCC 14028), *S. aureus* (ATCC 25923), *B. cerus* (ATCC 11778), *P. aeruginosa* (ATCC 9027), *C. freundii* (ATCC 8090), *E. aerogenes* (ATCC 13048) and *E. faecalis* (ATCC 29212) were evaluated. The average inhibition zones of extracts against the bacterial stains are tabulated in Table-2.

As shown in Table-2, the ethanolic extract of *T. catappa* kernel exhibited the highest inhibition (15.50 mm) against *C. freundii* and *E. faecalis* at 2000 µg/mL, whereas the least inhibition (12.50 mm) observed at 2000 µg/mL was against *S. aureus* and *E. coli*. All the tested concentrations have exhibited antibacterial activity against all the studied bacterial strains except *E. coli*. It has not exhibited any antibacterial activity at 500 µg/mL extract concentration compared to the diameter of the well (10 mm). The results revealed that the ethanolic extract of *T. catappa* kernel has potential antibacterial activity even though their activities are less than the selected positive control at 1000 µg/mL.

Mwangi *et al*. [17] showed that *T. catappa* plant material's methanolic extract exhibited superior antimicrobial activity against *S. aureus*, *P. aeruginosa* and *E. coli*. The study demonstrated that the aqueous extract of *T. catappa* exhibited stronger inhibitory activity against *B. subtilis* at all tested concentrations. Additionally, significant differences were observed in the inhibition zone diameters for the methanolic extract across all the microorganisms, particularly at 25 mg/mL and 100 mg/mL.

Nguyen *et al.* [16] reported that both hot-pressed oil and cold-pressed oils of *T. catappa* seeds showed inhibitory effects against *S. aureus*, *V. parahaemolyticus*, *E. coli*, *P. aeruginosa* and *B. cereus* bacterial strains. Another study [23] revealed that acetone, chloroform and petroleum ether leaf extracts of *T. catappa* showed remarkable antibacterial activity against *S. aureus* and *P. mirabilis*. Among them, the acetone extract produced the largest inhibition zone, approximately 31 mm, against both pathogens at a concentration of 10 µg/mL.

Total phenolic content and total flavonoid content: The total phenolic content (TPC) and total flavonoid content (TFC) of the ethanolic extract of *T. catappa* kernel were determined using the Folin-Ciocalteu method and the $AlCl_3$ colorimetric method, respectively. Gallic acid served as the reference compound for TPC, while quercetin was used for TFC. TPC and TFC were calculated using linear regression equations from their

TABLE-2
ZONES OF INHIBITION OF ETHANOLIC EXTRACT OF *Terminalia catappa* KERNEL AGAINST BACTERIAL STRAINS

Culture	Positive control (amoxicillin)	Negative control (distilled water)	Concentration (µg/mL)	Sample mean ± SD
Staphylococcus aureus (ATCC 25923)	46 mm	–	2000	12.50 ± 0.70
			1000	11.00 ± 1.61
			500	10.00 ± 2.10
Escherichia coli (ATCC 25922)	28 mm	–	2000	12.50 ± 0.70
			1000	10.00 ± 0.50
			500	10.00 ± 0.66
Pseudomonas aeruginosa (ATCC 9027)	18 mm	–	2000	14.50 ± 0.70
			1000	12.50 ± 0.50
			500	11.50 ± 0.35
Enterobacter aerogenes (ATCC 13048)	30 mm	–	2000	14.00 ± 1.10
			1000	11.50 ± 0.80
			500	10.00 ± 0.50
Bacillus cereus (ATCC 11778)	32 mm	–	2000	15.00 ± 0.92
			1000	13.00 ± 0.50
			500	10.00 ± 1.32
Salmonella enteric (ATCC 14028)	35 mm	–	2000	14.00 ± 1.41
			1000	10.50 ± 0.70
			500	10.00 ± 0.50
Citrobacter freundii (ATCC 8090)	23 mm	–	2000	15.50 ± 0.71
			1000	11.50 ± 1.12
			500	10.00 ± 0.50
Enterococcus faecalis (ATCC 29212)	20 mm	–	2000	15.50 ± 0.54
			1000	12.00 ± 1.50
			500	10.50 ± 0.71

Amoxicillin (1000 µg/mL) was used as the positive control and distilled water was used as the negative control. Data are expressed in mean ± Standard deviation (SD) (n = 2).

respective standard curves (R^2 = 0.9248 for gallic acid and R^2 = 0.9725 for quercetin).

As shown in Table-3, the ethanolic extract of *T. catappa* kernel contains both phenolic compounds and flavonoids, which are likely contributors to its potent antioxidant activity [26,39-42]. According to Krishnaveni *et al*. [26], the TPC and TFC of the aqueous extract of tropical almond kernel were reported as 26.33 ± 2.30 mg GAE/g dry weight and 10.66 ± 1.52 mg QE/g dry weight, respectively. Divya & Anand [40] assessed the TPC and TFC of the ethanolic extracts of ripe fruit, unripe fruit and leaves of *T. catappa*. The TPC values were reported as 117.10 ± 3.6 mg GAE/g for ripe fruit, 76.38 ± 2.24 mg GAE/g for unripe fruit and 285.77 ± 3.41 mg GAE/g for leaves. Similarly, the TFC values were 28.21 ± 0.83 mg QE/g, 6.75 ± 0.23 mg QE/g and 59.95 ± 4.83 mg QE/g for ripe fruit, unripe fruit and leaves, respectively.

TABLE-3
TPC AND TFC VALUES OF ETHANOLIC EXTRACT OF *Terminalia catappa* KERNEL

Sample	TPC (mg of gallic acid equivalent/g)	TFC (mg of quercetin equivalent/g)
Ethanolic extract of *Terminalia catappa* kernel	20.52 ± 1.20	3.92 ± 0.09

Data are expressed in mean ± SD (n = 3).

Oyeniran *et al*. [41] reported TPC and TFC of the aqueous leaf extract of tropical almonds as 23.95 ± 0.17 mg GAE/g of dry weight and 0.03 ± 0.00 mg QE/g of dry weight, respectively. Adefegha *et al*. [42] reported the TPC of the methanolic extracts of tropical almond hull and drupe were 7.00 ± 0.75 mg GAE/100 g and 4.00 ± 0.67 mg GAE/100 g of dry weight, respectively. The TFC in the same extracts was 4.10 ± 0.33 mg QE/100 g for hull and 1.35 ± 0.10 mg QE/100 g for drupe. Daram *et al*. [39] reported that the TPC of the ethanolic and aqueous extracts of *T. catappa* bark was 287 mg GAE/g and 175 mg GAE/g of dry weight, respectively. The TFC of these extracts was found to be 10.2 mg QE/g and 61.7 mg QE/g of dry weight, respectively.

Based on these findings, the ethanolic extracts of ripe fruit, unripe fruit, leaf and both ethanolic and aqueous extracts of tropical almond bark are significantly higher in total phenolic content (TPC) and total flavonoid content (TFC) compared to the ethanolic extract of tropical almond kernel. Ethanolic extract of tropical almond kernel has higher TFC than aqueous extract, although its TPC values are identical.

α-Amylase inhibitory activity: The *in vitro* α-amylase inhibitory activity of the ethanolic extract of *T. catappa* kernel was evaluated using a spectrophotometric method, with acarbose acting as reference drug. The IC_{50} values for the α-amylase inhibitory activity of the ethanolic extract of *T. catappa* kernel and the standard acarbose, which were found to be 147.77 ± 4.74 µg/mL and 42.23 ± 3.32 µg/mL, respectively. The results indicate that the ethanolic extract of the kernel has significant α-amylase inhibitory activity, though it is less potent than acarbose. These findings suggest that the ethanolic extract of *T. catappa* kernel could serve as a potential ingredient in natural antidiabetic formulations.

Adefegha *et al*. [42] reported that the methanolic extracts of tropical almond hull and drupe showed α-amylase inhibitory activity, with IC_{50} values of 66.56 ± 3.12 µg/mL and 10.11 ± 7.21 µg/mL, respectively. The study conducted by Nagappa *et al*. [43] investigated the antidiabetic effects of petroleum ether, methanol and aqueous extracts of *T. catappa* fruit on fasting blood sugar levels and biochemical parameters in alloxan-induced diabetic rats, showing significant antidiabetic activity at doses of 1/5 of their lethal doses.

Sun protection factor (SPF): The *in vitro* SPF of ethanolic extract of *T. catappa* kernel and commercially available reference sunscreen was evaluated using a spectrophotometric method [33]. The SPF values for both the extract and reference sunscreen were calculated using the Mansur equation and the results are summarized in Table-4. All the tests were triplicated (n = 3). The SPF values of the ethanolic extract of *T. catappa* kernel and the reference sunscreen were 5.27 ± 0.63 and 35.94 ± 0.56, respectively. The findings showed that the ethanolic extract of the kernel offers limited UV protection, with an SPF value of less than 10. A study by Indrisari *et al*. [44] also reported that the ethanolic (SPF = 3.68) and aqueous (SPF = 3.61) extracts of *T. catappa* leaf provided minimal protection against UV radiation.

TABLE-4
SPF VALUE OF ETHANOLIC EXTRACT OF *Terminalia catappa* KERNEL AND THE REFERENCE COMMERCIAL SUNSCREEN AT 2.0 mg/mL CONCENTRATION

Sample	SPF value ± SD
Ethanolic extract of *Terminalia catappa* kernel	5.27 ± 0.63
Reference sunscreen	35.94 ± 0.56

Data are expressed in mean ± SD (n = 3).

Conclusion

In this work, the ethanolic extract of *Terminalia catappa* kernel demonstrated strong antioxidant potential, with significant DPPH free radical scavenging activity (IC_{50} = 21.68 ± 1.97 µg/mL) and high phenolic and flavonoid contents. It also exhibited α-amylase inhibitory (IC_{50} = 147.77 ± 4.74 µg/mL) and anti-inflammatory (IC_{50} = 180.14 ± 3.99 µg/mL) activities, indicating its potential for use in natural antidiabetic and anti-inflammatory formulations subject to toxicity testing. Moreover, the extract showed antibacterial activity against a wide range of pathogens and minimal protection against UV radiation (SPF = 5.27 ± 0.63). These findings suggest that *T. catappa* kernel holds promise as a bioactive agent with diverse therapeutic benefits.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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