Indonesian Journal of Cancer Chemoprevention, October 2025
ISSN: 2088–0197
e-ISSN: 2355-8989

Cytotoxic Activity Against P388 Leukemia Cells and Cellular Antioxidant Capacity of *Theobroma cacao* L. Pod Husk Extract: A Dual Bioactivity Assessment

Fahrauk Faramayuda*, Nabila Cahya Kentama, Clara Sunardi, Dyah Nur Azizah, Endang Kumolowati

Faculty of Pharmacy, Universitas Jenderal Achmad Yani, Jl. Terusan Jenderal Sudirman, Cimahi 40531, West Java, Indonesia

Abstract

Cacao pod husk (*Theobroma cacao* L.), a major agro-industrial by-product representing more than 70% of cocoa fruit weight, is a rich source of bioactive secondary metabolites including flavonoids, polyphenols, tannins, alkaloids, and quinones. This study evaluated the cytotoxic activity of a methanol extract against P388 murine leukemia cells using the MTT assay, cellular antioxidant capacity via superoxide dismutase (SOD) activity and malondialdehyde (MDA) levels in hydrogen peroxide (H_2O_2)-induced human erythrocytes, and comprehensive phytochemical profiling with thin-layer chromatography (TLC) fingerprinting. Physicochemical characterization of the simplicia, performed to verify compliance with standardization parameters, revealed a moisture content of 2.49±0.70% v/w, total ash of 6.185±0.103% w/w, water-soluble extractive of 12.47±0.03% w/w, and ethanol-soluble extractive of 6.86±0.54% w/w. Phytochemical screening confirmed flavonoids, alkaloids, tannins, polyphenols, monoterpenes, sesquiterpenes, and quinones. Soxhlet extraction produced a higher yield (21.59%) than cold maceration (19.46%), supporting its use for subsequent bioactivity testing. At 53.8 µg/mL, the extract reduced MDA levels to a near-normal value (0.8780±0.0060 nmol/mL) and increased SOD activity (14.8493±0.0001 units/mL) to values higher than those obtained with the quercetin positive comparator, although no formal statistical comparison between groups was performed. The MTT assay yielded an IC_{50}>100 ug/mL (extrapolated estimate 106.64 ug/mL) against P388 leukemia cells, classifying the crude extract as inactive against this cell line. These findings indicate that *T. cacao* pod husk possesses notable cellular antioxidant activity accompanied by weak direct cytotoxicity at the crude-extract level, supporting its potential as a candidate for bioactivity-guided fractionation in future studies.

Keywords: *Theobroma cacao; cellular antioxidant capacity; superoxide dismutase; malondialdehyde; P388 leukemia cells.*

Submitted: May 22, 2026
Revised: August 04, 2026
Accepted: August 12, 2026

*Corresponding author: fahrauk.faramayuda@lecture.unjani.ac.id

INTRODUCTION

Cancer represents one of the most formidable global public health challenges of the twenty-first century. In 2022, an estimated 20 million new cancer cases were diagnosed and 9.7 million cancer-related deaths occurred worldwide (Bray, *et al.*, 2024). Hematologic malignancies, particularly leukemia, account for approximately 30% of all childhood cancer diagnoses in developing countries, with acute lymphoblastic leukemia (ALL) constituting 70–80% of pediatric leukemia cases in children under 15 years of age (Steliarova-Foucher, *et al*., 2017 and Bhakta, *et al*., 2019). The five-year overall survival rate for ALL in high-income countries now exceeds 90%, yet outcomes remain substantially lower in low- and middle-income countries due to limited access to optimal therapy (Pui, *et al*., 2018 Hunger and Mullighan, 2015).

Despite significant advances in chemotherapy, standard anthracycline-based regimens—most notably doxorubicin—are associated with severe and often irreversible cardiotoxicity, imposing a substantial long-term health burden on pediatric survivors (Zamorano, *et al*., 2016 Armenian, *et al*., 2017). These limitations have catalyzed a global resurgence of interest in plant-derived natural products as sources of structurally diverse lead compounds with potential anticancer activity and improved safety profiles (Newman and Cragg, 2020 Atanasov, *et al*., 2021). Indeed, more than 60% of currently approved anticancer drugs are derived from or inspired by natural product scaffolds, underscoring the continued importance of biodiversity as a source of therapeutic innovation (Harvey, *et al*., 2015).

The cacao tree (*Theobroma cacao* L., family Malvaceae, formerly Sterculiaceae) is one of the most economically significant tropical crops globally, primarily cultivated for its seeds (Campos-Vega, *et al*., 2018). However, the pod husk—constituting approximately 73% of the fresh fruit weight—is generated as a massive agro-industrial by-product and largely discarded, representing a significant environmental burden and an unexploited bioeconomy resource (Campos-Vega, *et al.*, 2018 Okiyama, *et al.*, 2017). Scientific characterization of *T. cacao* by-products, including pod husk and bean shell, has revealed a complex mixture of condensed flavonoids (anthocyanidins, catechins, leucoanthocyanidins), polyphenols, tannins, and alkaloids including theobromine (~0.4% dry weight) (Nzekoue, *et al*., 2020 Maleyki and Ismail, 2010). The pod husk also contains monoterpenes, sesquiterpenes, and quinones, all of which contribute to its broad spectrum of biological activities (Crozier, *et al*., 2011).

Oxidative—stress defined as an imbalance between reactive oxygen species (ROS) production and cellular antioxidant defense capacity—is recognized as a central driver of oncogenesis, tumor progression, and chemotherapy resistance (Pizzino, *et al*., 2017 Jomova, *et al*., 2023). At physiological concentrations, ROS serve as second messengers in intracellular signaling cascades; however, when overproduced, they damage DNA, proteins, and membrane lipids, promoting genomic instability and malignant transformation (Forman and Zhang, 2021 Schieber and Chandel, 2014). The redox environment within cancer cells is characteristically dysregulated, making antioxidant phytochemicals attractive as chemopreventive and adjuvant therapeutic agents (Rojo de la Vega, *et al*., 2017).

Malondialdehyde (MDA), a major end-product of polyunsaturated fatty acid peroxidation, serves as a widely validated biomarker of lipid peroxidation-mediated cellular damage (Tsikas, 2017; Yin, *et al*., 2011). Quantification of MDA via the thiobarbituric acid (TBA) colorimetric assay is one of the most widely used indices of oxidative cellular injury in biological systems (Ayala, *et al.*, 2014). Superoxide dismutase (SOD), which catalyzes the dismutation of superoxide radicals to hydrogen peroxide and molecular oxygen, constitutes the first line of enzymatic defense against ROS (Fukai and

Ushio-Fukai, 2011; Wang, *et al*., 2018). Depression of SOD activity is a hallmark of oxidative stress-induced cellular damage (Ighodaro and Akinloye, 2018). The *ex vivo* model employing H_2O_2-induced oxidative stress in human erythrocytes is a well-established, physiologically relevant platform for evaluating cellular antioxidant capacity of plant extracts, given the susceptibility of erythrocyte membranes to lipid peroxidation (Kurata, *et al*., 1993; Santi, *et al.*, 2011).

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction assay is the gold-standard colorimetric method for high-throughput assessment of antiproliferative activity, relying on the reduction of the yellow tetrazolium salt to purple formazan by metabolically active cells (Riss, *et al.*, 2016; Vichai and Kirtikara, 2006). Previous investigations on *T. cacao* pod husk documented potent DPPH radical-scavenging activity (IC_{50} 5.38 µg/mL) and preliminary cytotoxic activity against brine shrimp (LC_{50} 39.69 µg/mL), suggesting anticancer potential warranting evaluation in mammalian cancer cell models (Prayong, *et al.,* 2008).

This study aimed to characterize the pharmacognostic standard parameters of *T. cacao* pod husk simplicia, conduct phytochemical screening and TLC fingerprinting of both cold maceration and Soxhlet methanol extracts, quantify cellular antioxidant capacity via SOD activity and MDA levels using an *ex vivo* H_2O_2-induced erythrocyte model, and evaluate *in vitro* cytotoxic activity against P388 murine leukemia cells by the MTT assay.

MATERIALS AND METHODS

Plant Material and Simplicia Preparation

Cacao pod husks (*T. cacao* L.) were collected from community plantations in Desa Puspahiang, Tasikmalaya, West Java, Indonesia. The Herbarium Bandungense, Institut Teknologi Bandung (ITB) confirmed the botanical identity of the specimen as *Theobroma cacao* L. (family Malvaceae, formerly Sterculiaceae) (verification letter No. 590/I1.CO2.2/PL/2014). Ripe fruits were harvested; husks were separated, washed, cut into smaller pieces, and dried by indirect shade-drying to preserve thermolabile active constituents. The dried material was pulverized and sieved to obtain uniform simplicia powder.

Simplicia Characterization

Standard pharmacognostic parameters were determined according to Indonesian Pharmacopoeial standards. Macroscopic examination recorded morphological descriptors including color, odor, taste, shape, and dimensions. Microscopic analysis at 400× magnification using chloral hydrate was performed to identify diagnostic cellular elements. Physicochemical parameters determined included: total ash content, water-soluble ash, water-soluble extractive, ethanol-soluble extractive (95% ethanol), and moisture content by azeotropic toluene distillation. All determinations were performed in duplicate.

Extraction

Two parallel extraction methods were employed on ~300 g simplicia each using redistilled methanol. Cold maceration (301.01 g): immersed in methanol, stirred intermittently during the first 6 h, then left for 18 h; repeated three times until the filtrate was colorless. Soxhlet continuous extraction (300 g): placed in a cellulose thimble with 1.7 L methanol and extracted for 3 days at 8 h per day until the solvent in the siphon was colorless. Both extracts were concentrated under reduced pressure at 60°C using a rotary evaporator and further evaporated on a water bath to yield viscous crude extracts.

Phytochemical Screening

Qualitative phytochemical screening of simplicia powder and methanol extracts was performed using standard colorimetric methods.

Alkaloids were identified using Mayer's and Dragendorff's reagents after chloroform–HCl 2N partitioning. Flavonoids were detected by the magnesium–HCl reduction test with amyl alcohol partitioning, yielding red-to-orange coloration as a positive result. Quinones were detected by treatment with KOH 5%, tannins by 1% gelatin solution precipitation, and polyphenols by $FeCl_3$ 1% (blue-black coloration). Saponins were identified by vigorous shaking producing stable foam, steroids and triterpenoids by the Liebermann–Burchard reaction, and monoterpenes and sesquiterpenes by vanillin 10% in H_2SO_4 reagent.

Thin-Layer Chromatography (TLC) Fingerprinting

Methanol extracts were spotted on silica gel GF254 aluminum plates. The mobile phase n-hexane–ethyl acetate (6:4 v/v) was used; the development chamber was pre-saturated for 30 minutes. Plates were observed under UV light at 254 nm and 366 nm, and $AlCl_3$ 1% in ethanol was sprayed to detect flavonoids by their characteristic yellow fluorescence under UV 366 nm.

Cellular Antioxidant Assessment

Preparation of 10% RBC Suspension

Blood type O from a healthy, non-smoking male volunteer (not medicated for at least 2 days) was centrifuged at 3000 rpm for 5 minutes. The plasma was discarded and the erythrocyte pellet was washed three times with 0.9% NaCl solution (3× pellet volume), with re-centrifugation at 3000 rpm for 5 minutes after each wash. Ten mL of the washed pellet was resuspended in phosphate-buffered saline (PBS, pH 7.4) to 100 mL to yield a 10% RBC suspension.

Experimental Groups

Six groups in triplicate were used: (1) normal control, 2 mL RBC 10%; (2) negative control, 1 mL RBC 10% + 1 mL H_2O_2 200 mM; (3) positive comparator, 1 mL RBC 10% + quercetin 538 µg/mL + 1 mL H_2O_2 200 mM; (4) test group 1, extract 5.38 µg/mL + H_2O_2; (5) test group 2, extract 53.8 µg/mL + H_2O_2; and (6) test group 3, extract 538 µg/mL + H_2O_2. Extract solutions were prepared by dissolving the viscous extract in DMSO then diluting with distilled water.

Measurement of Malondialdehyde (MDA) Levels

For all treatment groups, the RBC suspension was pre-incubated with extract or quercetin at 37°C for 60 minutes, then H_2O_2 200 mM was added and incubation continued for a further 60 minutes at 37°C. After centrifugation (3000 rpm, 5 min), 0.25 mL supernatant was combined with 0.25 mL distilled water and 1.25 mL TCA 20%, then re-centrifuged. From the resultant supernatant, 1 mL was added to 0.5 mL TBA 0.67%, heated at 100°C for 30 minutes, cooled under running water, and absorbance read at 532.5 nm. MDA concentrations were interpolated from a tetraethoxypropane (TEP) calibration curve ($y = 0.058x - 0.0087$; $r = 0.9985$) (Tsikas, 2017; Santi, *et al.*, 2011).

Measurement of Superoxide Dismutase (SOD) Activity

SOD activity was determined by the adrenochrome assay based on inhibition of epinephrine autooxidation in alkaline medium. After treatment and oxidative induction as above, 1 mL supernatant was diluted with 7 mL distilled water; 1 mL of this dilution was mixed with 1 mL chloroform–96% ethanol (3:5 v/v) and centrifuged (3000 rpm, 5 min). From the aqueous phase, 50 µL was combined with 2900 µL carbonate buffer (pH 10.2) and 50 µL 0.02 M epinephrine. Absorbance was measured at 480 nm at minutes 1, 2, 3, and 4. SOD activity (units/mL) was calculated from the percentage inhibition of epinephrine autooxidation relative to the blank (Fukai and Ushio-Fukai, 2011; Wang, *et al.*, 2018; Ighodaro and Akinloye, 2018).

Indonesian Journal of Cancer Chemoprevention, October 2025
ISSN: 2088–0197
e-ISSN: 2355-8989

Cytotoxicity Assay — 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) Method

Cytotoxic activity was evaluated against P388 murine lymphocytic leukemia cells following the MTT protocol established by the Cancer Chemoprevention Research Center (CCRC), Universitas Gadjah Mada (Riss, *et al*., 2016). Cells were maintained in RPMI-1640 medium supplemented with 10% FBS and kanamycin at 37°C under 5% CO_2. Cells were seeded at 3×10^3 cells/well in 96-well flat-bottom plates and pre-incubated overnight for equilibration. Serial concentrations of both the Soxhlet and cold-maceration methanol extracts (0.1–100 µg/mL, triplicate) and doxorubicin as positive control were added and incubated for 48 h. MTT solution (0.5 mg/mL in PBS, 20 µL/well) was added; after 4 h, formazan crystals were solubilized with SDS 10% in 0.1 N HCl. Absorbance was read at 540 nm. IC_{50} values were derived from logarithmic regression using OriginPro 8.0. Extracts with IC_{50}>30 µg/mL are classified as inactive per established criteria (Vichai and Kirtikara, 2006; Prayong, *et al.*, 2008).

RESULTS

Simplicia Characterization

Botanical verification confirmed the test material as authentic *T. cacao* L. (family Malvaceae, formerly Sterculiaceae). Macroscopic examination of fresh cacao pod husks revealed ovoid-to-oblong fruits (length approx. 19 cm, width approx. 7 cm), yellow-green to yellow-orange in color, with a characteristic pungent odor and bitter taste (Figure 1A–B). After shade-drying and pulverization, the simplicia powder was blackish-brown in color (Figure 1C). Microscopic analysis identified stone cells of the epicarp and monocellular covering trichomes as the primary diagnostic elements (Figure 1D). Macroscopic characteristics are summarized in Table 1.

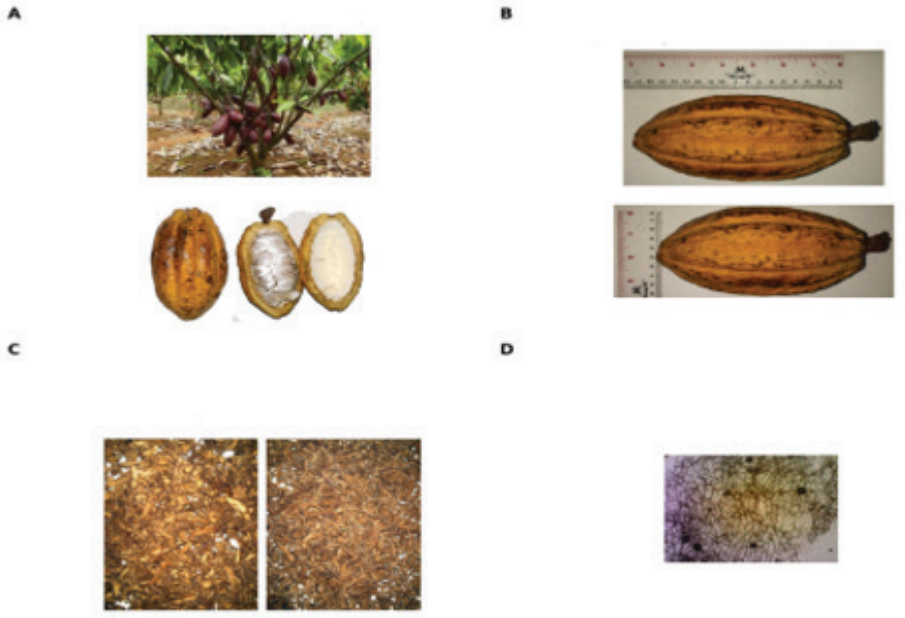


Figure 1. Macroscopic and microscopic characterization of *Theobroma cacao* pod husk. (A) Cacao plant and fresh pod husks from community plantations in Tasikmalaya, West Java, and a transverse section of the fresh husk. (B) Length (approx. 18 cm) and width (approx. 9 cm) measurement of the fresh husk. (C) Chopped fresh pod husk before drying (left) and dried simplicia (right). (D) Microscopic appearance of the simplicia powder showing epicarp stone cells and monocellular covering trichomes (chloral hydrate mounting medium, 400× magnification).

Table 1. Macroscopic characteristics of *T. cacao* pod husk and its simplicia.

Parameter	Fresh Husk	Dried Simplicia
Color	Yellow-green to yellow-orange	Blackish-brown
Odor	Characteristic, pungent	Characteristic, pungent
Taste	Bitter	Bitter
Shape	Ovoid to oblong	Irregular fragments
Size	Length approx. 19 cm, width approx. 7 cm	Variable powder

Standard physicochemical parameters are presented in Table 2 Moisture content (2.49±0.70% v/w) satisfied the pharmacopoeial requirement of <10%. The water-soluble extractive (12.47±0.03% w/w) substantially exceeded the ethanol-soluble extractive (6.86±0.54% w/w).

Extraction Yield

Soxhlet continuous extraction of 300 g simplicia over 3 days yielded a viscous dark extract with a yield of 21.59%. Cold maceration of 301.01 g simplicia yielded 19.46%, indicating a higher extraction efficiency for the Soxhlet method; the Soxhlet methanol extract was therefore selected for the cellular antioxidant assays, while phytochemical screening and the MTT assay were performed on both extracts for comparison.

Phytochemical Screening

Qualitative phytochemical screening (Table 3) revealed identical profiles across simplicia powder and both methanol extracts: flavonoids, alkaloids, tannins, polyphenols, monoterpenes, sesquiterpenes, and quinones were detected in all samples, while saponins and steroids/triterpenoids were absent.

Table 2. Standard pharmacognostic parameters of *T. cacao* pod husk simplicia.

No	Parameter	Result (mean±SD)	Unit	Reference/literature range
1	Total ash content	6.185±0.103	% w/w	No compendial monograph exists for *T. cacao* pod husk; general Indonesian Herbal Pharmacopoeia guidance for leaf/bark drugs was used as reference only
2	Water-soluble ash	4.90±0.17	% w/w	As above
3	Water-soluble extractive	12.47±0.03	% w/w	As above
4	Ethanol-soluble extractive	6.86±0.54	% w/w	As above
5	Moisture content (distillation)	2.49±0.70	% v/w	Indonesian Herbal Pharmacopoeia general limit: <10% v/w

Table 3. Phytochemical screening results of *T. cacao* pod husk simplicia and methanol extracts.

Compound Class	Simplicia	Maceration Extract	Soxhlet Extract
Flavonoids	+	+	+
Alkaloids	+	+	+
Tannins	+	+	+
Polyphenols	+	+	+
Monoterpenes & Sesquiterpenes	+	+	+
Quinones	+	+	+
Saponins	–	–	–
Steroids & Triterpenoids	–	–	–

(+): positive; (-): negative

Thin-Layer Chromatography (TLC) Fingerprinting

TLC analysis of the Soxhlet methanol extract using n-hexane–ethyl acetate (6:4 v/v) on silica gel GF254 revealed a fluorescent spot at R_f 0.73 under UV 366 nm (Figure 2). Upon spraying with $AlCl_3$ reagent, an intense yellow-fluorescing spot was confirmed at R_f 0.21, characteristic of flavonoid compounds. The maceration extract showed a corresponding spot at R_f 0.70.

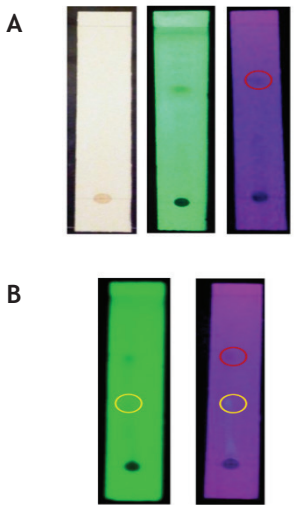


Figure 2. TLC profile of *T. cacao* pod husk methanol extract (mobile phase: n-hexane-ethyl acetate 6:4 v/v): A. upper panels, fluorescent spot at R_f 0.73 under UV 366 nm; B. lower panels, yellow-green fluorescence at R_f 0.21 after AlCl3 spray reagent under UV 366 nm, indicative of flavonoid compounds.

Cellular Antioxidant Capacity Malondialdehyde (MDA) Levels

The TEP calibration curve was linear over 0.25–2.0 nmol/mL ($y = 0.058x - 0.0087$; $r = 0.9985$; Figure 3).

H_2O_2 induction in the negative control markedly elevated MDA levels (1.6504±0.0066 nmol/mL) compared with the normal control (0.7888±0.0037 nmol/mL), confirming successful oxidative stress induction (Figure 4). All three test

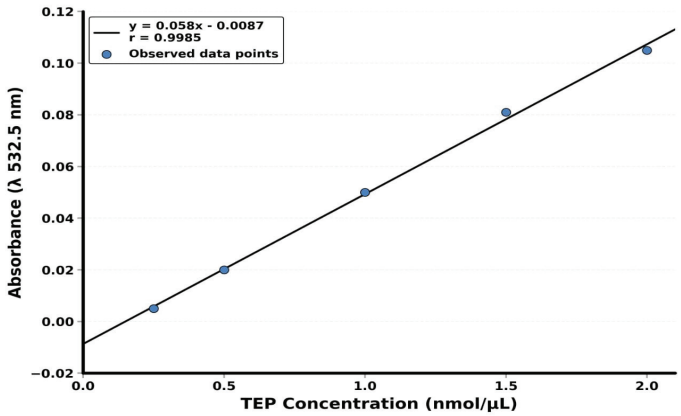


Figure 3. Calibration curve of tetraethoxypropane (TEP) standard at λ 532.5 nm. Linear regression: y = 0.058x − 0.0087; r = 0.9985.

concentrations reduced MDA relative to the negative control. Test group 2 (53.8 µg/mL) achieved a near-normal MDA level (0.8780±0.0060 nmol/mL), lower than that of the quercetin comparator (1.4991±0.0032 nmol/mL); no formal statistical test was performed to compare these two groups. Test group 3 (538 µg/mL) showed less reduction than test group 2, suggesting a non-linear dose-response relationship.

Superoxide Dismutase (SOD) Activity

SOD activity was severely depressed in the negative control (1.8362±0.0003 units/mL) relative to the normal control (3.6427±0.0009 units/mL) following H_2O_2 induction. Test group 2 (53.8 µg/mL) demonstrated the highest SOD activity (14.8493±0.0001 units/mL) among all groups, exceeding both the quercetin comparator (12.8356±0.0001 units/mL) and test group 1 (12.4247±0.0009 units/mL); again, no formal statistical comparison between groups was performed. Test group 3 (538 µg/mL) showed paradoxically lower SOD activity (4.0000±0.0007 units/mL) than groups 1 and 2. MDA and SOD results for all experimental groups are summarized numerically in the Supplementary Data; the values are illustrated in Figures 4 and 5.

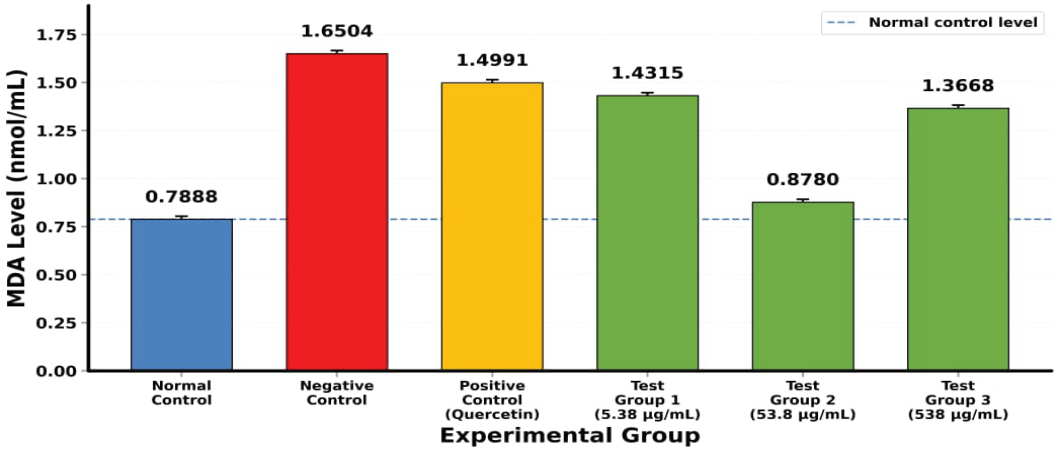


Figure 4. Mean MDA levels (nmol/mL) in 10% RBC suspension across experimental groups. Test group 2 (53.8 µg/mL) achieved the lowest, near-normal MDA level among the treated groups. Data are presented as mean +/- SD (n=3).

Indonesian Journal of Cancer Chemoprevention, October 2025
ISSN: 2088–0197
e-ISSN: 2355-8989

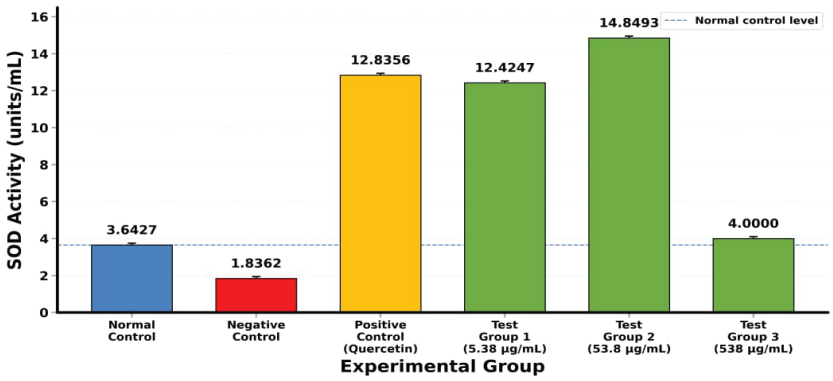


Figure 5. SOD activity (units/mL) in 10% RBC suspension across experimental groups. Test group 2 (extract 53.8 µg/mL) achieved the highest SOD activity among all groups. Data are presented as mean +/- SD (n=3).

Cytotoxic Activity Against P388 Leukemia Cells

Logarithmic regression analysis yielded an extrapolated IC_{50} of 106.64 ug/mL (*i.e.*, >100 ug/mL, the highest concentration tested) for the Soxhlet methanol extract against P388 leukemia cells, which is classified as inactive according to the widely applied threshold of IC_{50}>30 µg/mL for crude plant extracts (Vichai and Kirtikara, 2006; Prayong, *et al.*, 2008). Doxorubicin, used as the positive control, demonstrated strong cytotoxic activity (IC_{50}<5 µg/mL). The concentration–response data underlying this IC_{50} value (mean absorbance at 540 nm across triplicate wells, 0.1–100 µg/mL) are shown in Figure 6, together with the corresponding data for the cold-maceration extract (extrapolated IC_{50} of 112.65 ug/mL, *i.e.*, >100 ug/mL) for comparison.

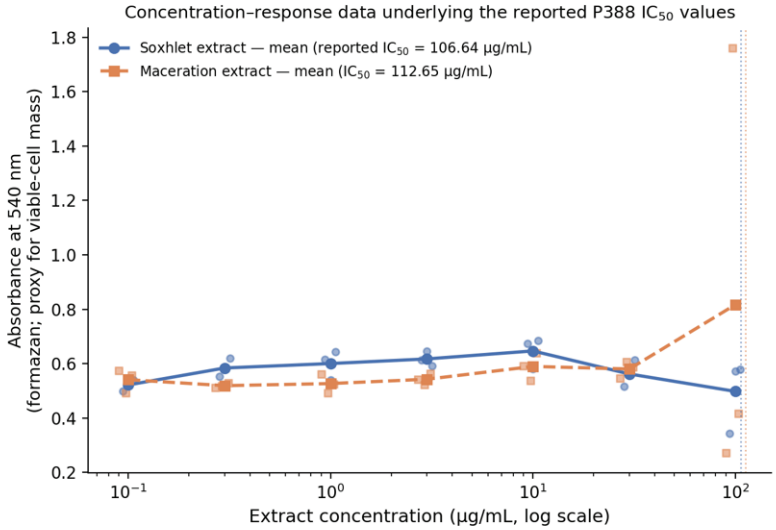


Figure 6. Concentration-response data underlying the reported P388 IC_{50} values for the *T. cacao* pod husk Soxhlet methanol extract (IC_{50}=106.64 µg/mL) and the cold-maceration methanol extract (IC_{50}=112.65 µg/mL). Points show individual triplicate absorbance readings at 540 nm; lines connect the concentration-wise means. Vertical dotted lines mark the corresponding IC_{50} values, previously determined by logarithmic regression (OriginPro 8.0). Both IC_{50} values lie above the highest concentration tested (100 ug/mL) and are therefore extrapolated estimates.

DISCUSSION

Pharmacognostic Standardization

The standardization of *T. cacao* pod husk simplicia establishes essential quality benchmarks for biological activity studies. Moisture content of 2.49±0.70% v/w confirmed adequate drying, well below the critical 10% threshold above which microbial proliferation and enzymatic hydrolysis of thermolabile constituents are initiated (Mukherjee, *et al*., 2017). The total ash value (6.185±0.103% w/w) falls within the range commonly reported for dried plant materials, although no compendial limit exists for *T. cacao* pod husk. It represents the sum of physiological minerals—including calcium, magnesium, and potassium—as well as non-physiological contaminants such as silica and soil particles.

The markedly higher water-soluble extractive content (12.47±0.03% w/w) relative to the ethanol-soluble extractive (6.86±0.54% w/w) indicates that the predominant compounds in *T. cacao* pod husk are polar, including flavonoid O-glycosides, hydrophilic polyphenols, and organic acids, consistent with reports on other Malvaceae (formerly Sterculiaceae) species and cocoa-derived matrices (Panche, *et al*., 2016; Tungmunnithum, *et al*., 2018; Fraga, *et al.*, 2019; Tsao, 2010".

Phytochemical Composition

The phytochemical profile of *T. cacao* pod husk—flavonoids, alkaloids, tannins, polyphenols, monoterpenes, sesquiterpenes, and quinones—was consistent across simplicia powder and both methanol extracts (Table 3), confirming that both extraction methods solubilized the same compound classes (Campos-Vega, *et al*., 2018; Nzekoue, *et al.*, 2020). The principal flavonoid constituents of *T. cacao*–catechins, epicatechins, and procyanidins–are potent radical scavengers that neutralize reactive oxygen species via the o-dihydroxyl group on the B-ring and chelate transition metal ions that would otherwise catalyze Fenton-type radical generation (Panche, *et al.*, 2016; Xu, *et al.*, 2019; Procházková, *et al*., 2011; Pietta, 2000). Tannins contribute antioxidant activity through protein precipitation and iron chelation, while the alkaloid theobromine (~0.4% dry weight) and the quinones detected here have been separately linked to cardiovascular, anti-inflammatory, and redox-active biological effects (Smeriglio, *et al*., 2017; Oo, *et al*., 2021; Bautista-Expósito, *et al.*, 2021; Aniszewski, 2015; Martínez-Pinilla, *et al*., 2015; Khader and Eckl, 2014; El-Far, *et al.*, 2018). Collectively, this multi-class phytochemical composition provides a plausible chemical basis for the cellular antioxidant activity observed in this study.

Extraction Efficiency and Thin-Layer Chromatography (TLC) Fingerprinting

The Soxhlet method yielded a higher extraction efficiency (21.59%) than cold maceration (19.46%), consistent with the thermodynamic advantage of continuous hot-solvent extraction for matrices with thick-walled, lignified cell structures such as cacao pod husk: continuous renewal of fresh solvent maintains a high concentration gradient, while moderate heating enhances solubility and facilitates solvent penetration through the cell wall (Do, *et al*., 2014; Chemat, *et al.*, 2012; Spigno, *et al.*, 2007). Although both methods solubilized the same compound classes (Table 3), the higher yield obtained by Soxhlet extraction supported its selection for subsequent testing.

TLC fingerprinting identified a flavonoid-class compound (R_f 0.21, showing yellow-green fluorescence under UV 366 nm after $AlCl_3$ spraying) as the major UV-active constituent of the Soxhlet extract. This fluorescence pattern is consistent with chelate-complex formation between Al^{3+} ions and the carbonyl/hydroxyl groups of flavonol–or flavone–type compounds, and the R_f value obtained is consistent with a moderately polar flavonoid aglycone or partially acylated glycoside (Stalikas, 2007; Saeed, *et al.*, 2012).

Cellular Antioxidant Activity: Malondialdehyde (MDA) and Superoxide Dismutase (SOD) as Biomarkers

The *ex vivo* H_2O_2-induced erythrocyte model used here is a well-established, physiologically relevant system for evaluating cellular antioxidant capacity, since erythrocytes are particularly susceptible to oxidative injury owing to their haemoglobin-iron content, their polyunsaturated-fatty-acid-rich membranes, and their inability to repair oxidative damage once it occurs (Kurata, *et al.*, 1993; Santi, *et al*., 2011; Niki, 2009; Frei, *et al.*, 1988; Mhaidat, *et al*., 2017). The marked elevation of MDA and depression of SOD activity in the negative control confirmed successful induction of oxidative stress, while the concentration-related reduction in MDA and restoration of SOD activity by the extract indicate that its constituents can intercept the lipid peroxidation chain reaction and support the enzymatic antioxidant defense system (Tsikas, 2017; Yin, *et al*., 2011; Ayala, *et al.*, 2014; Fukai and Ushio-Fukai, 2011; Wang, *et al*., 2018; Ighodaro and Akinloye, 2018).

Optimal Concentration and Dose-Response Analysis

The non-linear, hormetic dose–response relationship observed—with test group 2 (53.8 µg/mL) outperforming both the lower (5.38 µg/mL) and higher (538 µg/mL) test concentrations—is a well-documented phenomenon with plant polyphenols. At the intermediate concentration, the combined action of multiple antioxidant classes (flavonoids, polyphenols, tannins) is thought to achieve maximal ROS interception without mutual interference, consistent with the phytochemical-synergy hypothesis (Liu, 2004; Arts Hollman, 2005). At the highest concentration tested (538 µg/mL), the reduced antioxidant response is consistent with the known prooxidant–antioxidant duality of polyphenols at high doses: at elevated concentrations, phenolic compounds can reduce Fe^{3+} to Fe^{2+}, generating Fenton-reactive iron, and may also interfere with the epinephrine-based SOD assay itself (Galati O'Brien, 2004; Lambert, *et al.*, 2007). This concentration-dependent behavior has practical implications for selecting an optimal therapeutic concentration in future *in vivo* work, and should be confirmed with formal statistical comparison (*e.g.*, one-way ANOVA with post-hoc testing) among groups, which was not performed in the present study.

Cytotoxic Activity Against P388 Leukemia Cells

The extrapolated IC_{50} of 106.64 ug/mL (>100 ug/mL) obtained againstP388 murine leukemia cells classifies the crude Soxhlet methanol extract as inactive by the widely applied threshold of IC_{50}>30 µg/mL for crude plant extracts (Vichai and Kirtikara, 2006; Lichota Gwozdzinski, 2018; Gordaliza, 2007). This modest result should be interpreted in the context of the P388 model's aggressive growth profile and the fact that, in a crude extract, bioactive constituents are diluted by pharmacologically inert matrix components such as polysaccharides, chlorophylls, and waxes (Newman Cragg, 2020; Atanasov, *et al*., 2021; Harvey, *et al*., 2015). The flavonoid, tannin, alkaloid, and quinone constituents identified here have each been separately associated with antiproliferative mechanisms in leukemia cell lines—including cell cycle arrest, caspase activation, topoisomerase inhibition, and metalloproteinase suppression—in previous reports on structurally related compounds (Smeriglio, *et al*., 2017; Oo, *et al.*, 2021; Bautista-Expósito, *et al*., 2021; Aniszewski, 2015; Khader Eckl, 2014; El-Far, *et al*., 2018; Russo, *et al*., 2017; Murakami, *et al.*, 2008; Meiyanto, *et al*., 2012; Spagnuolo, *et al.*, 2018; Othman, *et al.*, 2007; Elmore, 2007; Hassan, *et al.*, 2014; Evan Vousden, 2001). Although these mechanisms cannot be confirmed from the present crude extract data alone, they provide a plausible rationale, together with the weak activity observed, for pursuing bioactivity-guided fractionation in future work,

potentially in combination with sub-therapeutic doses of doxorubicin to explore whether cardiotoxic dosing could be reduced (Meyer, *et al*., 1982; Cragg Pezzuto, 2016; Thakkar, *et al*., 2013; Yin, *et al*., 2013).

Implications for Agro-Industrial Waste Valorization

Beyond its pharmacological significance, the demonstrated cellular antioxidant activity of *T. cacao* pod husk extract underscores the broader potential of this agro-industrial by-product as a source of value-added bioactive ingredients. Indonesia is one of the world's largest cocoa producers, generating hundreds of thousands of tons of pod husks annually that are currently discarded or used as low-value animal feed (Campos-Vega, *et al.*, 2018; Okiyama, *et al.*, 2017). Valorization of pod husk as a pharmaceutical raw material or nutraceutical ingredient would contribute to sustainable bioeconomy goals, reduce environmental waste, and create economic value for smallholder farmers, in line with circular bioeconomy frameworks increasingly adopted by the food and pharmaceutical industries (Lim, 2013; Grasso, *et al.*, 2014; Dai Mumper, 2010; Rifai Saleh, 2020).

CONCLUSION

This study demonstrates that *T. cacao* pod husk is a pharmacognostically characterized, phytochemically rich agro-industrial by-product with notable cellular antioxidant activity. The Soxhlet methanol extract (yield 21.59%) contained flavonoids, alkaloids, tannins, polyphenols, monoterpenes, sesquiterpenes, and quinones. At 53.8 µg/mL, the extract reduced MDA levels to a near-normal value (0.878 nmol/mL) and increased SOD activity to 14.8493 units/mL in H_2O_2 induced human erythrocytes, values higher than those obtained with the quercetin comparator, although this difference was not confirmed by formal statistical testing. In contrast, the crude extract showed weak direct cytotoxicity against P388 murine leukemia cells (IC_{50} >100 ug/mL; extrapolated estimate 106.64 ug/mL; inactive by established criteria), which may reflect dilution of any active constituents within the crude matrix. Taken together, these findings support *T. cacao* pod husk primarily as a source of cellular antioxidant activity, with its modest basal cytotoxicity providing a rationale for future bioactivity-guided —fractionation including liquid–liquid partitioning, chromatographic isolation, and testing of isolated fractions against a panel of human leukemia cell lines—to identify and concentrate the constituents responsible for its anticancer potential.

ACKNOWLEDGMENT

The authors thank the Herbarium Bandungense, Institut Teknologi Bandung, for botanical verification of the plant specimen. The authors acknowledge the technical support provided by the Laboratory of Pharmacy, Faculty of Pharmacy, Universitas Jenderal Achmad Yani, and the assistance of community plantation farmers in Desa Puspahiang, Tasikmalaya. This research received no specific external funding.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Aniszewski, T., 2015, *Alkaloids: Chemistry, Biology, Ecology, and Applications,* 3rd ed. Amsterdam: Elsevier.

Armenian, S.H., Lacchetti, C., Barac, A., Carver, J., Constine, L.S., Denduluri, N., *et al*., 2017, Prevention and monitoring of cardiac dysfunction in survivors of adult cancers: American Society of Clinical Oncology clinical practice guideline, *Journal of Clinical Oncology,* **35**(8), 893-911.

Arts, I.C.W. and Hollman, P.C.H., 2005, Polyphenols

and disease risk in epidemiologic studies, *The American Journal of Clinical Nutrition*, **81** (1 Suppl), 317S-325S.

Atanasov, A.G., Zotchev, S.B., Dirsch, V.M., International Natural Product Sciences Taksforce, and Supuran, C.T., 2021, Natural products in drug discovery: advances and opportunities, *Nature Reviews Drug Discovery*, **20**(3), 200-216.

Ayala, A., Muñoz, M.F., and Argüelles, S., 2014, Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal, *Oxidative Medicine and Cellular Longevity,* **2014**, 360438.

Bautista-Expósito, S., Peñas, E., Frias, J., Dueñas, M., and Martínez-Villaluenga, C., 2021, Alkaloids in health and disease: structure-bioactivity relationships and new analytical approaches, *Food Research International*, **150**, 110775.

Bhakta, N., Force, L.M., Allemani, C., Atun, R., Bray, F., Coleman, M.P., *et al.*, 2019, Childhood cancer burden: a review of global estimates, *The Lancet Oncol*ogy, **20**(1), e42-e53.

Bray, F., Laversanne, M., Sung, H., Ferlay, J., Siegel, R.L., Soerjomataram, I., *et al.*, 2024, Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, *CA: a Cancer Journal for Clinicians*, **74**(3), 229-263.

Campos-Vega, R., Nieto-Figueroa, K.H., and Oomah, B.D., 2018, Cocoa (*Theobroma cacao* L.) pod husk: renewable source of bioactive compounds, *Trends in Food Science & Technology*, **81**, 172-184.

Chemat, F., Vian, M.A., and Cravotto, G., 2012, Green extraction of natural products: concept and principles, *International Journal of Molecular Sciences*, **13**(7), 8615-8627.

Cragg, G.M. and Pezzuto, J.M., 2016, Natural products as a vital source for the discovery of cancer chemotherapeutic and chemopreventive agents, *Medical principles and practice*, **25**(Suppl 2), 41-59.

Crozier, S.J., Preston, A.G., Hurst, J.W., Payne, M.J., Mann, J., Hainly, L., *et al.*, 2011, Cacao seeds are a 'Super Fruit': a comparative analysis of various fruit powders and products, *Chemistry Central Journal*, **5**, 5.

Dai, J. and Mumper, R.J., 2010, Plant phenolics: extraction, analysis and their antioxidant and anticancer properties, *Molecules*, **15**(10), 7313-7352.

Do, Q.D., Angkawijaya, A.E., Tran-Nguyen, P.L., Huynh, L.H., Soetaredjo, F.E., Ismadji, S., *et al.*, 2014, Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of Limnophila aromatica, *Journal of Food and Drug Analysis,* **22**(3), 296-302.

El-Far, A.H., Al Jaouni, S.K., Li, W., and Mousa, S.A., 2018, Protective roles of thymoquinone by inhibiting VEGF/VEGFR-2 signaling pathway in human hepatocellular carcinoma cells, *Environ Sci Pollut Res Int.*, **25**(28), 27899-27908.

Elmore, S., 2007, Apoptosis: a review of programmed cell death, *Toxicologic Pathology*, **35**(4), 495-516.

Evan, G.I. and Vousden, K.H., 2001, Proliferation, cell cycle and apoptosis in cancer, *Nature*, **411**(6835), 342-348.

Forman, H.J. and Zhang, H., 2021, Targeting oxidative stress in disease: promise and limitations of antioxidant therapy, *Nature Reviews Drug Discovery*, **20**(9), 689-709.

Fraga, C.G., Croft, K.D., Kennedy, D.O., and Tomas-Barberan, F.A., 2019, The effects of polyphenols and other bioactives on human health, *Food & Function*, **10**(2), 514-528.

Frei, B., Stocker, R., and Ames, B.N., 1988, Antioxidant defenses and lipid peroxidation in human blood plasma, *Proceedings of the National Academy of Sciences of the United States of America*, **85**(24), 9748-9752.

Fukai, T. and Ushio-Fukai, M., 2011, Superoxide dismutases: role in redox signaling, vascular function, and diseases, *Antioxidants & Redox*

Signaling, **15**(6), 1583-1606.

Galati, G. and O'Brien, P.J., 2004, Potential toxicity of flavonoids and other dietary phenolics: significance for their chemopreventive and anticancer properties, *Free Radical Biology & Medicine*, **37**(3), 287-303.

Gordaliza, M., 2007, Natural products as leads to anticancer drugs, *Clinical & Translational Oncology,* **9**(12), 767-776.

Grasso, S., Brunton, N.P., Lyng, J.G., Lalor, F., and Monahan, F.J., 2014, Healthy processed meat products-regulatory, reformulation and consumer challenges, *Trends in Food Science & Technology,* **39**(1), 4-17.

Harvey, A.L., Edrada-Ebel, R., and Quinn, R.J., 2015, The re-emergence of natural products for drug discovery in the genomics era, *Nature Reviews Drug Discovery,* **14**(2), 111-129.

Hassan, M., Watari, H., AbuAlmaaty, A., Ohba, Y., and Sakuragi, N., 2014, Apoptosis and molecular targeting therapy in cancer, *BioMed Research International*, **2014**, 150845.

Hunger, S.P. and Mullighan, C.G., 2015, Acute lymphoblastic leukemia in children, *The New England Journal of Medicine*, **373**(16), 1541-1552.

Ighodaro, O.M. and Akinloye, O.A., 2018, First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): their fundamental role in the entire antioxidant defence grid, *Alexandria Journal of Medicine*, **54**(4), 287-293.

Jomova, K., Raptova, R., Alomar, S.Y., Alwasel, S.H., Nepovimova, E., Kuca, K., *et al.*, 2023, Reactive oxygen species, toxicity, oxidative stress, and antioxidant: chronic, diseases and aging, *Archives of Toxicology*, **97**(10), 2499-2574.

Khader, M. and Eckl, P.M., 2014, Thymoquinone: an emerging natural drug with a wide range of medical applications, *Iranian Journal of Basic Medical Sciences,* **17**(12), 950-957.

Kurata, M., Suzuki, M., and Agar, N.S., 1993, Antioxidant systems and erythrocyte life-span in mammals, *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*, **106**(3), 477-487.

Lambert, J.D., Sang, S., and Yang, C.S., 2007, Possible controversy over dietary polyphenols: benefits vs risks, *Chemical Research in Toxicology*, **20**(4), 583-585.

Lichota, A. and Gwozdzinski, K., 2018, Anticancer activity of natural compounds from plant and marine environment, *International Journal of Molecular Sciences*, **19**(11), 3533.

Lim, T.K., 2013, *Edible Medicinal and Non-Medicinal Plants*. Vol. 5. Dordrecht: Springer.

Liu, R.H., 2004, Potential synergy of phytochemicals in cancer prevention: mechanism of action, *The Journal of Nutrition,* **134**(12 Suppl), 3479S-3485S.

Maleyki, M.J.A. and Ismail, A., 2010, Antioxidant properties of cocoa powder, *Journal of Food Biochemistry,* **34**(1), 111-128.

Martínez-Pinilla, E., Oñatibia-Astibia, A., and Franco, R., 2015, The relevance of theobromine for the beneficial effects of cocoa consumption, *Frontiers in Pharmacology*, **6**, 30.

Meiyanto, E., Hermawan, A., and Anindyajati, 2012, Natural products for cancer-targeted therapy: citrus flavonoids as potent chemopreventive agents, *Asian Pacific Journal of Cancer Prevention*, **13**(2), 427-436.

Meyer, B.N., Ferrigni, N.R., Putnam, J.E., Jacobsen, L.B., Nichols, D.E., and McLaughlin, J.L., 1982, Brine shrimp: a convenient general bioassay for active plant constituents, *Planta Medica*, **45**(5), 31-34.

Mhaidat, N.M., Abu-Zaiton, A.S., Alzoubi, K.H., *et al.*, 2017, Antioxidant properties of raw and cooked lentils on oxidative damage in human erythrocytes *in vitro*, *Journal of Clinical and Diagnostic Research*, **11**(4), BC14-BC17.

Mukherjee, P.K., Harwansh, R.K., Bahadur, S., Banerjee, S., Kar, A., Chanda, J., *et al.*, 2017, Development of Ayurveda-tradition to trend,

Journal of Ethnopharmacology, **197**, 10-24.

Murakami, A., Ashida, H., and Terao, J., 2008, Multitargeted cancer prevention by quercetin, *Cancer Letters,* **269**(2), 315-325.

Newman, D.J. and Cragg, G.M., 2020, Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019, *Journal of Natural Products*, **83**(3), 770-803.

Niki, E., 2009, Lipid peroxidation: physiological levels and dual biological effects, *Free Radical Biology & Medicine*, **47**(5), 469-484.

Nzekoue, F.K., Scheili, E., Caprioli, G., *et al.*, 2020, Cocoa bean shells (*Theobroma cacao* L.): going from a food by-product to a health-promoting ingredient, *Food Chemistry*, **310**, 125948.

Okiyama, D.C.G., Navarro, S.L.B., and Rodrigues, C.E.C., 2017, Cocoa shell and its compounds: applications in the food industry, *Trends in Food Science & Technology*, **63**, 103-112.

Oo, T.N., Smith, T.J., and Bhatt, D.L., 2021, Tannins and their bioactivities, *Molecules,* **26**(8), 2178.

Othman, A., Ismail, A., Ghani, N.A., and Adenan, I., 2007, Antioxidant capacity and phenolic content of cocoa beans, *Food Chemistry*, **100**(4), 1523-1530.

Panche, A.N., Diwan, A.D., and Chandra, S.R., 2016, Flavonoids: an overview, *Journal of Nutritional Science*, **5**, e47.

Pietta, P.G., 2000, Flavonoids as antioxidants, *Journal of Natural Products*, **63**(7), 1035-1042.

Pizzino, G., Irrera, N., Cucinotta, M., Pallio, G., Mannino, F., Arcoraci, V., *et al.*, 2017, Oxidative stress: harms and benefits for human health, *Oxidative Medicine and Cellular Longevity*, **2017**, 8416763.

Prayong, P., Barusrux, S., and Weerapreeyakul, N., 2008, Cytotoxic activity screening of some indigenous Thai plants, *Fitoterapia*, **79** (7-8), 598-601.

Procházková, D., Boušová, I., and Wilhelmová, N., 2011, Antioxidant and prooxidant properties of flavonoids, *Fitoterapia*, **82**(4), 513-523.

Pui, C.H., Yang, J.J., Bhakta, N., and Rodriguez-Galindo, C., 2018, Global efforts toward the cure of childhood acute lymphoblastic leukaemia, *The Lancet Child & Adolescent Health,* **2**(6), 440-454.

Rifai, L. and Saleh, F.A., 2020, A review on acrylamide in food: occurrence, toxicity, and mitigation strategies, *International Journal of Toxicology*, **39**(2), 93-102.

Riss, T.L., Moravec, R.A., Niles, A.L., Duellman, S., Benink, H.A., Worzella, T.J., *et al.*, 2016, Cell viability assays. In: Sittampalam GS, *et al.*, eds. Assay Guidance Manual. Bethesda: Eli Lilly/NIH.

Rojo de la Vega, M., Krajisnik, A., Zhang, D.D., and Wondrak, G.T., 2017, Targeting NRF2 for improved skin barrier function and photoprotection: Focus on the achiote-derived apocarotenoid bixin, *Nutrients*, **9**(12), 1371.

Russo, M., Milito, A., Spagnuolo, C., Carbone, V., Rosen, A., Monasi, P., *et al.*, 2017, CK2 and PI3K are direct molecular targets of quercetin in chronic lymphocytic leukaemia, *Oncotarget,* **8**(26), 42571-42587.

Saeed, N., Khan, M.R., and Shabbir, M., 2012, Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts Torilis leptophylla L., *BMC Complementary and Alternative Medicine*, **12**, 221.

Santi, A., Menezes, C., Duarte, M.M. F., Leitemperger, J., Lopes, T., and Loro, V.L., 2011, Oxidative stress biomarkers and acetylcholinesterase activity in human erythrocytes exposed to clomazone *in vitro*, *Interdisciplinary Toxicology*, **4**(3), 149-153.

Schieber, M. and Chandel, N.S., 2014, ROS function in redox signaling and oxidative stress, *Current Biology,* **24**(10), R453-R462.

Smeriglio, A., Barreca, D., Bellocco, E., and Trombetta, D., 2017, Proanthocyanidins and hydrolysable tannins: occurrence, biochemistry and biological activity, *Phytochemistry Reviews*, **16**(2), 337-354.

Spagnuolo, C., Moccia, S., and Russo, G.L., 2018, Anti-inflammatory effects of flavonoids in

neurodegenerative disorders, *European Journal of Medicinal Chemistry*, **153**, 105-115.

Spigno, G., Tramelli, L., and De Faveri, D.M., 2007, Effects of extraction time, temperature and solvent on concentration and antioxidant activity of grape marc phenolics, *Journal of Food Engineering*, **81**(1), 200-208.

Stalikas, C.D., 2007, Extraction, separation, and detection methods for phenolic acids and flavonoids, *Journal of Separation Science*, **30**(18), 3268-3295.

Steliarova-Foucher, E., Colombet, M., Ries, L.A.G., Moreno, F., Dolya, A., Bray, F., *et al.*, 2017, International incidence of childhood cancer, 2001-10: a population-based registry study, *The Lancet. Oncology*, **18**(6), 719-731.

Thakkar, A., Sutaria, D., Grandhi, B.K., Wang, J., and Prabhu, S., 2013, The molecular mechanism of action of aspirin, curcumin and sulforaphane combinations in the chemoprevention of pancreatic cancer, *Oncology Reports*, **29**(4), 1671-1677.

Tsao, R., 2010, Chemistry and biochemistry of dietary polyphenols, *Nutrients*, **2**(12), 1231-1246.

Tsikas, D., 2017, Assessment of lipid peroxidation by measuring malondialdehyde (MDA) and relatives in biological samples: analytical and biological challenges, *Analytical Biochemistry*, **524**, 13-30.

Tungmunnithum, D., Thongboonyou, A., Pholboon, A., and Yangsabai, A., 2018, Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: an overview, *Medicines*, **5**(3), 93.

Vichai, V. and Kirtikara, K., 2006, Sulforhodamine B colorimetric assay for cytotoxicity screening, *Nature Protocols*, **1**(3), 1112-1116.

Wang, Y., Branicky, R., Noë, A., and Hekimi, S., 2018, Superoxide dismutases: dual roles in controlling ROS damage and regulating ROS signaling, *The Journal of Cell Biology*, **217**(6), 1915-1928.

Xu, D., Hu, M.J., Wang, Y.Q., and Cui, Y.L., 2019, Antioxidant activities of quercetin and its complexes for medicinal application, *Molecules*, **24**(6), 1123.

Yin, H., Xu, L., and Porter, N.A., 2011, Free radical lipid peroxidation: mechanisms and analysis, *Chemical Reviews*, **111**(10), 5944-5972.

Yin, S.Y., Wei, W.C., Jian, F.Y., and Yang, N.S., 2013, Therapeutic applications of herbal medicines for cancer patients, *Evidence-Based Complementary and Alternative Medicine*, **2013**, 302426.

Zamorano, J.L., Lancellotti, P., Muñoz, D.R., Aboyans, V., Asteggiano, R., Galderisi, M., *et al.*, 2016, ESC Position Paper on cancer treatments and cardiovascular toxicity developed under the auspices of the ESC Committee for Practice Guidelines: The Task Force for cancer treatments and cardiovascular toxicity of the European Society of Cardiology (ESC), *European Heart Journal*, **37**(36), 2768-2801.