Food Research 8 (3) : 62 - 72 (June 2024)
Journal homepage: https://www.myfoodresearch.com

FOOD RESEARCH

RESEARCH PAPER

Antioxidant and toxicity activities of kayu manis hutan (*Cinnamomum iners* Reinw. Ex Blume Laureceae) leaf extracts

[1]Padzil, K.N.M., [1,2,*]Rukayadi, Y., [1]Abas, F., [1,4]Sanny, M. and [3,4]Abdul-Mutalib, N.A.

[1]*Department of Food Science and Technology, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia*
[2]*Natural Medicine and Product Research (NaturMeds) Laboratory, Institute of Bioscience, Universiti Putra Malaysia, 43400, Serdang, Selangor, Malaysia*
[3]*Department of Food Service and Management, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia*
[4]*Laboratory of Food Safety and Food Integrity, Institute of Tropical Agriculture and Security, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia*

Article history:
Received: 31 January 2023
Received in revised form: 9 March 2023
Accepted: 25 November 2023
Available Online: 8 May 2024

Keywords:
Antioxidant activity,
Cinnamomum iners,
Medicinal plant,
Toxicity activity

DOI:
https://doi.org/10.26656/fr.2017.8(3).365

Abstract

Cinnamomum iners Reinw. ex Blume Laureceae which is known in Malaysia as "kayu manis" has a wide range of traditional applications for certain plant parts, including the leaves, bark, and stem and it has pharmacological features, including anti-inflammatory and antibacterial activity. Unfortunately, there is a lack of reports regarding the antioxidant and toxicity activities of ethanolic *C. iners* crude leaf extracts. The objective of the study was to determine the total phenolic content and its antioxidant activities of ethanolic *C. iners* crude leaf extracts from two different age maturity, as well as its toxicity activity. The extracts were tested for total phenolic content and its antioxidant activities in terms of 2,2-diphenyl-1-picryllhydrazyl (DPPH) free radical scavenging- and ferric reducing antioxidant power (FRAP) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay. The toxicity assay was tested using brine shrimp (*Artemia salina*) lethality assay. The total phenolic content of the extracts for young and old leaves *C. iners* resulted in 248.06±12.77 and 351.11±8.62 mg GAE/g dw, respectively. The DPPH radical scavenging showed results for young and old leaf extracts were IC_{50} of 31.92 µg/mL and 27.62 µg/mL, respectively. Meanwhile, the value of FRAP for young and old leaf extracts were 8.574±0.21 and 9.155±0.36 TEAC mg/g dw, respectively. The ABTS antioxidant activity for the young and old leaf extracts were 305.23±7.02 and 316.86±8.22 Trolox equivalence mg/g dw, respectively. Based on the toxicity assay, both young and old leaf extracts exhibited LC_{50} at 19.627 and 13.201 mg/mL, respectively. The findings indicate that *C. iners* leaves crude extracts have exhibited high total phenolic content and antioxidant activity and the extracts were not toxic against brine shrimp. Thus, *C. iners* leaf extracts could be developed as potential natural antioxidant agents.

1. Introduction

Highly reactive chemicals called ROS are produced by living organisms as a result of normal cellular metabolism and environmental factors, such as air pollutants or cigarette smoke (Madkour, 2019) and possess the ability to harm components of cells, including proteins, lipids, carbohydrates, and nucleic acids, and change how those components function (Birben *et al.*, 2012; Sireesha and Rao, 2015). Reactive species (RS) are a general term that refers to a variety of species, some of which are free radicals, including reactive oxygen species (ROS), reactive sulphur species (RSS), reactive nitrogen species (RNS), and others. Each of these species has the potential to result in oxidative stress because their accumulation within the cell exceeds its ability to remove them (Dumanovic *et al.*, 2021). An example of reactive molecules such as hydroxyl radical (OH), superoxide anion (O_2^-), hydroxyl ion (OH^-), hydrogen peroxide (H_2O_2) and nitric oxide (NO) are respectively produced from molecular oxygen, free radicals and non-radical molecular forms (Sanchez, 2017).

Oxidative stress is a status of imbalance between antioxidants and oxidants following an interruption of the redox equilibrium resulting in molecular damage as a

*Corresponding author.
Email: *yaya_rukayadi@upm.edu.my*

eISSN: 2550-2166
Published by Rynnye Lyan Resources

consequence of the increased level of ROS within the cell (Jiang *et al.*, 2021). Neurological disorders, cancer, hypertension, atherosclerosis, diabetes, ischemia/ perfusion, acute respiratory distress syndrome, idiopathic pulmonary fibrosis, asthma and chronic obstructive pulmonary disease, are just a few pathological conditions and diseases that oxidative stress may be involved (Unsal *et al.*, 2020). Cell survival, activation, proliferation, and organ function all depend on control of the reducing and oxidising (redox) state of ROS (Salazar-Ramiro *et al.*, 2016).

Plants produce plant secondary metabolites, or phytochemicals, for a variety of purposes, such as UV protection, protection against infections and herbivores, boosting pollination chances with pigmentation, and improving the plant to be healthier and more resilient without directly compromising key functions like growth and reproduction (Tan and Lim, 2015). For example, phenolic, flavonoids, alkaloid and terpenoid compounds represent groups of plant secondary metabolites which are particularly significant since they may neutralise free radicals and serve as antioxidants (Ifeanyi, 2018), antimicrobial, antiviral (Ozcelik *et al.*, 2011), anticarcinogenic (Correa and Couto, 2016) and pharmacological effects (Al-Snafi, 2019).

Antioxidants, which are molecules that can slow down or stop other molecules from oxidising, protect cells through a number of mechanisms, such as the transformation of ROS into non-radical species, stopping the auto-oxidative chain reaction that ROS started, and lowering localised oxygen concentrations (Lourenco *et al.*, 2019). The cell's antioxidants cooperate to keep the body's redox equilibrium at its ideal level. This equilibrium is essential for creating the circumstances needed for cell signalling, which is crucial for regulating the expression of numerous genes, stress adaption, and cell homeostasis stability (Surai *et al.*, 2019). The intake of exogenous antioxidants, such as α-tocopherol, ascorbic acid, carotenoids, polyphenolic and flavonoids, which prevent free radical damage can be found in commonly consumed fruits, vegetables, as herbs, spices, seeds, beverages, cereals and other food products, may support the antioxidative defence (Rekha *et al.*, 2012).

The brine shrimp lethality assay is a crucial method for the early cytotoxicity assessment of plant extracts and other compounds based on their ability to kill lab-cultured larvae (nauplii). The nauplii were treated with various plant extract concentrations for 24 h (Sarah *et al*., 2017). The larvae (nauplii; singular nauplius), approximately 22 mm in length, are both big enough to be visible without a magnifying lens and small enough to hatch in a good amount of numbers without taking up much space in a lab. The efficacy of the extract was determined by counting the number of motile nauplii (Hamidi *et al.*, 2014). Lately, the cytotoxic action of bioactive compounds has been frequently evaluated using the lethality assay on brine shrimp (*Artemia salina*) (Al-Zabt *et al*., 2022). It is an early test for toxicity screening of plant extracts, heavy metals, fungal toxins, pesticides, cyanobacteria, and cytotoxicity testing of dental material (Suryawanshi *et al.*, 2020). It is an easy test to do since it does not require aseptic procedures, is economical, can readily use a large number of organisms for statistical validation, doesn't need specialised equipment, and only needs a small quantity of material (2-20 mg or less) (Sarah *et al.*, 2017).

Cinnamomum iners Reinw. Ex Blume Lauraceae is one of the 250 species from the genus Cinnamomum, belongs to the family Lauraceae, commonly known as wild cinnamon or ‘kayu manis hutan’ is one of the potential natural plants that has been traditionally used in treating different illnesses, adding flavour to food, and other products worldwide (Wahab *et al*., 2020). It is a small to average evergreen tree originating from Sri Lanka and is distributed widely in India, Malaysia, China, Philippines, Thailand and Indonesia (Arifullah *et al.*, 2014). There are ethnobotanical reports on *C. iners* reported by Udayaprakash *et al.* (2015), which provides medicinal and pharmacological properties of leaves, roots, stem bark and wood. *Cinnamomum iners* were previously reported to possess antimicrobial (Mustaffa *et al.*, 2011), antioxidant, antifungal (Anis *et al.*, 2012) and analgesic activity (Annegowda *et al.*, 2012) which have contributed to its medicinal value and potential in the treatment of various diseases. Hence, the research was carried out to evaluate the total phenolic content, antioxidants activities and the toxicity of ethanolic kayu manis hutan (*C. iners*) crude leaf extracts from two age leaves maturity in terms of 2,2-diphenyl-1-picryllhydrazyl (DPPH) free radical scavenging, ferric reducing antioxidant power (FRAP), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay and brine shrimp lethality assay.

2. Materials and methods

2.1 Plant material and extraction of Cinnamomum iners leaf

The fresh *C. iners* leaves employed in this study were obtained from Taman Pertanian Universiti, Universiti Putra Malaysia, Malaysia and it was collected in September 2021. The leaf maturity was chosen by the young and old leaves. The plant was identified by Dr. Khairil Mahmod from the Faculty of Agriculture, Universiti Putra Malaysia with the voucher number MY18646. The extraction of *C. iners* was done utilising the soaked method illustrated by Rukayadi *et al.* (2013).

https://doi.org/10.26656/fr.2017.8(3).365

The organic solvent used in the extraction of *C. iners* was ethanol (R and M Chemicals, 99.8%, United Kingdom). The leaves obtained were cleaned under a constant flow of water. The cleaned leaves were oven-dried at 65℃ for 48 hrs and were pulverized into fine powders using a blender (Waring, model 32 BL 80, New Hartford, USA) and approximately 100 g of each young and old leaf were soaked in 400 mL of ethanol at room temperature with occasional shaking for 48 hrs. The Whatman filter paper size No. 2 (Whatman International Ltd., Middlesex, England) was used to vacuum filter the plant extract. The ethanolic *C. iners* crude leaf extracts were then concentrated using a rotary vacuum evaporator (Heidolph VV2011, Schwabach, Germany) at 40°C and 100 revolutions per minute (rpm) for 3–4 hrs. For 48 hrs, the crude extracts were freeze-dried to remove water. The crude extract was then stored in a chiller at 4°C and the stock concentration at 100 mg/mL was prepared for further analysis.

2.2 Antioxidant activity

2.2.1 Total phenolic content

The crude extracts were evaluated for its total phenolic content (TPC) using the method reported by Singleton *et al.* (1999) and Goraya and Bajwa (2015), with slight modification by using 96-well round bottom microwell plates. Approximately 100 mL of 10% Folin–Ciocalteau's reagent (Sigma-Aldrich) (v/v) was mixed with a volume of 20 µL of the crude extract (10 mg/mL) and was let for 5 mins. After that, the solution was neutralized with 80 µL of 7.5% sodium carbonate (Sigma-Aldrich). The reaction mixture will then be incubated for 30 mins at 37°C and the absorbance was measured using microplate reader (UV-1650 PC, Shidmazu, Tokyo, Japan) at 750 nm. Gallic acid was used as a standard curve to obtain the linear equation for TPC determination. The content of the total phenolic compounds of ethanolic *C. iners* crude leaf extracts were calculated with the formula below and was expressed as mg/g gallic acid equivalent (GAE).

$$TPC = \frac{C1 \times V}{m}$$

where TPC = mg GAE/g (Gallic acid equivalent), C1 = gallic acid concentration established from the calibration curve in mg/mL, V = volume of extract in mL, and m = the weight of the plant extract in g.

2.2.2 2,2-diphenyl-1-picrylhydrazyl free radical scavenging assay

The ethanolic *C. iners* crude leaf extracts were evaluated with DPPH (Sigma-Aldrich) radical scavenging assay with slightly modification by using 96-well round bottom microwell plates (Tello *et al.*, 2018). 0.1 mM DPPH was prepared in a dark bottle in methanol. The first column in 96 microwell contained reagent blank (RB) (solvent used to dissolve the sample with and DPPH). The second column in 96 microwell contained sample blank (SB) (sample used and solvent used to dissolve the DPPH). For the third column,96 microwell plates contained the sample (sample and DPPH). The designs stated are for the first row and serial dilution was followed for each column until the last row of 96 microwell plates. The mixture was left to be incubated for 30 mins in a dark room at room temperature. The absorbance was measured using spectrophotometer TECAN, Infinite F200 Pro at 517 nm. IC_{50} is the inhibition concentration (IC) at half the concentration of the inhibition sample required to inhibit 50% of DPPH radical. The inhibition percentage of DPPH discolouration was calculated using the equation:

$$\text{Percentage of inhibition (\%)} = \frac{(\text{Absorbance of the control} - \text{Absorbance of the sample})}{\text{Absorbance of the control}} \times 100$$

2.2.3 Ferric reducing antioxidant power assay

The assay was carried out according to the method proposed by Benzie and Strain (1996) with slight modification (Abeysekera *et al.*, 2019) in 96-well microplate assay. Prior to experiment, the 300 mM acetate buffer (pH 3.6), 10 mM TPTZ solution, and 20 mM $FeCl_3.6H_2O$ (10:1:1 v/v/v) were mixed to create the working reagent for the FRAP assay. This mixture was then heated to 37°C. A solution of 10 mM TPTZ in 40 mM HCl was mixed to create the TPTZ solution. Two hundred microlitre reaction volume comprising of 150 µL of functioning FRAP reagent, 30 µL of acetate buffer, and 20 µL of leaf extracts containing 0.5 mg/mL were incubated at room temperature (30±2°C) for 8 mins and the absorbance was recorded at 700 nm using UV–visible spectrophotometer. Results were expressed as mg of trolox equivalence per gram of dry weight cinnamon leaf.

2.2.4 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) assay

According to Banglao *et al.* (2020), the ABTS radical cation ($ABTS^+$) was prepared by mixing 4 mL of each solution of 7 mM ABTS stock solution and 2.5 mM potassium persulfate. The preparation of 7 mM ABTS stock solution was prepared by dissolving 32 mg of ABTS in 4 mL of distilled water and 2.5 mM potassium persulfate solution was prepared by dissolving 13.2 mg of potassium persulfate in 10 mL of distilled water. Prior to use, both solutions were stored at room temperature in the dark for 12 to 16 hrs. After that, methanol was added to the ABTS+ solution to dilute it until its absorbance at 734 nm was 0.70±0.02. Approximately 100 µL of diluted ABTS+ solution was combined with 100 µL of leaf extracts at concentrations of 62.5, 125, 250, 500, and

https://doi.org/10.26656/fr.2017.8(3).365

1000 µg/mL before being incubated for 15 mins at room temperature in the dark. At a wavelength of 515 nm, the reaction mixture's absorbance was measured, and the results were reported as trolox equivalent antioxidant capacity (TEAC).

2.3 Brine shrimp lethality assay

Toxicity analysis using brine shrimp lethality assay was done according to Wong *et al.* (2021). Commercial sea salt and sterile distilled water were combined in a basin to create an artificial seawater solution (38 g/L) for brine shrimp hatching. Using sodium hydroxide (1 N), the pH of the produced seawater was brought to 8.5. Thereafter, for 48 hrs at room temperature (22°C), dried encysted eggs (50 g) of brine shrimp (*Artemia salina*) were immediately added to the seawater solution. Upon hatching, all nauplii were drawn to the brighter area of the hatching container by a 60-watt table light (SOFTONE, Philips), and the adult nauplii were then collected using a micropipette. Using a sterile saline solution, a two-fold dilution procedure was used to provide a range of saline concentrations between 0.122 and 250 mg/mL. Each test tube received twenty nauplii, which were then incubated for 24 hrs at room temperature (22°C) under light. In contrast, sterile saline water was utilised as the negative control, while potassium dichromate was employed as a positive control with a range of 0.078 to 10.000 mg/mL. Every test tube's number of surviving nauplii were counted and monitored at intervals of 1 hr. The evaluation was done in three separate runs. The formula used to determine the mortality rate of nauplii was calculated as:

$$\text{Percentage of mortality (\%)} = \frac{\text{Number of dead nauplii}}{\text{Total nauplii}} \times 100$$

The sample concentration in logarithms was plotted against the mean mortality percentage (%) on the graph. Based on the presented curve, the lethal concentration (LC_{50}) for 50% of mortality was estimated.

2.4 Statistical data analysis

The data were variance-analyzed (ANOVA) using Minitab software (version 20.0) for Windows (Minitab Inc.) application software to determine the statistically significant differences between the means for each treatment. The significant difference was determined using Tukey's test with a 95% confidence interval ($p<0.05$). Data from repeated analyses are presented as means with standard deviations (SD) using Excel software.

3. Results and discussion

3.1 Yield of Cinnamomum iners leaf extracts

The extracts and preparations of medicinal plants are used for experimental purposes or for direct human consumption as herbal medicine. The idea of preparing medicinal plants for use in experiments include the timely and appropriate harvesting of the plant, professional authentication, suitable drying, and grinding. When applicable, the bioactive ingredient is then extracted, fractionated, and isolated. It also includes measuring the quantity and quality of bioactive substances (Abubakar and Haque, 2020). As it is important to separate and characterise the desired phytochemicals from the plant components, extraction is a crucial initial step in the investigation of herbal/ medicinal plants. To achieve a homogeneous sample and optimise the kinetics of analytical extraction, the process includes steps like pre-washing, drying of plant samples or freeze-drying, and grinding to increase the surface area that is in contact with the solvent system (Fonmboh *et al.*, 2020).

One of the most important among them is the solvent. Antioxidants have been extracted from foods and medicinal plants using a range of solvents. The chemical makeup and polarity of the antioxidant chemicals to be extracted are taken into consideration while choosing the solvents. The majority of the antioxidants found in phenolics, flavanoids, and anthocyanins are hydrosoluble. Water, ethanol, methanol, propanol, acetone, and their aqueous mixes are only a few examples of polar and moderately polar solvents that are frequently employed for extraction (Xu *et al.*, 2017). In this study, the dried *C. iners* young and old leaves were extracted by using ethanol and yielded crude extracts of viscous liquid and dark brown for both extracts. The extraction yields of two extracts are shown in Table 1, where the highest percentage of yield obtained per 100 g of dried *C. iners* leaves was observed in young leaves at 17.46% followed by 11.72% for old leaves.

Table 1. Yield of *C. iners* extract in ethanol with two different leaves maturity.

Leaves maturity	Initial Weight (g)	Final Weight (g)	Yield Recovery (%)
Young	100	17.46	17.46
Old	100	11.72	11.72

Ethanol is considered safe for people to consume as a solvent for natural ingredients used in food and natural medicine. Absolute ethanol and aqueous ethanol have been used as good solvent for polyphenol extraction (Do *et al.*, 2014; Hikmawanti *et al.*, 2021). According to Siddhuraju and Becker (2003), ethanol (70%) was found to be the best solvent for the extraction of antioxidant

https://doi.org/10.26656/fr.2017.8(3).365

compounds from moringa leaves. It has been reported by Altemimi *et al.* (2017) that in comparison to acetone, water, and methanol extract, the ethanolic extracts of Ivorian plants extracted larger levels of phenolics. Also, the same was reported, *Mammea longifolia* buds extracted in both methanol and aqueous ethanol showed antioxidant activity, while aqueous ethanol had a greater level of antioxidant activity.

3.2 Total phenolic content of ethanolic Cinnamomum iners crude leaf extracts

The scavenging role of phenolic compounds helps to slow and limit the synthesis of lipids by inhibiting the growth of oxidative chains. Phenolic compounds are one of the numerous types of phytochemicals with health-promoting properties and activities (Dhalaria *et al*., 2020). Secondary metabolites known as phenolic compounds are abundant throughout the plant kingdom and have complex structures and roles (Swallah *et al.*, 2020). By using the Folin-Ciocalteu method to determine the total phenolic content of two crude extracts was reported as gallic acid equivalent. The total phenolic contents of ethanolic *C. iners* crude leaf extracts for young and old leaves were 248.06±12.77 and 351.11±8.62 mg GAE/g as shown in Table 2. Based on the data collected, the ethanolic *C. iners* crude old leaf extract has higher TPC compared to young leaves. According to Nobosse *et al.* (2018) reported that the *Moringa oleifera* Lam., 60 days old leaf extract was found to have high TPC value of 3.97±0.17 compared to 30 old days young leaf extract with a TPC value of 3.32±0.37. In consensus with the report done by Kristiningrum *et al.* (2016), the total phenolic of Robusta and Arabica coffee leaves was higher in old leaves compared to young leaves. The main factors influencing their antioxidant activity are the ability to form hydrogen bonds, chemical structure, capability of metal ions chelation and reduction, kinetic solvents effect, adduct formation, mechanism of antioxidant reaction reduction potential and capability of antioxidant enzyme activation (Olszowy, 2019).

Table 2. TPC for ethanolic *C. iners* crude leaf extracts

Ethanolic *C. iners* leaf extract	Total phenolic content (mg GAE/g extracts)
Young leaf	248.06±12.77^{B}
Old leaf	351.11±8.62^{A}

Values are presented as mean±SD of replications (n = 3×2). Values with different superscripts are statistically significantly different at p≤0.05 using Tukey's range test between different leaf of the same solvent used. GAE: Gallic acid equivalent.

3.3 Antioxidant activity of ethanolic Cinnamomum iners crude leaf extracts

3.3.1 2,2-diphenyl-1-picrylhydrazyl (radical scavenging assay

The DPPH method is a simple, fast, sensitive, and reproducible method for testing antioxidant activity, where DPPH is a free radical that is stable at room temperature and is often used to assess the antioxidant activity of plant extracts or compounds (Baliyan *et al.*, 2022; Khairunnisa *et al*., 2022). It is estimated that the amount of extracts dispersed in ethanol will inhibit the DPPH radical by 50%. This shows that the value of IC_{50} decreases with increasing antioxidant activity (Anis *et al.*, 2012). An antioxidant is a substance which is capable of slowing down the rate of oxidation in an autoxidizable material or neutralising free radicals. The word "antioxidant" originally referred particularly to a substance that stops oxygen from being consumed. Antioxidant substances are crucial for protecting health (Kebede and Emire, 2019).

The potential of natural antioxidants to neutralise free radicals is frequently assessed using the DPPH radical scavenging technique. The colour of DPPH, a stable nitrogen-based free radical, changes from violet to yellow when it is reduced by either the mechanism of hydrogen- or electron-transfer. Antioxidants and therefore, radical scavengers are substances that can carry out this process (Adjimani and Asare, 2015). By supplying hydrogen atoms or by donating electrons, phenolic compounds with antioxidant properties can quench DPPH through a free-radical attack on the DPPH molecule, turning it into a colourless or bleached product (i.e., 2,2-diphenyl-1-hydrazine or substituted analogous hydrazine), a stable diamagnetic molecule (Mathew *et al.*, 2015).

At different stages of maturation, ethanolic crude leaf extracts from *C. iners* demonstrated significant DPPH free radical scavenging activity. In Table 3, the result showed the calculation of the inhibitory concentration and the IC_{50}. The crude extracts of young and old leaves showed significant DPPH radical scavenging activity, with inhibition of 88.91±2.58 and

Table 3. DPPH free radical scavenging assay for ethanolic *C. iners* crude leaf extracts.

Ethanolic *C. iners* crude extract	Percentage of inhibition (%)	IC_{50} value (µg/mL)
Young leaf	88.91±2.58^{B}	31.92
Old leaf	90.08±3.61^{B}	27.62
Trolox	94.04±0.13^{A}	2.18

Values are presented as mean±SD of replications (n = 3×2). Values with different superscripts are statistically significantly different at p≤0.05 using Tukey's range test between different leaf of the same solvent used.

https://doi.org/10.26656/fr.2017.8(3).365

90.08±3.61% at 10 mg/mL, respectively. With regard to IC_{50} value, both young (31.92 µg/mL) and old leaves (27.62 µg/mL) crude extracts exhibited lower antioxidant activity than standard Trolox (2.18 µg/mL). According to Udayaprakash *et al.* (2015), the antioxidant activity of methanolic *C. iners* leaves showed better free radical scavenging activity at 15 µg/mL due to the presence of strong biologically active components such as phenols and polyphenols which play an important role against free radicals. The majority of the compounds evaluated in this study's DPPH assay were easy and quick methods for determining their antioxidant activity. It was found that the old leaves crude extract exhibited slightly higher antioxidant activity compared to young leaf extracts.

3.3.2 Ferric reducing antioxidant power assay

From a medical perspective, Fe^{3+} has a significant impact on a number of damaging oxidation processes that occur within the human body. Ferric-reducing antioxidant power (FRAP), a colorimetric approach that makes use of antioxidants' capacity to reduce the colourless Fe^{3+} to Fe^{2+}, is one of the techniques used to evaluate the reduction capacity. In an acidic media, the colorless $[Fe^{3+}\text{-}(2,4,6\text{-Tris}(2\text{-pirydyl})\text{-s-triazine})_2]^{3+}$ transforms into the intensely blue-colored complex of $[Fe^{2+}\text{-}(TPTZ)_2]^{2+}$ (Wojtunik-Kulesza, 2020). The changes in absorption can be detected at 593 nm at low pH. As a result, the change in absorbance is directly correlated with the reaction mixture's total or combined reducing power of the antioxidants that donate electrons (Sherikar and Mahanthesh, 2015). In this test, standard solutions were used to obtain standard curves, which were $FeSO_4.7H_2O$ and trolox. FRAP value of ethanolic *C. iners* crude leaf extracts was calculated and it was found to be 8.574±0.21 TEAC mg/g dw for young leaves and 9.155±0.36 TEAC mg/g dw for old leaves as stated in Table 4. The FRAP value of the ethanolic *C. iners* crude leaf extracts was significantly lower than trolox standard. FRAP assay is widely used in the evaluation of the antioxidant component in dietary polyphenols (Abdelwahab *et al.*, 2017). The FRAP test is affordable, the preparation of the reagents is easy, the outcomes are highly repeatable, and the process is simple and quick.

Table 4. FRAP assay for ethanolic *C. iners* crude leaf extracts.

Ethanolic *C. iners* leaf extract	Trolox Equivalence (mg/g dw sample)
Young leaf	$8.574±0.21^{B}$
Old leaf	$9.155±0.36^{A}$

Values are presented as mean±SD of replications (n = 3×2). Values with different superscripts are statistically significantly different at p≤0.05 using Tukey's range test between different leaf of the same solvent used.

3.3.3 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay

ABTS free radical scavenging test or trolox equivalent antioxidant capacity (TEAC) is a test method that mainly studies the electron transfer of antioxidants. It is based on antioxidants' ability to scavenge the ABTS radical cation, which is produced when ABTS is oxidised. In the ABTS assay, the activity of the sample extracts that have been examined is given as the millimolar equivalent of the Trolox solution, which has an antioxidant capacity equal to 1 g of the extract's dry matter (Yang *et al.*, 2012). As shown in Table 5, the ABTS value of ethanolic *C. iners* crude young and old leaf extract was calculated and it was found to be 305.23±7.02 and 316.86±8.22 trolox equivalence (mg/gw), respectively. In addition, the percentage of inhibition for both young and old leaves from ethanolic *C. iners* crude leaf extracts were 90.58±1.33% and 92.71±0.17%, respectively. According to a report done by Brodowska *et al.* (2016), the *Cinnamomum cassia* bark extracts and essential oil showed high ABTS values of 251.22±0.07 and 238.87±0.32 TEAC mg/L at 5 µg/L. The GCMS analysis of the essential oil showed a high percentage of (*E*)-Cinnamaldehyde phytochemical constituents which contributed to the high antioxidant activity. Another study done by Liang *et al.* (2022), reported that the *C. cassia* essential oil showed 90.35±0.48% inhibition when the concentration is 75 µg/mL indicating strong antioxidant capacity. In consensus the study done by Abeysekera *et al.* (2019), reported that the mature leaf of Ceylon cinnamon (*C. zeylanicum*) had the highest antioxidant activity in terms of ABTS (422.46±14.03 mg Trolox equivalents per g of extract) compared to young leaf (227.63±20.45 mg Trolox equivalents per g of extract). Yang *et al.* (2012) reported that the *C. cassia* leaves exhibited an ABTS value of 297.34 mmol Trolox/g lower compared to the bark of *C. cassia.*

Table 5. ABTS assay for ethanolic *C. iners* crude leaf extracts.

Ethanolic *C. iners* crude extract	Trolox Equivalence (mg/g dw sample)	Percentage of inhibition (%)
Young leaf	$305.23±7.02^{A}$	$90.58±1.33^{A}$
Old leaf	$316.86±8.22^{A}$	$92.71±0.17^{A}$

Values are presented as mean±SD of replications (n = 3×2). Values with different superscripts are statistically significantly different at p≤0.05 using Tukey's range test between different leaf of the same solvent used.

3.4 Brine shrimp lethality assay

The brine shrimp lethality assay is effective, affordable, take a minimal quantity of material, and may produce results rapidly. It can be used to identify the bioactivity of crude extracts (Ramli *et al.*, 2020). BSLA

https://doi.org/10.26656/fr.2017.8(3).365

is designed as a preliminary screening to investigate the toxicity of crude plant extract at different concentrations. According to the current study, hatched nauplii are special for the initial cytotoxicity investigation. Before beginning this test, a number of parameters need to be taken into account. If there is a salt solution present, the egg will hatch into nauplii. Salt solution concentrations range (2-4%). Instead of iodinized salt or reagent grade salt, sea salt produces the greatest results. The pH adjustment of the water is important for hatching the eggs with the optimal pH range of 8.0±0.5. To prevent nauplii lethality brought on by a pH decrease during incubation, the pH should be regulated using sodium hydroxide or sodium carbonate. The growth of the nauplii is started when the salt solution enters the eggs. The floating eggs take oxygen directly from the air.

Nauplius will appear at room temperature 20 to 30 h after being wet. The nauplii hatched after 24–30 h of intense aeration and constant lighting for the 36–48 hrs that they were incubated at room temperature (28–30°C). The plastic tube that extends to the bottom of the jar is bubbling with air to keep the eggs moving continuously. During the study period, the nauplii do not receive food. The effects of the plant extract or starvation may be to account for the nauplii's mortality. A control sample made up only of nauplii is also used to verify the plant extract's ability to cause death. Nonetheless, newly hatched nauplii can continue to feed on their yolk sac for up to 48 hrs without nourishment. The mortality endpoint of this bioassay is defined as the absence of controlled forward motion during 30 sec of observation (Abiola and Aiyelaagbe, 2023). Calculations were made to determine the nauplii's lethality percentage for each concentration and control.

Table 6 shows the LC_{50} of the ethanolic *C. iners* crude leaf extracts to brine shrimps 24 hrs after treatment. The current study has shown that 20 mg/mL of ethanolic *C. iners* crude leaf extracts had an LC_{50} value of 19.627 mg/mL for young leaves and 13.201 mg/mL for old leaves. The LC_{50} of BSLA obtained from the standard curve was plotted in Figures 1 (a and b) and standard potassium dichromate was shown to be toxic with its LC_{50} at 0.075 mg/mL. According to Wong *et al.* (2021), the LC_{50} value of less than 1 mg/mL indicates significantly harmful, whereas a higher value shows the crude plant extract is not considerably toxic. The findings showed that the extract of *C. iners* leaves was non-toxic, therefore it is safe for human consumption. Nevertheless, potassium dichromate had lower LC_{50} values than *C. iners* crude extracts, indicating that it was more toxic towards brine shrimps. Because of its well-known toxicity level, potassium dichromate is frequently employed as a positive control in BSLA (Ramli *et al.*, 2020). The findings were in line with earlier research by Abdul Wahab and Hussain (2021) that showed LC_{50} value of ethyl acetate *C. iners* bark extracts was 3370.13 µg/mL which is above 1.00 mg/mL indicating its non-toxicity.

Table 6. Toxicity analysis of ethanolic *C. iners* crude leaf extracts

Ethanolic *C. iners* crude leaf extracts	Lethality concentration (LC_{50}) (mg/mL)
Young leaf	19.627
Old leaf	13.201
Potassium Dichromate ($K_2Cr_2O_7$)	0.075

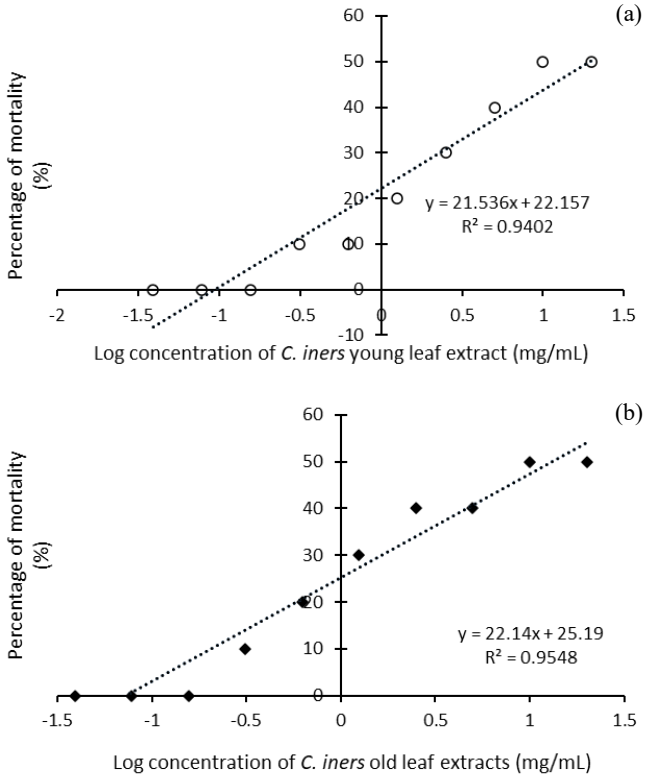

Figure 1. Representative of the toxicity analysis ethanolic *C. iners* crude leaf extracts of (a) young leaves and (b) old leaves versus percentage of mortality (%).

4. Conclusion

In conclusion, the ethanolic *C. iners* crude leaf extracts for young and old leaves have exhibited high total phenolic content and showed good antioxidant activities. From the discovery, both young and old leaf extracts were shown to be non-toxic. The extracts exhibit a protective activity against the oxidative stress and are safe for usage in medical or food application. It has been demonstrated that the ethanolic extracts of the crude leaves of *C. iners* to have pharmacological activities and might be explored as viable candidates for the natural antioxidant agent. Additional antioxidant testing should also be performed to further enhance its potential as an effective antioxidant for the treatment of diseases brought on by uncontrolled oxidative stress.

https://doi.org/10.26656/fr.2017.8(3).365

Conflict of interest

The authors declare no conflict of interest. The authors alone are responsible for the content of the paper.

Acknowledgements

The author wishes to convey his sincere thanks to the University Putra Malaysia for allowing us to perform the study and tests. We also want to express our gratitude to everyone who helped with this study, both directly and indirectly.

References

Abdelwahab, S.I., Mariod, A.A., Taha, M.M.E., Zaman, F.Q., Abdelmageed, A.H.A., Khamis, S., Sivasothy, Y. and Awang, K. (2017). Chemical composition and antioxidant properties of the essential oil of *Cinnamomum altissimum* Kosterm (Lauraceae). *Arabian Journal of Chemistry*, 10(1), 131-135. https://doi.org/10.1016/j.arabjc.2014.02.001

Abdul Wahab, I.R. and Hussain, M.F. (2021). Brine shrimp lethality test of various *Cinnamomum iners* (Lauraceae) barks extracts. *Journal of Tropical Resources and Sustainable Science*, 6(2), 573. https://doi.org/10.47253/jtrss.v6i2.573

Abeysekera, W.P.K.M., Arachchige, S.P.G., Abeysekera, W.K.S.M., Ratnasooriya, W.D. and Medawatta, H.M.U.I. (2019). Antioxidant and glycemic regulatory properties potential of different maturity stages of leaf of Ceylon cinnamon (*Cinnamomum zeylanicum* Blume) *in vitro. Evidence-Based Complementary and Alternative Medicine*, 2019, 2693795. https://doi.org/10.1155/2019/2693795

Abubakar, A.R. and Haque, M. (2020). Preparation of medicinal plants: basic extraction and fractionation procedures for experimental purposes. *Journal of Pharmacy and Bioallied Sciences*, 12(1), 1-10. https://doi.org/10.4103/jpbs.JPBS_175_19

Adjimani, J.P. and Asare, P. (2015). Antioxidant and free radical scavenging activity of iron chelators. *Toxicology Reports*, 2, 721-728. https://doi.org/10.1016/j.toxrep.2015.04.005

Abiola, J.L. and Aiyelaagbe, O.O. (2023). Phytochemical, antimicrobial and cytotoxic activities of *Strophanthus sarmentosus* DC. *Biology, Medicine and Natural Product Chemistry*, 12(1), 119-126.

Al-Snafi, A.E. (2019). Constituents and pharmacological effects of *Leontice leontopetalum* - a review. *To Chemistry Journal*, 3, 103-109.

Al-Zabt, A.M., Dek, M.S.P., Ramli, N.S. and Rukayadi, Y. (2022). Toxicity evaluation of the ethanolic *Jambu bol* [*Syzygium malaccense* (L.) Merr. and Perry] leaves extract and mechanisms underlying its antibacterial action. *Malaysian Journal of Microbiology,* 18(5), 505-513.

Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D.G. and Lightfoot, D.A. (2017). Phytochemicals: extraction, isolation, and identification of bioactive compounds from plant extracts. *Plants*, 6(4), 42-65. https://doi.org/10.3390/plants6040042

Anis, Z., Sulaiman, O., Hashim, R., Mehdi, S.H. and Ghalib, R.M. (2012). Radical scavenging activity, total phenol content and antifungal activity of C*innamomum iners* wood. *Iranica Journal of Energy and Environment*, 3, 74-78. https://doi.org/10.5829/idosi.ijee.2012.03.05.12

Annegowda, H.V., Gooi, T.S., Awang, S.H.H., Alias, N.A. and Mordi, M.N. (2012). Evaluation of analgesic and antioxidant potent of various extracts of *Cinnamomum iners* bark. *International Journal of Pharmacology*, 8(3), 198-203. https://doi.org/10.3923/ijp.2012.198.203

Arifullah, M., Vikram, P., Chiruvella, K.K., Shaik, M.M. and Ripain, I.H.B.A. (2014). A review on Malaysian plants used for screening of antimicrobial activity. *Annual Research and Review in Biology*, 4(13), 2088-2132. https://doi.org/10.9734/ARRB/2014/8258

Baliyan, S., Mukherjee, R., Priyadarshine, A., Vibhuti, A., Gupta, A., Pandey, R.P. and Chang, C.M. (2022). Determination of antioxidants by DPPH radical scavenging activity and quantitative phytochemical analysis of *Ficus religiosa. Molecules*, 27(4), 1326-1345. https://doi.org/10.3390/molecules27041326

Banglao, W., Thongmee, A., Sukplang, P. and Wanakhachornkrai, O. (2020). Determination of antioxidant, anti-aging and cytotoxicity activity of the essential oils from *Cinnamomum zeylanicum*. *Journal of Microbiology, Biotechnology and Food Sciences*, 10(3), 436-440. https://doi.org/10.15414/jmbfs.2020.10.3.436-440

Benzie, I.F.F. and Strain, J.J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power", the FRAP assay. *Analytical Biochemistry*, 239(1), 70-76. https://doi.org/10.1006/abio.1996.0292

Birben, E., Sahiner, U.M., Sackesen, C., Erzurum, S. and Kalayci, O. (2012). Oxidative stress and antioxidant defence. *World Allergy Organization Journal*, 5(1), 9-19. https://doi.org/10.1097/WOX.0b013e3182439613

Brodowska, K.M., Brodowska, A.J., Smigielski, K. and Lodyga-Chruscinska, E. (2016). Antioxidant profile of essential oils and extracts of cinnamon bark (*Cinnamomum cassia*). *European Journal of*

Biological Research, 6(4), 310-316.

Correa, M.G. and Couto, J.S. (2016). Anticancer properties of *Psidium guajava* - a mini-review. *Asian Pacific Journal of Cancer Prevention*, 17(9), 4199-4204.

Dhalaria, R., Verma, R., Kumar, D., Puri, S., Tapwal, A., Kumar, V., Nepovimora, E. and Kuca, K. (2020). Bioactive compounds of edible fruits with their anti-aging properties: A comprehensive review to prolong human life. *Antioxidants*, 9(11), 1123. https://doi.org/10.3390/antiox9111123

Do, Q.D., Angkawijaya, A.E., Tran-Nguyen, P.L., Huynh, L.H., Soetaredjo, F.E., Ismadji, S. and Ju, Y.H. (2014). Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatic. Journal of Food and Drug Analysis*, 22(3), 296-302. https://doi.org/10.1016/j.jfda.2013.11.001

Dumanovic, J., Nepovimova, E., Natic, M., Kuca, K. and Jacevic, V. (2021). The significance of reactive oxygen species and antioxidant defense system in plants: a concise overview. *Frontiers in Plant Science*, 11, 552969. https://doi.org/10.3389/fpls.2020.552969

Fonmboh, D.J., Abah, E.R., Fokunang, T.E., Herve, B., Teke, G.N., Rose, N.M., Borgia, N.N., Fokunang, L. B., Andrew, B.N., Kaba, N., Bathelemy, N. and Ntungwen, F.C. (2020). An overview of methods of extraction, isolation and characterization of natural medicinal plant products in improved traditional medicine research. *Asian Journal of Research in Medical and Pharmaceutical Sciences*, 9(2), 31-57. https://doi.org/10.9734/ajrimps/2020/v9i230152

Goraya, R.K. and Bajwa, U. (2015). Enhancing the functional properties and nutritional quality of ice cream with processed amla (Indian gooseberry). *Journal of Food Science and Technology*, 52(12), 7861-7871. https://doi.org/10.1007/s13197-015-1877-1

Hamidi, R., Jovanova, B. and Panovska, T.K. (2014). Toxicological evaluation of the plant products using brine shrimp (*Artemia salina* L.) model. *Macedonian Pharmaceutical Bulletin*, 60(1), 9-18. https://doi.org/10.33320/maced.pharm.bull.2014.60.01.002

Hikmawanti, N.P.E., Fatmawati, S. and Asri, A.W. (2021). The effect of ethanol concentrations as the extraction solvent on antioxidant activity of katuk (*Sauropus androgynus* (L.) Merr.) leaf extracts. *IOP Conference Series: Earth and Environmental Science*, 755, 012060. https://doi.org/10.1088/1755-1315/755/1/012060

Ifeanyi, O.E. (2018). A review on free radicals and antioxidants. *International Journal of Current Research in Medical Sciences*, 4(2), 123-133.

Jiang, S., Liu, H. and Li, C. (2021). Dietary regulation of oxidative stress in chronic metabolic diseases. *Foods*, 10(8), 1854-1872. https://doi.org/10.3390/foods10081854

Kebede, M. and Emire, S.A. (2019). Application of antioxidant in food processing, industry: options to improve the extraction yields and market value of natural products. *Advances in Food Technology and Nutritional Sciences*, 5(2), 38-48. https://doi.org/10.17140/AFTNSOJ-5-155

Khairunnisa, K.Q., Febriyanti, R.M. and Muhaimin, M. (2022). Phytochemistry and pharmacological potentials of *Premna serratifolia* L.: traditional medicinal plant used by local people in Kalimantan. *Indonesian Journal of Biological Pharmacy*, 2(3), 178-188 https://doi.org/10.24198/ijbp.v2i3.43537

Kristiningrum, N., Cahyanti, Y.N. and Wulandari, L. (2017). Determination of total phenolic content and antioxidant activity in methanolic extract of robusta and arabica coffee leaves, presented at International Conference on Medicine and Health Sciences (ICMHS). United Kingdom.

Liang, D., Feng, B., Li, N., Su, L., Wang, Z., Kong, F. and Bi, Y. (2022). Preparation, characterization, and biological activity of *Cinnamomum cassia* essential oil nano-emulsion. *Ultrasonics Sonochemistry*, 86, 106009. https://doi.org/10.1016/j.ultsonch.2022.106009

Lourenco, S.C., Moldao-Martins, M. and Alves, V.D. (2019). Antioxidants of natural plant origins: from sources to food industry applications. *Molecules*, 24 (22), 4132. https://doi.org/10.3390/molecules24224132

Madkour, L.H. (2019). Function of reactive oxygen species (ROS) inside the living organisms and sources of oxidants. *Pharmaceutical Sciences and Analytical Research Journal*, 2(2), 180023.

Mathew, S., Abraham, T.E. and Zakaria, Z.A. (2015). Reactivity of phenolic compounds towards free radicals under in vitro conditions. *Journal of Food Science and Technology*, 52, 5790-5798. https://doi.org/10.1007/s13197-014-1704-0

Mustaffa, F., Indurkar, J., Ismail, S., Shah, M. and Mansor, S.M. (2011). An antimicrobial compound isolated from *Cinnamomum iners* leaves with activity against methicillin-resistant *Staphylococcus aureus*. *Molecules*, 16(4), 3037-3047. https://doi.org/10.3390/molecules16043037

Nobosse, P., Fombang, E.N. and Mbofung, C.M.F.

(2018). Effects of age and extraction solvent on phytochemical content and antioxidant activity of fresh *Moringa oleifera* L. leaves. *Food Science and Nutrition*, 6(8), 2188-2198. https://doi.org/10.1002/fsn3.783

Olszowy, M. (2019). What is responsible for antioxidant properties of polyphenolic compounds from plants? *Plant Physiology and Biochemistry*, 144, 135-143. https://doi.org/10.1016/j.plaphy.2019.09.039

Ozcelik, B., Kartal, M. and Orhan, I. (2011). Cytotoxicity, antiviral and antimicrobial activities of alkaloids, flavonoids, and phenolic acids. *Pharmaceutical Biology*, 49(4), 396-402. https://doi.org/10.3109/13880209.2010.519390

Ramli, S., Son, R., Shaari, K. and Rukayadi, Y. (2020). Toxicity analysis of *Syzygium polyanthum* (Wight) Walp. leaf extract and its stability against different pH and temperature. *Emirates Journal of Food and Agriculture*, 32(6), 461-468. https://doi.org/10.9755/ejfa.2020.v32.i6.2116

Rekha, C., Poornima, G., Manasa, M., Abhipsa, V., Devi, J.P., Kumar, H.T.V. and Kekuda, T.R.P. (2012). Ascorbic acid, total phenol content and antioxidant activity of fresh juices of four ripe and unripe citrus fruits. *Chemical Science Transaction*, 1(2), 303-310. https://doi.org/10.7598/cst2012.182

Rukayadi, Y., Lau, K.Y., Zainin, N.S., Zakaria, M. and Abas, F. (2013). Screening antimicrobial activity of tropical edible medicinal plant extracts against five standard microorganisms for natural food preservative. *International Food Research Journal*, 20(5), 2905-2910.

Salazar-Ramiro, A., Ramirez-Ortega, D., Cruz, V.P., Hernandez-Pedro, N.Y., Gonzalez-Esquivel, D.F., Sotelo, J. and Pineda, B. (2016). Role of redox status in development of glioblastoma. *Frontiers in Immunology*, 7, 156. https://doi.org/10.3389/fimmu.2016.00156

Sanchez, C. (2017). Reactive oxygen species and antioxidant properties from mushrooms. *Synthetic and Systems Biotechnology*, 2(1), 13-22. https://doi.org/10.1016/j.synbio.2016.12.001

Sarah, Q.S., Anny, F.C. and Misbahuddin, M. (2017). Brine shrimp lethality assay. *Bangladesh Journal of Pharmacology*, 12, 186-189. https://doi.org/10.3329/bjp.v12i2.32796

Sherikar, A.S. and Mahanthesh, M.C. (2015). Evaluation of aqueous and methanolic extract of leaves of *Epipremnum aureum* for radical scavenging activity by DPPH method, total phenolic content, reducing capacity assay and FRAP assay. *Journal of Pharmacognosy and Phytochemistry*, 4(4), 36-40.

Siddhuraju, P. and Becker, K. (2003). Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (*Moringa oleifera* lam.) leaves. *Journal of Agricultural and Food Chemistry*, 51(8), 2144-2155. https://doi.org/10.1021/jf020444+

Singleton, V.L., Orthofer, R. and Lamuela-Raventos, R.M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology*, 299, 152-178. https://doi.org/10.1016/S0076-6879(99)99017-1

Sireesha, K. and Rao, S.P. (2015). Oxidative stress and diabetes: an overview. *Asian Journal Pharmaceutical Clinical Research*, 8(1), 15-19.

Surai, P.F., Kochish, I.I., Fisinin, V.I. and Kidd, M.T. (2019). Antioxidant defence systems and oxidative stress in poultry biology; an update. *Antioxidants*, 8(7), 235-271. https://doi.org/10.3390/antiox8070235

Suryawanshi, V.S., Yadav, A.R. and Mohite, S.K. (2020). Toxicological assessment using brine shrimp lethality assay and antimicrobial activity of *Capparis grandis*. *Journal of University of Shanghai for Science and Technology*, 22(11), 746-760.

Swallah, M.S., Sun, H., Affoh, R., Fu, H. and Yu, H. (2020). Antioxidant potential overviews of secondary metabolites (polyphenols) in fruits. *International Journal of Food Science*, 2020, 9081686. https://doi.org/10.1155/2020/9081686

Tan, J.B.L. and Lim, Y.Y. (2015). Critical analysis of current methods for assessing the *in vitro* antioxidant and antibacterial activity of plant extracts. *Food Chemistry*, 172, 814-822. https://doi.org/10.1016/j.foodchem.2014.09.141

Tello, J.C.C., Flores, H.E.M., Romo, M.G.G., Ramirez, J.S.P., Molina, A.S., Cortez, O.A., Camacho, M.C.B. and Lopez, J.O.R. (2018). Quantification 151 of phenolic compounds and in vitro radical scavenging abilities with leaf extracts from two varieties of *Psidium guajava* L. *Antioxidant*, 7(3), 34. https://doi.org/10.3390/antiox7030034

Udayaprakash, N.K., Ranjithkumar, M., Deepa, S., Sripriya, N., Al-arfaj, A.A. and Bhvaneswari, S. (2015). Antioxidant, free radical scavenging and GC-MS composition of *Cinnamomum iners* Reinw. ex Blume. *Industrial Crops and Products*, 69, 175-179. https://doi.org/10.1016/j.indcrop.2015.02.018

Unsal, V., Dalkiran, T., Cicek, M. and Kolukcu, E. (2020). The role of natural antioxidants against reactive oxygen species produced by cadmium toxicity: a review. *Advanced Pharmaceutical Bulletin*, 10(2), 184-202. https://doi.org/10.34172/

apb.2020.023

Wahab, I.A., Jaliuddin, A.F. and Anuar, N.A. (2020, December). Mosquito Repellency Effects of The Essential Oils from *Cinnamomum iners* Leaves and Barks. In *IOP Conference Series: Earth and Environmental Science*, 596, 012079. https://doi.org/10.1088/1755-1315/596/1/012079

Wojtunik-Kulesza, K.A. (2020). Approach to optimization of FRAP methodology for studies based on selected monoterpenes. *Molecules*, 25(22), 5267. https://doi.org/10.3390/molecules25225267

Wong, J.X., Ramli, S. and Chen, S.N. (2021). Assessment of toxic effect of *Centella asiatica* extract and its application as natural preservative in fresh-cut mango, pear and cabbage. *Journal of Food Processing and Preservation*, 45(10), e15824. https://doi.org/10.1111/jfpp.15824

Xu, D.P., Li, Y., Meng, X., Zhou, T., Zhou, Y., Zheng, J., Zhang, J.J. and Li, H.B. (2017). Natural antioxidants in foods and medicinal plants: extraction, assessment and resources. *International Journal for Molecular Sciences*, 18(1), 96-128. https://doi.org/10.3390/ijms18010096

Yang, C.H., Li, R.X. and Chuang, L.Y. (2012). Antioxidant activity of various parts of C*innamomum cassia* extracted with different extraction methods. *Molecules*, 17(6), 7294. https://doi.org/10.3390/molecules17067294

