Antibacterial and antioxidant activities in various parts of *Artocarpus lacucha* Buch. Ham. ethanolic extract

DEWI PERTIWI[1,2], RIKA HARTATI[1], ELIN JULIANTI[3] and IRDA FIDRIANNY[1]

[1]Department of Pharmaceutical Biology, School of Pharmacy, Bandung Institute of Technology, Bandung, West Java 40132; [2]Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, North Sumatra 20155; [3]Department of Pharmacochemistry, School of Pharmacy, Bandung Institute of Technology, Bandung, West Java 40132, Indonesia

Received May 3, 2023; Accepted October 9, 2023

DOI: 10.3892/br.2024.1755

Abstract. *Artocarpus lacucha* is an endemic plant to North Sumatera, Indonesia. This plant has pharmacological activities, including acting as an antioxidant and antibacterial. The aim of the present study was to analyze the antibacterial and antioxidant activities, and determine the flavonoid compounds from four parts of *A. lachuca*, namely leaves, barks, twigs and fruits. Antioxidant activity was investigated using the 2,2-diphenyl 1-picrylhydrazyl (DPPH) and cupric reducing antioxidant capacity (CUPRAC) methods. Antibacterial activity was analyzed using disk diffusion and microdilution methods. Several flavonoids, such as luteolin-7-O-glucoside, rutin, quercetin, kaempferol and apigenin, were determined using high performance liquid chromatography. Based on the antioxidant activity test results using the DPPH method, the bark ethanolic extract provided the highest antioxidant capacity, while the CUPRAC method indicated that the twig ethanolic extract had the highest antioxidant capacity. The antibacterial activity test results demonstrated that at a low concentration of 750 μg/disk the bark ethanolic extract obtained the highest inhibition zone and minimum inhibitory concentration level against six of nine pathogenic bacteria. Therefore, *A. lachuca* bark ethanolic extract could be potentially developed as antioxidant and antibacterial agents.

Correspondence to: Dr Rika Hartati, Department of Pharmaceutical Biology, School of Pharmacy, Bandung Institute of Technology, 10 Ganesa Street, Bandung, West Java 40132, Indonesia
E-mail: rika@fa.itb.ac.id

Abbreviations: AEAC, ascorbic acid equivalent antioxidant capacity; CUPRAC, cupric reducing antioxidant capacity; DNA, deoxyribose nucleic acid; DPPH, 2,2-diphenyl 1-picrylhydrazyl; GAE, gallic acid equivalent; HPLC, high performance liquid chromatography; MBC, minimum bactericidal concentration; MIC, minimum inhibitory concentration; TFC, total flavonoid content; TPC, total phenolic content; QE, quercetin equivalent; ROS, reactive oxidative stress

Key words: antioxidant, antibacterial, *Artocarpus lacucha*, flavonoids

Introduction

Medicinal plants have substances in their parts that can be utilized for therapeutic purposes or precursors that are beneficial for drug synthesis. There is a difference between medicinal plants that have therapeutic properties with constituents that have been scientifically established and plants that are used medicinally but have not been thoroughly investigated or only used traditionally (1). Numerous plants have been widely used in the health care system for a number of years as traditional medicine in various developing countries (2,3). A number of plants have also been beneficial for health, although scientific data to confirm their efficacy is limited. Plants should fulfill several requirements including toxicity and safety before being used as a medicinal plant or as drugs derived from nature that have medicinal properties (1).

Based on previous data there are 35,000-70,000 plant species used in traditional medicine around the world (4), and ~10,000 of these plants are used for both food and medicine. Mobe (*Artocarpus lacucha*) is a plant from the *Artocarpus* genus that is traditionally used by communities in North Sumatra and Thailand for stomachaches, liver problems, headaches, worming and wound-healing (5,6). *A. lacucha* contains flavonoid compounds that are thought to provide antioxidant and antimicrobial activities. *A. lacucha* also provides antiglycation, anthelmintic effects and cancer cell inhibitory activity (7,8).

Current research on antioxidant and antibacterial activities in plants has increased due to phenolic compounds that possess these activities. The presence of phenolic compounds was the basis for the present study (9,10). Phenolic compounds can scavenge free radicals for cell protection against reactive oxidative stress (ROS) (11-15). If the amount of ROS in the body exceeds the antioxidants the oxidative stress will emerge and the excess ROS will damage the components of lipids, proteins and DNA (16-19).

Studies have increasingly focused on protecting the cell against the effects of ROS and reactive nitrogen species to understand the mechanism of action for various antioxidants (20-22). In addition, studies regarding the roles of oxygen-derived pro-oxidants and antioxidants that have important roles in several clinical conditions (such as inflammation,

atherosclerosis, gastrointestinal disorder and cardiovascular disease) and normal metabolism have currently been increasingly conducted (20-22). Antioxidants exhibit pro-oxidant activity that depends on a particular condition (21,22). The aim of the present study was to determine the antibacterial and antioxidant activity of the leaves, bark, twigs and fruits of *A. lachuca* and determine the levels of several flavonoid compounds.

Materials and methods

Plant, materials and instruments. Fresh leaves, barks, twigs and fruits of *A. lacucha* were collected from Laguboti, Simalungun, Sumatera Utara, Indonesia. The plant sample was successfully identified by the Indonesian Institute of Sciences with the serial number: 2027/IPH.1.01/If.07/VIII/2017. The materials used in the present study were: Ethanol 96%; methanol pro-analysis (Merck KGaA); methanol for high-performance liquid chromatography (HPLC; Merck KGaA); distilled water; 2,2-diphenyl 1-picrylhydrazyl (DPPH; Sigma-Aldrich; Merck KGaA); neocuproine (Sigma-Aldrich; Merck KGaA); copper(II) chloride (Merck KGaA); ascorbic acid (Merck KGaA); Folin-Ciocalteu reagent (Merck KGaA); quercetin (ROTH; gallic acid (Sigma-Aldrich; Merck KGaA); Mueller Hinton agar (MHA; HiMedia Laboratories, LLC); nutrient agar (Oxoid; Thermo Fisher Scientific, Inc.); Mueller Hinton broth (MHB; Oxoid); chloramphenicol disk (Oxoid); and dimethyl sulfoxide. Bacterial cultures used in the present study were all purchased from American Type Culture Collection and included: *Pseudomonas aeruginosa* (cat. no. 9027); *Escherichia coli* (cat. no. 8939); *Salmonella typhi* (cat. no. 6539); *Methicillin resistant Staphylococcus aureus* (MRSA; cat. no. BAA-44); *Staphylococcus aureus* (cat. no. 6538); *Cutibacterium acnes* (cat. no. 11827); *Bacillus subtilis* (cat. no. 6633); *Staphylococcus epidermidis* (cat. no. 12228); and *Streptococcus mutans* (cat. no. 25175). The instruments used in the present study were UV-Vis spectrophotometer DU 720 (Beckman Coulter, Inc.), HPLC UFLC Prominence (Shimadzu Corporation) and Laminar Air Flow (Telstar AV-100; Azbil Corporation).

Extract preparation. The powdered leaves, barks, twigs and fruits of *A. lacucha* (300 g) were refluxed with 3 liters of 96% ethanol a total of three times. The filtrate was collected and evaporated at 50˚C for 5 h until a viscous fraction was produced.

Phytochemical screening. Phytochemical screening was conducted using a standard method for alkaloids, phenols, flavonoids, tannins, coumarin, saponins and steroids/triterpenoids detections as previously described (23).

Total phenolic content (TPC). Gallic acid was used as a standard for TPC and the previously described method was followed (24). Briefly, 50 μl of the extract sample was added to 500 μl of 10% Folin-Ciocalteu reagent and 400 μl of 1 M Na_2CO_3, and then incubated at 25˚Cfor 15 min before the sample was measured with a UV-vis spectrophotometer at a wavelength of 765 nm. The TPC was expressed as g of gallic acid equivalents (GAE)/100 g extract (g GAE/100 g extract).

Total flavonoid content (TFC). Quercetin was used as a standard for TFC and the previously described method was followed (25). Briefly, 100 μl of extract was added to 300 μl of methanol pro-analysis, 20 μl of 10% $AlCl_3$, 20 μl of sodium acetate and 560 μl of distilled water, and then incubated at 25˚C for 15 min and measured with a UV-vis spectrophotometer at a wavelength of 415 nm. TFC was expressed as g of quercetin equivalent (QE)/100 g of extract (g QE/100 g extract).

Antioxidant activities

DPPH method. Ascorbic acid was used as a standard for antioxidant activity, following the previously described method (26) with modifications. The 12.5 μl of 10.000 μg/ml extract was added to 125 μl methanol pro-analysis and 750 μl of 50 μg/ml DPPH (purple powder in methanol pro-analysis), and then incubated at 25˚C for 30 min and the absorbance was measured with a UV-vis spectrophotometer at a wavelength of 517 nm. Antioxidant activity was expressed by the antioxidant capacity equivalent to ascorbic acid [mg ascorbic acid equivalent antioxidant capacity (AEAC)/g extract].

Cupric ion reducing antioxidant capacity (CUPRAC) method. Ascorbic acid was used as a standard for antioxidant activity, following the previously described method (27) with modifications. The 12.5 μl of 10.000 μg/ml extract was added to 237.5 μl of 77.08 mg/ml ammonium acetate and 750 μl of CUPRAC solution [mixture of 1,705 μg/ml $CuCl_2$ and 1,562 μg/ml neocuproine (in a 1:1 ratio)], and then incubated at 25˚C for 30 min and the absorbance was measured with a UV-vis spectrophotometer at a wavelength of 450 nm. Antioxidant activity was expressed by the antioxidant capacity equivalent to ascorbic acid (mg AEAC/g extract).

Determination of a number of flavonoids using the HPLC method

HPLC conditions. A number of flavonoids were analyzed using the HPLC method. A reversed phase of HPLC method with a LiChrosper® (Merck KGaA) 100 RP-C18 5 μm column (100 mm, 4 mm inner diameter), water (solution A) and methanol (solution B), and a linear gradient mode were designed in accordance with 40-60% solution B for 5 min, then linear gradient mode B to 70% on 10th min, linear gradient mode B to 40% on 15th min. The column temperature was 30˚C, the flow rate was set at 1 ml/min, the injection volume was 20 μl, and UV-vis detector was used at 360 nm.

Standard solutions. Standard solutions of luteolin 7-O-glucoside, rutin, quercetin, kaempferol and apigenin were prepared and analyzed with HPLC using 20 μl of each standard (50 μg/ml).

Extract sample preparation. For each sample, 100 mg of dry extract was dissolved in 10 ml of methanol for HPLC analysis. Subsequently, 20 μl of the solution was injected for HPLC.

Antibacterial activity

Disk diffusion. Bacterial suspension (300 μl; ~1x10^8 cells) was pipetted on a sterile Petri dish, before pouring 15 ml of MHA medium until homogenous and solid. The disk containing 15 μl of extract with 5, 10, 15 and 20% concentrations were placed on the media. DMSO was used as a negative control

and chloramphenicol was used as a positive control. The plates were incubated at 37˚C for 18-24 h, before the clear zones were measured using a Vernier-caliper.

Microdilution. The microdilution was carried out by adding 100 μl MHB into each well of a 96-well sterile microplate with 100 μl extract in the first column until homogenous. From these wells in the first column, 100 μl sample was taken and added to the wells in the next column. The dilution was repeated as aforementioned until reaching the well with the lowest concentration that gave a clear zone of inhibition. Subsequently, 10 μl of bacterial suspension was added into each well after the absorbance on a UV-vis spectrophotometer at wavelength 625 nm was measured to be 0.08-0.13. The MHB was used as a negative control and chloramphenicol was used as a positive control. The microplate was incubated at 37˚C for 18-24 h and the turbidity level was observed in each well. After obtaining the minimum inhibitory concentration (MIC) value, non-turbid samples were streaked on MHA media and incubated at 37˚C for 24 h. Inhibited bacterial growth was observed to obtain the minimum bactericidal concentration (MBC).

Statistical analysis. Data were statistically analyzed using Minitab® (version 21; https://dti.itb.ac.id/minitab/) software. Two-way analysis of variance (ANOVA), followed by Tukey's post hoc test, and Pearson correlation was used to analyze the collected data. $P<0.05$ was considered to indicate a statistically significant difference.

Results and discussion

Phytochemical constituent. There were seven groups of compounds identified in the phytochemical screening, namely alkaloids, flavonoids, phenols, saponins, tannins, steroid/triterpenoid and coumarins, as presented in Table I. The four parts of the *A. lacucha* plant, namely leaves, barks, twigs and fruits positively contained flavonoids, phenols, saponins, tannins and coumarins. This result was similar to the phytochemical screening of the *Artocarpus* genus in the study by Buddhisuharto *et al* (28).

Total phenolic and flavonoid contents. The total phenolic and flavonoid contents are presented in Table II. Based on the table, the highest TPC was observed in barks, while the highest TFC was obtained from leaves. Based on the statistical analysis using ANOVA-Tukey in Table II, a significant difference was revealed in the TPC values between: i) Leaves vs. barks and fruits; ii) barks vs. twigs and fruits; and iii) twigs vs. fruits, while no significant difference was revealed in leaves vs. twigs with $P<0.05$. A significant difference was also revealed in the TFC values between leaves vs. barks, twigs and fruits, while no significant difference was revealed between barks vs. twigs and fruits with $P<0.05$.

Folin-Ciocalteu reagent reduction by phenolic compounds under alkaline conditions is the basis for the Folin-Ciocalteu test, as a phosphomolybdic acid/phosphotungstic acid complex is reduced to produce a blue chromophore with a maximum absorption at 765 nm (29). This test is beneficial for determining the TPC, based on the simplicity, reproducibility and robustness of the test. However, this test is sensitive to pH, temperature and reaction time (30).

Table I. Phytochemical constituent in four parts of the *Artocarpus lacucha* crude drug.

	Crude drug			
Compounds	Leaves	Barks	Twigs	Fruits
Alkaloids	-	-	-	-
Flavonoids	+	+	+	+
Phenols	+	+	+	+
Saponins	+	+	+	+
Tannins	+	+	+	+
Steroid/triterpenoid	-	-	-	-
Coumarin	+	+	+	+

+, detected; -, undetected.

Table II. Total phenol and flavonoids content.

Parts of the plant	Total phenol (g GAE/100 g)	Total flavonoids (g QE/100 g)
Leaves	4.75±0.51[a]	17.13±1.77[b]
Barks	10.14±0.72[c]	1.81±0.15[d]
Twigs	5.41±0.51	2.84±0.17
Fruits	0.94±0.11[e]	1.66±0.25[f]

Values are presented as means ± SD from at least six experimental repeats. Total phenol content is presented as g of GAE/100 g sample. Total flavonoid content is presented as g of QE/100 g sample. [a]No significant difference between leaves vs. twigs. [b]$P<0.05$ between leaves vs. barks, twigs or fruits. [c]$P<0.05$ between barks vs. leaves, twigs or fruits. [d]No significant difference between barks vs. twigs or fruits. [e]$P<0.05$ between fruits vs. leaves or twigs. [f]No significant difference between fruits vs. twigs. GAE, gallic acid equivalent; QE, quercetin equivalent.

Phenolic compounds can scavenge reactive oxygen without causing other oxidative reactions. Thus, TPC evaluation is currently one of the standard tests for phytochemical studies to determine the antioxidant activity in an extract because 5,7,3',4'-hydroxy-substituted flavonoids are considered to have efficient radical scavenging capacity (31,32). This was in accordance with previous results on the direct association between phenolic and flavonoid contents on biological activity of plant extracts (33-36).

Antioxidant activity. The antioxidant activity is presented in Table III. The highest antioxidant activity using the DPPH method was revealed in barks, while the highest antioxidant activity using the CUPRAC method was revealed in twigs.

From the DPPH method in Table III, a significant difference ($P<0.05$) was revealed in: i) Leaves vs. barks, twigs and fruits; ii) barks vs. twigs and fruits; and iii) twigs vs. fruits. In the CUPRAC method, a significant difference ($P<0.05$) was revealed in fruits vs. leaves, barks and twigs, while no

Table III. Antioxidant activity of *Artocarpus lacucha* using DPPH and CUPRAC methods.

	Methods	
Parts of the plant	DPPH (mg AEAC/g extract)	CUPRAC (mg AEAC/g extract)
Leaves	6.94±0.11[a]	80.76±11.74[b]
Barks	7.19±0.10[c]	91.32±3.77[d]
Twigs	6.16±0.06[e]	92.53±1.00
Fruits	6.34±0.06	28.66±7.99[f]

Values are presented as means ± SD from at least six experimental repeats. [a]P<0.05 between leaves vs. barks, twigs or fruits. [b]No significant difference between leaves vs. barks or twigs. [c]P<0.05 between barks vs. twigs or fruits. [d]No significant difference between bark vs. twigs. [e]P<0.05 between twigs vs. fruits. [f]P<0.05 between fruits vs. leaves, barks or twigs. DPPH, 2,2-diphenyl 1-picrylhydrazyl; CUPRAC, cupric reducing antioxidant capacity; AEAC, ascorbic acid equivalent antioxidant capacity.

Table IV. Pearson's correlation test on total phenol, total flavonoids and antioxidant activity.

	Pearson's correlation coefficient (r)	
Antioxidant parameters	Total phenol content	Total flavonoids content
DPPH		
Leaves	0.935[a,b]	0.726[c]
Barks	0.971[a,b]	0.961[a,b]
Twigs	0.955[a,b]	0.748[c]
Fruits	0.905[a,d]	0.980[a,b]
CUPRAC		
Leaves	0.952[a,b]	0.761[c]
Barks	0.948[a,b]	0.853[c,d]
Twigs	0.902[a,d]	0.949[a,b]
Fruits	0.757[c]	0.850[c,d]

[a]Very strong correlation. [b]P<0.01. [c]Strong correlation. [d]P<0.05. n=6. DPPH, 2,2-diphenyl 1-picrylhydrazyl; CUPRAC, cupric reducing antioxidant capacity.

significant difference was revealed in leaves vs. barks and twigs nor in barks vs. twigs.

DPPH is a water-insoluble and stable compound, and the latter two properties of DPPH are responsible for its use as a radical model in various antioxidant studies (37-39). DPPH can accept electrons or hydrogen radicals to become a stable molecule (38,39). DPPH has a purple color (at 515-517 nm in an alcohol solution), but can turn to a yellow color when the counterparts, N radicals, are reduced (37-39). Thus, the higher the antioxidant activity, the stronger the reagent decolorization. The DPPH method is simple, quick to perform, cost-effective and efficient, thereby becoming the most commonly used method for antioxidant screening of constituents, mixtures, extracts or biological matrices (38,39).

Additionally, the CUPRAC method was originally used to determine the total antioxidant activity and has been modified for various measurement methods (such as calculating the IC_{50} or the antioxidant capacity in an antioxidant). Antioxidant activity is based on the reduction of Cu^{2+} to Cu^{+}. Similar to other tests, ligands are used to form cuprum-ligand complexes to facilitate absorbance measurements. Neocuproine (2,9-pimethyl-1,10 phenanthroline) is a ligand commonly used in the CUPRAC test (35). This method has been applied to various matrices containing lipophilic and hydrophilic antioxidants (39,40).

L-ascorbic acid, also known as vitamin C, is a water-soluble antioxidant that has a role as an electron donor, which can neutralize and reduce ROS, protecting the cells from free radical damage (41,42). As an antioxidant, ascorbic acid can directly react with free radicals, either with or without enzyme catalysts, and form mono-dehydroascorbic and/or dehydroascorbic acids (19).

Pearson's correlation test on total phenolics, total flavonoids and antioxidant activity. The correlation results of total phenolics, total flavonoids and antioxidant activity are presented in Table IV. Schober *et al* (43) categorized the interpretation of correlation based on the value obtained as: i) If the correlation value is 0.00-0.09 the parameters are in a negligible correlation; ii) a correlation value of 0.10-0.39 is a weak correlation; iii) a correlation value of 0.40-0.69 is a moderate correlation; iv) a correlation value of 0.70-0.89 is a strong correlation; and v) a correlation value of 0.90-1.00 is an extremely strong correlation. From Table IV, the correlation of the TPC with DPPH and CUPRAC in leaves is extremely strong, while the TFC with DPPH and CUPRAC in leaves is strongly correlated. The correlation of the TPC with DPPH and CUPRAC as well as the TFC with the DPPH method in barks is extremely strong, while the TFC with the CUPRAC method in barks was strongly correlated. An extremely strong correlation of the TPC with DPPH and CUPRAC was revealed in twigs, while the TFC with DPPH in twigs was strongly correlated and with CUPRAC in twigs was extremely strong. In fruits, extremely strong correlations of the TPC and TFC was revealed with the DPPH method, while a strong correlation was revealed with the CUPRAC method. Gaweł-Bęben *et al* (44) considered that the high content of phenolic compounds in plant extracts was often correlated with their significant antioxidant activity.

Determination of several flavonoids using HPLC. Several flavonoids identified using HPLC are presented in Table V. The standards were three major flavonols (namely, kaempferol, rutin and quercetin) and two major flavones (apigenin and luteolin) in plants (45). The *Artocarpus* genus was reported to contain kaempferol (46), rutin (47,48), quercetin (46,48), luteolin and apigenin (49). The highest levels of luteolin-7-glucoside, quercetin, kaempferol and apigenin were revealed in leaves, while the highest levels of rutin was revealed in barks. Luteolin-7-O-glucoside was undetected in barks, rutin was undetected in leaves, kaempferol was undetected in barks and twigs, and apigenin was undetected in fruits. This may occur due to low levels in each part, and thus remained undetected.

Table V. Luteolin-7-O-glucoside, rutin, quercetin, kaempferol and apigenin contents in each part of the plant.

	Marker in sample (%)				
Sample	Luteolin-7-O-glucoside	Rutin	Quercetin	Kaempferol	Apigenin
Leaves	3.67	UD	0.62	0.23	0.16
Barks	UD	0.31	0.13	UD	0.03
Twigs	0.18	0.08	0.21	UD	0.08
Fruits	0.19	0.09	0.15	0.14	UD

UD, undetected.

It is important to ensure all analytes have separated completely; however, it is not always easy to achieve, especially in the case of natural products (50-52), which are known to contain >50 different types of phenols and flavonoids. In the present case, finding a gradient method capable of achieving complete separation for all constituents is almost impossible since a number of these phenolic compounds are similar in chemical composition and polarity (53). However, in the present study, orientation (a search for a suitable system and mobile phase) was carried out to determine the appropriate analyte separation method so that well separated analytes were obtained when the levels of several flavonoid compounds present in the sample were determined.

Antibacterial activity. Antibacterial activity, as an inhibition zone diameter, is presented in Table VI and the MIC and MBC values are presented in Table VII. From these results, *A. lacucha* has an increased antibacterial effect against Gram-positive bacteria, namely MRSA, *S. aureus, C. acnes, B. subtilis, S. epidermidis* and *S. mutans*. This was in line with previous studies, which reported that *Artocarpus integra* (47,54) and *Artocarpus altilis* (55) had antibacterial activity and demonstrated a higher activity on Gram-positive bacteria compared with Gram-negative bacteria.

This may be due to the different constituents and organization of the cell membrane (56). Gram-negative bacteria have an outer phospholipid membrane called the lipopolysaccharide component structure. Therefore, the cell wall has a reduced permeability to antimicrobial agents. Gram-positive bacteria are more sensitive to antimicrobial agents, as they only have an outer and ineffective peptidoglycan layer. Therefore, Gram-negative bacteria have a more complex outer layer compared with Gram-positive bacteria, which is capable of resisting the antimicrobial agents to a greater extent compared with Gram-positive bacteria (57).

However, an inhibition diameter for the fruit extract was obtained with disc diffusion and MICs were revealed for Gram-negative bacteria, namely *P. aeruginosa, E. coli* and *S. typhi*. High concentrations of 2,250 and 3,000 μg/disk fruit extract produced small inhibition diameter zones of 6.80±0.40 mm and 7.4±0.00 mm against *P. aeruginosa*, respectively; 7.43±0.05 mm and 7.46±0.05 against *E. coli*, respectively; and 6.40±0.00 and 7.40±0.00 against *S. typhi*, respectively. As a positive control, chloramphenicol provided antibacterial activity based on the inhibition zone diameter and MIC against all nine species of bacteria. Chloramphenicol is a broad-spectrum antibiotic that can be used for Gram-positive and Gram-negative bacteria, although it is most commonly used for treating *S. typhi* bacterial infection.

Antioxidants. Plants are a source of a number of important components, such as phenolic compounds that can scavenge free radicals and reduce oxidative stress (58-60). Compounds belonging to the phenolic group that provide antioxidant effects are flavonoids, phenolic acids, lignans and stilbenes. The properties of these compounds are used by plants as defense mechanisms against the adverse effects of UV radiation, temperature and mechanical damage, as well as releasing important chemical defenses through their specific physiological actions against herbivores and insects (60-65). In addition, phenolic compounds can prevent adverse changes in living organisms by reacting directly with fatty acid oxidation products. They also prevent deterioration of organoleptic and sensory characteristics in food products (66-68).

Plants have been demonstrated to produce phenolics in response to oxidative stress (69,70), including flavonoids. Flavonoids are a well-known class of phenolic compounds with antioxidant properties (71). In addition to being antioxidants, flavonoids also function as scavenger chemical controls due to ROS exposure. This dual role applies to the large number of free OH-groups, especially 3-OH and the higher reactivity of flavonoid is observed in hydroxyl groups with oxidants (65,72,73). Currently, it is considered that phenolics and flavonoids function to promote health as anti-inflammatory and antioxidant agents, thus protecting the body against chronic diseases (74-76). In addition, these secondary metabolites also provide protection to plants against abiotic and biotic stresses (77,78).

Free radicals are compounds or molecules that have one or more unpaired electrons in its outermost orbit, causing them to become very reactive in order to find bonds, such as by attacking and binding to the electrons of molecules (17,18,79).

Antioxidants are substances from food or in the body that can delay, control or prevent oxidative stress at low concentrations, thus reducing the deterioration of food and the spread of degenerative diseases in the body. Antioxidants that fit this definition include free radical scavengers, singlet oxygen quenchers, inactivators of peroxides and other ROS, metal ion chelators, secondary oxidation product quenchers, and pro-oxidative enzyme inhibitors (80). Currently, the use

Table VI. Inhibition zone in disk diffusion method.

Parts of the plant	Concentration (μg/disk)	Inhibition zone (mm)[a]								
		PA	EC	ST	MRSA	SA	CA	BS	SE	SM
Leaves	750	UD	UD	UD	UD	UD	UD	UD	UD	UD
	1,500	UD	UD	UD	UD	UD	UD	UD	UD	UD
	2,250	UD	UD	UD	UD	UD	UD	UD	UD	UD
	3,000	UD	UD	UD	UD	UD	UD	UD	UD	UD
Barks	750	UD	UD	UD	10.20±0.00	10.40±0.69	9.80±0.34	7.27±0.37	8.20±0.17	8.85±0.26
	1,500	UD	UD	UD	11.23±0.11	11.10±0.17	10.63±0.12	7.77±0.06	10.22±0.49	11.02±0.83
	2,250	UD	UD	UD	11.60±0.17	11.90±0.25	11.10±0.83	8.50±0.65	11.03±0.30	11.43±0.29
	3,000	UD	UD	UD	13.23±0.11	12.68±0.20	13.23±0.35	10.18±0.75	11.15±0.87	12.33±0.11
Twigs	750	UD	UD	UD	8.63±0.11	9.00±0.17	8.95±0.57	7.40±0.26	7.22±0.03	7.20±0.10
	1,500	UD	UD	UD	9.83±0.05	10.10±0.09	10.52±0.08	7.53±0.08	8.12±0.08	8.13±0.06
	2,250	UD	UD	UD	10.93±0.11	10.20±0.86	10.70±0.17	9.08±1.02	8.17±0.23	8.90±0.55
	3,000	UD	UD	UD	11.87±0.25	10.40±0.61	11.51±0.29	9.92±0.86	8.33±0.06	9.95±0.09
Fruits	750	UD	UD	UD	UD	UD	UD	UD	UD	UD
	1,500	UD	UD	UD	UD	UD	UD	UD	UD	UD
	2,250	6.80±0.40	7.43±0.05	6.40±0.00	6.60±0.00	7.27±0.08	8.15±0.26	7.47±0.03	7.27±0.06	10.17±1.05
	3,000	7.4±0.00	7.46±0.05	7.40±0.00	7.20±0.10	12.57±0.64	11.20±0.57	10.87±0.32	11.17±0.06	12.90±0.66
Chloramphenicol	30	10.10±0.00	16.1±0.00	17.0±0.00	23.6±0.00	21.53±1.32	19.83±0.38	30.97±0.16	26.08±0.73	22.62±0.14
DMSO	750	UD	UD	UD	UD	UD	UD	UD	UD	UD

[a]Mean values of inhibition zones ± SD. UD, undetected; PA, *Pseudomonas aeruginosa*; EC, *Escherichia coli*; ST, *Salmonella typhi*; MRSA, *Methicillin resistant Staphylococcus aureus*; SA, *Staphylococcus aureus*; CA, *Cutibacterium acnes*; BS, *Bacillus subtilis*; SE, *Staphylococcus epidermidis*; SM, *Streptococcus mutans*.

Table VII. Minimum inhibition concentration and minimum bactericidal concentration.

A, Minimum inhibition concentration

	% (w/v)								
Parts of the plant	PA	EC	ST	MRSA	SA	CA	BS	SE	SM
Leaves	UD	UD	UD	UD	UD	UD	UD	UD	UD
Barks	UD	UD	UD	0.16	1.25	2.50	1.25	2.50	1.25
Twigs	UD	UD	UD	0.31	1.25	2.50	1.25	1.25	2.50
Fruits	2.50	2.50	2.50	1.25	2.50	6.25	5.00	6.25	3.13
Chloramphenicol	$6.25x10^{-3}$	$3.91x10^{-4}$	$1.95x10^{-4}$	$7.81x10^{-4}$	$1.56x10^{-3}$	$3.91x10^{-4}$	$1.95x10^{-4}$	$7.81x10^{-4}$	$1.95x10^{-4}$
MHB	UD	UD	UD	UD	UD	UD	UD	UD	UD

B, Minimum bactericidal concentration

	% (w/v)								
Parts of the plant	PA	EC	ST	MRSA	SA	CA	BS	SE	SM
Leaves	UD	UD	UD	UD	UD	UD	UD	UD	UD
Barks	UD	UD	UD	UD	2.50	UD	UD	UD	UD
Twigs	UD	UD	UD	UD	2.50	UD	UD	5.00	UD
Fruits	UD	2.50	2.50	UD	5.00	UD	UD	12.50	UD
Chloramphenicol	0.05	UD	UD	UD	UD	UD	UD	$1.25x10^{-2}$	UD
MHB	UD	UD	UD	UD	UD	UD	UD	UD	UD

UD, undetected; PA, *Pseudomonas aeruginosa*; EC, *Escherichia coli*; ST, *Salmonella typhi*; MRSA, *Methicillin resistant Staphylococcus aureus*; SA, *Staphylococcus aureus*; CA, *Cutibacterium acnes*; BS, *Bacillus subtilis*; SE, *Staphylococcus epidermidis*; SM, *Streptococcus mutans*; MHB, Mueller Hinton broth.

of antioxidant compounds has been growing rapidly along with an increasing value of free radical activity against several degenerative diseases such as cancer, cardiovascular disease, atherosclerosis and aging (22,81).

Antioxidants obtained from food are useful to eliminate excessive ROS in the body. Antioxidants will be utilized to protect biomolecules from being oxidized. Thus, it is important to investigate whether there is a correlation between the strong antioxidants and oxidative stress level (82,83). Phenolic compounds are abundant in plants and exhibit antimicrobial (84-86) and antioxidant (34) properties, thus can be applied for further usage in pharmaceutics, cosmetics and natural food preservatives (86,87,88).

The direct reaction that occurs between DPPH and antioxidants has been used to measure the antioxidant activity (50) and high percentage of DPPH radical-scavenging compound indicates the excellent antioxidant activity (36). In the present study, the antioxidant capacity using DPPH and CUPRAC methods indicates that an increased antioxidant activity will produce an increased antibacterial activity. However, *A. lacucha* is known to have antibacterial activity, thus a further study is necessary to determine the mechanism and location of the antibacterial action.

Antibacterials. Antioxidant compounds in plants can act as antibacterials in several ways, such as by inhibiting oxidation in several areas of the cell, damaging the membrane structure leading to cell nutrient leakage and blocking the binding sites of DNA gyrase for DNA coiling, thus negatively affecting the growth of the cell (89).

By reviewing the literature, Kuete (90) classified the MIC values to indicate a significant activity if <100 μg/ml, to have good activity if 100-625 μg/ml and to have low activity if >625 μg/ml. In the present study, low antibacterial activity was revealed with the MIC value of 0.16% (barks against MRSA) and the results of other extracts demonstrated low antibacterial activity based on their MIC values. Similar observations for MIC values with minor variations have also been observed in other studies (54-57,90-94). However, Ouchari *et al* (95) reported that the antimicrobial inhibition zones were grouped into four categories, namely weak (<5 mm), moderate (5-10 mm), strong (10-20 mm) and highly strong (>20-30 mm) antimicrobial activities. In the present study, the inhibition zone was classified as moderate to strong (95) for the bark and twig extracts against MRSA, *S. aureus*, *C. acnes*, *B. subtilis*, *S. epidermidis* and *S. mutans* at concentrations starting from 750 μg/disc, as well as for the fruit extract against *P. aeruginosa*, *E. coli*, *S. typhi*, MRSA, *S. aureus*, *C. acnes*, *B. subtilis*, *S. epidermidis* and *S. mutans* at concentrations starting from 2,250 μg/disc. This was based on the results of the disc diffusion method, and thus the bark and twig extracts can be considered as a potential for further investigation and development as an antibacterial agent. However, the disc diffusion method has limitations, as polar compounds diffuse from the disk to the agar medium easily, while semi-polar and non-polar compounds will remain undiffused (96).

Based on the mechanism of action to inhibit the growth of microorganisms, Khameneh *et al* (97), Radji (98), Kapoor (99) and Reygaert (100) stated that antibacterials can be classified as: First, cell wall synthesis inhibitors. The bacterial cell wall is important for maintaining the structure of bacterial cells. Substances that can damage the cell wall will lyse the cell wall and affect the shape and structure of the cell, thereby killing the bacterial cell (97-100). Second, cell membrane disruptors. The cell membrane functions to regulate nutrients and metabolites inside and outside the cell (97-100). A number of antibacterials can disrupt the cell membrane and negatively affect the growth of the bacterial cell including lipopeptides (100) such as polymyxins (97). Third, nucleic acid biosynthesis disruptors. The process of DNA replication in bacterial cells is an important cycle in the cell. A number of antibacterials can interfere with nucleic acid metabolism, thus affecting the entire growth phase of bacteria, including quinolons (97,99,100) such as ciprofloxacin (97). Fourth, protein synthesis inhibitors. In protein synthesis, the DNA is transcribed into mRNA and the mRNA is translated continuously into protein (97-100). Antibacterials can inhibit these processes, so that protein synthesis will be disrupted, such as macrolides, aminoglycosides, tetracyclins, chloramphenicol and oxazolidinones (97,99,100).

In various studies it has been reported that *A. lacucha* has pharmacological activities, such as acting as an antimicrobial (101), as a treatment for skin ailments (102), antiglycation (103) and traditionally this plant has been used by communities in Thailand for tapeworm infections (5), as an anti-inflammatory, to relieve stomachaches, headaches and fevers (6,101). In North Sumatera, Indonesia, the fruit is used as seasoning in food. Lakoochins A and B, the isolated compounds from *A. lacucha*, have antimycobacterial activity (5) and the isolated compound cathecin has antinociceptive activity (6). It has also been reported by Islam *et al* (6) that *A. lacucha* extracts demonstrated a low toxicity profile in acute toxicity tests. In a previous study, it has also been concluded that leaves extract from this plant have a strong antioxidant activity and the potential to inhibit bacterial growth (104).

In conclusion, *A. lacucha* has the potential to be developed as a natural antioxidant with improved antibacterial properties against Gram-positive bacteria compared with Gram-negative bacteria. The present study indicated that a number of the compounds in *A. lacucha*, namely from flavonoids, support the antioxidant and antibacterial activities. Therefore, further investigations should be carried out in the *Artocarpus* genus in order to potentially find new natural resources.

Acknowledgements

Not applicable.

Funding

This work was supported by Beasiswa Pendidikan Indonesia (grant no. 202101121470), Center for Higher Education Fund (BPPT), Ministry of Education, Culture, Research and Technology, and the Indonesia Endowment Fund for Education Agency (LPDP), Ministry of Finance of the Republic of Indonesia.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

DP designed and performed the experiments, analyzed the data and wrote the manuscript. RH and EJ designed the experiments. IF designed the experiment and wrote the manuscript. DP and IF confirm the authenticity of all the raw data. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Sofowora A, Ogunbodede E and Onayade A: The role and place of medicinal plants in the strategies for disease prevention. Afr J Tradit Complement Altern Med 10: 210-229, 2013.
2. Giday K, Lenaerts L, Gebrehiwot K, Yirga G, Verbist B and Muys B: Ethnobotanical study of medicinal plants from degraded dry afromontane forest in northern Ethiopia: Species, uses and conservation challenges. J Herb Med 6: 96-104, 2016.
3. Kpodar MS, Lawson-Evi P, Bakoma B, Eklu-Gadegbeku K, Agbonon A, Aklikokou K and Gbeassor M: Ethnopharmacological survey of plants used in the treatment of diabetes mellitus in south of Togo (Maritime Region). J Herb Med 5: 147-152, 2015.
4. Shikov AN, Pozharitskaya ON, Makarov VG, Wagner H, Verpoorte R and Heinrich M: Medicinal plants of the Russian pharmacopoeia; their history and applications. J Ethnopharmacol 154: 481-536, 2014.
5. Puntumchai A, Kittakoop P, Rajviroongit S, Vimuttipong S, Likhitwitayawuid K and Thebtaranonth Y: Lakoochins A and B, new antimycobacterial stilbene derivatives from *Artocarpus lakoocha*. J Nat Prod 67: 485-486, 2004.
6. Islam S, Shajib MS, Rashid RB, Khan MF, Al-Mansur MA, Datta BK and Rashid MA: Antinociceptive activities of *Artocarpus lacucha* Buch-ham (Moraceae) and its isolated phenolic compound, catechin, in mice. BMC Complement Altern Med 19: 214, 2019.
7. Gautam P and Patel R: *Artocapus lakoocha* Roxb: An overview. Eur J Compl Integr Med 1: 10-11, 2014.
8. Songoen W, Phanchai W, Brecker L, Wenisch D, Jakupec W, Pluempanupat W and Schinnerl J: Highly aromatic flavan-3-ol derivatives from palaeotropical *Artocarpus lacucha* Buch.-Ham possess radical scavenging and antiproliferative properties. Molecules 26: 1078, 2021.
9. Thakur R, Yadav K and Khadka KB: Study of antioxidant, antibacterial and anti-inflammatory activity of cinnamon (*Cinamomum tamala*), ginger (*Zingiber officinale*) and turmeric (*Curcuma longa*). Am J Life Sci 1: 173-277, 2013.
10. Al-Rifai A, Aqel A, Al-Warhi T, Wabaidur SM, Al-Othman ZA and Badjah-Hadj-Ahmed AY: Antibacterial, antioxidant activity of ethanolic plant extracts of some convolvulus species and their DART-ToF-MS profiling. Evid Based Complement Alternat Med 2017: 5694305, 2017.
11. Song JM, Lee KH and Seong BL: Antiviral effect of catechins in green tea on influenza vírus. Antivir Res 68: 66-74, 2005.
12. Cushnie TPT and Lamb AJ: Antimicrobial activity of flavonoids. Int J Antimicrob Agents 26: 343-356, 2005.
13. Da Silva MA, Cardoso CAL, Vilegas W and dos Santos LC: High-performance liquid chromatographic quantification of flavonoids in Eriocaulaceae species and their antimicrobial activity. Molecules 14: 4644-4654, 2009.
14. Ferguson PJ, Kurowska E, Freeman DJ, Chambers AF and Koropatnick DJ: A flavonoid fraction from cranberry extract inhibits proliferation of human tumor cell lines. J Nutr 134: 1529-1535, 2004.
15. Russo A, Cardile V, Sanchez F, Troncoso N, Vanella A and Garbarino JA: Chilean propolis: Antioxidant activity and antiproliferative action in human tumor cell lines. Life Sci 76: 545-558, 2004.
16. Lobo V, Patil A, Phatak A and Chandra N: Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn Rev 4: 118-126, 2010.
17. Halliwell B and Gutteridge JMC: Free radicals in biology & medicine. Fifth edition. Oxford University Press, 2015.
18. Rani V and Yadav UCS: Free radicals and human health and disease. Springers, 2015.
19. Zulaikhah ST: The role of antioxidant to prevent free radicals in the body. Sains Med 8: 39-45, 2017.
20. Kurutas EB: The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: Current state. Nutr J 15:71, 2016.
21. Sharifi-Rad M, Anil Kumar NV, Zucca P, Varoni EM, Dini L, Panzarini E, Rajkovic J, Tsouh Fokou PV, Azzini E, Peluso I, *et al*: Lifestyle, oxidative stress, and antioxidants: back and forth in the pathophysiology of chronic diseases. Front Physiol 11: 694, 2020.
22. Vona R, Pallota L, Cappelletti M, Severi C and Matarrese P: The impact of oxidative stress in human pathology: Focus on gastrointestinal disorders. Antioxidants (Basel) 10: 201, 2021.
23. Fransworth NR: Biological and phytochemical screening of plants. J Pharm Sci 55: 225-276, 1996.
24. Pourmorad F, Hosseinimehr SJ and Shahabimajd N: Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. Afr J Biotechnol 5: 1142-1145, 2006.
25. Chang CC, Yang MH, Wen HM and Chern JC: Estimation of total flavonoid content in propolis by two complementary colometric methods. J Food Drug Anal 10: 178-182, 2002.
26. Celep E, Charehsaz M, Akyüz S, Acar ET and Yesilada E: Effect of in vitro gastrointestinal digestion on the bioavailability of phenolic components and the antioxidant potentials of some Turkish fruit wines. Food Res Int 78: 209-215, 2015.
27. Ozyürek M, Bektaşoğlu B, Güçlü K, Güngör N and Apak R: Simultaneous total antioxidant capacity assay of lipophilic and hydrophilic antioxidants in the same acetone-water solution containing 2% methyl-cyclodextrin using the supric reducting antioxidant capacity (CUPRAC) method. Anal Chim Acta 630: 28-39, 2008.
28. Buddhisuharto AK, Pramastya H, Insanu M and Fidrianny I: An updated review of phytochemical compounds and pharmacology activities of *Artocarpus* genus. Biointerface Res Appl Chem 11: 14898-14905, 2021.
29. Magalhães LM, Segundo MA, Reis S and Lima JLFC: Methodological aspects about in vitro evaluation of antioxidant properties. Anal Chim Acta 613: 1-19, 2008.
30. Karadag A, Ozcelik B and Saner S: Review of methods to determine antioxidant capacities. Food Anal Methods 2: 41-60, 2009.
31. Tsimogiannis DI and Oreopoulou V: The contribution of flavonoid C-ring on the DPPH free radical scavenging efficiency. A kinetic approach for the 3',4'-hydroxy substituted members. Innov Food Sci Emerg Technol 7: 140-146, 2006.
32. Platzer M, Kiese S, Tybussek T, Herfellner T, Schneider F, Schweiggert-Weisz U and Eisner P: Radical scavenging mechanisms of phenolic compounds: A quantitative structure-property relationship (QSPR) study. Front Nutr 9: 882458, 2022.
33. Zunjar V, Mammen D and Trivedi BM: Antioxidant activities and phenolics profiling of different parts of *Carica papaya* by LCMS-MS. Nat Prod Res 29: 2097-2099, 2015.
34. Rice-Evans CA, Miller NJ and Paganga G: Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radic Biol Med 20: 933-956, 1996.
35. Stanković MS, Stefanović O, Čomić L, Topuzović M, Radojević I and Solujić S: Antimicrobial activity, total phenolic content and flavonoid concentrations of Teucrium species. Cent Eur J Biol 7: 664-671. 2012.

36. Al-Abd NM, Nor ZM, Mansor M, Azhar F, Hasan MS and Kassim M: Antioxidant, antibacterial activity, and phytochemical characterization of *Melaleuca cajuputi* extract. BMC Complement Altern Med 15: 385, 2015.
37. Islam ME, Parvin MS and Islam MR: Antioxidant and hepatoprotective activity of an ethanol extract of *Syzygium jambos* (L.) leaves. Drug Discov Ther 6: 205-211, 2012.
38. Romulo A: The principle of some in vitro antioxidant activity methods: Review. IOP Conf Ser Earth Environ Sci 426: 012177, 2020.
39. Dontha S: A review on antioxidant methods. Asian J Pharm Clin Res 9: 14-32, 2016.
40. Munteanu IG and Apetrei C: Analytical methods used in determining antioxidant activity: A review. Int J Mol Sci 22: 3380, 2021.
41. Siswanto, Setyawati B and Ernawati F: Role of several micro-substances in immunity. Nutr Eng 36: 57-64, 2013.
42. Pehlivan FE: Vitamin C: An antioxidant agent. In: Hamza AH (ed), Vitamin C. IntechOpen Croatia, pp23-35, 2017.
43. Schober P, Boer C and Schwarte LA: Correlation coefficients: Appropriate use and interpretation. Anesth Analg 126: 1763-1768, 2018.
44. Gaweł-Bęben K, Kukula-Koch W, Hoian U and Czop M: Characterization of *Cistus x incanus* L. and *Cistus ladanifer* L. extracts as potential multifunctional antioxidant ingredients for skin protecting cosmetics. Antioxidants (Basel) 9: 202, 2020.
45. Miean KH and Mohamed S: Flavonoid (myricetin, quercetin, kaempferol, luteolin, and apigenin) content of edible tropical plants. J Agric Food Chem 49: 3106-3112, 2001.
46. Devi M, Sunaryo MA and Mansoor A: Antioxidant analysis of *Artocarpus heterophyllus* drink. IOP Conf Ser Earth Environ Sci 733: 012087, 2021.
47. Sharma A, Gupta P and Verma AK: Preliminary nutritional and biological potential of *Artocarpus heterophyllus* L. shell powder. J Food Sci Technol 52: 1339-1349, 2015.
48. Jalal TK, Khan AYF, Natto HA, Rasad MSBA, Arifin Kaderi M, Mohammad M, Johan MF, Omar MN and Abdul Wahab R: Identification and quantification of quercetin, a major constituent of *Artocarpus altilis* by targeting related genes of apoptosis and cell cycle: In vitro cytotoxic activity against human lung carcinoma cell lines. Nutr Cancer 71: 792-805, 2019.
49. Arriffin NM, Jamil S, Basar N, Khamis S, Abdullah SA and Lathiff SMA: Phytochemical studies and antioxidant activities of *Artocarpus scortechinii* King. Rec Nat Prod 11: 299-303, 2017.
50. Kečkeš S, Gašić U, Ćirković Veličković T, Milojković-Opsenica D, Natić M and Tešić Z: The determination of phenolic profiles of Serbian unifloral honeys using ultra-high-performance liquid chromatography/high resolution accurate mass spectrometry. Food Chem 138: 32-40, 2013.
51. Tomás-Barberán FA, Martos I, Ferreres F, Radovic BS and Anklam E: HPLC flavonoid profiles as markers for the botanical origin of European unifloral honeys. J Sci Food Agric 81: 485-496, 2001.
52. Yao L, Datta N, Tomás-Barberán FA, Ferreres F, Martos I and Singanusong R: Flavonoids, phenolic acids and abscisic acid in Australian and New Zealand Leptospermum honeys. Food Chem 81: 159-168, 2003.
53. Mizzi L, Chatzitzika C, Gatt R and Valdramidis V: HPLC Analysis of phenolic compounds and flavonoids with overlapping peaks. Food Technol Biotechnol 58: 12-19, 2020.
54. Eve A, Aliero AA, Nalubiri D, Adeyemo RO, Akinola SA, Pius T, Nabaasa S, Nabukeera S, Alkali B and Ntulume I: In vitro antibacterial activity of crude extracts of *Artocarpus heterophyllus* seeds against selected diarrhoea-causing superbug bacteria. ScientificWorldJournal 2020: 9813970, 2020.
55. Kamal T, Muzammil A and Nor Oma M: Evaluation of antimicrobial activity of *Artocarpus altilis* on pathogenic microorganisms. Sci Ser Data Rep 4: 41-48, 2012.
56. Negi PS, Jayaprakasha GK and Jena BS: Antibacterial activity of the extracts from the fruit rinds of *Garcinia cowa* and *Garcinia pedunculata* against food borne pathogens and spoilage bacteria. LWT Food Sci Tech 41: 1857-1861, 2008.
57. Alonso R, Fernandez-Aranguiz A, Colom K, Herreras A and Cisterna R: Profile of bacterial isolates and antimicrobial susceptibility: Multicenter study using a one-day cut-off. Rev Esp Quimioter 13: 384-393, 2002 (In Spanish).
58. Huyut Z, Beydemir Ş and Gülçin İ: Antioxidant and antiradical properties of selected flavonoids and phenolic compounds. Biochem Res Int 2017: 7616791, 2017.
59. Ziarno M, Kozłowska M, Ścibisz I, Kowalczyk M, Pawelec S, Stochmal A and Szleszyński B: The effect of selected herbal extracts on lactic acid bacteria activity. Appl Sci 11: 3898, 2021.
60. Kozłowska M, Ścibisz I, Zaręba D and Ziarno M: Antioxidant properties and effect on lactic acid bacterial growth of spice extracts. Cyta J Food 13: 573-577, 2015.
61. War AR, Paulraj MG, Ahmad T, Buhroo AA, Hussain B, Ignacimuthu S and Sharma HC: Mechanisms of plant defense against insect herbivores. Plant Signal Behav 7: 1306-1320, 2012.
62. Onofrejová L, Vašíčková J, Klejdus B, Stratil P, Misurcová L, Krácmar S, Kopecký J and Vacek J: Bioactive phenols in algae: The application of pressurized-liquid and solid-phase extraction techniques. J Pharm Biomed Anal 51: 464-470, 2010.
63. Dai J and Mumper RJ: Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. Molecules 15: 7313-7352, 2010.
64. Maadane A, Merghoub N, Ainane T, El Arroussi H, Benhima R, Amzazi S, Bakri Y and Wahby I: Antioxidant activity of some Moroccan marine microalgae: Pufa profiles, carotenoids and phenolic content. J Biotechnol 215: 13-19, 2015.
65. Mehmood A, Javid S, Khan MF, Ahmad KS and Mustafa A: In vitro total phenolics, total flavonoids, antioxidant and antibacterial activities of selected medicinal plants using different solvent systems. BMC Chem 16: 64, 2022.
66. Kozłowska M, Żbikowska A, Szpicer A and Półtorak A: Oxidative stability of lipid fractions of sponge-fat cakes after green tea extracts application. J Food Sci Technol 56: 2628-2638, 2019.
67. Kozłowska M, Zbikowska A, Marciniak-Lukasiak K and Kowalska M: Herbal extracts incorporated into shortbread cookies: Impact on color and fat quality of the cookies. Biomolecules 9: 858, 2019.
68. Kozłowska M, Ścibisz I, Przybył JL, Laudy AE, Majewska E, Tarnowska K, Małajowicz J and Ziarno M: Antioxidant and antibacterial activity of extracts from selected plant material. Appl Sci 12: 9871, 2022.
69. O'Sullivan AM, O'Callaghan YC, O'Grady MN, Queguineur B, Hannify D, Troy DJ, Kerry JP and O'Brien NM: In vitro and cellular antioxidant activities of seaweed extracts prepared from five brown seaweeds harvested in spring from the west coast of Ireland. Food Chem 126:1064-1070, 2011.
70. Minhas AK, Hodgson P, Barrow CJ and Adholeya A: A review on the assessment of stress conditions for simultaneous production of microalga lipids and carotenoids. Front Microbiol 7: 546, 2016.
71. Hernández I, Alegre L, Van Breusegem F and Munné-Bosch S: How relevant are flavonoids as antioxidants in plants? Trends Plant Sci 14: 125-132, 2009.
72. Nijveldt RJ, Van Nood EL, Van Hoorn DE, Boelens PG, Van Norren K and Van Leeuwen PA: Flavonoids: A review of probable mechanisms of action and potential applications. Am J Clin Nutr 74: 418-425, 2001.
73. Korkina LG and Afanas'ev IB: Antioxidant and chelating properties of flavonoids. Adv Pharmacol 38: 151-163, 1997.
74. Alqahtani AS, Ullah R, and Shahat AA: Bioactive constituents and toxicological evaluation of selected antidiabetic medicinal plants of Saudi Arabia. Evid Based Complement Alternat Med 2022: 7123521, 2022.
75. Shahidi F and Yeo J: Bioactivities of phenolics by focusing on suppression of chronic diseases: A review. Int J Mol Sci 19: 1573, 2018.
76. Ullah R, Alsaid MS, Shahat AA, Nasher AA, Al-mishary AA, Adnan M and Tariq A: Antioxidant and hepatoprotective effects of methanolic extracts of *Zilla spinosa* and *Hammada elegans* against carbon tetrachloride-induced hepatotoxicity in rats. Open Chem 16: 133-140, 2018.
77. Pagare S, Bhatia M, Tripathi N, Pagare S, and Bansal YK: Secondary metabolites of plants and their role: Overview. Curr Trends Biotechnol Pharm 9: 293-304, 2015.
78. Sarker U, Oba S, Ercisli S, Assouguem A, Alotaibi A and Ullah R: Bioactive phytochemicals and quenching activity of radicals in selected drought-resistant *Amaranthus tricolor* vegetable amaranth. Antioxidants (Basel) 11: 578, 2022.
79. Murray RK, Granner DK and Roadwel VW: Harper's Illustrated Biochemistry. In: Wulandari N, Randy L, Dwijayanti L, Liena, Dany F and Rachman LY (eds). Harper Biochemistry. 27th edition. Jakarta: EGC, 2009.
80. Shahidi F, Alasalvar C and Liyana-Pathirana CM: Antioxidant phytochemicals in hazelnut kernel (*Corylus avellana* L.) and hazelnut byproducts. J Agric Food Chem 55: 1212-1220, 2007.
81. Yoon J, Ham H, Sung J, Kim Y, Choi Y, Lee JS, Jeong HS, Lee J and Kim D: Black rice extract protected HepG2 cells from oxidative stress-induced cell death via ERK1/2 and Akt activation. Nutr Res Pract 8: 125-131, 2014.

82. Huang D, Ou B and Prior RL: The chemistry behind antioxidant capacity assays. J Agric Food Chem 53: 1841-1856, 2005.
83. Serafini M, Bugianesi R, Maiani G, Valtuena S, De Santis S and Crozier A: Plasma antioxidants from chocolate. Nature 424: 1013, 2003.
84. Ullah R and Alqahtani AS: GC-MS analysis, heavy metals, biological, and toxicological evaluation of *Reseda muricata* and *Marrubium vulgare* methanol extracts. Evid based Complement Altern Med 2022: 2284328, 2002.
85. Silva NCC and Fernandes Júnior A: Biological properties of medicinal plants: A review of their antimicrobial activity. J Venom Anim Toxins Incl Trop Dis 16: 402-413, 2010.
86. Bouarab-Chibane L, Forquet V, Lantéri P, Clément Y, Léonard-Akkari L, Oulahal N, Degraeve P and Bordes C: Antibacterial properties of polyphenols: Characterization and QSAR (quantitative structure-activity relationship) models. Front Microbiol 10: 829, 2019.
87. Kočevar Glavač N and Lunder M: Preservative efficacy of selected antimicrobials of natural origin in a cosmetic emulsion. Int J Cosmet Sci 40: 276-284, 2018.
88. Jirakitticharoen S, Wisuitiprot W, Jitareerat P and Wongs-Aree C: Phenolics, antioxidant and antibacterial activities of immature and mature *Blumea balsamifera* leaf extracts eluted with different solvents. J Trop Med 2022: 1-12, 2022.
89. Naqvi SAR, Nadeem S, Komal S, Naqvi SAA, Mubarik MS, Qureshi SY, Ahmad S, Abbas S, Zahid M, Khan NUH, *et al*: Antioxidants: Natural antibiotics. Antioxidants 2019: 1-17, 2019.
90. Kuete V: Potential of Cameroonian plants and derived products against microbial infections: A review. Planta Med 76: 1479-1491, 2010.
91. Thuille N, Fille M and Nagl M: Bactericidal activity of herbal extracts. Int J Hyg Environ Health 206: 217-221, 2003.
92. Tsai PJ, Tsai TH and Ho SC: In vitro inhibitory effects of rosemary extracts on growth and glucosyltransferase activity of *Streptococcus sobrinus*. Food Chem 105: 311-316, 2007.
93. Mostafa AA, Al-Askar AA, Almaary KS, Dawoud TM, Sholkamy EN and Bakri MM: Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases. Saudi J Biol Sci 25: 361-366, 2018.
94. Gonemali DF, Lin J, Miao W, Xuan J, Charles F, Chen M and Hatab SR: Antimicrobial properties and mechanism of action of some plant extracts against food pathogens and spoilage microorganisms. Front Microbiol 9: 1639, 2018.
95. Ouchari L, Boukeskasse A, Bouizgarne B and Ouhdouch Y: Antimicrobial potential of actinomycetes isolated from the unexplored hot Merzouga desert and their taxonomic diversity. Biol Open 8: bio035410, 2019.
96. Eloff JN: Avoiding pitfalls in determining antimicrobial activity of plant extracts and publishing the results. BMC Complement Altern Med 19: 106, 2019.
97. Khameneh B, Iranshahy M, Soheili V and Fazly Bazzaz BS: Review on plant antimicrobials: A mechanistic viewpoint. Antimicrob Resist Infect Control 8: 118, 2019.
98. Radji M: Microbiology textbook: Guide for pharmacy and medical students. EGC, Jakarta, pp179-199, 2011.
99. Kapoor G, Saigal S and Elongavan A: Action and resistance mechanisms of antibiotics: A guide for clinicians. J Anaesthesiol Clin Pharmacol 33: 300-305, 2017.
100. Reygaert WC: An overview of the antimicrobial resistance mechanisms of bacteria. AIMS Microbiol 4: 482-501, 2018.
101. Pandey A and Bhatnagar SP: Preliminary phytochemical screening and antimicrobial studies on *Artocarpus lakoocha* Roxb. Anc Sci Life 28: 21-24, 2009.
102. Jagtap UB and Bapat VA: Artocarpus: A review of its traditional uses, phytochemistry and pharmacology. J Ethnopharmacol 129: 142-166, 2010.
103. Povichit N, Phrutivorapongkul A, Suttajit M and Leelapornpisid P: Antiglycation and antioxidant activities of oxyresveratrol extracted from the heartwood of *Artocarpus lakoocha* Roxb. Maejo Int J Sci Technol 4: 454-461, 2010.
104. Sitorus P, Muhammad M, Satria D and Nasri: Antibacterial and antioxidant activities of ethanol extract of *Artocarpus lacucha* Buch-Ham leaves. Food Res 7: 360-364, 2023.