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Evaluation of Antioxidant and Antimicrobial Activities of Ethanol Extracts of Three Kinds of Strawberries

Semeneh Seleshe[1], Jong Seok Lee[2], Sarah Lee[2], Hye Jin Lee[2], Ga Ryun Kim[2], Joohong Yeo[2], Jong Yea Kim[3], and Suk Nam Kang[1]

[1]Department of Animal Resource, Daegu University, Gyeongbuk 38453, Korea
[2]National Institute of Biological Resources, Incheon 22689, Korea
[3]Department of Food Science and Biotechnology, Kangwon National University, Gangwon 24341, Korea

ABSTRACT: The antioxidant and antimicrobial activities of three kinds of strawberry ethanol extracts from *Robus corchorifolius* L. f. (RCL), *Rubus parvifolius* L. var. parvifolius (RPL), and *Duchesnea chrysantha* Miq. (DCM) were investigated. The RPL was highest ($P<0.05$) in phenolic, flavonoid, and anthocyanin contents. 2,2-Diphenyl-1-picrylhydrazyl and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) radical scavenging activities of RPL and DCM extracts were higher than that of RCL ($P<0.05$). Hydrogen peroxide scavenging activity of RPL was high compared to DCM and RCL ($P<0.05$). RCL exhibited a significant ($P<0.05$) potent antioxidant activity in nitric oxide radical inhibition. Inhibition diameter zone (nearest mm) of extracts against the test bacteria ranged from 11.5 in RCL to 12.5 in DCM against *Staphylococcus aureus*, from 10.5 in RCL to 13.5 in DCM against *Streptococcus pneumoniae*, from 8.5 in DCM to 10.5 in RCL against *Escherichia coli*, and the same inhibition of 10 mm in three of the extracts against *Klebsiella pneumoniae*. However, there was no inhibition against fungi *Aspergillus niger* and *Candida albicans*. Three of the extracts had the same minimum inhibitory concentration values of 12.50, 12.50, and 6.25 μg/mL against *S. aureus*, *K. pneumoniae*, and *S. pneumoniae*, respectively. On the other hand, MIC values of 12.50, 12.50, and 6.50 μg/mL were recorded for RPL, DCM, and RCL against *E. coli*, respectively. The result of present study revealed that extracts from three kinds of strawberries could be potential candidates as antioxidant and antimicrobial sources for functional food industries.

Keywords: strawberry, antioxidant, antimicrobial, NO inhibition, minimum inhibitory concentration

INTRODUCTION

Oxidation is one of the major causes of chemical spoilage, resulting in rancidity and/or deterioration of the nutritional quality, color, flavor, texture, and safety of foods (1). Hence, antioxidants have been widely used as food additives to provide protection against oxidative degradation of foods (2). The main characteristics of an antioxidant is its ability to trap free radicals (3). Antioxidants retard the progress of many chronic diseases as well as lipid peroxidation (4). A number of synthetic antioxidants have been added to foodstuffs but, because of toxicity issues (5) and their adverse reaction such as carcinogenicity (6), their use is being restricted (5). Attention has therefore been directed toward the development/isolation of natural antioxidants from botanical sources, especially edible plants that replace synthetic antioxidants. The spoilage and poisoning of foods by microorganisms is also a problem that has not yet been brought under adequate control despite the range of robust preservation techniques available. Alike the application of synthetic antioxidants in foods, consumers are increasingly avoiding foods prepared with preservatives of chemical origin, and natural alternatives are therefore needed to achieve sufficiently long shelf-life of foods and a high degree of safety with respect to foodborne pathogenic microorganisms (7). As a result, there has been an increasing interest in different medicinal and dietary plants for their antioxidant and antimicrobial potential because the antioxidant compounds are related to human health, as well as to pharmaceutical and food industries. It has been suggested that fruits, vegetables, and plants are the main source of antioxidants in the diet (8). Antioxidant compounds of plant sources like phenolic acids, polyphenols, and flavonoids scavenge free radicals such as peroxide, hydroperoxide, or lipid peroxyl and thus inhibit oxida-

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Correspondence to Suk Nam Kang, Tel: +82-53-850-6726, E-mail: whitenightt@hanmail.net

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tive mechanisms (3). Moreover, the use of plant extracts, with known antimicrobial properties, can be of great significance in therapeutic treatments. Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant (9). These products are known as the active substances part of essential oils, for example phenolic compounds and tannins (10,11). Strawberries are popular due to their desirable sweet taste and attractive aroma, with smooth texture and red color. There were more than 600,000 acres and 3.9 million tons of strawberries produced worldwide in 2005 (12). Strawberries are a very good source of dietary fiber, iodine, and folate and are a good source of copper, potassium, biotin, phosphorus, magnesium, vitamin B6, and omega-3 fatty acids (13). Moreover, they are a rich source for ascorbic acid and contain a diverse range of polyphenols (14,15). Oliveira et al. (16) indicated that strawberries could be beneficial for pharmaceutical applications or food supplements. According to Katherine et al. (17), the functionality of strawberries is highly associated with their cultivars. The aim of this research was to determine the antioxidant and antimicrobial potential of extracts from three kinds of strawberries: *Robus corchorifolius* L. f. (RCL), *Rubus parvifolius* L. var. parvifolius (RPL), and *Duchesnea chrysantha* Miq. (DCM).

MATERIALS AND METHODS

Extract preparation

Dried samples of three kinds of strawberries were supplied by the National Institute of Biological Resources (Incheon, Korea). Each sample (100 g) was extracted with 1,000 mL of 70% ethanol at room temperature for 24 h. The extraction process was repeated three times. The extracted materials were filtered with Whatman No. 3 filter paper (Whatman International Ltd., Kent, UK), concentrated with a rotary evaporator (N-3000, Eyela, Tokyo, Japan), and freeze-dried using a freeze dryer (Biotron, Bucheon, Korea). The extracts were dissolved in dimethyl sulfoxide (DMSO) for analysis.

Measurement of total phenolic content

Total phenolic content was determined with the Folin-Ciocalteu reagent according to a procedure described by Singleton and Rossi (18). Briefly, 0.5 mL of the diluted sample was reacted with 2.5 mL of 0.2 mol/L Folin-Ciocalteu reagent for 4 min, and then 2 mL saturated sodium carbonate solution (about 75 g/L) was added into the reaction mixture. The absorbance readings were taken at 760 nm after incubation at room temperature for 2 h. Gallic acid was used as a reference standard, and the results were expressed as milligram gallic acid equivalent (mg GAE)/g dry weight.

Measurement of total flavonoid content

Total flavonoid was determined using the method of Meda et al. (19) with minor modifications. In brief, 0.25 mL of sample (1 mg/mL) was added to a tube containing 1 mL of double-distilled water. Next, 0.075 mL of 5% $NaNO_2$, 0.075 mL of 10% $AlCl_3$, and 0.5 mL of 1 M NaOH were added at 0, 5, and 6 min, sequentially. Finally, the volume of the reacting solution was adjusted to 2.5 mL with double-distilled water. The absorbance of the solution at a wavelength of 410 nm was detected using spectrophotometer (Ultrospec 2100 pro, Amersham Pharmacia Biotech Co., Piscataway, NJ, USA). Quercetin was used as standard to quantify the total flavonoid content. Results were expressed in milligram quercetin equivalents (mg QE)/g dry weight.

Measurement of total anthocyanins

Total anthocyanin was measured using the pH differential method indicated by Giusti and Wrolstad (20). Two flasks were filled with 1 mL of extract each. The first flask was diluted with 4 mL of pH 1.0 buffer (potassium chloride, 0.025 M) and the second one diluted with pH 4.5 buffer (sodium acetate, 0.4 M), separately. Absorbance was measured at 520 and 700 nm in pH 1.0 and 4.5 buffers. A molar extinction coefficient of 26,900 L/cm/mol and a molecular weight of 449.2 were used for anthocyanin calculations. Results were expressed as mg of cyanidin 3-glucoside equivalents (mg CE) per g dry weight.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The free radical scavenging activity of samples (0.05 to 1 mg/mL in DMSO) was measured using the method of Brand-Williams et al. (21) with some modifications. L-ascorbic acid was used as a standard. The inhibition percentage was calculated from the following equation:

$$\% \text{ Inhibition} = \frac{A_0 - A_1}{A_0} \times 100$$

where A_0 is the absorbance of the control and A_1 is the absorbance of the sample and the standard compound.

The radical scavenging activity of the tested samples is expressed as the IC_{50} value. The absorbance was measured by a spectrophotometer at 517 nm.

2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical scavenging assay

The ABTS radical scavenging was conducted using the method indicated by Re et al. (22). Briefly, the ABTS radical cation ($ABTS^+$) was produced by reacting 7 mM stock solution of ABTS with 2.45 mM potassium persulfate

and allowing the mixture to stand in the dark, at room temperature, for 12 h before use. The $ABTS^+$ solution was diluted with methanol to an absorbance of 0.70±0.02 at 734 nm. Next, 2.85 mL of this $ABTS^+$ solution was added to 0.15 mL of different concentrations of the samples, and the decrease in absorbance at 734 nm was observed after mixing for up to 10 min. The radical scavenging activity of the tested samples is expressed as the IC_{50} value. The percentage inhibition of $ABTS^+$ was calculated using the formula:

$$\% \text{ Inhibition} = \frac{A_0 - A_1}{A_0} \times 100$$

where A_0 is the absorbance of the control and A_1 is the absorbance of the sample and the standard compound.

Nitric oxide (NO) scavenging assay

NO radical inhibition was estimated by the Griess Ilosvay reaction according to Hyoung (23) with slight modifications. Briefly, Griess Ilosvay reagent was modified using naphthyl ethylene diamine dihydrochloride (0.1% w/v) instead of 1-naphthylamine (5%). The reaction mixture (3 mL) containing sodium nitroprusside (10 mM, 2 mL), phosphate buffer saline (0.5 mL), and the extract (0.2～1.0 mg/mL) were incubated at 25°C for 150 min. After incubation, 0.5 mL of the reaction mixture was mixed with 1 mL sulfanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 min for completion of the reaction process of diazotization. Then, 1 mL of naphthyl ethylene diamine dihydrochoride was added, mixed and was allowed to stand for 30 min at 25°C. The absorbance was measured at 546 nm. Butylated hydroxyanisole was used as a standard. The percent inhibition was calculated using the formula:

$$\text{Scavenging effect } (\%) = \frac{A_0 - A_1}{A_0} \times 100$$

where A_0 is the absorbance of the control and A_1 is the absorbance of the sample and the standard compound.

Antimicrobial test using the disc diffusion method

The samples were dissolved in DMSO and filtered through 0.45 μm Millipore membrane filters. The microbial strains used in the experiment were purchased as lyophilized samples from the Microbial Resource Center, Korea Research Institute of Bioscience and Biotechnology, Daejeon, Korea. Gram-positive bacteria [*Staphylococcus aureus* subsp. *aureus* (KCTC 1929) and *Streptococcus pneumoniae* (KCTC 5412)], Gram-negative bacteria [*Klebsiella pneumoniae* subsp. *pneumoniae* (KCTC 2208) and *Escherichia coli* (KCTC 1682)], and fungi [*Aspergillus niger* (KCTC 6971) and *Candida albicans* (KCTC 7007)] were used for antimicrobial tests of the extracts. The obtained strains were inoculated with trypticase soy broth and incubated at 37°C for 24 h. The antimicrobial test was then carried out using the disc diffusion method by Bauer et al. (24). One hundred μL of each bacterial suspension contained 10^8 CFU/mL, and each fungal culture was standardized to 10^5 CFU/mL and was spread on nutrient agar medium. Then, 10 to 80 μL (with 10 mg/mL stock samples) per disk was impregnated into 8 mm diameter sterile discs (Toyo Roshi Kaisha, Ltd., Tokyo, Japan), and the disks were allowed to dry for 24 h in the dark at room temperature. The impregnated discs were placed on the inoculated agar and incubated at 37°C for 24 h for clinical bacterial strains and 96 h for fungal strains. Antimicrobial activity was evaluated by measuring the zone of inhibition against the tested organisms. Each assay in this experiment was repeated two times.

Determination of the minimum inhibitory concentration (MIC)

The MIC values were defined as the lowest concentration of the extracts that inhibited the growth of the micro-organism. MIC of extracts was evaluated with resazurin based microtiter dilution assay as follow: under aseptic conditions, 96 well microtitre plates (Tarsons Products Pvt. Ltd., Kolkata, India) were used, and the first wells of the microtiter plate were filled with 100 μL of test materials (from 1,000 μg/mL extract stock solution). The 2nd to 10th wells of the microtitre plates were filled with 50 μL of sterile water. Two fold serial dilution (throughout 2nd to 10th wells) was achieved by transferring 50 μL test material from the first wells to the subsequent wells (the next well) of the same row so that each well had 50 μL of test material in serially descending concentrations. From the 10th wells, 50 μL was removed. The working solution of extracts (100 μg/mL) was diluted out across a 96-well in a two-fold serial dilution to give final testing concentrations of 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39, 0.195, and 0.098 μg/mL. Each microtiter plate had a set of 2 controls: (a) a control with test organism without test extract as positive control (11th wells) and (b) a control with all solutions except test organism (12th wells) to confirm that no contamination occurred while preparing the plate. Then, a volume of 20 μL was taken from bacterial and fungal suspensions (test organisms) and added to each well. The plates were incubated in a temperature controlled incubator at 37°C for 24 h for bacteria and 48 h for fungi. After the period of incubation, 80 μL resazurin dyes was added and re-incubated for 2 h for color development. Finally, the color change in the well was observed visually. The inhibitory concentration was indicated by the blue coloration of the wells following the addition of resazurin. A change of color from blue to red indicated the

Table 1. Total polyphenol, flavonoid, and anthocyanin contents of ethanol extracts from different kinds of strawberries

	RCL	RPL	DCM
Total polyphenol (mg GAE/g)	123.95±3.24[b]	177.11±3.94[a]	118.91±3.20[c]
Total flavonoid (mg QE/g)	11.44±0.42[c]	26.09±2.36[a]	20.47±0.14[b]
Total anthocyanin (mg CE/g)	24.56±4.02[b]	66.00±5.31[a]	36.44±3.80[b]

Data represent mean±standard deviation.
Different letters (a-c) within the row indicate significant differences at $P<0.05$.
RCL, *Robus corchorifolius* L. f.; RPL, *Rubus parvifolius* L. var. parvifolius; DCM, *Duchesnea chrysantha* Miq.
GAE, gallic acid equivalent; QE, quercentin equivalent; CE, cyanidin-3-glucoside equivalent.

presence of live micro-organisms. All the experiments were performed in triplicates. The average values were calculated for the MIC of test material.

Statistical analysis

The data were analyzed using the General Linear Model's procedure using SAS version 8.1 (SAS Institute, Cary, NC, USA). The significance of differences among means of treatments was determined. Duncan's multiple range test ($P<0.05$) was done to compare variations between samples.

RESULTS AND DISCUSSION

Total phenolic, total flavonoid, and total anthocyanin contents of extracts

The health benefits attributed to polyphenols in the diet have stimulated research to investigate the total antioxidant activity of different fruits and vegetables. Strawberries are a good source of dietary antioxidants (25). In this research, as shown in Table 1, the ethanolic extracts from three different kinds of strawberries showed significant differences in their total phenolic, total flavonoid, and total anthocyanin contents ($P<0.05$). It is known that phenolic compounds are important plant constituents because of their free radicals scavenging ability facilitated by their hydroxyl group, and the total phenolic concentration could be used as basis for rapid screening of antioxidant activity (26). Of the three different kinds of strawberries extracts, a significant ($P<0.05$) total phenolic content was found in RPL extract; in their total phenolic content, extracts were ranked as follows: RPL (177.11 mg GAE/g) > RCL (123.95 mg GAE/g) > DCM (118.91 mg GAE/g). Flavonoids are one of the most diverse and widespread group of compounds and are probably the most important natural phenolics. These compounds possess a broad spectrum of chemical and biological activities including radical scavenging properties (27). The investigation for total flavonoid content of three different kinds of strawberries extracts showed a significant ($P<0.05$) content in the RPL extract (26.09 mg QE/g) as compared to both DCM and RCL extracts (11.44 and 20.47 mg QE/g, respectively) (Table 1). The order of total anthocyanin content of the three extracts were ranked as follows: RPL (66.00 mg CE/g) > DCM (36.44 mg CE/g) > RCL (24.56 mg CE/g). The extract from RPL had the highest ($P<0.05$) amount of antioxidants compared to RCL and DCM (Table 1).

Antioxidant activity of extracts

The DPPH radical and ABTS radical cation scavenging assays are widely used to determine the radical scavenging ability of samples (21,22). Therefore, the radical scavenging activities of ethanol extracts of strawberries were determined by these assays. Extracts had a similar trend of antioxidant activities under three concentrations levels (200, 100, and 50 μg/mL) used. Ethanolic extracts from three kinds of strawberries were significantly ($P<0.05$) different in their DPPH radical scavenging activities (Table 2). In the presence of 200 μg/mL concentration, the free DPPH radical inhibitions of extracts were ranked as follows: DCM (86.65%) > RPL (85.67%) > RCL (80.70 %). The IC_{50}, the concentration of extract required to

Table 2. Antioxidant capacities and nitric oxide (NO) inhibition of ethanol extracts from different kinds of strawberries

(unit: %)

Con. (μg/mL)	RCL	RPL	DCM
DPPH			
200	80.70±0.64[b]	85.67±0.17[a]	86.65±0.09[a]
100	62.15±1.90[b]	70.57±1.66[a]	75.80±0.63[a]
50	29.78±0.96[b]	45.17±1.54[a]	48.66±1.29[a]
0	0.00	0.00	0.00
ABTS			
200	88.51±4.52[b]	93.12±0.14[a]	93.06±0.07[a]
100	74.37±3.00[b]	79.67±0.06[a]	80.80±1.19[a]
50	49.16±2.59	47.39±1.59	48.88±0.85
0	0.00	0.00	0.00
NO inhibition			
1,000	68.11±0.78[a]	63.07±0.99[b]	62.28±0.59[b]
500	50.24±1.97[a]	40.99±1.66[c]	42.80±0.90[b]
250	38.38±0.35[a]	26.73±2.07[b]	22.83±1.45[c]

Data represent mean±standard deviation.
Different letters (a-c) within the row indicate significant differences at $P<0.05$.
DPPH, 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity; ABTS, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) radical scavenging activity.
RCL, RPL, and DCM are described in Table 1.

Table 3. IC_{50} of antioxidant capacities of ethanol extracts from different kinds of strawberries (unit: μg/mL)

	RCL	RPL	DCM	Standard (Ascorbic acid)
DPPH	52.80±3.23^{b}	46.69±4.21^{c}	67.85±1.32^{a}	1.64±0.38^{d}
ABTS	53.86±5.13^{a}	51.38±1.11^{a}	49.73±1.12^{b}	8.84±0.67^{c}

Data represent mean±standard deviation.
Different letters (a-d) within the row indicate significant differences at $P<0.05$.
DPPH, 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity; ABTS, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) radical scavenging activity.
RCL, RPL, and DCM are described in Table 1.

quench 50% of test radical, is inversely proportional to the scavenging activities of the extracts. In their increasing DPPH IC_{50}, extracts were order as follow: ascorbic acid (1.64 μg/mL)< RPL (46.69 μg/mL)< RCL (52.80 μg/mL)< DCM (67.85 μg/mL) (Table 3). In the ABTS assay, radicals are deactivated by single electron transfer. This method is common and simple for fast assessment of antioxidant activity of various plants extracts (28). The ethanolic extracts of three kinds of strawberries exhibited strong ABTS radicals scavenging activities as shown in Table 2. Extracts from RPL and DCM had a significant higher ($P<0.05$) scavenging activity than RCL. The descending order of ABTS radical scavenging activity of extracts was as follows: RPL (93.12%), DCM (93.06%)> RCL (88.51%). The IC_{50} of ABTS values of the standard (ascorbic acid), and ethanol extracts of DCM, RPL, and RCL were 8.84, 49.73, 51.38, and 53.86 μg/mL, respectively (Table 3). NO is a diffusible free radical, which plays many important roles as an effector molecule in diverse biological systems including vasodilation, neuronal messenger, and antimicrobial and antitumor activities (29). The result for NO radical scavenging activity of three kinds of strawberries is presented in Table 2. Extracts had significant differences ($P<0.05$) in their NO radical scavenging activity; among the three types of extracts, RCL had the highest scavenging activity at 1 mg/mL sample concentration. The NO radical scavenging ability (percentage) of extracts were ranked in descending order as follows: RCL (68.11%)> RPL (63.07%)> DCM (62.28%).

Disc diffusion antimicrobial activity of extracts

In present study, the antibacterial activity of extracts from three kinds of strawberries against four strains of bacteria; two Gram-positive (*S. aureus* and *S. pneumoniae)* and two Gram-negative (*K. pneumoniae* and *E. coli)* were evaluated using the disc diffusion assay. The inhibitory activity examined was at four concentration levels of samples: 800, 400, 200, and 100 μg/disc as shown in Table 4. The inhibitory activity of extracts showed a similar trend at different concentrations for the same test organisms, and their effectiveness increased as the sample concentration increased from 200 μg/disc. At the lowest concentration of 100 μg/disc, the extracts exhibited no inhibition for all test organisms. A comparison of the inhibitory effect among samples was done with 800 μg/mL concentration. Extracts from three kinds of strawberries exhibited potent antimicrobial activity against Gram-pos-

Table 4. The antimicrobial activities of ethanol extracts from different kinds of strawberries against 6 microorganisms

Con. (μg/disc)	Test organisms					
	SA	SP	KP	EC	AN	CA
RCL						
800	11.52±0.35^{Ac}	10.51±0.71^{Ac}	10.02±0.01^{Ba}	10.52±0.31^{Aa}	8.00±0.00	8.00±0.00
400	10.01±0.35^{Ac}	9.04±0.35^{Cc}	9.54±0.01^{Bb}	10.03±0.30^{Aa}	8.00±0.00	8.00±0.00
200	9.00±0.01^{Bc}	9.01±0.71^{Bb}	9.03±0.35^{Bb}	9.53±0.02^{Aa}	8.00±0.00	8.00±0.00
100	8.00±0.01	8.00±0.00	8.00±0.00	8.00±0.00	8.00±0.00	8.00±0.00
RPL						
800	12.03±0.21^{Bb}	12.52±0.56^{Ab}	10.01±0.25^{Ca}	9.50±0.56^{Db}	8.00±0.00	8.00±0.00
400	10.53±0.15^{Bb}	11.52±0.62^{Ab}	10.00±0.06^{Ca}	9.03±0.32^{Db}	8.00±0.00	8.00±0.00
200	10.01±0.05^{Aa}	10.02±0.12^{Aa}	10.05±0.24^{Aa}	8.05±0.42^{Bb}	8.00±0.00	8.00±0.00
100	8.00±0.00	8.00±0.00	8.00±0.00	8.00±0.00	8.00±0.00	8.00±0.00
DCM						
800	12.51±0.35^{Ba}	13.51±0.18^{Aa}	10.03±0.08^{Ca}	8.53±0.08^{Dc}	8.00±0.00	8.00±0.00
400	11.53±0.62^{Ba}	12.03±0.52^{Aa}	10.02±0.38^{Ca}	8.51±0.04^{Dc}	8.00±0.00	8.00±0.00
200	9.53±0.43^{Bb}	10.02±0.16^{Aa}	10.01±0.46^{Aa}	8.00±0.00^{Cb}	8.00±0.00	8.00±0.00
100	8.00±0.00	8.00±0.00	8.00±0.00	8.00±0.00	8.00±0.00	8.00±0.00

Data represent mean±standard deviation.
Means in column (a-c) are significant different within the same concentration and microorganism.
Means in row (A-D) are significant different with the different microorganisms within the same concentration.
RCL, RPL, and DCM are described in Table 1.
SA, *Staphylococcus aureus*; SP, *Streptococcus pneumoniae*; KP, *Klebsiella pneumoniae*; EC, *Escherichia coli*; AN, *Aspergillus niger*; CA, *Candida albicans*.

itive bacteria, *S. aureus*, and the inhibitory activity was significantly different ($P<0.05$) from other bacteria. It is known that *S. aureus* is one of the most common Gram-positive bacteria causing food poisoning. Its source is not food itself but humans who contaminate foods after they have been processed (7). Extracts from three kinds of strawberries affected this bacterium. Based on their effectiveness, they can be ranked as DCM> RPL> RCL with zone of inhibition of 12.5, 12.0, and 11.5 mm, respectively. Similarly, the extracts were found to possess high antimicrobial activity against Gram-positive *S. pneumoniae* and were significantly ($P<0.05$) varying in their antimicrobial activities. From three kinds of strawberry extracts, DCM and RPL had a significant ($P<0.05$) powerful inhibitory activity against *S. pneumoniae* compared to RCL with 13.5, 12.5, and 10.5 mm zone of inhibition, respectively. *S. pneumonia* or *Streptococcus pneumoniacoccus*, is a member of the genus *Streptococcus*. This Gram-positive bacteria is α-hemolytic and a bile soluble aerotolerant anaerobe (30). Extracts significantly differed ($P<0.05$) in the inhibitory effect against the two Gram-negative bacteria tested. Three kinds of strawberry extracts had the same inhibitory effect against *K. pneumoniae* with 10 mm zone of inhibition. The inhibition effect of extracts against *E. coli* was also significantly varying ($P<0.05$) from one to another. Based on their inhibitory effect, extracts can be ranked as RCL> RPL> DCM having the zone of inhibition of 10.5, 9.5, and 8.5 mm, respectively. In the present study, extracts were observed to have a more potent inhibitory activity against Gram-positive bacteria than Gram-negative and were inactive against fungi. This finding is agreement with previous studies conducted on different plants for antimicrobial activity (31,32). The reason for the difference in sensitivity between Gram-positive and Gram-negative bacteria may be related to their cell wall structure. The resistance of Gram-negative bacteria towards antibacterial substances may be due to the outer phospholipidic membrane carrying structural lipopolysaccharide components of a selective barrier to the hydrophilic solution (33). The antifungal effect of extracts was tested against two fungi strains, *A. niger* and *C. albicans*, and the results are shown in Table 4. Extracts from three kinds of strawberries did not have inhibitory activity against the tested fungi; this calls for further study using concentrations above 800 μg/disc which was used in the present study. Based on the antimicrobial activity results of the present study, it can be suggested that extracts from RCL, RPL, and DCM can be beneficial candidates as antibacterial sources for functional food industries. Moreover, their inhibitory activity was specially promising for potential against Gram-positive bacteria, *S. pneumoniae* and *S. aureus*.

MIC of extracts

In microbiology, the MIC is the lowest concentration of an antimicrobial (like an antifungal, antibiotic, or bacteriostatic) drug that will inhibit the viable growth of a microorganism after overnight incubation (34). The MICs for strawberry extracts against the examined bacterial strains and fungi are presented in Table 5. All three kinds of strawberry extracts (RPL, DCM, and RCL) exhibited marked antimicrobial potential after determining their MIC against the tested bacteria. The results showed that MIC of different extracts of strawberry against bacterial strains ranged from 6.25 μg/mL to 12.5 μg/mL and no inhibition was observed for fungi up to the highest concentration, 50 μg/mL. In this study, the extracts from RPL, DCM, and RCL had the same growth inhibitory activity for the two Gram-positive bacteria, *S. pneumoniae* and *S. aureus*, with MIC values of 6.25 and 12.5 μg/mL, respectively. MICs are defined as the lowest concentration of antimicrobial that will inhibit the visible growth of a micro-organism after overnight incubation. *S. pneumoniae* was found to be more susceptible to the action of all three kinds of strawberries; this bacterium can be inhibited with RPL, DCM, and RCL extracts at the same MIC values of 6.25 μg/mL concentration. Comparing the MIC values of extracts, the lowest value for the Gram-negative bacteria, *E. coli*, was exhibited by the RCL sample at 6.25 μg/mL concentration. Extracts from RPL and

Table 5. Minimum inhibitory concentration (MIC) values of ethanol extracts from different kinds of strawberries against 6 microorganisms

Test organisms	MIC (μg/mL)		
	RPL	DCM	RCL
Staphylococcus aureus	12.50±0.08	12.50±0.06	12.50±0.12
Streptococcus pneumoniae	6.25±0.25	6.25±0.12	6.25±0.08
Escherichia coli	12.50±0.24	12.50±0.06	6.25±0.24
Klebsiella pneumoniae	12.50±0.06	12.50±0.24	12.50±0.06
Aspergillus niger	>50.00	>50.00	>50.00
Candida albicans	>50.00	>50.00	>50.00

Data represent mean±standard deviation.
RCL, RPL, and DCM are described in Table 1.
>50: Inhibition is not found until up to 50 μg/mL.

DCM had the same MIC of 12.5 μg/mL for *E. coli*. For the other Gram-negative bacteria, *K. pneumoniae*, all three kinds of strawberries exhibited the same MIC values of 12.5 μg/mL of extracts. In the present study, the MIC values at 6.25 μg/mL concentration could be achieved against *S. pneumoniae* by the three extracts and against *E. coli* from RCL extract. For two bacteria strains, *S. aureus* and *K. pneumoniae*, the three extracts could exhibit the same MIC values at 12.5 μg/mL concentrations. The difference in MIC of extracts against different strains of bacteria is indicative of the importance of selecting specific kinds of strawberry extracts for target bacteria. For fungi, it was not possible to determine MIC values, due to the absence of, or only weak, antifungal activities up to 50 μg/mL samples concentration used in the current study. However, extracts could have MIC values as the concentrations get stronger.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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