Comparative antibacterial activities of *Garcinia cowa* and *Piper sarmentosum* extracts against *Staphylococcus aureus* and *Escherichia coli* with studying on disc diffusion assay, material characterizations, and batch experiments

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Research article

Using extracted plants is an attractive option because they consist of good chemical properties against different types of bacteria, and the idea of a form modification in beads may conveniently apply to the disinfection of wastewater systems. *Garcinia cowa* and *Piper sarmentosum* leaves were extracted for the synthesis of bead materials and studied material characterizations. A disc diffusion assay, batch experiments, adsorption isotherms, adsorption kinetics, and desorption experiments were studied for investigating the bacteria removal efficiencies of materials. *G. cowa* and *P. sarmentosum* leaves were prepared in powder (GCP and PSP), ethanol extracted (EGC and EPS), and synthesized bead materials (GCB and PSB). GCB had a higher surface area than PSB whereas the particle size and pore size were smaller than PSB. GCP and PSP had heterogeneous cracking surfaces whereas GCB and PSB had sphere-shaped and rough surfaces. Carbon (C), oxygen (O), calcium (Ca), and functional groups of O-H, C-H, N-H, C-O, and C-Cl were found in GCP, PSP, GCB, and PSB. Both extracted and beaded materials demonstrated high antibacterial activities on *Staphylococcus aureus* and *Escherichia coli* by a disc diffusion assay, and GCB demonstrated high bacteria removals on both bacteria types by almost 100% by batch experiments. Freundlich isotherm and a pseudo-second-order kinetic model are good fit models for explaining the adsorption pattern and mechanism of GCB related to a physiochemical adsorption and chemisorption mechanism. Moreover, GCB could reuse more than 3 cycles, so it is possible to use GCB for disinfection in a wastewater treatment system.
1. Introduction

The release of wastewater with bacteria contaminations from industrial, agricultural, and community activities into water resources may cause many human diseases such as typhoid fever, diarrhea, and septicaemia from water consumption [1, 2, 3]. Especially, *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) represented as Gram-positive and Gram-negative bacteria are concerned a case of creating many human diseases above [4, 5], and *E. coli* is part of fecal coliform bacteria which is one of the indicators of water quality assessment [6]. Therefore, contaminated water by bacteria should be recommended to treat below water quality standards before use.

The commonly disinfectant methods in wastewater systems are ozone, ultraviolet light (UV), and chlorine [7]. Among these methods, chlorination is popularly used because of its cost-effective and easy operation; however, this method also leaves unwanted odor and residual chlorine which may affect organisms in the water. Therefore, many studies have investigated alternative choices instead of the use of chlorine. Various metal oxides of zinc oxide (ZnO), titanium dioxide (TiO₂), copper (II) oxide (CuO), and magnesium oxide (MgO) including silver nanoparticles [8, 9, 10, 11, 12] were obtained much attention with high antibacterial efficiencies in several Gram-positive and Gram-negative bacteria; however, positive and negative effects of uses of these materials should be weighted. Metal oxides may accidentally release effluents that may affect the environment, so the applications of these metal oxides need careful use for a safe purpose. As a result, another option of uses of extracted plants against bacteria is an appropriate idea to deal with bacteria removal in water after wastewater treatment.

*Mentha cordifolia*, *Sechium edule* Sm, *Piper belle*, *Linnophila aromatic*, *Oxismium sanctum*, *Pitiecellodium dulce*, *Cratoxyllum formosum*, *Polygonum odoratum*, *Garcinia cowa*, and *Piper sarmentosum* are attractive plants because of their proper chemical properties such as rutin, catechin, hydroxychavicol, and vitexin [13, 14, 15, 16]. As a result, they may inhibit many types of bacteria such as *Staphylococcus epidermidis*, *Bacillus cereus*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Acetobacter aceti*, *Enterobacter aerogenes*, *S. aureus*, and *E. coli* [17, 18, 19, 20, 21]. Since *Garcinia cowa* (*G. cowa*) and *Piper sarmentosum* (*P. sarmentosum*) consists of phenols, flavonoids, alkaloids, xanthones, vitexin, terpenoids, and tannin [20, 22, 23, 24, 25], they were appropriate selected for examining the abilities to inhibit *S. aureus* and *E. coli*. Therefore, the current study attempted to synthesize bead materials of the extracted *G. cowa* and *P. sarmentosum* for investigating their antibacterial against *S. aureus* and *E. coli* by batch tests and to investigate the feasibility of application in the wastewater treatment system.

The current research aimed to extract *G. cowa* and *P. sarmentosum* with ethanol and synthesize *G. cowa* beads (GCB) and *P. sarmentosum* beads (PSB) for the antibacterial studies against *S. aureus* and *E. coli*. Their characteristics on particle size, size of surface area, pore size, pore volume, surface morphology, chemical element, and the functional group were identified by particle size analysis (PSA), Brunauer-Emmett-Teller (BET), Field Emission Scanning Electron Microscopy and Focus Ion Beam (FESEM-FIB), Energy Dispersive X-ray Spectrometer (EDX), and Fourier Transform Infrared Spectroscopy (FTIR), respectively. Antibacterial efficiencies were investigated by a disc diffusion assay and batch experiments. Furthermore, the adsorption pattern and mechanism of the material were investigated to understand which models good explained by studying adsorption isotherms and kinetics. Finally, the desorption experiments explored whether the material could reuse.

2. Materials and methods

2.1. Raw materials

Raw materials were leaves of *Garcinia cowa* (*G. cowa*) in Clusiaceae and *Piper sarmentosum* (*P. sarmentosum*) in Piperaeace which were bought at a local market in Khon Kaen province, Thailand.

2.2. Chemicals

All chemical reagents were analytical grades (AR) and used without purification. The culture media were nutrient agar (NA) and nutrient broth (NB) (HiMedia Laboratories, India). 99.9% Ethanol (*C₂H₅OH*) (RCI Labscan, Thailand) was used as a solvent for plant extraction. 99.5% Dimethyl sulfoxide (*C₆H₅OS*) or DMSO (SDFCL, India), streptomycin (Sigma-Aldrich, Germany), and gentamicin (Sigma-Aldrich, Germany) were used for a disc diffusion assay. For the bead formation, sodium alginate (Na-alginate) and calcium chloride (*CaCl₂*) (KEMAXUS, Australia) were used. 0.65% Nitric acid (*HNO₃*) (Merck, Germany) and 1% sodium hydroxide (*NaOH*) (RCI Labscan, Thailand) were used for pH adjustments.

2.3. Microorganism

*Staphylococcus aureus* (DMST 562) and *Escherichia coli* (DMST 4212) were used as microorganisms and DMST represents the Department of Medical Sciences, Ministry of Public Health, Bangkok, Thailand.

2.4. Bacteria water sample preparation

The dilute concentrations in the range of 10⁴–10⁷ CFU/mL from 10⁸ CFU/mL of *S. aureus* and *E. coli* by sterile deionized water were used for preparing water samples.

2.5. Material synthesis

The material synthesis based on a method by P. Ngamsurach and P. Praipipat [26], and a flow chart of synthesis methods of six materials (GCP, PSP, EGC, EPS, GCB, and PSB) was demonstrated in Figure 1(a) and (b).

Firstly, *G. cowa* powder (GCP) and *P. sarmentosum* powder (PSP) were prepared by washing *G. cowa* and *P. sarmentosum* leaves with tap water, drying them in a hot air oven (Binder, FED 53, Germany) at 50 °C for 12 h, grounding to powder by the blender, and then sieving size of 125 μm. Next, the extracted *G. cowa* (EGC) and *P. sarmentosum* (EPS) were obtained from the mixing of 10 g of GCP or PSP with 100 mL of ethanol in a 250 mL Erlenmeyer flask by an orbital shaker (GFL, 3020, Germany) of 200 rpm for 24 h at ambient temperature. Next, the samples were filtered by a vacuum pump, and then they were evaporated by a rotary evaporator (BUCHI, RE-111 Rotavapor, Switzerland) at 50 °C. After that, they were freeze-dried (LaboGene, Scanvac, Denmark) and kept at 4 °C. For the synthesis of *G. cowa* beads (GCB) and *P. sarmentosum* beads (PSB), EGC or EPS was added into 2% w/v Na-alginate solution, and they were homogenously mixed by a hot plate (Ingenieurburo CAT, M. Zipperer GmbH, M 6, Germany) of 60 °C with constant stirring of 250 rpm for 30 min. Then, they were added into a 50 mL glass syringe of 3 mm diameter, dropped by drop into 0.1 M CaCl₂ solution, and soaked in the CaCl₂ solution for 24 h. After that, bead materials were filtrated and rinsed with deionized water (DI). Then, they were air-dried and kept in a desiccator before use.

Note that: The concentration ratio for preparing plant extract is indicated by how many grams of EGC or EPS is added to 2% w/v Na-alginate. For preparing of 200 mg/mL concentration, 0.2 g of EGC or EPS was added to 1 mL of 2% w/v Na-alginate [26].

2.6. Characterization of materials

Materials in powder and beads were characterized to identify the particle size, size of surface area, pore volume, pore size, surface morphology, chemical composition, and chemical functional group. For the particle size analysis (PSA), a Zetasizer Nano (Malvern, Zetasizer Nano ZS, UK) was used which 0.1 g of sample was added to 30 mL of polyethylene bottle containing 10 mL DI water and sonicated at ambient temperature. Then, they were transferred to a cuvette for a particle size
Figure 1. A flow chart of synthesis methods of six materials (a) GCP, EGC, and GCB (b) PSP, EPS, and PSB.
analysis. Brunauer-Emmett-Teller (BET) (Bel, Bel Sorp mini II, Japan) by isothermal nitrogen gas (N₂) adsorption-desorption at 77.3 K and degas temperature of 80 °C for 24 h was used to analyze the size of surface area, pore volume, and pore size. For the investigations of surface morphology and chemical composition, Field Emission Scanning Electron Microscopy and Focus Ion Beam (FESEM-FIB) with Energy Dispersive X-Ray Spectrometer (EDX) (FEI, Helios NanoLab G3 CX, USA) was used which the samples were mounted on aluminum stubs by double-side carbon tapes and coated by gold-coater for 4 min by a 108 auto Sputter Coater with thickness controller MTM-20 model (Cressington, Ted Pella Inc, USA) with analyzing at a 10 kV accelerating voltage. Fourier Transform Infrared Spectroscopy (FTIR) (Bruker, TENSOR27, Hong Kong) in a range of 4000-600 cm⁻¹ with a resolution of 4 cm⁻¹ and 16 scans over the entire covered range was used to identify chemical functional group [26].

2.7. Disc diffusion assay

The method of disc diffusion assay is based on the study of Ngamsurach, P and Praipipat, P [27]. Paper disc, GCB, and PSB were used for a disc diffusion assay shown in Figure 2 including three steps, and the details were explained below. For GCB and PSB, step 2 was skipped.

Step 1. Preparing of bacteria concentration, a 0.5 McFarland standard was used for preparing of bacteria concentration of 10⁶ CFU/mL of S. aureus and E. coli.

Step 2. Preparing of test solution, 10% dimethyl sulfoxide (DMSO) was used as a solvent. EGC or EPS was added to the solvent in concentrations of 100–400 mg/mL.

Step 3. The antibacterial tests, paper disc, GCB, and PSB were used, and the details are explained below:

Step 3.1. Paper disc

For each plate test, a three-dimension swab technique was used for applying bacteria into nutrient agar and putting four pieces of paper disc soaked the test solution in step 2 in a plate test.

Step 3.2. GCB and PSB

For each plate test, a three-dimension swab technique was used for applying bacteria into nutrient agar and putting four pieces of GCB or PSB in a plate test.

After that, the plates were taken in an incubation oven (Termaks, KBP 6087, Norway) at 37 °C for 24 h. The results were reported by measuring the inhibition zone. The average diameters of the inhibition zones (mm) (mean ± SD) were reported, and the results were confirmed by triplicate experiments. For the controls, paper discs soaked with 30 μg/mL of streptomycin and gentamicin were used as positive controls of S. aureus and E. coli, respectively whereas paper discs soaked with 50 μL of 10% DMSO were used as negative controls of S. aureus and E. coli. Finally, the bead material with higher antibacterial activity was selected for investigating batch experiments [26].

2.8. Batch experiments

The bead materials (GCB or PSB) which demonstrated high antibacterial activity in a disc diffusion assay were used for batch tests, adsorption isotherms, adsorption kinetics, and desorption experiments.

For batch tests, the differences in dosage, contact time, pH, and concentration of GCB or PSB were investigated for bacteria removal efficiencies on S. aureus and E. coli, and the details were clearly explained below:

2.8.1. Effect of dose

The effect of dose was investigated which dose displayed the highest bacteria removal efficiency of GCB or PSB on S. aureus and E. coli, and 0.1, 0.2, 0.3, and 0.4 g of GCB or PSB were used to explore the optimum dose. A sample volume of 100 mL, a bacteria concentration of 10⁶ CFU/mL, a shaking speed of 200 rpm, a contact time of 6 h, and pH 7 were applied as the control condition.

2.8.2. Effect of contact time

The optimum dose of GCB or PSB from 2.8.1 was used to investigate the bacteria removal efficiency of GCB or PSB for the effect of contact time which 1, 2, 3, 4, 5, 6, 7, and 8 h were used to find the optimum contact time of GCB or PSB. A sample volume of 100 mL, a bacteria concentration of 10⁶ CFU/mL, a shaking speed of 200 rpm, and pH 7 were applied as the control condition.

2.8.3. Effect of pH

The optimum dose and contact time of GCB or PSB from 2.8.1 and 2.8.2 were used to investigate the bacteria removal efficiency of GCB or PSB for the effect of pH which pHs of 5, 7, and 9 were used to investigate the optimum pH of GCB or PSB. A sample volume of 100 mL, a bacteria concentration of 10⁶ CFU/mL, and a shaking speed of 200 rpm were applied as the control condition.

2.8.4. Effect of concentration

The optimum dose, contact time, and pH of GCB or PSB from 2.8.1, 2.8.2, and 2.8.3 were examined for the bacteria removal efficiency of GCB or PSB for the effect of concentration which bacteria concentrations of 10¹, 10⁵, 10⁶, and 10⁷ CFU/mL were used to study the optimum concentration of GCB or PSB. The control condition was a sample volume of 100 mL and a shaking speed of 200 rpm.

The optimum condition was selected from the highest bacteria removal efficiency of the lowest value of each factor. Triplicate experiments were conducted and reported the average values. The plate count technique was used for data analysis, and the percentage of bacteria removal efficiency is calculated following Eq. (1):

\[
\text{Bacteria removal efficiency} (%) = \frac{C_0 - C_e}{C_0} \times 100
\]

where \( C_0 \) is the final bacteria concentration (CFU/mL), and \( C_e \) is the initial bacteria concentration (CFU/mL).

2.9. Adsorption isotherms

The interaction of bacteria in the solution with GCB or PSB can be described by using linear and nonlinear adsorption isotherms of Langmuir, Freundlich, and Temkin following to equations in Table 1.

where \( q_e \) is the adsorption capacity of bacteria on GCB or PSB at equilibrium (CFU/g), and \( q_m \) is the maximum number of adsorbed bacteria on GCB or PSB (CFU/g). \( C_e \) is the final bacteria concentration (CFU/mL), and \( K_L \) is the Langmuir adsorption constant (L/CFU) or Freundlich adsorption constant (CFU/g) (L/CFU)¹/n and \( n \) is the constant depicting the adsorption intensity [26]. \( R \) is the universal gas constant (8.314 J/mol K), and \( T \) is the absolute temperature (K), \( b_1 \) is the constant related to the heat of adsorption (J/mol), and \( b_2 \) is the equilibrium binding constant corresponding to maximum binding energy (L/CFU) [28]. Graphs of Langmuir, Freundlich, and Temkin isotherms were plotted by linear plotting graphs of \( C_e/q_e \) versus \( C_e \), versus \( C_e \), and \( q_e \) versus \( n \), respectively whereas their nonlinear plotting graphs were plotted by \( q_e \) versus \( C_e \).

For isotherm tests, the optimum dose of GCB or PSB was applied with various concentrations of S. aureus and E. coli from 10¹ to 10⁷ CFU/mL. Moreover, a sample volume of 100 mL, a contact time of 6 h, pH 7, a temperature of 25 °C, and a shaking speed of 200 rpm were applied as the control condition [26].

2.10. Adsorption kinetics

The adsorption kinetic is used to determine the rate of adsorption to time or explain the adsorption mechanism of the adsorbent. Linear and nonlinear of a pseudo-first-order kinetic model, pseudo-second-order
Adsorption kinetics and intra-particle diffusion model were investigated characteristic constants of sorption following to equations in Table 1.

where \( q_e \) and \( q_t \) (CFU/g) are the adsorption capacities of bacteria on GCB or PSB at equilibrium and at the time \( t \), respectively. \( k_1 \) (min\(^{-1}\)), \( k_2 \) (g/(CFU-min)), and \( k_i \) (g/(CFU-min\(^{0.5}\))) are the reaction of rate constants of pseudo-first-order kinetic model, pseudo-second-order kinetic model [26], and intra-particle diffusion model, respectively. \( C_i \) is the constant value that gives an idea about the thickness of the boundary layer (mg/g) [26]. Graphs of the linear pseudo-first-order kinetic model, pseudo-second-order kinetic model, and intra-particle diffusion model were plotted by \( \ln(q_e-q_t) \) versus time \( t \), \( t/q_e \) versus time \( t \), and \( q_t \) versus time \( t^{0.5} \) at the difference of initial bacteria concentrations, respectively. Finally, their nonlinear plotting graphs were plotted by \( q_t \) versus time \( t \).

For kinetic tests, the optimum dose of GCB or PSB was used with the control condition of \( S. aureus \) and \( E. coli \). For kinetic tests, the optimum dose of GCB or PSB was used with the control condition of \( S. aureus \) and \( E. coli \). Desorption tests were performed with \( 10^6 \) CFU/mL concentration of \( S. aureus \) and \( E. coli \), and a shaking speed of 200 rpm [26].

### 2.11. Desorption experiment

Desorption experiments of three adsorption-desorption cycles based on a method of P. Ngamsurach and P. Praipipat [26, 27] were designed to investigate the possible reusability of GCB or PSB. Used GCB or PSB was added to a 250 mL Erlenmeyer flask containing 100 mL of 0.01 M HNO\(_3\) solutions, and then they were shaken at 200 rpm for 2 h. Then, they were washed with DI water, air-dried at ambient temperature, and ready for next cycle use. Eq. (2) is used for calculating the percentage of desorption efficiency.

\[
\text{Desorption (\%)} = \left( \frac{q_d}{q_e} \right) \times 100 \tag{2}
\]

where \( q_d \) is the number of bacteria desorbed (CFU/mL) and \( q_e \) is the number of bacteria adsorbed (CFU/mL).

### 3. Results and discussion

#### 3.1. The physical characteristic of raw materials

The physical characteristics of \( G. cowa \) and \( P. sarmentosum \) in leaf, powder, and beads were illustrated in Figure 3(a, b, c, d, e, f). For \( G. cowa \), a leaf is a simple, opposite, oblong-shape, acute or obtuse-apex shape, acute, cuneate or attenuate-base shape, and entire-leaf margin. Normally, \( G. cowa \) leaves are deep-green color whereas young leaves are light-green or reddish-purple green color [35] illustrated in Figure 3(a). \( G. cowa \) powder (GCP) was brown color shown in Figure 3(b) while \( G. cowa \) beads (GCB) were black color beads demonstrated in Figure 3(c). For \( P. sarmentosum \), a leaf is a simple, alternate, heart-shaped, acuminate-apex shape, cordate-base shape, and entire-leaf margin. Leaves have a waxy surface and light to dark green color [35, 36] presented in Figure 3(d). \( P. sarmentosum \) powder (PSP) was olive-green color displayed in Figure 3(e) while \( P. sarmentosum \) beads (PSB) were black color beads shown in Figure 3(f).

| Adsorption isotherms | Linear | Nonlinear | Reference |
|----------------------|--------|-----------|-----------|
| Langmuir isotherm    | \( C_e/q_e = 1/q_m K_L + C_s/q_m \) | \( q_e = q_m K_L C_s / (1 + K_L C_s) \) | [29] |
| Freundlich isotherm  | \( \log q_e = \log K_F + 1/n \log C_s \) | \( q_e = K_F C_s^{1/n} \) | [30] |
| Temkin isotherm      | \( q_e = RT/b_t \ln A_t + RT/b_1 \ln C_s \) | \( q_e = RT/b_t \ln A_t C_s \) | [31] |
| Adsorption kinetics  | \( \ln (q_e - q_t) = \ln q_e - K_1 t \) | \( q_t = q_e (1 - e^{-K_1 t}) \) | [32] |
| Pseudo-first-order kinetic model | \( t/q_t = 1/k_{eq} + t/q_a \) | \( q_t = k_2 t^{0.5} (1 + q_e k_2 t) \) | [33] |
| Pseudo-second-order kinetic model | \( t/q_t = 1/k_{eq} + t/q_a \) | \( q_t = k_2 t^{0.5} (1 + q_e k_2 t) \) | [33] |
| Intra-particle diffusion model | \( q_t = k_d t^{0.5} + C_i \) | \( q_t = k_d t^{0.5} (1 + q_e k_2 t) \) | [34] |

Figure 2. A disc diffusion assay of the paper disc (EGC and EPS) and bead materials (GCB and PSB) on \( S. aureus \) and \( E. coli \).
3.2. Characterizations of antibacterial materials

3.2.1. Physicochemical properties of GCB and PSB by PSA and BET analysis

The physicochemical properties of GCB and PSB by PSA and BET analysis were reported in Table 2. For the particle size analysis (PSA), the diameters of GCB and PSB were 2402 ± 144.7 and 3353 ± 215.2 nm, respectively which PSB demonstrated a higher particle size than GCB. For BET Analysis, sizes of the surface area of GCB and PSB were 0.7129 and 0.6798 m²/g, respectively which GSB presented a higher surface area than PSB. For pore volumes, they were 0.2045 and 0.2331 cm³/g for GCB and PSB, respectively which PSB demonstrated a higher pore volume than GCB. The pore sizes of GCB and PSB were 0.0196 and 0.0233 nm, respectively which PSB represented a higher pore size than GCB corresponded to the results of particle size. Moreover, their pore sizes were classified as microporous size (≤2 nm) following the classification of IUPAC [37]. Normally, a material that has a higher surface area and smaller pore size will highly adsorb the pollutant than another [28]. Therefore, since GCB had a higher surface area and smaller pore size than PSB, GCB might better adsorb or remove S. aureus and E. coli than PSB.

3.2.2. FESEM-FIB and EDX analysis

FESEM-FIB with 5,000X magnification was used to investigate the morphology structures of GCP and PSP, and the morphology structures of GCB and PSB were explored through FESEM-FIB with 10,000X magnification. GCP and PSP were heterogeneous cracking or flake surfaces shown in Figure 4(a) and (c) whereas GCB and PSB had uneven and coarse surfaces shown in Figure 4(e) and (h). Moreover, the shapes of GCB and PSB were spherical shape with 65X magnification shown in Figure 4(f) and (i).

Figure 4(b, d, g, j) represented the chemical elements of GCP, GCB, PSP, and PSB by EDX analysis. Carbon (C), oxygen (O), and calcium (Ca) were the main chemical elements observed in all materials while chloride (Cl) was detected in all of them except GCP. Potassium (K) and copper (Cu) were found in GCP and PSP while sodium (Na) was observed in GCB and PSB which might be from Na-alginate in a bead formation. In addition, only PSP detected magnesium (Mg). The percentages by weight of each chemical element of GCP, GCB, PSP, and PSB were also demonstrated in Figure 4(b, d, g, j), and their three main compositions ranged from high to low as C > O > Ca. For K, GCB and PSB had 0.6 wt% and 7.6 wt%. For Na, GCB and PSB had 1.1 wt% and 1.8 wt%. For Mg, GCB had 0.5 wt% and 0.4 wt%. Finally, PSP had 0.7 wt% of Mg. Therefore, the changing material from a powder to a bead form affects the mass percentages of chemical elements by the increase of O, Ca, and Cl and decrease of C. In addition, the use of CaCl2 in a bead formation might result in the increase of Ca and Cl in bead materials.

3.2.3. FTIR analysis

Figure 5(a, b, c, d) illustrated chemical functional groups of GCP, GCB, PSP, and PSB in a range of 4000–600 cm⁻¹ by FTIR analysis. Five main functional groups of O–H (carboxylic acid), C–H (alkane or aldehyde or methyl or alkene or benzene), N–H (amine I or amide II), C–O (ether and ester), and C–Cl (chloride compound) were realized in all materials whereas C¼O (ester) was found in GCP, PSP, and PSB except GCB. Although they shared main functional groups mentioned above, their wavenumbers of each functional group of O–H (3600–3200 cm⁻¹), C–H (3000–2800 or 2900–1450 or 1375–800 cm⁻¹), C–O

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Table 2. The particle sizes, sizes of surface area, pore volumes, and pore sizes of GCB and PSB by PSA and BET analysis.

|        | GCB        | PSB        |
|--------|------------|------------|
| Diameter (nm) (±SD) | 2402 ± 144.7 | 3353 ± 215.2 |
| PDI    | 0.726      | 0.579      |
| Surface area (m²/g)* | 0.7129 | 0.6798 |
| Pore volume (cm³/g)** | 0.2045 | 0.2331 |
| Pore size (nm)*** | 0.0196 | 0.0233 |

* MultiPoint BET.
** Total pore volume for pores with Radius.
*** Average pore radius.
For GCP, the chemical functional groups observed through O–H (carboxylic acid) at 3286.04 cm$^{-1}$, C–H (alkane) at 2918.41 cm$^{-1}$, C–H (aldehyde) at 2856.47 cm$^{-1}$, C–H (methyl) at 1441.67 cm$^{-1}$, C–H (benzene) at 821.76 cm$^{-1}$, C–O (ester) at 1030.30 cm$^{-1}$, and C–Cl (chloride compound) at 762.81 and 665.72 cm$^{-1}$ [39] shown in Figure 5(a). For GCB, the chemical functional groups illustrated through O–H (carboxylic acid) at 3320.44 cm$^{-1}$, C–H (alkane) at 2921.60 cm$^{-1}$, C–H (aldehyde) at 2854.54 cm$^{-1}$, C–H (methyl) at 1421.70 cm$^{-1}$, C–H (alkene) at 987.18 cm$^{-1}$, N–H (amine I) at 1595.85 cm$^{-1}$, C–O (ether and ester) at 1116.74 and 1088.06 cm$^{-1}$, and C–Cl (chloride compound) at 622.26 cm$^{-1}$ shown in Figure 5(b). For PSP, the chemical functional groups examined through O–H (carboxylic acid) at 3285.36 cm$^{-1}$, C–H (alkane) at 2918.64 cm$^{-1}$, C–H (aldehyde) at 2852.61 cm$^{-1}$, C–H (methyl) at 1442.15 cm$^{-1}$, C–H (benzene) at 829.98 cm$^{-1}$, C–O (ester) at 1737.28 cm$^{-1}$, N–H (amine I) at 1620.60 cm$^{-1}$, C–O (ether and ester) at 1318.88, 1195.10, 1058.99, and 1025.11 cm$^{-1}$, and C–Cl (chloride

Figure 4. The morphology structures of G. cowa and P. sarmentosum in (a, c) powder (e, h) beads, (f, i) shape, and chemical compositions of G. cowa and P. sarmentosum in (b, d) powder, (g, j) beads.
compound) at 782.16 cm\(^{-1}\) shown in Figure 5(c). Finally, the chemical functional groups of PSB determined through O-H (carboxylic acid) at 3344.84 cm\(^{-1}\), C-H (alkane) at 2923.95 cm\(^{-1}\), C-H (aldehyde) at 2896.98 cm\(^{-1}\), C-H (methyl) at 1432.91 cm\(^{-1}\), C-H (benzene) at 819.01 cm\(^{-1}\), C=O (ester) at 1733.60 cm\(^{-1}\), N-H (amine I) at 1602.74 cm\(^{-1}\), N-H (amide II) at 1511.33 cm\(^{-1}\), C-O (ether and ester) at 1023.94 cm\(^{-1}\), and C-Cl (chloride compound) at 740.86 cm\(^{-1}\) shown in Figure 5(d).

3.3. Results of disc diffusion assay

A general method for the antibacterial examination by extracted plants is a disc diffusion assay. This study attempted to investigate how many of the antibacterial efficiencies of the paper disc (EGC and EPS) and bead materials (GCB and PSB) on S. aureus and E. coli were. The disc diffusion assay results are reported in Table 3.

![Figure 5. FTIR spectrum of G. cowa and P. sarmentosum in (a, c) powder (b, d) beads, respectively.](image-url)
For controls, the inhibition zones of positive controls were $16.1 \pm 0.2$ mm for *S. aureus* and $15.5 \pm 0.3$ mm for *E. coli*, respectively whereas their negative controls were 0 mm.

For the paper disc, the inhibition zones in various EGC concentrations from 100-400 mg/mL on *S. aureus* and *E. coli* were 7.3 ± 0.1, 7.5 ± 0.1, 8.2 ± 0.3, 8.5 ± 0.3 mm for *S. aureus*, and 7.3 ± 0.3, 7.5 ± 0.1, 7.6 ± 0.3, 7.8 ± 0.3 mm for *E. coli*, and the average of four concentrations of EGC on *S. aureus* and *E. coli* were 7.9 ± 0.6 and 7.6 ± 0.2 mm, respectively. Moreover, the inhibition zones in various EPS concentrations from 100-400 mg/mL on *S. aureus* and *E. coli* were 7.0 ± 0.1, 7.2 ± 0.1, 7.8 ± 0.3, 7.9 ± 0.3 mm for *S. aureus*, and 7.0 ± 0.4, 7.1 ± 0.1, 7.5 ± 0.5, 7.7 ± 0.2 mm for *E. coli*, and the average of four concentrations of EPS on *S. aureus* and *E. coli* were 7.5 ± 0.4 and 7.3 ± 0.3 mm, respectively. Therefore, the average inhibition zones of EGC and EPS represented a similar trend by the increase of antibacterial efficiency with the increase of EGC and EPS concentration, and EGC presented higher antibacterial activity on *S. aureus* and *E. coli* than EPS.

For bead materials, the inhibition zones in various GCB concentrations from 100-400 mg/mL on *S. aureus* and *E. coli* were 3.3 ± 0.5, 3.5 ± 0.1, 4.6 ± 0.1, 5.1 ± 0.8 mm for *S. aureus*, and 3.0 ± 0.0, 3.4 ± 0.1, 4.6 ± 0.3, 5.0 ± 0.0 mm for *E. coli*, and the average of four concentrations of GCB on *S. aureus* and *E. coli* were 4.1 ± 0.9 and 4.0 ± 1.0 mm, respectively. In addition, the inhibition zones in various PSB concentrations from 100-400 mg/mL on *S. aureus* and *E. coli* were 3.5 ± 0.1, 3.6 ± 0.1, 3.7 ± 0.3, 3.8 ± 0.3 mm for *S. aureus*, and 3.4 ± 0.3, 3.5 ± 0.0, 3.6 ± 0.1, 3.7 ± 0.1 mm for *E. coli*, and the average of four concentrations of GCB on *S. aureus* and *E. coli* were 3.7 ± 0.1 and 3.6 ± 0.1 mm, respectively. Therefore, the average inhibition zones on GCB and PSB also represented

### Table 3. Disc diffusion assay on *S. aureus* and *E. coli* by paper disc (EGC and EPS) and bead materials (GCB and PSB).

| Bacteria | Positive control | Negative control | The average diameters of the inhibition zones (mm) (mean ± SD) |
|----------|------------------|------------------|---------------------------------------------------------------|
|          |                  |                  | Paper disc                                                    |
|          |                  |                  | EGC | EPS | GCB | PSB |
| *S. aureus* | 16.1 ± 0.2 | 0 | 100 mg/mL | 7.3 ± 0.1 | 7.0 ± 0.1 | 3.3 ± 0.5 | 3.5 ± 0.1 |
|          |                  |                  | 200 mg/mL | 7.5 ± 0.1 | 7.2 ± 0.1 | 3.5 ± 0.3 | 3.6 ± 0.1 |
|          |                  |                  | 300 mg/mL | 8.2 ± 0.3 | 7.8 ± 0.3 | 4.6 ± 0.1 | 3.7 ± 0.3 |
|          |                  |                  | 400 mg/mL | 8.5 ± 0.3 | 7.9 ± 0.3 | 5.1 ± 0.8 | 3.8 ± 0.3 |
| *E. coli* | 15.5 ± 0.3 | 0 | 100 mg/mL | 7.3 ± 0.3 | 7.0 ± 0.4 | 3.0 ± 0.0 | 3.4 ± 0.3 |
|          |                  |                  | 200 mg/mL | 7.5 ± 0.1 | 7.1 ± 0.1 | 3.4 ± 0.1 | 3.5 ± 0.0 |
|          |                  |                  | 300 mg/mL | 7.6 ± 0.3 | 7.5 ± 0.5 | 4.6 ± 0.3 | 3.6 ± 0.1 |
|          |                  |                  | 400 mg/mL | 7.8 ± 0.3 | 7.7 ± 0.2 | 5.0 ± 0.0 | 3.7 ± 0.1 |

Figure 6. The results of batch tests of GCB in (a) dose, (b) contact time, (c) pH, and (d) concentration on *S. aureus* and *E. coli*.
a similar trend by the increase of antibacterial efficiency with the increase of GCB and PSB concentration, and GCB presented higher antibacterial activity on *S. aureus* and *E. coli* than PSB.

For the comparison of disc diffusion assay results by paper disc (EGC and EPS), and bead materials (GCB and PSB), they demonstrated a similar trend of the increase of antibacterial efficiency with the increase of concentration. Because their average inhibition zones were almost 300 mg/mL, it was a suitable concentration to inhibit *S. aureus* and *E. coli* which was the same optimum concentration of extract plants found in our previous studies [26]. Furthermore, both EGC and GCB represented a higher antibacterial activity on *S. aureus* and *E. coli* than EPS and PSB which were similarly found in other studies [13, 20, 22, 40], so *G. cowa*

Figure 7. The plotting graphs of (a, b, c, d, e, f) linear and (g, h) nonlinear adsorption isotherms of GCB on *S. aureus* and *E. coli*.
could inhibit both bacteria types than P. sarmentosum. Moreover, these results corresponded to the results of PSA and BET analysis that GCB might highly inhibit S. aureus and E. coli than PSB. In our previous study [26], we used different plant types of Cratoxylum formosum and Polyg- onum odoratum in bead materials (CFB and POB) and found both bead materials could high be against E. coli than S. aureus whereas CFB illustrated a higher inhibition zone than POB. For comparing the highest antibacterial activity by bead materials (CFB and GCB) of both studies, CFB illustrated the higher antibacterial activity than GCB on S. aureus and E. coli which might result from the different main chemical extractions or chemical functional groups of both plants.

The possible mechanisms of the paper disc (EGC and EPS) and bead materials (GCB and PSB) to inhibit S. aureus and E. coli might be from the main substances of extracted G. cowa (phenolic acid, flavonoids, and charmonague) and P. sarmentosum (phenolic acid, flavonoids, tannic acid, gallic acid, quercetin, naringin, and alkaloids) [41, 42] in paper discs and bead materials penetrated cell wall of bacteria, then it results in malfunction of bacteria cells [26]. Consequently, the cell dies.

However, the average antibacterial efficiency from paper disc (EGC and EPS) to GCB tests on S. aureus was decreased to 41.25% and 47.24% for E. coli which might be the material test effect. Two factors which were the size of test materials and Na-alginabe in bead materials might result in the higher antibacterial of the paper disc than bead materials. Since the paper disc of 6 mm had a larger diameter than the glass syringe of 3 mm, the extracted plant solutions might be absorbed into the paper disc more than the bead materials similar reported by another study [27]. In addition, the bead formation by sodium alginate might affect the releasing the extracted plant to antibacterial activity. Therefore, batch experiments were designed to confirm the bacteria removal efficiencies of GCB on S. aureus and E. coli in this study.

### 3.4. Batch experiments

**3.4.1. Effect of dose**

Figure 6(a) illustrated the effect of GCB dose on bacteria removal efficiencies. Bacteria removal efficiencies of GCB on S. aureus and E. coli were increased from 96.50 to 99.99% and 96.50–100%, respectively with the increase of dosages from 0.1 to 0.4 g. Moreover, bacteria removal efficiencies of GCB on S. aureus and E. coli were constant after 0.3 g, so 0.3 g was the optimum dose of GCB against S. aureus and E. coli for the next experiment of contact time effect.

**3.4.2. Effect of contact time**

Figure 6(b) represented the effect of contact time from 1 to 8 h of GCB on bacteria removal efficiencies. For S. aureus, bacteria removal efficiencies of GCB were higher than 97% in all contact times, and they found that bacteria removal efficiencies of GCB were 100% from 6 to 8 h. As a result, 6 h was a suitable contact time because it was the lowest value for 100% bacteria removal efficiency of GCB. For E. coli, bacteria removal efficiencies of GCB were increased from 97.10 to 100% when the contact times were increased from 1 to 8 h. Then, they demonstrated a constant value of 100% after 3h. Thus, 3h was the optimum contact time of GCB on E. coli. Therefore, the conditions of GCB on S. aureus and E. coli were 0.3 g, 6 h and 0.3 g, 3 h, respectively for studying of pH effect.

**3.4.3. Effect of pH**

The changing of pH conditions might be another affecting factor to bacteria removal efficiencies of GCB on S. aureus and E. coli, so pH values of 5, 7, and 9 are good choices for representing acidic, neutral, and alkaline conditions. Figure 6(c) presented the effect of pH on bacteria removal efficiencies of GCB on S. aureus and E. coli. For S. aureus, the results illustrated all pH values represented almost 100% bacteria removal efficiencies, and they also found a little increase in bacteria removal efficiencies from pH 5 to 9 in the range of 99.82–99.96%. For E. coli, the results demonstrated pH 7 demonstrated 100% bacteria removal efficiencies whereas pH 5 and 9 represented the same bacteria removal efficiencies of 99.99%. As a result, since they showed little difference in bacterial removal efficiencies in pH values of 5, 7, and 9, the changing of pH values did not affect to bacteria removal efficiencies of GCB on both bacteria types. In the case of safe water quality, pH 7 was a preferred value. Therefore, the conditions of GCB on S. aureus and E. coli were 0.3 g, 6 h, pH 7 and 0.3 g, 3 h, pH 7 for investigating of concentration effect.

**3.4.4. Effect of concentration**

Figure 6(d) demonstrated the effect of concentration from $10^4$ to $10^7$ CFU/mL of GCB on bacteria removal efficiencies. The result represented high bacteria removal efficiencies of GCB on both bacteria types almost 100% at concentrations from $10^4$ to $10^6$ CFU/mL, and then they were a little decreased. Therefore, the concentration of $10^6$ CFU/mL was confirmed as the suitable concentration against S. aureus and E. coli.

In summary, all factors of dose, contact time, and concentration affected bacteria removal efficiencies in S. aureus and E. coli whereas pH did not affect GCB efficiencies. Finally, the optimum conditions of GCB were 0.3 g, 6 h, pH 7, $10^6$ CFU/mL for S. aureus and 0.3 g, 3 h, pH 7, $10^6$ CFU/mL for E. coli, and they could be treated almost 100% in 100 mL of contaminated water.

### 3.5. Isotherm study

Figure 7(a, b, c, d, e, f, g, h) demonstrated the results of linear and nonlinear Langmuir, Freundlich, and Temkin isotherms of GCB on S. aureus and E. coli, and Table 4 also reported their equilibrium isotherm parameters.

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**Table 4. The equilibrium isotherm parameters of GCB on S. aureus and E. coli.**

| Isotherm Type   | Linear Parameters | Nonlinear Parameters |
|-----------------|-------------------|----------------------|
|                 | $S$. a. | $E$. c. | $S$. a. | $E$. c. |
| Langmuir         | $q_m$ (×10$^6$ CFU/g) | 5.00 | 5.00 | 5.04 | 5.04 |
|                  | $K_L$ (L/CFU) | 0.0002 | 0.0003 | 0.0001 | 0.0002 |
|                  | $R^2$ | 0.70 | 0.80 | 0.94 | 0.96 |
| Freundlich       | $K_F$ (×10$^6$ (CFU/g) (L/CFU)$^{1/n}$) | 4.27 | 6.23 | 4.77 | 6.96 |
|                  | $1/n$ | 0.66 | 0.68 | 0.68 | 0.67 |
|                  | $R^2$ | 0.99 | 0.99 | 1 | 1 |
| Temkin           | $b_T$ ($\times 10^6$ J/mol) | 8.26 | 8.26 | 8.89 | 7.76 |
|                  | $A_T$ (L/CFU) | 0.26 | 0.37 | 0.21 | 0.28 |
|                  | $R^2$ | 0.59 | 0.66 | 0.58 | 0.66 |
For linear isotherms, the Langmuir maximum adsorption capacity ($q_m$) of GCB on *S. aureus* and *E. coli* had a similar value of $5.00 \times 10^5$ CFU/g which meant GCB had the same inhibition on both bacteria types, and Langmuir adsorption constants ($K_L$) were 0.0002 and 0.0003 L/CFU, respectively. Freundlich adsorption constants ($K_F$) GCB on *S. aureus* and *E. coli* were $4.27 \times 10^6$ and $6.23 \times 10^6$ (CFU/g) (L/CFU)$^{1/n}$, respectively, and their $1/n$ values were 0.66 and 0.68, respectively. $1/n < 1$ means the increase in concentration results in a decrease in adsorption capacity [26]. For Temkin isotherm, by value of GCB on *S. aureus* and *E. coli* had a similar value of $8.26 \times 10^6$ J/mol, and their $A_T$ values were 0.26 and
0.37 L/CFU, respectively meant GCB had a stronger energy equilibrium on E. coli than S. aureus [28]. Since the higher $R^2$ closely to 1 is used to decide the best fit of the isotherm model, Freundlich corresponded to these studies with $R^2$ of 0.99 higher than Langmuir and Temkin isotherms in both bacteria types.

For nonlinear isotherms, the Langmuir maximum adsorption capacity ($q_m$) of GCB on S. aureus and E. coli had a similar value of $5.04 \times 10^6$ CFU/g which meant GCB had the same inhibition on both bacteria types, and Langmuir adsorption constants ($K_L$) were 0.0001 and 0.0002 L/CFU, respectively. Freundlich adsorption constants ($K_F$) GCB on S. aureus and E. coli were $4.77 \times 10^6$ and $6.96 \times 10^6$ (CFU/g) (L/CFU)$^{1/n}$, respectively, and their $1/n$ values were 0.68 and 0.67, respectively. $1/n < 1$ means the increase in concentration results in a decrease in adsorption capacity [43]. For Temkin isotherm, $b_T$ values of GCB on S. aureus and E. coli were $8.89 \times 10^8$ and $7.76 \times 10^8$ J/mol, respectively, and their $A_T$ values were 0.21 and 0.28 L/CFU, respectively meant GCB had a stronger energy equilibrium on E. coli than S. aureus [44]. Since the higher $R^2$ closely to 1 is used to decide the best fit of the isotherm model, Freundlich corresponded to these studies with $R^2$ of 1 higher than Langmuir and Temkin isotherms in both bacteria types.

The plotting graphs of both linear and nonlinear isotherms with various models could guarantee the best fit model of actual experiments without error analysis [45,46,48,49], and the above results of linear and nonlinear isotherms were agreed with Freundlich isotherm because of higher $R^2$ than others which meant the adsorptions of GCB on S. aureus and E. coli were heterogeneous adsorptions relating to a process of physiochemical adsorption [27].

### 3.6. Kinetic study

The adsorption kinetic is normally used to describe the rate of adsorption to time or the adsorption mechanism of the adsorbent [47]. Figure 8(a, b, c, d, e, f, g, h) demonstrated the results of the linear and nonlinear pseudo-first-order kinetic model, pseudo-second-order kinetic model, and intra-particle diffusion model of GCB on S. aureus and E. coli. Their adsorption kinetic parameters are reported in Table 5.

![Adsorption and desorption of GCB on (a) S. aureus and (b) E. coli.](image-url)
For linear kinetics, the adsorption capacities ($q_e$) of a pseudo-first-order kinetic model for GCB on $S. aureus$ and $E. coli$ were $38.17 \times 10^6$ and $0.26 \times 10^6$ CFU/g, respectively which meant GCB had strong inhibition on $S. aureus$ than $E. coli$, and $k_1$ were 0.02 and 0.01 min$^{-1}$, respectively. The adsorption capacities ($q_e$) of a pseudo-second-order kinetic model for GCB on $S. aureus$ and $E. coli$ had the same value of $333.33 \times 10^6$ CFU/g, and their $k_2$ were $0.002 \times 10^6$ and $0.09 \times 10^6$ g/(CFU-min), respectively. For the intra-particle diffusion model, $k_1$ of GCB on $S. aureus$ and $E. coli$ were $6.04 \times 10^6$ and $5.69 \times 10^6$ CFU/g(min$^{-0.5}$), respectively, and their $C_i$ values were $237.89 \times 10^6$ and $245.43 \times 10^6$ CFU/g, respectively. Since the higher $R^2$ closely to 1 is used to decide the best fit of the kinetic model, a pseudo-second-order kinetic model corresponded to these studies with $R^2$ of 1 higher than other kinetic models in both bacteria types.

For the nonlinear kinetic model, the adsorption capacities of a pseudo-first-order kinetic model ($q_e$) for GCB on $S. aureus$ and $E. coli$ were $41.18 \times 10^6$ and $0.28 \times 10^6$ CFU/g, respectively which meant GCB had strong inhibition on $S. aureus$ than $E. coli$, and $k_1$ were 0.22 and 0.10 min$^{-1}$, respectively. The adsorption capacities ($q_e$) of a pseudo-second-order kinetic model for GCB on $S. aureus$ and $E. coli$ were $332.25 \times 10^6$ and $337.09 \times 10^6$ CFU/g, respectively, and their $k_2$ were $0.004 \times 10^6$ and $0.10 \times 10^6$ g/(CFU-min), respectively. For the intra-particle diffusion model, the $k_1$ value of GCB on $S. aureus$ and $E. coli$ were $6.98 \times 10^6$ and $6.57 \times 10^6$ CFU/g(min$^{-0.5}$), respectively, and their $C_i$ values were $225.59 \times 10^6$ and $233.85 \times 10^6$ CFU/g, respectively. Since the higher $R^2$ closely to 1 is used to decide the best fit of the kinetic model, a pseudo-second-order kinetic model corresponded to these studies with $R^2$ of 1 higher than other kinetic models in both bacteria types.

Comparing graphs of linear and nonlinear kinetics is used to confirm data analysis to protect data mistranslation similarly to adsorption isotherm [45,46,48,49]. The results of linear and nonlinear kinetics agreed with a pseudo-second-order kinetic model because of higher $R^2$ than others which meant the adsorptions of GCB on $S. aureus$ and $E. coli$ were chemical adsorption with physicochemical interactions [27].

### 3.7. Desorption experiment

In the case of industrial applications, cost-effective, potential, and reuse materials are preferred, so adsorption-desorption experiments are normally required. This study designed the desorption experiment to confirm material reusability by running of adsorption-desorption of GCB on $S. aureus$ and $E. coli$ in 3 cycles which bacteria on GCB were removed by 0.01 M HNO$_3$ solution. The results of adsorption-desorption of GCB on both bacteria types are demonstrated in Figure 9(a) and (b) which confirmed the high reusability of GCB on $S. aureus$ and $E. coli$ in a range of $89 \pm 0.4–100 \pm 0.0\%$ for the bacteria adsorption and $86 \pm 0.2–100 \pm 0.0\%$ for the bacteria desorption, respectively. Therefore, GCB was a high potential material against $S. aureus$ and $E. coli$ for more than 3 cycles, and it is possible to further applications in the wastewater treatment system.

### 4. Conclusions

$G. cowa$ powder (GCP) and $P. sarmentosum$ powder (PSP) were extracted and used to synthesize antibacterial materials of $G. cowa$ beads (GCB) and $P. sarmentosum$ beads (PSB). The surface area of GCB had higher than PSB whereas particle size and pore size of GCB were smaller than PSB. GCP and PSP were heterogeneous surfaces whereas GCB and PSB had spherical shapes with coarse surfaces. The three main chemical elements of GCP, GSB, PSF, and PSB were carbon (C), oxygen (O), and calcium (Ca). Five main functional groups of O-H, C-H, N=H, C-O, and C-Cl were found in all materials. Results of the paper disc (EGC and EGS) and bead materials (GCB and PSB) confirmed that the extracted $G. cowa$ (EGC) and $P. sarmentosum$ (EPS) had potential inhibitions on $S. aureus$ and $E. coli$; however, EGC and GCB had higher antibacterial activities against both bacteria types than EPS and PSB. Thus, GCB was chosen to investigate bacteria removal efficiencies on both bacteria types by batch experiments. In batch experiments, the optimum conditions of GCB on $S. aureus$ and $E. coli$ with almost 100% bacteria removals were 0.3 g, 6 h, pH 7, 10$^6$ CFU/mL and 0.3 g, 3 h, pH 7, 10$^6$ CFU/mL, respectively. Freundlich isotherm and pseudo-second-order kinetic model well explained the adsorption pattern and rate of adsorption to time of GCB on $S. aureus$ and $E. coli$. Furthermore, GCB could reuse for more than 3 cycles, so it is possible to apply GCB for the disinfection of a wastewater treatment system.

Various bacteria types need to investigate for appropriate application in real wastewater and confirm whether GCB is a potential material to deal with a variety of conditions of bacteria-contaminated wastewater. Moreover, several extract plants are recommended to investigate and compare which one has the high potential to inhibit $S. aureus$ or $E. coli$.

### Declarations

**Author contribution statement**

Pimploy Ngamsurach: Performed the experiments; Wrote the paper. Pornsawal Praipipat: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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### Data availability statement

Data will be made available on request.

**Declaration of interests statement**

The authors declare no conflict of interest.

### Additional information

No additional information is available for this paper.

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