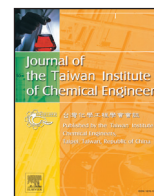




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Exploring community evolutionary characteristics of microbial populations with supplementation of *Camellia* green tea extracts in microbial fuel cells

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ABSTRACT

This first-attempt study deciphered combined characteristics of species evolution and bioelectricity generation of microbial community in microbial fuel cells (MFCs) supplemented with *Camellia* green tea (GT) extracts for biomass energy extraction. Prior studies indicated that polyphenols-rich extracts as effective redox mediators (RMs) could exhibit significant electrochemical activities to enhance power generation in MFCs. However, the supplementation of *Camellia* GT extract obtained at room temperature with significant redox capabilities into MFCs unexpectedly exhibited obvious inhibitory effect towards power generation. This systematic study indicated that the presence of antimicrobial components (especially catechins) in GT extract might significantly alter the distribution of microbial community, in particular a decrease of microbial diversity and evenness. For practical applications to different microbial systems, pre-screening criteria of selecting biocompatible RMs should not only consider their promising redox capabilities (abiotic), but also possible inhibitory potency (biotic) to receptor microbes. Although *Camellia* tea extract was well-characterized as GRAS energy drink, some contents (e.g., catechins) may still express inhibition towards organisms and further assessment upon biotoxicity may be inevitably required for practice.

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1. Introduction

In face of gradual exhaustion of fossil energy shortage around the globe, biomass energy is considered to be the most green and sustainable alternative with the environmental friendliness for worldwide utilization [1–4]. Among myriads of bioresources of biomass energy, microbial fuel cells (MFCs) were effective electrochemical systems to convert chemical energy for bioelectricity generation via simultaneous waste biotreatment and product biosynthesis [5–7]. However, the relatively low capacity of bioelectricity generation still greatly limited its potentials for practical applications due to the low electron transfer efficiency [8]. To overcome this disadvantage, exogenous supplementation of redox mediators (RMs) was considered to effectively improve the electron transfer capability for augmenting power generation [9,10]. Regarding such electroactive RMs, several pioneer works selected artificially synthesized compounds for feasibility study [11–13]. For example, aromatic compounds with promising electrochemically active functional

groups (e.g., -OH and -NH₂) at *ortho* or *para* positions could exhibit significant redox capabilities to effectively enhance power-generating capabilities in MFCs [11]. Compared with amino group, hydroxyl group sometimes owned the more reversible and stable electrochemical activity for electron-shuttling [14]. However, for green sustainability using synthetic compounds as RMs may still introduce several inevitable concerns for practice (e.g., inhibitory potency for biocompatibility towards biological systems) [15,16]. Therefore, natural resources or products were recently considered to replace such artificially synthesized compounds for sustainable applications. Several studies clearly revealed that appropriate supplementation of natural products abundant in electrochemically active substances could significantly promote simultaneous bioelectricity generation and wastewater treatment in MFCs [17–20]. Recently, Xu et al. [20] extracted natural herbal substances abundant in anthocyanins to effectively increase nearly threefold efficiency of bioelectricity generation in MFCs. From the perspective of chemical structure, in fact these bioelectricity-stimulating substances were mostly polyphenols, which are abundant in natural plants [17–20].

Regarding polyphenols in natural plants, over some thousand years, *Camellia* green tea (GT) has been used as a traditional drink in China and India and become popular all over the world. In particular,

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UK has become one of the world's greatest tea consumer since eighteenth century [21]. Based upon the degree of tea fermentation, *Camellia* tea can be classified as GT (0%), yellow tea (10%–30%), Oolong tea (30%–80%) and red tea (80%–100%) [22]. Medicinal values of tea have been well known (e.g., “Cha Jing” (Tea Bible) by Lu Yu of the Tang Dynasty), but potential health properties of tea polyphenols indicated anticancer, antioxidant validated scientifically within these 3–4 decades [23]. Modern medicine used the theory of free radical and immunity to elucidate such specific effects of *Camellia* tea on human health. In summary, tea contained not only the essential nutrients of human body, but also the medicinal ingredients beneficial to the restoration of human health under certain pathological conditions [24]. In fact, tea polyphenols as the main antioxidant components in *Camellia* tea has been mentioned in medical literature. For instance, Bag et al. [25] and Henning et al. [26] pointed out that tea polyphenols could prevent cancer by regulating the expression of genetic aberrations occurring in targeted DNA, modified histones and micro RNAs. Ding et al. [27] also further indicated that Pu'er tea had a significant hypoglycemic effect on diabetic rats induced by streptozotocin. Chen et al. [28] even pointed out that theaflavin-3,3'-digallate (TF) in Puer and black tea could effectively inhibit SARS-CoV 3C-like protease. That is, tea polyphenols may be a potential source of anticancer and antiviral drugs with fewer side effects and lower prices. Moreover, recent studies also deciphered that abundant tea polyphenols in tea extract sufficiently provided a reliable source of electrochemically active RMs [29,30].

Considering stimulation of effective bioenergy extraction, the protagonist dealing with bioelectricity production in MFCs is electroactive microbes or mixed consortia, that effectively release electrons from oxidation of organic matter [20]. Of course, the bioactivities of the electricity-generating microorganisms (e.g., biofilm forming, electron-transporting characteristics) would directly influence the power-generating efficiency of MFCs [17,19]. Due to some inhibitory/antagonistic interactions to be taken place, it was not possible to guarantee that all of natural substances are appropriate sources to be natural RMs. This might also explain why the extracellular metabolites of *Chlorella* with significant redox capacities sometimes could not significantly stimulate the bioelectricity production of MFCs as previously mentioned [31]. If natural extracts exhibited significant redox-mediating capabilities, they may also possibly express toxicity potency to the receptor organisms. Therefore, significant redox capability cannot be the sole screening criterion to select electrochemically active natural RMs for bioenergy applications. For example, as literature mentioned [29,30], some tea polyphenols owned anti-inflammatory and bacteriostatic effects (e.g., noticeable inhibition on *Typhoid bacillus*, *Paratyphoid bacillus*, *Yellow haemolytic staphylococcus*, *Streptococcus aureus* and *Dysentery bacillus*). Some studies have also shown that tea polyphenols had a strong killing effect on *Streptococcus mutans* in the oral cavity [32]. Therefore, tea polyphenols have also been widely used in industrial preparation of toothpaste. That is, such strong antibacterial effects of components in tea polyphenols may also have negative effects on bacteria in MFCs.

As prior work indicated, different methods of extraction could result in different levels of antioxidant/ electron transfer capabilities (e.g., H₂O extracted > EtOH extracted) [33]. Moreover, many medicinal herbs may not be appropriate to be extracted in low temperature, rather than at high temperature. In fact, high temperature by macerated and heat-dried or processing heating could attenuate contents of some toxic species and sometimes even reduce power of side effects in medication. To clearly reveal whether such phenomena may also be taken place in MFCs, *Camellia* GT was selected herein as study material using water and methanol as solvents of extraction. Furthermore, to reveal the novelty of this study and to compare results of high-temperature extraction in prior studies, low-temperature extraction was intentionally selected to inspect the electrochemical activity and/or antibacterial characteristics of *Camellia* green tea

extract. After centrifugation and freeze drying, extracted powders of *Camellia* GT obtained from the extraction of different solvents were harvested for comparison. In addition, electrochemical properties of the extracts were characterized via electrochemical measures to evaluate whether their electrochemical potential activities could be considered as RMs. Quantitative analyses of bioelectricity generation and community ecology in MFCs with the supplementation of these electrochemical active extracts were further implemented to quantitatively present such inhibitory or stimulating responses to affect the performance of bioenergy-extracting processes in MFCs.

2. Materials and methods

2.1. Preparation of tea extracts

To achieve maximal extraction efficiency, 5.0 g GT sample was ground into powder and screened through the particle size sieve (diameter of 40 μ m). In addition, the final solid/liquid ratio (S/L) was set as 5 g/50 mL to have identical basis for comparison. To have comparative assessment with prior studies, the deionized water and 80% methanol (MEOH) aqueous solutions were intentionally used as solvents of extraction at room temperature in total volume of 50 mL with continuous stirring for 12 h [33–35]. Supernatants of GT extracts were obtained via centrifugation at 13,000 rpm, 25 °C for 10 min. Harvested supernatants were then purified via 0.2 μ m filters (Millipore Millex®-GS 0.22 μ m filter unit) to eliminate residual particles. After refrigeration under the condition of –80 °C for overnight, frozen extracts were placed into the freezer dryer for 48 h and then pulverized. As the resultant powder may be light-sensitive and were then placed in dried, dark brown glass containers to avoid deliquesce and illumination.

2.2. High performance liquid chromatography (HPLC) analysis

For comparison upon components and contents of tea extracts obtained via different extraction solvents, water extract and methanol extract of *Camellia* GT at the same mass concentration were analyzed by HPLC. The HPLC system was accomplished with a Chromaster-5110 single pump, Chromaster-5260 auto sampler, and Chromaster-5420 UV–VIS Detector (Hitachi High-Tech Sci. Corp., Japan) using an InertSustain C18 column (5 μ m, 4.6 \times 250 mm, GL Sciences Inc., Japan) under a mode of gradient eluent. The gradient used was mobile phases A (3% acetic acid solution, HAc: water =3:97) and mobile phases B (methanol), where the percentage of mobile phases A was changed over time as follows: 0–1 min, 100%; 1–28 min, 100–37%; 28–33 min, 37–100%. The rejection volume was 10 μ L with the flow rate of 1 mL min⁻¹. The chromatographic separation was completed in 33 min for each sample. The separated components were detected at 280 nm using the Chromaster-5420 UV–VIS Detector. In fact, as Cabrera et al. [36] recommended, this is more reliable, rapid and simpler method of HPLC for simultaneous determination of catechins, gallic acids (GA) and caffeine (CAF).

2.3. Cyclic voltammetric (CV) analysis

To assess the electrochemical characteristics of tea extracts, comparative CV analysis upon these candidate redox mediators was carried out through electrochemical workstation (ALS/DY2325 BI-POTENTIostat, Taiwan). A glassy carbon electrode (0.07 cm²; CH Instruments Inc., SA) polished with 0.05 μ m alumina polish was used as the working electrode. Quadrant platinum electrode (6.08 cm²) was served as the counter electrode and was soaked in hydrogen peroxide (H₂O₂) prior to use. As the reference electrode, a Hg/Hg₂Cl₂ electrode was filled with saturated KCl(aq) to maintain electrochemical stability and reproducibility. Prior to analysis, the test solutions were purged with nitrogen 15 min for removal of residual oxygen. The symmetric

scan range from -1.5 to $+1.5$ V were carried out with a scanning rate of 10 mV s^{-1} . As the direct parameter to assess the redox capacity, closed curve area of redox potential (i.e., $\text{Area} = \int (i_h - i_r) dV$) were determined with Origin 8. Considering data calculation, V_H , V_L represented the CV scanning voltages of $+1.5$ V and -1.5 V, respectively; i_h , i_r denoted the oxidation currents and the reduction currents at specific scan voltage, respectively. Moreover, 100 cycles of CV scan were conducted to verify the electrochemical reversibility and stability of redox-mediating characteristics.

2.4. MFC construction

Membrane-free air cathode single-chamber MFCs (SC-MFCs) were constructed in cylindrical tubes made by polymethyl methacrylate (PMMA) (cell sizing ID = 54 mm, $L = 95$ mm) with the working volume of ca. 230 mL (i.e., $\frac{\pi(5.4 \text{ cm})^2}{4} \times (9.5 + 2 \times 0.3) \text{ cm} \approx 231.3 \text{ mL}$). Porous carbon cloth (CeTech) (without waterproofing catalyst) with a projected area of ca. 22.9 cm^2 (i.e., $\pi \times 2.7^2$) on one side was used as anode electrodes. The air cathode was almost identical to the anode in size and consisted of a polytetrafluoroethylene (PTFE) diffusion layer (CeTech) on the air-facing side. Detailed procedures of bacterial cultures for MFCs (e.g., bacterial acclimation, cell immobilization, bioelectricity stimulation) were described elsewhere [18,20]. To guarantee stable and reproducible electrochemical characteristics to be fully expressed, seeding microbe-*Shewanella haliotis* was used to inoculate open system LB-based MFCs for at least 1 month acclimation. Then, two domesticated microbial cultures (i.e., consortia A and consortia B) with high electricity-generating capacities were adopted as study MFC platforms for comparison. To explore the electrochemical influences of different *Camellia* GT extracts on the same MFC, the same microbial culture (e.g., consortia A) was seeded to two blank MFCs for comparison (e.g., marked as MFC-A1 and MFC-A2).

2.5. Power generation measurement

Experimental data of electric current (I_{MFC}) and voltage (V_{MFC}) were automatically collected with a data acquisition system (DAS 5020; Jiehan Technology Corporation, Taiwan). For comparison with prior results, the external resistance of microbial fuel cells was intentionally set at $1 \text{ K}\Omega$. Power density and current density of MFCs were calculated with the formulae below:

$$P_{\text{density}} = \frac{V_{\text{MFC}} \times I_{\text{MFC}}}{A_{\text{anode}}},$$

$$I_{\text{density}} = \frac{I_{\text{MFC}}}{A_{\text{anode}}},$$

where V_{MFC} and I_{MFC} could be directly measured with linear sweep voltammetry supported by a work station for electric chemistry analysis (Jiehan 5600, Jiehan Technology Corporation, Taiwan). The parameter A_{anode} was the actual working area of the graphite anode.

2.6. Microbial community analysis

(a) Sample preparation, library construction and sequencing: For total genomic DNA extractions, samples of bacterial solutions (ca. 50 mL) in MFC-A and MFC-B were taken and then separated with a high-speed centrifugation (10,000 rpm for 10 mins) to harvest bottom bacterial "precipitate". DNA was extracted using a QIAamp® DNA Stool Mini Kit (Qiagen, Valencia, CA, USA) to obtain an $\text{OD}_{260}/\text{OD}_{280}$ ratio between 1.8–2.0 for analysis. After library construction, samples were mixed with MiSeq Reagent Kit v3 (600-cycle) and loaded onto a MiSeq cartridge, then a 2×300 bp paired-end sequencing run was performed using the MiSeq platform (Illumina, San Diego, CA, USA).

(b) Operational Taxonomic Units (OTUs) analysis: The paired-end raw FASTQ reads generated from Illumina MiSeq platform were filtered using Bowtie 2. Trimmomatic was used to remove sequences with average $\text{QV} < 20$ to produce clean reads. Low-quality tails and primers were then trimmed and filtered based on length using Mothur to produce filtered tags. USEARCH was used to remove PCR chimeras to produce effective tags and to construct OTUs at 97% sequence identity.

3. Results and discussion

3.1. Chemical composition analysis

For solid-liquid extraction, apparently the composition and content of the extract are directly associated to the solvent to be used. In this study, two types of solvents- pure water and 80% methanol were selected to extract *Camellia* GT at room temperature and the chemical composition and content of the obtained solid extract powder would also be greatly different. Therefore, chemical constituents of water extract and methanol extract of *Camellia* GT were quantitatively analyzed by HPLC method for comparative assessment. According to Jiang et al. [37], *Camellia* green tea extracts mainly contain tea polyphenols and alkaloids, while major contents of tea polyphenols are phenolic acids and catechins. Therefore, representative substances of these three categories were selected for standard quantitative analysis (i.e., gallic acid (GA), epigallocatechin gallate (EGCG, catechin) and caffeine (CAF, alkaloid)). According to the HPLC spectra in Fig 1(A, B and C), the fingerprint peaks of standard GA, EGCG and CAF was responded at the retention time peak at 8 min, 17 min and 18 min, respectively, indicating that the green tea extracts contained these above-mentioned standards. As comparison of relative contents of three standard substances in the two different green tea extracts, the peak height of HPLC could be approximately represented as the relative contents of the three standard substances since the mass concentration of the prepared tea extract was identical. As indicated in Fig 1 (D), the content of CAF in these two extracts was nearly equal, while the content of GA in the water extraction was relatively higher than that in the methanol extraction. However, as Table 1 indicated, the content of EGCG was higher in MEOH than that in water, since EGCG was more soluble in MEOH than in water. In fact, Ahmad Muhamud and Amran [38] indicated that EGCG contents in *Camellia sinensis* GT were 0.9347 (MEOH extract) and 0.6705 (water extract) mg/mL. Furthermore, Oh et al. [39] also mentioned that MEOH extract of GT could obtain 60–580 g major catechins/kg dry extract, but water extract was only 385 g major catechins/kg dry extract for 85 °C extraction. In addition, extraction through pure organic solvents was found to yield the highest content of catechins. As indicated in Fig. 1, some unknown substances at the retention time of 3 min and 21 min were exhibited; however, their contents in the water extraction were also relatively small. This was owing to the different solubility of these substances in two solvents with different polarity, which resulting in the different content in the extracts [17].

To conduct a detailed quantitative analysis of the standards in the two extracts, HPLC analysis was implemented upon the pure standards with different concentrations ranged from 10 mg L^{-1} to 500 mg L^{-1} for calibration [40]. As shown in Fig 2, the retention time peak of these three standards increased with the increase in concentration of GA, EGCG and CAF. The linear relationship between the integral area of the retention time peak and the concentration of the three standards was shown in Fig 2(D). As indicated in the calibration line, the concentration of these three standards and the integral area of corresponding retention time peak showed a well-correlated linear relationship. The corresponding concentrations of GA, EGCG and CAF in the two extracts could be quantitatively determined by matching the integral area of corresponding retention time peak with the standard calibration line. The corresponding concentrations of these three

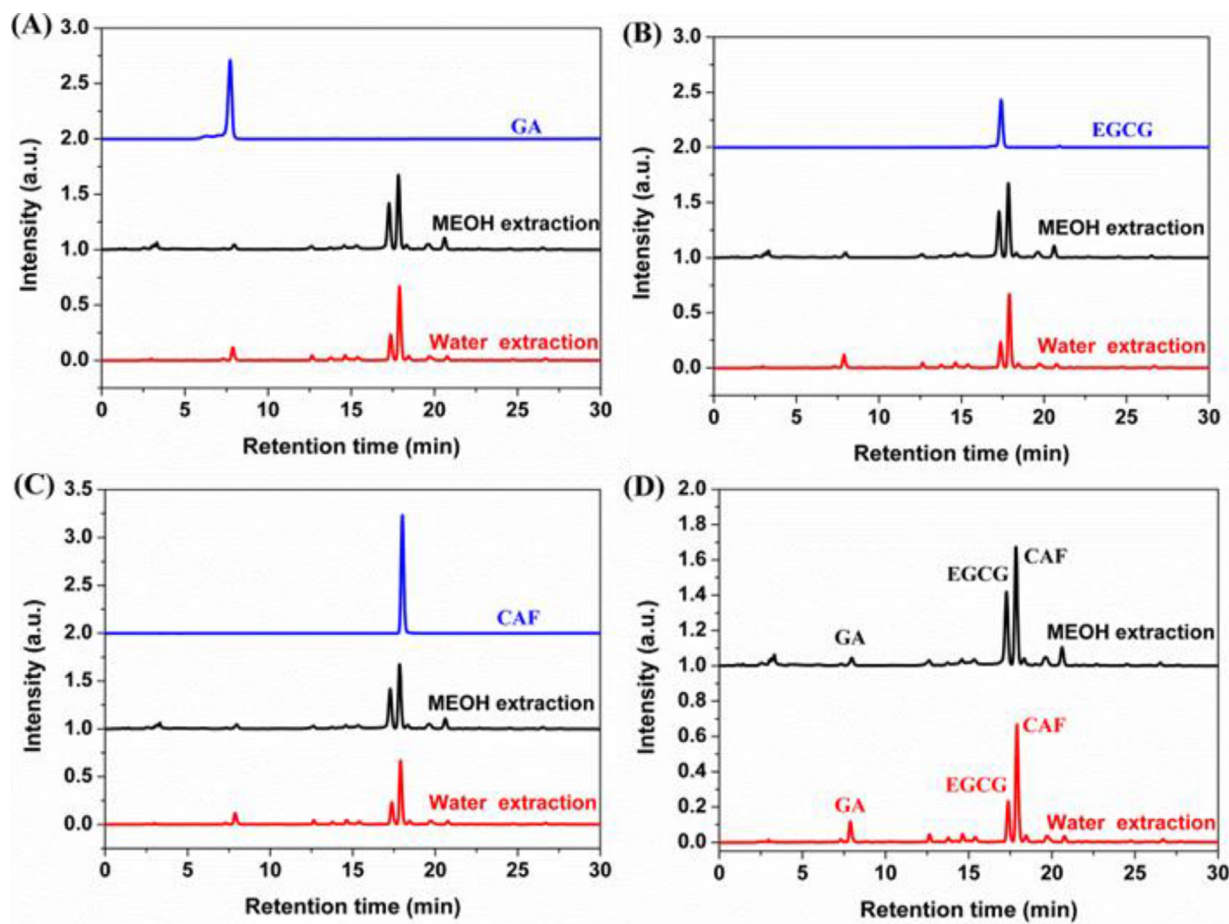


Fig. 1. HPLC analysis of (A) GA (B) EGCG (C) CAF compared with tea extract and (D) HPLC analysis of tea extracts between water extract and MEOH extract. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

standards in the water extraction and MEOH extraction were listed in Table 1. According to the detailed data in the Table 1, the contents of CAF in the two extracts were respectively 77 mg L^{-1} and 71 mg L^{-1} nearly at the same level. The content of GA in the water extraction was nearly 4 times of that in MEOH extraction, while the content of EGCG was only ca. 50%. These indicated that the contents of these three standards and other unknown substances in the two extracts also exhibited significantly different. This considerable difference might result in diverse outcomes of electrochemical and antioxidant activities as revealed in details afterwards.

3.2. Electrochemical capability assessment

To reveal the electrochemical capability of water extract and MEOH extract from green tea (e.g., reversibility and stability of oxidation and reduction potential peaks), 100 cycles CV scanning of these candidate redox mediators with the same mass concentration was also carried out [11]. As indicated in Fig 3, both the water extract and MEOH extracts of GT exhibited significant redox potential peaks, which directly reflected the bioenergy-stimulating capability. Regarding the electrochemical reversibility, the redox peaks of water extract tended to be stabilized after serial CV scanning (i.e., repeated reduction and oxidation) and

both oxidation peaks and reduction peaks could be clearly revealed. However, the redox peaks of MEOH extract seemed to show significant electrochemical instability of CV profiles and gradually attenuated. This was likely due to antioxidant and/or anti-reductants compositions present in most of extracted contents. Therefore, it could be seen that the water extract owned more significant electroactive capabilities than MEOH extract for bioenergy extraction. Since both the extracts were mixtures of phenolic acids and catechins, especially GA and EGCG, the redox capability should be the overall responses present of the electrochemical components. In fact, the redox capabilities of both GA and EGCG were also evaluated by CV inspections under the same scanning conditions for comparison. As shown in Fig 4, both GA and EGCG owned the chemical structure with three consecutive hydroxyl groups attached to the benzene ring. However, both redox capabilities of GA and EGCG were exhibited in significant differences. The CV profile of GA emerged significant redox potential peaks with higher stable reversibility. However, the redox capability of EGCG tended to be attenuated possibly due to electrochemical instability. This might indicate that GA owned more significant electrochemical capability than EGCG for electron-shuttling catalysis. Moreover, this seemed to explain why the redox capability of water extract was greater than that of MEOH extract. The water extract contained higher amount of electrochemically convertible GA, leading to the difference of electrochemical capability from MEOH extract.

Table 1

The measured concentrations of GA, EGCG and CAF in the water extraction and MEOH extraction.

Extracts	GA	EGCG	CAF
MEOH extraction	7 mg L^{-1}	122 mg L^{-1}	77 mg L^{-1}
water extraction	26 mg L^{-1}	68 mg L^{-1}	71 mg L^{-1}

3.3. Bioenergy performance analysis

As prior studies [11] indicated, aromatic compounds with electron-shuttling functional groups (e.g., *ortho*- or *para*-dihydroxyl ($-\text{OH}$) substituent(s)) could act as RMs to enhance efficiency of simultaneous

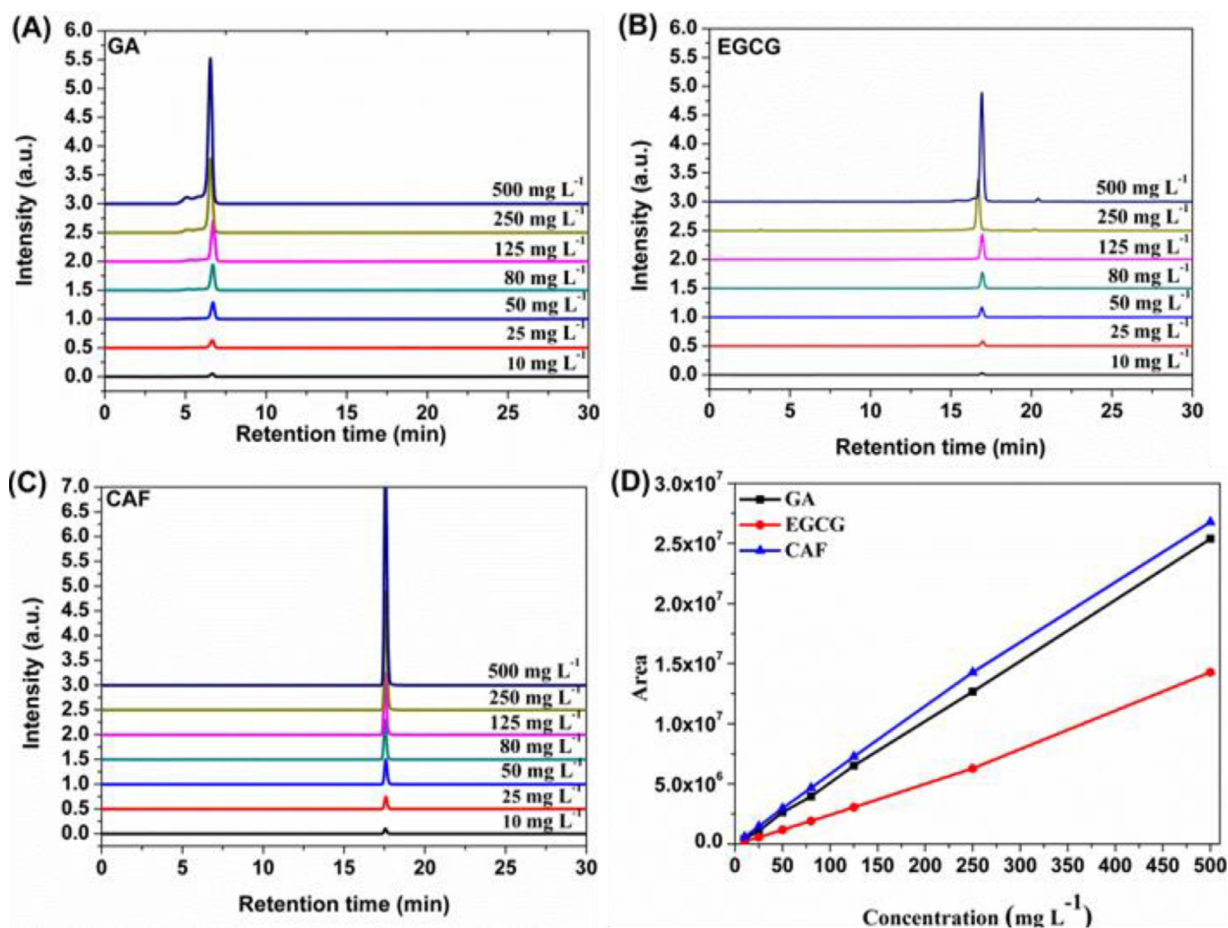


Fig. 2. Peak retention time of standard (A) GA (B) EGCG and (D) CAF by HPLC at different concentrations and (D) the summary diagram of calibration curve.

wastewater treatment and bioelectricity generation in MFCs. According to Chen et al. [17–19], RMs could be reversibly inter-converted between reduced and oxidized forms of intermediates to enhance electron transfer phenomena between electron donor(s) and electron acceptor(s) for augmenting electricity generation. Therefore, the application of two green tea extracts into MFCs should clearly present whether such electrochemical activities could be stably expressed by microbes. This would be reflected in the remarkable electron-shuttling phenomenon, improving the bioelectricity-augmenting performance

of MFCs [17]. Thus, water extract and MEOH extract were supplemented to MFCs with the same mass concentration to evaluate power-generating capacity for comparison. Aware that nearly identical mass concentration of tea extract simply suggested that the same weight of *Camellia* tea (biomass) was used for comparison. In fact, two kinds of MFCs inoculated with different mixed consortia of bacteria (i.e., MFC-A and MFC-B) were carried out to verify whether the expression of electrochemical RMs in MFCs is still controlled by the electroactive-bacterial populations. However, supplementation of both water

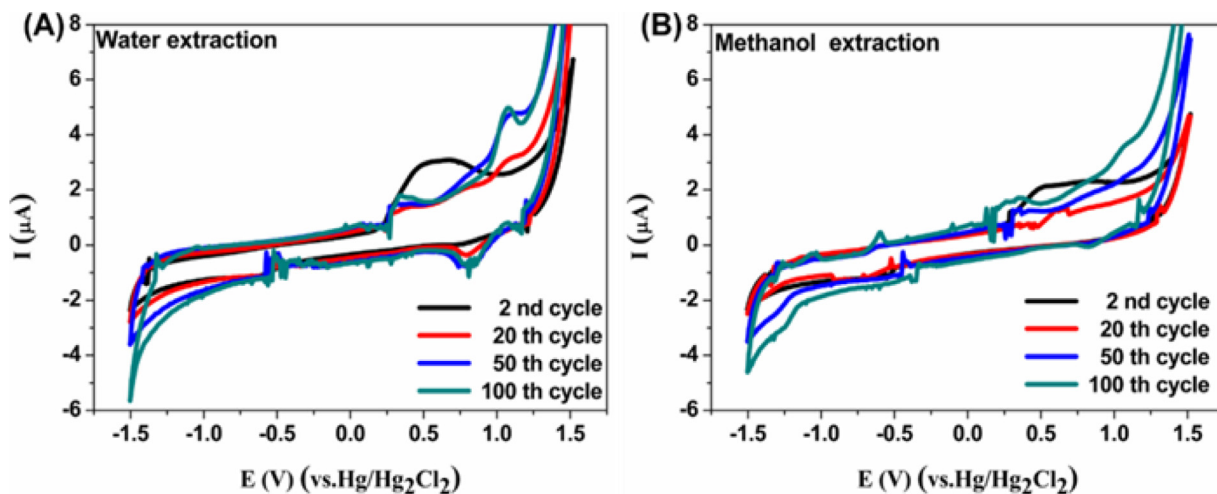


Fig. 3. Comparison CV profiles of (A) water extract and (B) MEOH extract of green tea at different scan cycle. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

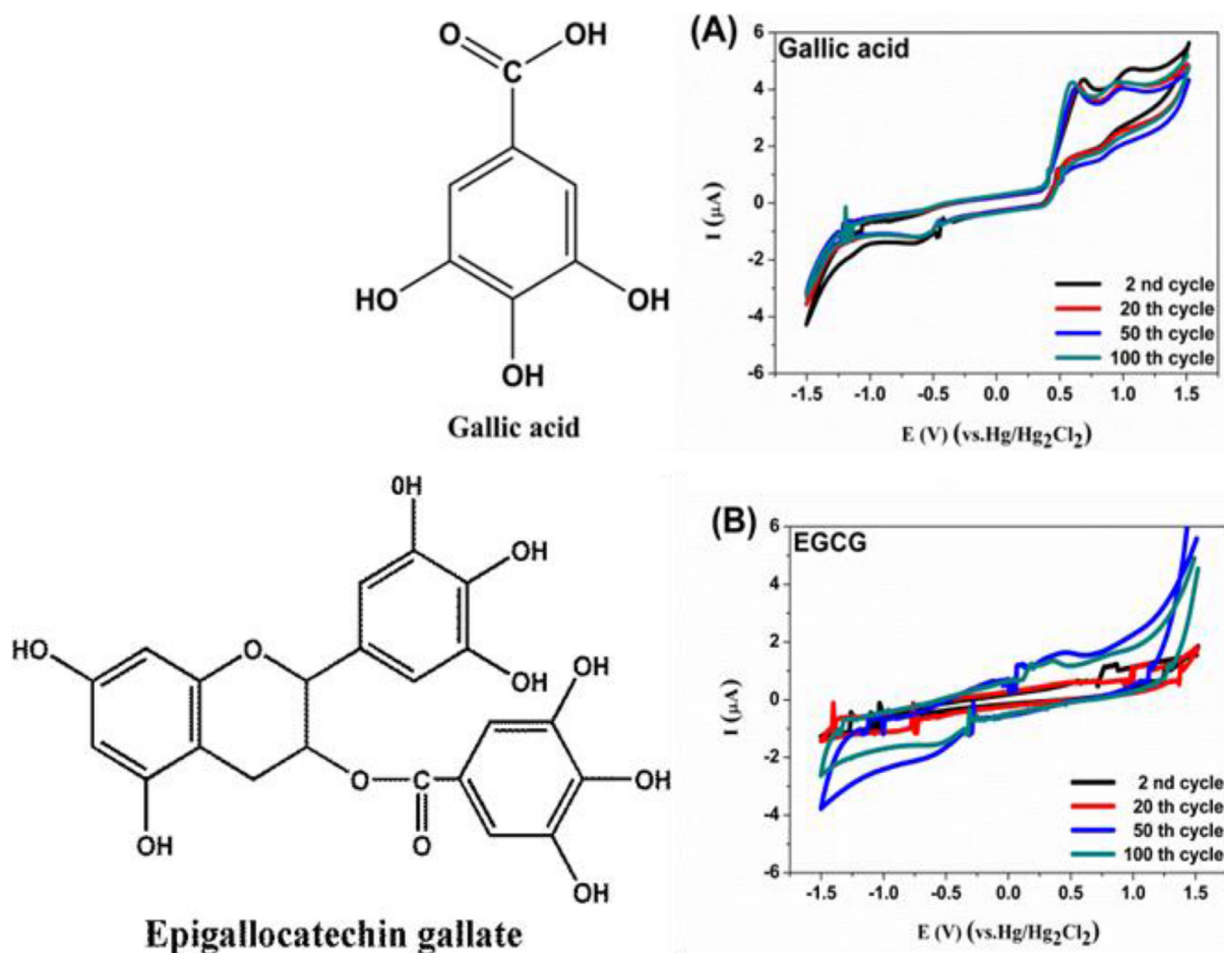


Fig. 4. The chemical structure and comparison CV profiles at different scan cycle of standard (A) GA and (B) EGCG.

extract and MEOH extract did not enhance the power generation of MFCs as anticipated. On the contrary, significant inhibitory effects were observed in both MFC-A and MFC-B. As shown in Fig 5, the twice successive supplementation of both water extract and MEOH extract obviously repressed the power production of MFC-A and MFC-B. Regarding the power generation capacity, the power density of MFC-A1 and MFC-B1 respectively decreased from 14 to 18 mW m^{-2} to 10 and 9 mW m^{-2} with the successive supplementation of water extract. Of course, dose concentration strongly influenced the degree of inhibitory potency expression since higher dose would trigger more severe adverse effects to be taken place to the receptor organisms. That was why power density of 2nd was even lower than 1st (Fig. 5). This also suggested that different levels of inhibitory responses would be resulted owing to the difference of microbial community. Furthermore, addition of MEOH extract tended to dramatically inhibit the power generation of MFC-A2 and MFC-B2, respectively decreased from 15 to 19 mW m^{-2} to 5 and 3 mW m^{-2} . These result directly suggested that the inhibition of MEOH extract was even more considerable than that of water extract. Since the sources of water extract and MEOH extract were the same green tea, the apparent inhibitory differences were most likely owing to the differences in the composition and content of the extract due to the different solvents and temperatures of extraction as discussed afterwards.

3.4. Microbial community analysis

As the two *Camellia* green tea extracts contained chemical components with strong antibacterial activities, applications to MFCs clearly expressed inhibitory effect on power generation, directly affecting

electrochemical activities of the electrogenic microorganism to be exhibited in MFCs. To clearly decipher such inhibitory effect of green tea extract on bacterial strains in MFCs, microbial community analysis of MFCs before and after supplementation of MEOH extract was implemented [41]. The changes of microbial community in MFCs were analyzed from the levels of phylum, class, order and family (Fig 6). Regarding MFC-A, from the phylum level, the original flora could be mainly divided into *Proteobacteria* and *Firmicutes*, and the two phyla accounted for 48.21% and 51.78%, respectively, in a relatively even distribution state (i.e., community in higher biodiversity). However, after the supplementation of MEOH extract, the proportion of the *Proteobacteria* increased significantly (up to 85.60%), while the *Firmicutes* could not adapt to such environmental stress, leading to significant loss of cell viability (decreased to 14.40%). The same result could be found in the microbial community analysis of MFC-B. The *Proteobacteria* and *Firmicutes* respectively changed from 42.73% and 57.27% to 67.00% and 33.00%, respectively. In addition, such phenomenon could also be observed in the classification of class and order levels. From the more detailed classification of the family, in addition to the obvious changes of microbial community in MFC-A, *Clostridiales* UC and *Clostridiaceae-1* strains seemed to become extinct with the supplementation of MEOH extract. In MFC-B, except for dying out of *Clostridiaceae-1*, *Carnobacteriaceae* strains were also eliminated from the population owing to the addition of MEOH extract. These results indicated that these strains were unlikely to resist such supplementation of MEOH extract.

Considering community distribution diversity, Shannon's Diversity Index (H') was adopted herein as performance index to characterize species (OTUs) diversity in this bacterial community,

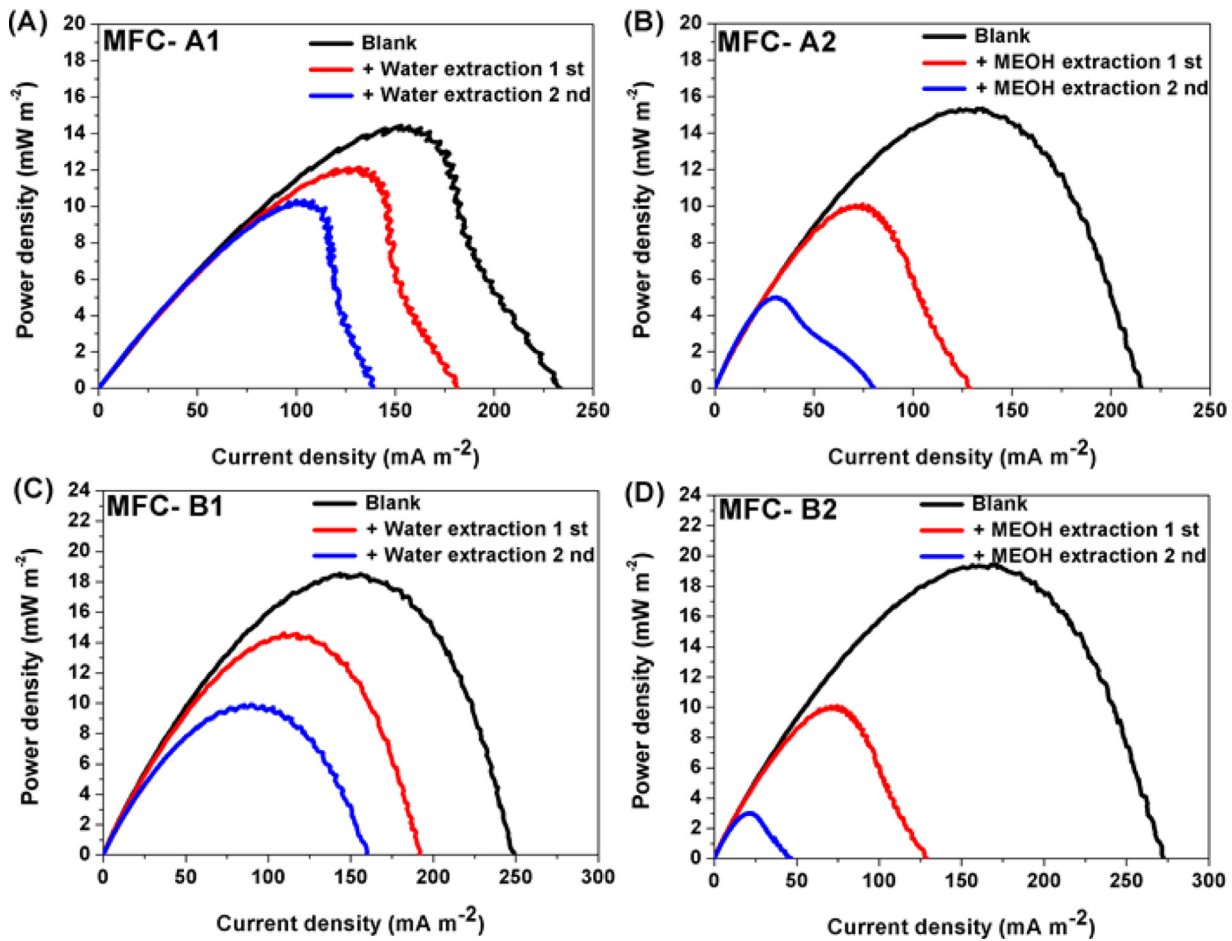


Fig. 5. Power density profiles of (A) MFC-A1 and (C) MFC-B1 supplemented with water extract and (B) MFC-A2 and (D) MFC-B2 supplemented with MEOH extract.

suggesting both abundance and evenness of the species (OTUs) in the population [42]. Thus, the Shannon's Diversity Index for comparative inspection was calculated by the following formula

$$H'_{\text{Shannon}} = -\sum_{i=1}^{S_{\text{obs}}} P_i \ln P_i,$$

where S_{obs} is total number of species (OTUs) and P_i is the fraction of the total number of individuals in a particular genotype or taxon i . Moreover, values for H' ranged from 0 (low diversity) to 5 (high diversity) could directly reflect the abundance level of bacterial community. In addition, to express species evenness of bacterial community, Shannon's Evenness Index (also known as Pielou's

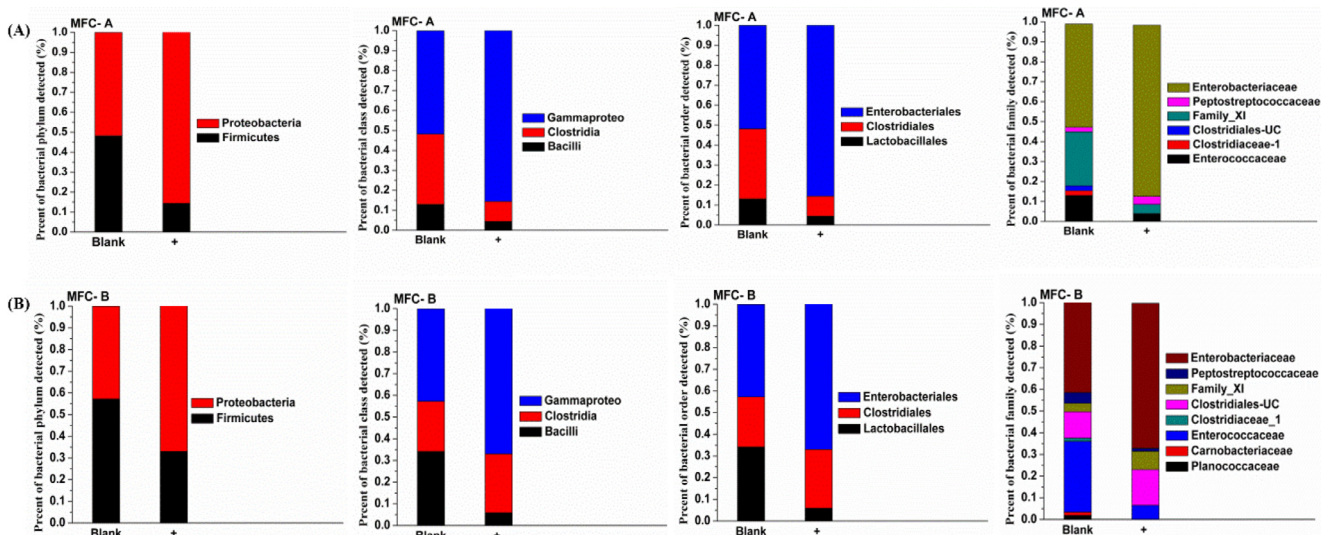


Fig. 6. Classification of the 16S rRNA gene sequences of clone library from (A) MFC-A and (B) MFC-B: Phylum, Class, Order and Family level category of total bacteria (from left to right).

Evenness Index (J)) was also calculated from the following formula

$$J = \frac{H'}{H'_{max}}$$

where H'_{max} is the maximum possible value of Shannon's Diversity Index. The values for J ranged from 0 (low evenness) to 1 (high evenness) could directly reflect the uniform distribution of bacterial community. In bacterial community, if the distribution of every species was likely equally, the H'_{max} could be calculated as

$$H'_{max} \cong - \sum_{i=1}^{S_{obs}} \frac{1}{S} \ln \frac{1}{S} = \ln S,$$

As indicated in these calculated indices (Table 2), continuous increases of Shannon's Diversity Index (H') and decreases of Shannon's Evenness Index (J) from phylum to family in both MFC-A and MFC-B suggested that the bacterial community tended to be highly diverse with low evenness if more specific levels were considered. This might suggest that some species populations were possibly on the verge of extinction as well. Furthermore, comparing the two different bacterial communities before and after the supplementation of MEOH extract, both the Shannon's Diversity Index and Shannon's Evenness Index exhibited significant decrease. This result was consistent with significant extinction of species as microbial community analysis indicated.

3.5. Mechanism exploration

To elucidate such different inhibitory outcomes of GT extracts, comparative analysis on prior studies and literature was carried out. As aforementioned, evidently contents of GA, EGCG and several unknown compositions in the water extract and MEOH extract were significantly different. As indicated in literature [30,31,41], EGCG owned apparent inhibitory effects on bacterial species (e.g., *Staphylococcus aureus*, *Proteus bacillus vulgaris*, *Salmonella typhosa*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Oral streptococcus*, *E. coli*, *Stenotrophomonas maltophilia*). Moreover, EGCG could even express significant antibacterial activity toward food poisoning bacteria and plant pathogenic bacteria. Therefore, due to the higher content of EGCG in GT extract, the greater inhibitory potency towards the bacterial populations was revealed. In addition to EGCG, there were many chemical components with the similar chemical structure (e.g., orthodihydroxyl bearing aromatic compounds EC, EGC, ECG, GA, TF) in GT extracts. According to Zuo et al. [34] and Yao et al. [35], catechins in tea extracts can also include epigallocatechin (EGC), epicatechin gallate (ECG), and epicatechin (EC), which are likely corresponding to the unidentified peaks in the HPLC results. Similar to EGCG, these catechins had high antioxidant capacities and also revealed very strong antibacterial activities. Furthermore, GT extracts obtained through room temperature extraction could also lead to higher contents of inhibitory chemical species than those from higher temperature (e.g., 65 °C) extracts. These findings all supported that the presence of antibacterial components in GT extract may directly alter the distribution of microbial community, further decreasing the power-generating capabilities of MFCs possibly due to reduction of electroactive populations in the community.

Table 2

List of calculation results of Shannon's Diversity Index (H') and Shannon's Evenness Index (J) in microbial community analysis.

Level	Shannon's Diversity Index (H')				Shannon's Evenness Index (J)			
	MFC-A		MFC-B		MFC-A		MFC-B	
	Blank	+	Blank	+	Blank	+	Blank	+
Phylum	0.999	0.594	0.985	0.915	0.999	0.594	0.985	0.915
Class	1.406	0.723	1.542	1.139	0.887	0.456	0.973	0.718
Order	1.406	0.723	1.542	1.139	0.887	0.456	0.973	0.718
Family	1.768	0.770	1.911	1.537	0.684	0.385	0.739	0.547

However, such strong inhibitory effect of tea extract seemed to be different from our prior findings [17,18]. From the comparison upon experimental methods, the extraction temperature seems to be the main-effect reason to evolve such a difference. In fact, different degree of inhibition caused by changes in temperature of extraction were also found in natural products (e.g., medicinal herbs). As a matter of fact, many medicinal herbs may not be appropriate to be extracted at low temperature (e.g., room temperature). According to the practices in Herbal Medicine, macerated and heat-dried or processing (páozhì) under higher temperature heating (65–85 °C) could attenuate some inhibitory chemical species and sometimes even lose power of side effects in medication. For example, artemisinin (Qinghaosu) was found to be effectively against malaria. However, this finding was due to “accidental” modification of the extraction at low temperature by the Youyou Tu's group [42]. After their further separation of acid extract, the natural extract indeed contain very promising antimalarial activity that was even much stronger than well-known chloroquine for clinical medication to patients with malaria. Moreover, this phenomenon was popularly observed in extracts of active compositions in medicinal herbs to against disease. As revealed in Oh et al. [39], ethanol extract of GT at 20 °C owned potent antimicrobial activity against all five test pathogens, compared to water extract at 80 °C. That is, ethanol extracts contained higher concentrations of inhibitory chemicals to pathogens than water extracts. This may be due to the favorable solubility of antimicrobial compounds in organic alcohols (e.g., methanol, ethanol) much higher than that in water.

In summary, it is noted that a Chinese saying “every medicine has its side effect” (yào jí shì dú). As this study and literature [1–9] indicated, evidently there were at least three crucial conditions to affect inhibition potency of GT to bacteria as follows: (1) temperature of extraction (e.g., room, higher temperature), (2) solvent of extraction (e.g., water, ethanol or methanol) and (3) concentration of extract (e.g., powder, concentrated solution). They all affected the role of GT to be either “medicine” (yào) or “poison” (dú). In addition, herbal tolerance and susceptibility of test organism also strongly influenced the responses to be in either “toxicity” or medication. These all strongly suggested that why macerated and heat-dried or processing (páozhì) is of great importance to clinical medication. The novelty of this MFC study was to depict a promising platform to evaluate possible herbal species for its bioenergy-extracting potential without sacrifice of living mice and animals in practice. The scope also pointed out some information of great importance on the threshold criteria of “toxicity” for the characteristic of *Camellia* tea extract (e.g., when and how it may be “drug” or “poison” to what receptor organisms?) [43–52].

4. Conclusion

Camellia green tea extract obtained from “inappropriate” extraction procedures (e.g., room temperature extraction) might be inhibitory to reduce the power generation of MFCs compared to bioelectricity stimulation by supplement of higher temperature-extracted green tea. Evidently, the main components of green tea extracted by pure water and MEOH showed significant difference in concentration and composition, which could directly lead to the differences in inhibitory potency and biocompatibility. Although the extracts obtained by different solvents (i.e., water and MEOH) owned significant redox capabilities, the application in MFCs unexpectedly still exhibited considerable inhibitory effect. Microbial community analysis showed that the supplementation of green tea extract significantly altered the distribution of microbial community, especially the decrease of microbial diversity and evenness. Therefore, in practical application of different microbial culture systems, selecting appropriate RMs should not only consider their excellent redox-mediating characteristics, but also the inhibitory responses for feasibility evaluation as screening criterion.

Declaration of Competing Interest

The authors report no conflict of interest. The authors alone are responsible for the content and writing of the paper.

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