31P-am072

Unveiling Bioenergy Stimulating Capabilities of Metabolites from *Citrus microcarpa* Peels and Pulps as Medicated Diet of Sustainable Energy Resource

Po-Wei Tsai[1,#], Timothy Jen R. Roxas[2,#], Lemmuel L. Tayo[2], Yi-Ru Lin[1], Chung-Chuan Hsueh[3], Bor-Yann Chen[3,*]

[1] Department of Medical Science Industries, College of Health Sciences, Chang Jung Christian University, Tainan 711, Taiwan
[2] School of Chemical, Biological, and Materials Engineering and Sciences, Mapúa University, 1002 Metro Manila, Philippines
[3] Department of Chemical and Materials Engineering, National I-Lan University, I-Lan 260, Taiwan
[#]These authors have equally contributed to this study
* Corresponding author

Introduction

The development of renewable energy production requires the increased demand for the discovery of sustainable sources of energy. Microbial-fuel cells (MFCs) can serve as a bioenergy-evaluating platform to screen for biomass capable of bioelectricity amplification through the use of electroactive bacterial species or consortia. In the Philippines, *Citrus microcarpa* peels and pulps are considered agricultural wastes that contain abundance of flavonoids, which possess an array of biological activities. Herein, the exploration of power augmenting capabilities and investigation of ESs species in *C. microcarpa* extracts was implemented for bioenergy applications to circular economy. Agricultural wastes were extracted using hot water and ethanol using a rotary evaporator and was freeze dried subsequently. The phytochemical and antioxidant activity analysis of the extracts were carried out using an ELISA microplate reader. Exogenous supplementation of the extracts in MFCs were used to study the bioenergy-generating capabilities of the agricultural wastes. Cyclic voltametric analysis of at least 50 cycles was carried out to reveal electrochemical characteristics of such herbal extracts. The ethanol extracts of the *C. microcarpa* peels and pulps exhibited superior bioelectricity-stimulation in MFCs.

Results

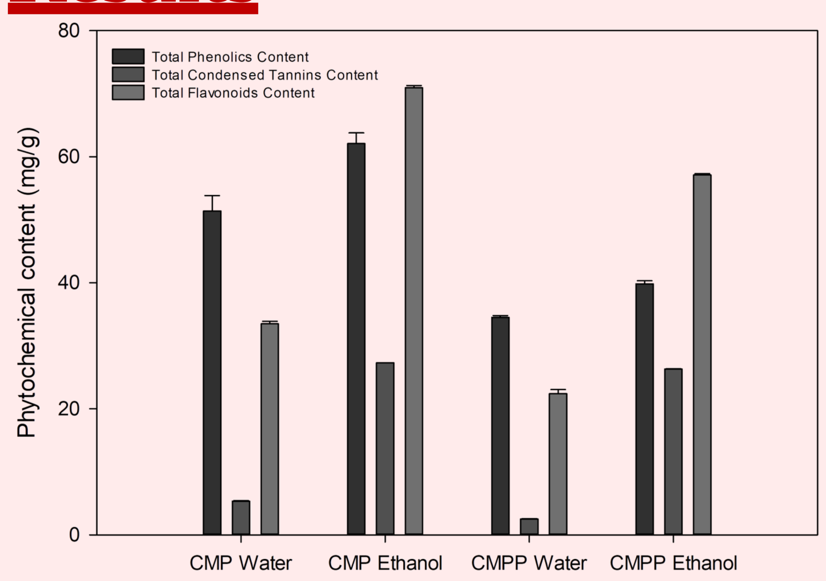


Fig. 1. Phytochemical content of different Philippine *C. microcarpa* extracts.

Table 1. Comparative list of phytochemical constituents of *C. microcarpa* (CM) extracts.

Sample extract	Total polyphenol content (GA mg/g)	Total condensed tannins content (catechin mg/g)	Total flavonoids content (rutin mg/g)
CM peels water extract	51.39±2.46	5.34±0.08	33.47±0.40
CM peels ethanol extract	62.10±1.69	27.31±0.00	70.95±0.35
CM peels and pulps water extract	34.53±0.27	2.53±0.04	22.38±0.66
CM peels and pulps ethanol extract	39.38±0.49	26.30±0.04	57.10±0.23

As shown in Table 1 and Fig. 1, analyses of the phytochemical constituents of the Philippine *C.*. Comparison of total polyphenols showed that the ethanol extracts of the calamansi peel (CMP) (62.10±1.69 GA mg/g) and calamansi peel and pulp (CMPP) (39.38±0.49 GA mg/g) contained higher amounts of the phytonutrient than their water extract constituents. Ethanolic extracts CMP (27.31±0.00 catechin mg/g) and CMPP (26.30±0.04 catechin mg/g) still contained the highest number of condensed tannins than samples extracted by using hot water. Results of the TPC and TCTC analysis, it is apparent that the ethanol extracts of the CMP (70.95±0.35 rutin mg/g) and CMPP (57.10±0.23 rutin mg/g) contained significantly more flavonoids than water extracts (Table 1).

Table 2. Comparative list of antioxidant activity of the *C. microcarpa* (CM) extracts. CMP = *C. microcarpa* peels; CMPP = *C. microcarpa* peels and pulps; W = water extract; E = ethanol extract.

Sample	DPPH IC_{50} (mg/mL)	FRAP (Trolox mg/g)
CMP-W	8.70±0.23	30.40±0.72
CMP-E	6.62±0.18	36.55±0.56
CMPP-W	Not detected	22.17±1.10
CMPP-E	14.27±0.34	30.83±0.14
Ascorbic acid	0.067±0.003	

For the results of the DPPH assay, it has been determined that the ethanol extracts of CMP (6.62±0.18 mg/mL) and CMPP (14.27±0.34 mg/mL) showed more promising antioxidant activity than their water extract counterparts, with values reaching up to 8.70±0.23 mg/mL for CMP and no antioxidant activity detected for CMPP water extracts (Table 2). The results of the FRAP assay rankings of the sample extracts in Trolox mg/g is as follows: CMP EtOH (36.55±0.56) > CMPP EtOH (30.83±0.14) > CMP water (30.40±0.72) > CMPP water extracts (22.17±1.10) (Table 2).

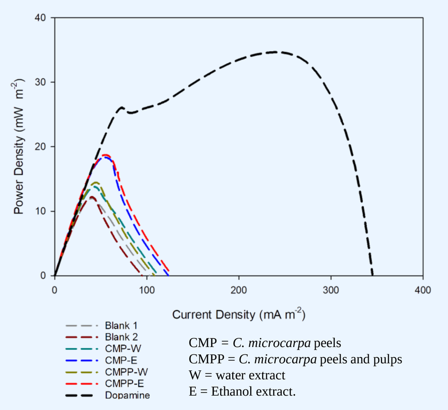
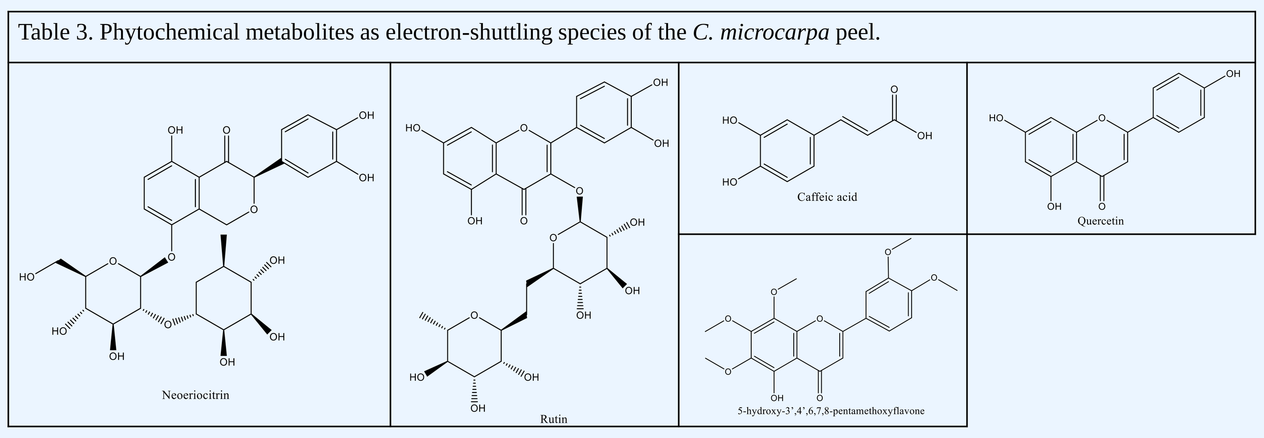


Fig. 2. Comparative graphs of power generation of different Philippine *C. microcarpa* extracts using microbial fuel cells (MFC) as the bioenergy evaluating platform.

Table 3. Phytochemical metabolites as electron-shuttling species of the *C. microcarpa* peel.



The ranking of the bioelectricity-generation (i.e., power density (PD) in mW m-2) of the *C. microcarpa* extracts (Fig. 2), where the subscript denotes the amplification factors with respect to blank, are as follows: dopamine (34.972.89±0.36) > CMPP-E (18.771.56±0.01) ~ CMP-E (18.401.53±0.10) > CMPP-W (14.471.21±0.01) > CMP-W (13.841.15±0.04) > blank 2 (12.231.02±0.06) > blank 1 (12.00). Table 3 has been showed that the *C. microcarpa* peels and pulps contain at least five ES species, which could have contributed to the ~50% increase in power generation of MFCs, namely, caffeic acid, rutin, neoeriocitrin, quercetin, and 5-hydroxy-3',4',6,7,8-pentamethoxyflavone. These compounds are all ortho-dihydroxyl substituent bearing aromatics; hence, are considered as ESs.

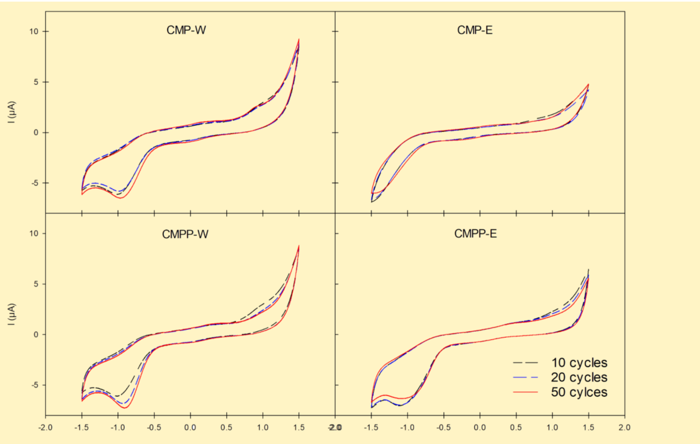


Fig. 3. Cyclic voltammograms of the different *C. microcarpa* samples at 10, 20, and 50 cycles. CMP = *C. microcarpa* peels; CMPP = *C. microcarpa* peels and pulps; W = water extract; E = ethanol extract.

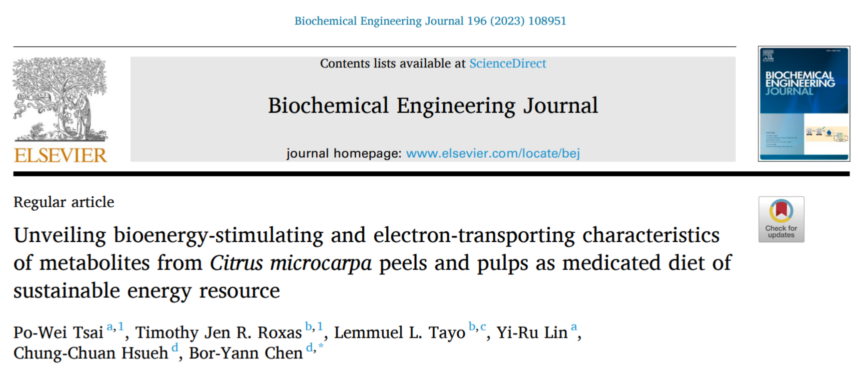
Table 4. Areas enclosed under the CV loop

Sample	10 cycles	20 cycles	50 cycles
CM peels water extract	6.39	6.30	6.97
CM peels ethanol extract	3.75	3.57	3.97
CM peels and pulps water extract	6.43	6.56	6.79
CM peels and pulps ethanol extract	6.61	6.49	6.18

From Fig. 3, it is learned that at neutral pH, the *C. microcarpa* agricultural waste extracts did not exhibit neither antioxidant (as shown by the lack of significant oxidation peaks) nor electron-shuttling activities; however, strong anti-reductant activity was observed for the CMP water extract, and CMPP water and ethanol extracts, as indicated by the reduction peaks. Table 4 shows the areas (in μW) enclosed by the CV loop, where: CM peel water and pulp water (6.59±0.03) ~ CM peel water (6.55±0.13) ~ CM peel and pulp ethanol (6.43±0.05) > CM peel ethanol (3.76±0.04). Aside from the mentioned, analysis of the CV curves at 10, 20, and 50 cycles indicates the stability of the samples at neutral pH (Table 4) and persistence of reduction peaks (Fig. 3), indicating the potential of *C. microcarpa* extracts as reusable anti-reductants in industrial contexts.

Conclusions

Using microbial fuel cells (MFCs) as a bioenergy evaluating platform, it was determined that Philippine *C. microcarpa* agricultural wastes are able to exhibit bioenergy stimulation in electroactive bacteria *S. haliotis* (WLP72). The ethanol extracts of CMP and CMPP were most promising in bioelectricity generation compared to their water extract constituents. Similarly, as DPPH and FRAP assays indicated, the ethanol extracts displayed better antioxidant activity than hot water extracts. Comparative analysis of the phytochemicals of these ethanol extracts also reveal an abundance of polyphenols and flavonoids, which can be correlated to the power amplification capabilities and antioxidant activity of the extracts. Since serial CV scanning at 50 cycles in neutral pH shows that the extracts did not exhibit ES activity, it is suggested that the exploration of the CV profiles of the extracts at more alkaline conditions be implemented. Nevertheless, the results show that the Philippine calamansi agricultural wastes can contribute to the development of circular economy as biomass-based energy sources and ingredients for reusable anti-reductants useful in the industrial setting.



Biochemical Engineering Journal 196 (2023) 108951
Contents lists available at ScienceDirect
Biochemical Engineering Journal

Regular article

Unveiling bioenergy-stimulating and electron-transporting characteristics of metabolites from *Citrus microcarpa* peels and pulps as medicated diet of sustainable energy resource

Po-Wei Tsai, Timothy Jen R. Roxas, Lemmuel L. Tayo, Yi-Ru Lin, Chung-Chuan Hsueh, Bor-Yann Chen