SHORT COMMUNICATION

IDENTIFICATION OF CHEMICAL CONSTITUENTS FROM *THEOBROMA CACAO* L. LEAVES AS A POTENTIAL COCOA LEAVES BEVERAGE IN COMPARISON WITH COMMERCIAL GREEN TEA VIA LC-QTOF-MS/MS ANALYSIS

Zainal Baharum[1*], Nurul Aishah Mohd Tamizi[2*], Saiful Nizam Tajuddin[1] & Ahmad Kamil Mohd Jaaffar[3]

[1] Division of Biotechnology, Cocoa Innovation & Technology Centre, Malaysian Cocoa Board, Lot Pt 12621, Nilai Industrial Park, 71800 Nilai, Negeri Sembilan, Malaysia;

[2]Bio Aromatic Research Centre of Excellent, Universiti Malaysia Pahang, Lebuhraya Tun Razak Gambang, 26300 Kuantan, Pahang, Malaysia;

[3]Biotechnology Division, 5th, 6th & 7th Floor, Wisma SEDCO, Lorong Plaza Wawasan, Off Coastal Highway, Locked Bag 211, 88999 Kota Kinabalu, Sabah.

**Corresponding author: zainal@koko.gov.my & aishah.syh@gmail.com*

Malaysian Cocoa J. 14: 1-4 (2022)

ABSTRACT - *Apart from the cocoa beans, other non-edible cocoa plant parts also contain bioactive compounds that are beneficial for health. In the present study, the chemical profiles of cocoa leaves were obtained via liquid chromatography quadrupole time of flight mass spectrometry (LC-QTOF-MS/MS) in comparison with the commercial green tea and cocoa shell beverage. Based on the data processing revealed the cocoa leaves composed of catechins compounds that were detected in commercial green tea, in addition with the alkaloid and tannins groups of compounds were detected, which could be an added value for the development of cocoa leaves as a potential health beverage. The various bioactive compounds found in cocoa leaves and their pharmacological properties had been summarized, thus serving as a reference material for their usage as health beverage.*

Key words: *Theobroma cacao*, leaves, beverage, LC-QTOF-MS/MS

INTRODUCTION

Tea, a dried leaf-infused beverage derived from the leaves of *Camellia sinensis* L. has become the world's second most popular beverage after water consumed every day. Green tea, black tea and oolong tea are the three major forms of tea which are categorized based on level of fermentation. Green tea is non-fermented and mainly consists of polyphenols, amino acids, theanine, proanthocyanidins and caffeine. The optimum consumption of green tea is associated with beneficial health effects such as anticancer, antioxidant, anti-diabetic, anti-obesity, hepatoprotective, neuroprotective and so forth (Huang et al., 2014; Prasanth et al., 2019; Suzuki et al., 2012).

Theobroma cacao L., popularly known as cocoa tree is the plant in which chocolate is made. The high polyphenol content of cocoa,
coupled with its widespread presence in many food items, render this food of particular

interest from the nutritional and health viewpoints. Currently, cocoa's health aspect has focused on the bean to develop the product in the form of chocolate and cosmetic ingredients only but no other non-edible cocoa plant parts. Meanwhile, studies reported that other non-edible cocoa plant parts such as cocoa leaf, cocoa husk and cocoa shell contain bioactive compounds that possess potential benefits to human health (Nidhi et al., 2015; Kumah et al., 2020; Zainal et al., 2014). The present study intends to provide the information about the chemical profiles in cocoa leaves obtained by liquid chromatography quadrupole time of flight mass spectrometry (LC-QTOF-MS/MS).

MATERIALS AND METHODS

Sample collection

Cocoa leaves were harvested from five states in Malaysia, including Durian Tunggal (Melaka), Sungai Pelek (Selangor), Sungai Ruan (Pahang), Rembau (Negeri Sembilan), and Tawau (Sabah). The cocoa leaves were collected manually and selected based on youthfulness and position in the tree. The samples were transported to the laboratory in sealed plastic bags and then washed with distilled water. Commercial green tea (Cameron Valley) and commercial cocoa husk beverage (Lees Cocoa Artisan Chocolate) were bought from the local shop as comparison.

Sample preparation and extraction

The cocoa leaves were subjected to a drying process in an oven at 48°C, for one hour, in order to remove about 30% of their original moisture. Then, the leaves were manually rolled to increase the leaves surface for better moisture removal. Then, the leaves

were dried in an oven at a temperature between 90°C for another one hour to reduce the water content to 4%. Subsequently, the dried leaves were ground using a commercial blender. The ground cocoa leaves, and commercial tea were weighed and dissolved in methanol at 1.0 mg/ml, and filtered through a syringe filter (0.45 µm) for further analysis.

Liquid Chromatography Quadrupole Time-of-Flight Mass Spectrometry (LC-QTOF-MS/MS) analysis

The LC-QTOF-MS/MS analysis was performed on Vion IMS LCQTOF MS/MS) with ACQUITY UPLC HSS T3 Column, 100Å, 1.8 µm, 2.1 mm x 30 mm (Waters, US) with a gradient solvent system of water and methanol (90:10). The flow rate was maintained at 0.5mL/min, and the injection volume was set to 10 µL. Chromatograms were acquired between 190 to 500 nm at resolution 1.2 nm using the PDA detector. The mass spectra were recorded across the range of m/z 50-1000 nm in the negative ionization mode (ESI) at the cycle 0.2 s/cycle scan rate. The mass spectrometric conditions were as follows: capillary voltage was 3.00kV, desolvation gas (N2) flow rate was 600 L/h, and source temperature was set at 120°C.

RESULTS AND DISCUSSIONS

The result for LC-QTOF-MS/MS analysis of compounds in cocoa leaves from Sg. Ruan, Sg. Pelek, Durian Tunggal, Rembau and Tawau, commercial green tea and cocoa husk beverages were depicted in Table 1.

Table 1. Chemical composition of cocoa leaves and commercial teas by LC-QTOF-MS/MS analysis

Compounds	Cocoa leaves					Commercial teas	
	Sg Ruan	Sg Pelek	Durian Tunggal	Rembau	Tawau	Green tea	Cocoa husk
Alkaloid							
Dehydrocorydaline				/			/
Magnocurarine			/	/			
Nicotine			/				
Flavonoid							
(-)-Epicatechin						/	
(-)-Epigallocatechin						/	
3,5,6-Trihydroxy-3',4',7-trimethoxyflavone			/		/		
3,5,6-Trihydroxy-4',7-dimethoxyflavone		/		/			
3-Hydroxy baicalein					/		
5,7-Dihydroxy-6,8-dimethyl-3-(2'-hydroxy-3',4'-methylenedioxy-benzyl)chromone				/	/	/	/
6-Formyl-isoophiopogonanone A		/	/	/	/	/	/
Catechin-(4α→8)-catechin	/	/		/		/	
Cinchonain Ib			/				
Cnidimol F		/				/	
Epicatechin gallate (Epicatechin -3-O-gallate)						/	
Epigallocatechin						/	
Epigallocatechin-3-O-gallate						/	
Gallocatechin(4α→8)-epicatechin	/	/	/	/	/	/	/
Genistein_1					/		
Irisflorentin	/	/	/	/	/	/	
Isoirigenin			/		/		
Jaceosidin		/		/			
Leucocyanidin						/	

Noririsflorentin	/			/			
Procyanidin A2	/	/	/	/	/		
Procyanidin B2	/	/		/		/	
Scutellarein		/	/		/		
Polyphenol (tannin)							
Arecatannin A1	/	/		/			
Total compound	**7**	**11**	**10**	**13**	**10**	**13**	**4**

Note: (/) indicates the compound was detected in respective samples via LC-QTOF-MS/MS analysis

Phenolic and alkaloid bioactive compounds are among the secondary metabolites which are very useful for curing many diseases such as tumour, diabetes, heart related illness, cancer and infectious diseases.

Flavonoid, one of the most widespread groups of phenolic compounds found in cocoa, were the main class of compounds characterised in cocoa leaves samples and commercial green tea. 6-formyl-isoophiopogonanone A, gallocatechin(4α→8)-epicatechin, procyanidin A2 and irisflorentin were detected in almost all cocoa leaves samples. The chemical structures of flavonols (epicatechin and catechin) and procyanidins are important for their antioxidant activity (Verstraeten et al., 2004). Gallocatechin(4α→8)-epicatechin and 6-formyl-isoophiopogonanone have been a subject of extensive studies for their antioxidant properties (Kumar & Nayak, 2020; Shi et al., 2016). A study showed that irisoflorentin improves α-synuclein accumulation and attenuates 6-OHDA-induced dopaminergic neuron degeneration, thus indicating its potential as anti-parkinsonian drug candidate (Chen et al., 2015).

The other detected compounds in cocoa leaves samples were magnocurarine and dehydrocorydaline which are alkaloid groups Previous studies showed that magnocurarine and dehydrocorydaline possess anticancer effects (Bala et al., 2017; Xu et al., 2012). A study by Jin et al. (2021) reported the antibacterial effect of polymeric proanthocyanidin, one of them was Arecatannin A1. These compounds were detected in cocoa leaves samples but not in commercial green tea. Catechins were the major compounds detected in commercial green tea which showed the best antioxidant activity. Some of the catechins compounds also appeared in cocoa leaves samples, in addition with the alkaloid and tannins groups of compounds were detected, which could be an added value for the development of cocoa leaves as a potential health beverage.

CONCLUSIONS

The data obtained from the present study showed that several bioactive compounds detected in commercial green tea also appeared in cocoa leaves green tea which justified its potential as a healthy cocoa leaves beverage. Further research is needed to ascertain the health benefits of cocoa leaves prior to being developed as a new health beverage.

ACKNOWLEDGEMENTS

The author would like to thank Mrs. Rasma Suzielawanis Ismail, Assistant Research Officer of the Malaysian Cocoa Board for helping in conducting research activities. Special gratitude extended to Malaysian Cocoa Board for funding the research under Temporary Research Fund. Authors would like to acknowledge Bioaromatic Research Center, Universiti Malaysia Malaysia Pahang for consultation and expertise advice to complete the research work.

REFERENCES

Chen, Y. M., Liu, S. P., Lin, H. L., Chan, M. C., Chen, Y. C., Huang, Y. L., ... & Fu, R. H. (2015). Irisflorentin improves α-synuclein accumulation and attenuates 6-OHDA- induced dopaminergic neuron degeneration, implication for Parkinson's disease therapy. *Biomedicine*, **5(1)**: 1-9.

Hammerstone, J. F., Lazarus, S. A., Mitchell, A. E., Rucker, R., & Schmitz, H. H. (1999). Identification of procyanidins in cocoa (*Theobroma cacao*) and chocolate using high-performance liquid chromatography/mass spectrometry. *Journal of agricultural and food chemistry*, **47(2)**: 490-496.

Huang, J., Wang, Y., Xie, Z., Zhou, Y., Zhang, Y., & Wan, X. (2014). The anti-obesity effects of green tea in human intervention and basic molecular studies. *European journal of clinical nutrition*, **68(10)**: 1075-1087.

Jin, S., Doi, A., Kuroda, T., Zhang, G., Hatano, T., & Chen, G. (2012). Polyphenolic constituents of *Cynomorium songaricum* Rupr. and antibacterial effect of polymeric proanthocyanidin on methicillin-resistant *Staphylococcus aureus*. *Journal of*

agricultural and food chemistry, **60(29)**: 7297-7305.

Kumah, P., Nunoo, L. E., & Bismark, O. (2020). Anti-microbial effect of cocoa leaf extracts on *Botryodiplodia theobromae*; leading causative organism of crown rot disease of banana (*Musa acuminata*). *Asian Journal of Agricultural and Horticultural Research*, **7(3):** 40-50.

Kumar, V., & Nayak, S. K. (2020). Homoisoflavonoids: Isolation, chemical synthesis strategies and biological activities. *Journal of Pharmaceutical Sciences and Research*, **12(8)**: 1046-1055.

Nidhi, S., Datta, S., Dey, A., Chowdhury, A. K., & Abraham, J. (2015). Antimicrobial activity and cytotoxicity of *Theobroma cacao* extracts. *Scholars Research Library*, **7(7)**: 287-294.

Prasanth, M. I., Sivamaruthi, B. S., Chaiyasut, C., & Tencomnao, T. (2019). A review of the role of green tea (*Camellia sinensis*) in antiphotoaging, stress resistance, neuroprotection, and autophagy. *Nutrients*, **11(474)**: 1-24.

Shi, M., Nie, Y., Zheng, X. Q., Lu, J. L., Liang, Y. R., & Ye, J. H. (2016). Ultraviolet B (UVB) photosensitivities of tea catechins and the relevant chemical conversions. *Molecules*, **21(10)**: 1345.

Suzuki, Y., Miyoshi, N., & Isemura, M. (2012). Health-promoting effects of green tea. Proceedings of the Japan Academy. *Series B, Physical and biological sciences*, *88*(3), 88–101.

Verstraeten, S. V., Oteiza, P. I., & Fraga, C. G. (2004). Membrane effects of cocoa procyanidins in liposomes and Jurkat T cells. *Biological research*, **37(2)**: 293-300.

Xu, Z., Chen, X., Fu, S., Bao, J., Dang, Y., Huang, M., ... & Wang, Y. (2012). Dehydrocorydaline inhibits breast cancer cells proliferation by inducing apoptosis in MCF-7 cells. *The American Journal of Chinese Medicine*, **40(01)**: 177-185.

Zainal, B., Abdah, M. A., Taufiq-Yap, Y. H., Roslida, A. H., & Rosmin, K. (2014). Anticancer agents from non-edible parts of *Theobroma cacao*. *Natural Products Chemistry & Research*, **2(4)**: 1-8.