

DNA barcoding and High-Performance Liquid Chromatography as golden standards of authentication in *Blumea balsamifera* and *Ehretia microphylla* products

ALYSSA MARIE A. LOLA^{1,2,3,4,*}, SAM DOMINIC A. BINAG^{1,4,5}, NIÑA KATHRYN G. ALFECHÉ^{1,4,5},
GRECEBIO JONATHAN D. ALEJANDRO^{1,4,5}

¹Graduate School, Thomas Aquinas Research Center, University of Santo Tomas. Manila, Metro Manila, 1008, Philippines. Tel.: +63-2-7315396, Fax.: +63-2-7409732, *email: aalola@ust.edu.ph

²Research Center for the Natural and Applied Sciences, Thomas Aquinas Research Center, University of Santo Tomas. Manila, Metro Manila, 1008, Philippines

³Graduate School of Science, Tokyo Metropolitan University, Minami-Osawa, Hachioji-shi, Tokyo 192-0397, Japan

⁴Makino Herbarium, Tokyo Metropolitan University, Minami-Osawa, Hachioji-shi, Tokyo 192-0397, Japan

⁵Department of Biological Sciences, College of Science University of Santo Tomas. Manila, Metro Manila, 1008, Philippines

Manuscript received: 26 September 2024. Revision accepted: 11 March 2025.

Abstract. *Lola AMA, Binag SDA, Alfeche NKG, Alejandro GJD. 2025. DNA barcoding and High-Performance Liquid Chromatography as golden standards of authentication in Blumea balsamifera and Ehretia microphylla products. Biodiversitas 26: 1258-1270.* In the Philippines, the Department of Health (DOH) has included *Blumea balsamifera* (*sambong*) and *Ehretia microphylla* (*tsaang-gubat*) among the top ten medicinal plants in the country. *Sambong* is known for treating urinary stones, while *tsaang-gubat* is used for helping increase the motility of intestines. Despite their known healing effects, there remains a lack of strict implementation for the approval of these products to be commercially available. This study aimed to employ DNA barcoding and High-Performance Liquid Chromatography (HPLC) by creating a Standard Reference Material (SRM) for both methods. Eight blindly selected *sambong* herbal medicinal products (HMPs), and six blindly selected *tsaang-gubat* HMPs are authenticated using the stated methods. DNA barcoding reveals that samples BB4 and EMP1 are cases of substitution for the products containing *Oryza glaberrima* and *Cymbopogon citratus*, respectively. HPLC analysis also reveals that all *sambong* derived HMPs contain quercetin, except for samples BB2, BB4, and BMP3, whereas for the *tsaang-gubat* herbal products, only sample EMP1 does not contain rosmarinic acid as its active compound. Dissidence in the results indicates that both protocols should be used in coordination as standard authentication tools to promote accuracy of the results and to provide safety to all the consumers. Furthermore, DNA barcoding authenticates the actual plant material used while HPLC checks the presence of active compounds.

Keywords: DNA barcoding, HMPs, HPLC, *sambong*, *tsaang-gubat*

INTRODUCTION

The Philippine Department of Health (DOH) has recognized ten medicinal plants as effective and alternative sources of medicine (Tupas and Gido 2021). Among these herbal plants are *Blumea balsamifera* (L.) DC. (*sambong*) and *Ehretia microphylla* Lam. (*tsaang-gubat*), which have gained popularity due to their accessibility, availability, and commonality. Traditionally, both plants are used as decoctions in several regions of the country. They are also commonly available in the market as tea, capsule, and tablet. With their known curable properties, the Philippine Institute of Traditional and Alternative Health Care (PITAHC) has reported the processing of 10-15 million tablets derived from *sambong* and *tsaang-gubat* yearly (de Guzman 2013). The former or *sambong* is recommended by the Philippine Kidney Transplant Institute (PKTI) as diuretic treatment for kidney stones (Montealegre and De Leon 2016; Rasonabe et al. 2023). Traditional local healers also use *sambong* to treat different conditions such as cough, fever, influenza, dysentery, sore eyes/throat, malaria, boils, infected umbilical cords, and tuberculosis (Maramba-

Lazarte et al. 2020). *Tsaang-gubat* is traditionally utilized to treat diarrhea, spasms, inflammation, gastrointestinal and biliary colic (Mageswari and Karpagam 2015). Moreover, its decoction tea is found to be effective in improving the symptoms of allergic rhinitis and may be used as an alternative to loratadine (Umali and Chua 2017). Legaspi and Bagaosian (2020) unveil the phytochemical constituents of *tsaang-gubat* which include flavonoids, phenolics, triterpenes, and alkaloids. Despite the increasing popularity of these derived herbal medicinal products (HMPs) in the Philippines, there remains a lack of strict regulation of the production of HMPs in the country (Penecilla and Magno 2011). This poses a great threat to consumers as they are not safeguarded from the ill effects of adulteration, contamination, and substitution.

The authenticity of the source material ensures the quality and effectiveness of the herbal products. HMPs contain a mixture of active ingredients from plant parts or plant materials (Tarmizi et al. 2021). Despite the World Health Organization (WHO) emphasizing the importance of using authentic raw plant material, studies have shown that a great portion of products available in the market

contain substitutes and unlabeled fillers (Buddhachat et al. 2015; Olivar et al. 2016; Pedales et al. 2016; Ichim et al. 2019). Industrial-scale protocols are available to test the authenticity of these products. High-Performance Liquid Chromatography (HPLC) has been the golden standard used by the Food and Drug Administration (FDA) of the United Kingdom and the United States to monitor the quality of products. It utilizes well-characterized marker compounds to set specifications for raw materials, standardize botanical preparations during all aspects of manufacturing processes, and obtain stability profiles (Upton et al. 2020). Results from HPLC analysis provide “chromatographic fingerprints” that allow the manufacturing of consistent herbal products. However, HPLC only authenticates at the level of the active compound. Mishra et al. (2016) have provided instances indicating the ease of product adulteration through replicated marker compounds and through external factors that affect the specific compound. This suggests the need for species level authentication of HMPs.

Hebert et al. (2003) have proposed a species authentication system that utilizes a standard region of the genome. This system, termed DNA barcoding, is a reliable tool since genetic information is not affected by external factors (Mishra et al. 2016). However, DNA Barcoding has been less successful in the authentication of plant material due to two factors: (i) limited agreement of gene regions used for barcoding, and (ii) low DNA yield from powdered plant material (Hollingsworth et al. 2011). Nevertheless, several studies (Newmaster et al. 2013; Buddhachat et al. 2015; Vassou et al. 2015; Olivar et al. 2016; Pedales et al. 2016; Alfeche et al. 2019) have shown its use in the identification of plant material from HMPs. Results of these studies consistently show that there is a rampant adulteration in herbal products, warranting the need to include DNA Barcoding in industrial-scale authentication protocols. This study, therefore, aimed to utilize DNA barcoding and HPLC to authenticate *B. balsamifera* and *E. microphylla* derived HMPs sold in Philippine markets. Both DNA barcoding and HPLC are utilized in this study to test incongruences regarding the authenticity of the sampled products. The results of the study promote strict adherence to standard authentication protocols that safeguard consumer health.

MATERIALS AND METHODS

Wild sample collection of *Blumea balsamifera* and *Ehretia microphylla*

Five *B. balsamifera* and seven *E. microphylla* samples were collected from various localities in the Philippines (Table 1). These collections served as the wild samples that were used in the construction of the SRM. Figure 1 illustrates the collected *B. balsamifera* and *E. microphylla* from the wild. Most of these were obtained from private properties and the Bureau of Plant and Industry, Manila. Multiple specimens of each species were considered to represent the extent of genetic variation. Leaf samples of the individual plants were placed in zip-lock bags with silica-gel beads for DNA Barcoding (Semagn 2014). Herbarium specimens were prepared for accessioning at the University of Santo Tomas Herbarium (USTH) and for authentication at the Philippine National Herbarium (PNH Control No. 16-07-870). Table 1 illustrated the collection information. A kilogram of leaf samples each of CB17-070 (*B. balsamifera*) and CB17-067 (*E. microphylla*) were collected and air-dried for HPLC analysis.

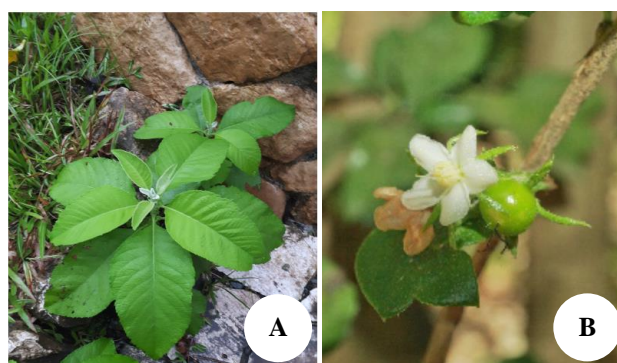


Figure 1. Wild samples of the collected. A. *B. balsamifera* (Photo taken by NKG Alfeche); B. *E. microphylla* (Photo taken by SDA Binag)

Table 1. Collection information of *B. balsamifera* and *E. microphylla* samples

Species name	Sample code	Collection locality	Usth accession number	Genbank accession numbers	
				ITS	trnH-psbA
<i>Blumea balsamifera</i>	16-605	Davao del Sur	USTH013666	OP602345	OP584405
	16-606	Polillo Island, Quezon	USTH013667	OP602346	OP584406
	16-607	Bureau of Plant Industry, Malate, Manila	USTH013668	OP602347	OP584407
<i>Ehretia microphylla</i>	CB17-070	Davao Oriental	USTH014281	OP602348	OP584408
	SB17-002	Tumauini, Isabela	USTH014182	OP602349	OP584409
	16-600	Orani, Bataan	USTH013525	OP604158	OP584399
	16-601	Bureau of Plant Industry, Malate, Manila	USTH013541	OP604159	OP584400
	16-602	Valenzuela City	USTH013523	OP604160	OP584401
	16-603	Pantok, Rizal	USTH013663	OP604161	OP584402
	CB17-067	Davao Oriental	USTH014279	OP604162	OP584404
	UG17-900	Aklan	USTH014280	OP604163	OP584403
	17-019	Alcoy, Cebu	USTH014144	OP604164	Not sequenced

Herbal product sampling

Eight and six blindly selected *B. balsamifera* and *E. microphylla* derived HMPs, respectively, were selected from different markets in the Philippines. The identities of these products were concealed to show anonymity, and were labeled randomly as BB1, BB2, BB3, BB4, BMP1, BMP2, BMP3, BMP4, EM1, EM2, EM3, EM4, EMP1, and EMP2.

DNA extraction, amplification, purification, and sequencing of wild samples and HMPs of *B. balsamifera* and *E. microphylla*

The total genomic DNA from silica-gel dried leaf samples of the wild samples and from powdered HMPs was extracted using DNeasy Plant Mini kit (Qiagen, Hilden, Germany), following the manufacturer's protocol. The ITS, *matK*, *rbcL*, and *trnH-psbA* barcodes were generated to test their efficiencies in discriminating *B. balsamifera* and *E. microphylla* from their closest relatives. Amplification was accomplished in 25 μ L PCR aliquots of 15.3 μ L dH₂O, 2.5 μ L 10X PCR buffer, 2.0 μ L 25 mM MgCl₂, 1.5 μ L 2 mM dNTPs, 1.0 μ L of 10 mM forward and reverse primers, respectively, 0.2 μ L KAPA Taq DNA polymerase, and 1.0 μ L DNA. Reactions were performed in Biometra TGradient Thermocycler. Primer information and amplification protocols were shown in Table 2. PCR amplicons were checked on 1% TBE agarose gel and were purified using Qia-Quick PCR purification kit (Qiagen, Hilden, Germany), following the manufacturer's protocol. Purified products were sent to MACROGEN South Korea for bidirectional sequencing. Sequences were then assembled and edited using Codon Code Aligner v5.1.5.

Establishment of a Standard Reference Material (SRM) of *B. balsamifera* and *E. microphylla* wild samples

A multi-locus/tiered dataset was prepared using 78 gene accessions of 13 closely related *Blumea* species, including accessions for *Ligularia* sp. as the outgroup, and 26 gene accessions of closely related *Ehretia* species, including accession for *Bourreria* sp. as the outgroup. GenBank sequences of species closely related to *B. balsamifera* and *E. microphylla* and the collected wild samples for each species were used in the establishment of an SRM. GenBank accession numbers of the newly generated sequences of the wild *B. balsamifera* and *E. microphylla* are shown in Table 1. Variable and parsimony-informative sites and resolutions of species were computed to provide data regarding the strengths of the utilized barcoding loci. Inter- and intraspecific

Kimura-2-Parameter (K2P) distances were computed using MEGA 7.0 to measure the extent of genetic variation (Kumar et al. 2016). The SRMs were established by constructing Maximum Likelihood (ML) trees using the K2P distances in MEGA 7.0.

Authentication of *B. balsamifera* and *E. microphylla* derived HMPs using DNA barcoding

Sufficient DNA quality and quantity were extracted from the HMPs following the standard DNA extraction protocols (Table 3). Amplification of the ITS, *trnH-psbA*, *matK*, and *rbcL* loci was then conducted. Authentication through DNA Barcoding was followed using BLASTn and ML tree reconstruction criteria. The generated sequences were queried using the BLASTn algorithm (<https://blast.ncbi.nlm.nih.gov>). An herbal product was considered to pass the BLASTn criterion if the BLAST search resulted in *B. balsamifera* or *E. microphylla* as its top hit with an e-value cut-off <0.01. The generated sequences were also incorporated within the established SRM tree. An herbal product was considered to pass the ML tree criterion if the sequences nested within a strongly supported (BS>70%) monophyletic clade with the authentic *B. balsamifera* and *E. microphylla* samples. If both criteria were met, an herbal product was considered authentic in terms of the presence of the actual plant material.

Table 3. Spectrophotometric results* of the *B. balsamifera* and *E. microphylla* derived HMPs showing its DNA concentration and purity

Product code	Concentration (μ g/ μ L)	A260/A280
BB1	0.045	2.004
BB2	0.099	2.011
BB3	0.017	1.983
BB4	0.024	2.019
BMP1	0.021	2.016
BMP2	0.018	1.856
BMP3	0.016	1.878
BMP4	0.019	1.589
EM1	0.011	1.426
EM2	0.049	1.906
EM3	0.015	1.747
EM4	0.060	1.692
EMP1	0.021	1.848
EMP2	0.019	1.730

Note: *Average of three trials per sample

Table 2. PCR primers and amplification protocols

Gene region	Primer name	Primer sequence (5' \rightarrow 3')	References
<i>trnH-psbA</i>	<i>trnH</i> <i>psbA</i>	CGCGCATGGTGGATTACAATCC GTTATGCATGAACGTAATGCTC	Lv et al. 2020
ITS	5 4	GGAAGTAAAAGTCGTAACAAGG TCCTCCGCTTATTGATATGC	Alfeche et al. 2019
<i>matK</i>	1RKIM-f 3FKIM-r	ACCCAGTCCATCTGGAAATCTTGGTTC CGTACAGTACTTTTGTGTTTACGAG	Kuzmina et al. 2012
<i>rbcL</i>	1F 724R	ATGTCAACCACAAACAGAAAC TCGCATGATCCTGCAGTAGC	Fay et al. 1997

Authentication of *B. balsamifera* and *E. microphylla* derived HMPs using HPLC analysis

Standard HPLC profiles were created following protocols of Nessa et al. (2005) and Toralba et al. (2015) for *B. balsamifera*, and Makino et al. (2003) and Wang et al. (2003) for *E. microphylla*. The chromatographic profiles of the targets, quercetin (QA) and rosmarinic acid (RA), were obtained using the Shimadzu LC-20AT, a Photodiode Array (PDA) detector, CBM-20A, and a C-18 Shimadzu column. These profiles were created to specifically detect the presence of QN for *B. balsamifera* and RA for *E. microphylla*. Approximately 200 mg of powdered samples from the wild samples and herbal products were used. *B. balsamifera* derived samples were extracted for 6 hours with 20 mL methanol in glass-stopped vessels on IKA®C-MAG HS7 hot plate with magnetic stirring at 40°C followed by filtration using Whatman® Filter Paper grade 602 h ½ and further extraction with 10 mL methanol for three times. The extract was evaporated to dryness in an Eyela®N-1200B rotary evaporator at 40°C. For *E. microphylla* collected wild and HMPs, these were dissolved in 100 mL ethanol/water (30:70, v/v) with sonication using Daikan® Labtech Power Ltd. Sonic410 for 40 minutes followed by filtration and further extraction using 80 mL ethanol-water with sonication for 20 minutes.

All extracts from wild and HMP samples of *sambong* and *tsaang-gubat* were evaporated to dryness in an Eyela® N-1200B rotary evaporator at 40°C. Five mg of the solid residue was then weighed and mixed with 5 mL methanol for the wild *sambong* and derived HMPs, and 5 mL ethanol/water (30:70, v/v) for wild *tsaang-gubat* and derived HMPs to produce a 1000 ppm mixture. The mixtures were then filtered using an Agilent® 0.45 µm nylon syringe filter and 20 µL of the resulting mixtures were injected into the HPLC for analysis. Three trials for each sample were done for the consistency of data.

For QN, the mobile phase consisted of methanol-0.5% phosphoric acid in water (50:50, v/v). The flow rate was held constant at 0.9 mL/min. For RA, solvents used for separation were 0.1% orthophosphoric acid in water (v/v) (eluent A) and 0.1% orthophosphoric acid in methanol (v/v) (eluent B). The gradient used was: 0-10 min, linear gradient from 40% to 50% B; 10-15 min, linear gradient from 50% to 60% B, maintained at 60% B until 25 min. The flow rate was 1.0 mL/min and the detection wavelength at 330 nm.

The chromatographic peaks of QN in both wild *B. balsamifera* and RA in wild *E. microphylla*, together with their respective HMPs, were confirmed by comparing their retention times (t_R) with that of their respective reference standards. The herbal product was deemed authentic if it showed similar HPLC profiles with the standards for QN and RA. The standard chromatographic profile of QN shows a distinct peak at a retention time of 20-30 minutes (Toralba et al. 2015; Jirakitticharoen et al. 2022) while RA exhibits a distinct peak at a retention time of 16-18 minutes, following the protocols of Makino et al. (2003).

To further validate chromatographic peaks, standards of QN or RA were used to spike wild samples and herbal products of *B. balsamifera* or *E. microphylla*, respectively. From the 100 ppm standards of QN and RA, 50 µL was taken. This was thoroughly mixed with 50 µL of the 1000 ppm samples of either wild samples or HMPs. A 20 µL of the mixture was injected into the HPLC instrument. The chromatographic profiles were then assessed to confirm the presence of the active compounds in the tested HMPs.

RESULTS AND DISCUSSION

Establishment of the Standard Reference Material (SRM) herbal barcode library of *B. balsamifera* and *E. microphylla* wild samples

An ideal barcode must be easily amplified, possess high variable sites, high parsimony-informative sites, high interspecific K2P distances, low intraspecific K2P distances, and high resolution of species. Table 4 shows the values for the amplification and sequencing success of the four loci which are used to describe the versatility of the primer amplified over a wide range of species. All the primers were successfully amplified (100%) in both *B. balsamifera* and *E. microphylla*, with ITS having the longest aligned length of 553 bp for *B. balsamifera* and *matK* with the longest aligned length of 719 bp for *E. microphylla*. ITS provided the best values for variable sites in both *B. balsamifera* (29.66%) and *E. microphylla* (37.76%). It also gave the most parsimony-informative characters, 27.49% for *B. balsamifera* and 31.82% for *E. microphylla*. The *trnH-psbA* marker had the second-highest parsimony-informative values with 7.89% and 7.08% for both species, respectively. Both *matK* and *rbcL* had the lowest variable and parsimony-informative sites for both species. This suggests that ITS and *trnH-psbA* can be readily amplified for both species and both loci provide universality while *matK* and *rbcL*, though successfully sequenced, are not enough to provide species discrimination.

The pairwise sequence divergences indicate the primer's discriminatory power. Wilcoxon signed-rank and two-sample tests performed in SPSS (IBM) confirmed the significant differences between inter- and intraspecific Kimura-2-parameter (K2P) distances of the investigated primers. The K2P interspecific distances depict how close different species are to each other, while the K2P intraspecific distances show the closeness of individuals within the same species. For the barcode to be ideal, it should have a significantly higher interspecific divergence to distinguish one species from another, and not the individuals of the same species (Galimberti et al. 2019). As illustrated in Table 5, each of the candidate barcodes has shown a higher interspecific distance compared to its intraspecific distance. Among the barcodes tested, the mean interspecific divergences were the highest in ITS (0.1048 and 0.2397).

Table 4. Properties of the four candidate DNA barcoding loci for *A. B. balsamifera* and *B. E. microphylla*

Properties	ITS	matK	rbcL	trnH-psbA
<i>B. balsamifera</i>				
Number of sequences	33	6	6	33
Aligned length (bp)	553	251	560	393
Variable sites (%)	29.66	1.99	2.68	9.67
Parsimony-informative characters (%)	27.49	1.99	0	7.89
Mean interspecific K2P distance	0.1048 ± 0.0062	0.0193 ± 0.0018	0.0083 ± 0.0007	0.0838 ± 0.0111
Mean intraspecific K2P distance	0.0026 ± 0.0011	0.0026 ± 0.0018	0	0
Resolution of species (%)	100.00	66.67	100.00	84.62
<i>E. microphylla</i>				
Number of sequences	7	6	7	6
Aligned length (bp)	286	719	443	339
Variable sites (%)	37.76	4.03	1.13	8.55
Parsimony-informative characters (%)	31.82	4.03	1.13	7.08
Mean interspecific K2P distance	0.2397 ± 0.0109	0.0418 ± 0	0.0083 ± 0.0007	0.0838 ± 0.0111
Mean intraspecific K2P distance	0.0035 ± 0.0028	0	0	0
Resolution of species (%)	100.00	100.00	100.00	100.00

Note: ^aEntries shown in bold are considered the best performing for a particular parameter

Table 5. Wilcoxon two-sample test for inter- and intra-specific divergences of four barcodes

Barcode	Number of inter specific	Mean inter specific	Number of intra specific	Mean intra specific	Wilcoxon W	P value
<i>B. balsamifera</i>						
ITS	669	0.1048	72	0.0026	2712.50	0.000
matK	21	0.0192	45	0.0000	1057.50	0.000
rbcL	7	0.0273	21	0.0000	231.00	0.000
trnH-psbA	673	0.0235	71	0.0000	2905.00	0.000
<i>E. microphylla</i>						
ITS	46	0.2397	32	0.0028	528.00	0.000
matK	14	0.0418	22	0.0000	253.00	0.000
rbcL	47	0.0083	31	0.0000	511.50	0.000
trnH-psbA	46	0.0838	32	0.0000	528.00	0.000

For *Blumea*, ITS had the highest interspecific divergence, followed by *rbcL*, *psbA-trnH*, and *matK* ($p < 0.05$) (Table 6), while for *Ehretia*, ITS had the highest interspecific divergence, followed by *trnH-psbA*, *matK*, and *rbcL* ($p < 0.05$). For the intraspecific divergence of *Blumea*, the lowest was for *trnH-psbA*, *matK*, and *rbcL* (0), followed by ITS (0.0026) (Table 6), while for *Ehretia*, all markers obtained a value of zero. Thus, ITS and *trnH-psbA* provided the best discriminatory power over the other candidate barcodes.

The resolution of the species was tested using BLASTn and the tree construction criterion which established the Standard Reference Material (SRM) herbal barcode library for *B. balsamifera* and *E. microphylla*. For a barcode to be efficient, it should be able to identify samples down to the species level. Single locus analysis following BLASTn criteria was able to resolve the species identity to a certain degree. Both ITS and *trnH-psbA* were able to identify all wild *Blumea* samples (100%) while ITS, *matK*, and *trnH-psbA* were able to resolve all the wild *Ehretia* samples

(100%) down to genus level only. Tree construction criterion utilizing single locus also resolved the species to a certain degree. ITS and *matK* were able to resolve the individuals of *B. balsamifera* to a monophyletic clade with BS=100%, while *trnH-psbA* BS=99%, and *rbcL* BS=92%. ITS and *trnH-psbA* resolved the individuals of *E. microphylla* to a monophyletic clade with BS=100%, while *matK* and *rbcL* had BS=99%. The multi-locus/tiered approach utilizing the four loci successfully provided distinctness of *B. balsamifera* and *E. microphylla* clades with both forming strongly supported (BS=100%) monophyletic groups (Figure 2). Evident in both SRMs is the distinctness of *B. balsamifera* and *E. microphylla* from their close allies as conspecific samples form strongly supported (BS=100%) monophyletic groups (highlighted in yellow). The concatenated dataset also supported stronger bootstrap values. This indicates that the established SRM can be used to associate unknown samples to *B. balsamifera* and *E. microphylla* when DNA barcoding protocols are followed.

Table 6. BLASTn results for the authentication of plant derived herbal products

Product code	ITS			<i>trnH-psbA</i>		
	BLASTn ID	e-value	% identity	BLASTn ID	e-value	% identity
BB1	<i>Blumea balsamifera</i> L.	0.0	99	<i>Blumea balsamifera</i> L.	0.0	98
BB2	<i>Blumea balsamifera</i> L.	0.0	99	<i>Blumea balsamifera</i> L.	0.0	99
BB3	<i>Blumea balsamifera</i> L.	0.0	99	<i>Blumea balsamifera</i> L.	0.0	99
BB4	<i>Oryza glaberrima</i> Steud.	1e-131	97	<i>Oryza glaberrima</i> Steud.	1e-131	97
BMP1	<i>Blumea balsamifera</i> L.	0.0	99	<i>Blumea balsamifera</i> L.	0.0	99
BMP2	<i>Blumea balsamifera</i> L.	0.0	99	<i>Blumea balsamifera</i> L.	0.0	99
BMP3	<i>Blumea balsamifera</i> L.	0.0	99	<i>Blumea balsamifera</i> L.	0.0	96
BMP4	<i>Blumea balsamifera</i> L.	0.0	97	<i>Blumea balsamifera</i> L.	0.0	99
EM1	<i>Ehretia microphylla</i> Lam.	0.0	95	<i>Ehretia laevis</i> Roxb.	1e-135	93
EM2	<i>Ehretia microphylla</i> Lam.	0.0	97	<i>Ehretia laevis</i> Roxb.	2e-153	96
EM3	<i>Ehretia microphylla</i> Lam.	0.0	97	<i>Ehretia laevis</i> Roxb.	3e-136	94
EM4	<i>Ehretia microphylla</i> Lam.	0.0	97	<i>Ehretia laevis</i> Roxb.	3e-143	95
EMP1	<i>Cymbopogon citratus</i> Stapf.	0.0	98	<i>Cymbopogon citratus</i> Stapf.	0.0	99
EMP2	<i>Ehretia microphylla</i> Lam.	0.0	99	<i>Ehretia laevis</i> Roxb.	3e-140	94

Note: ^aEntries shown in yellow are considered substitutions observed for the selected medicinal products

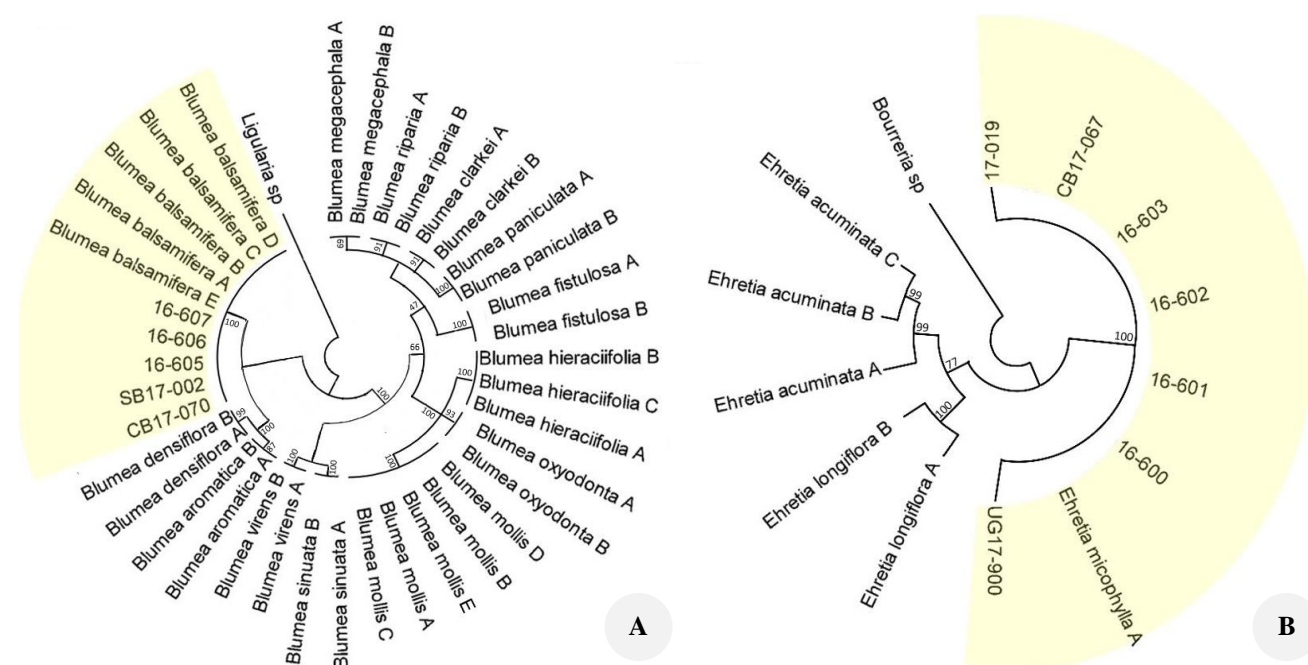


Figure 2. Standard reference material (SRM) herbal barcode library consensus tree (ML analysis) for combined ITS, *matK*, *rbcL*, and *trnH-psbA* dataset of A. 94 gene accessions of *Blumea* species and B. 38 gene accessions of *Ehretia* species. Highlighted are the A. *B. balsamifera* and the B. *E. microphylla* clades. Numbers on nodes are bootstrap (BS) support values

Authentication of *B. balsamifera* and *E. microphylla* derived HMPs following DNA barcoding

Authentication of 14 blindly selected *B. balsamifera* and *E. microphylla* derived HMPs followed the BLASTn and Maximum-Likelihood (ML) Tree reconstruction criteria. In terms of primer universality, both *sambong* and *tsaang-gubat* derived HMPs provided 28 good-quality sequences for both ITS and *trnH-psbA*. However, problems were experienced in the PCR amplification of *matK* and *rbcL* for the HMPs. This does not necessarily point to *matK* and *rbcL* as unsuitable loci, but unsuccessful amplifications may have been due to primer mismatch, degradation of the DNA material, or due to inhibition of polymerase. As Newmaster et al. (2013) stated, difficulties are often

encountered based on the primers used. Table 6 reveals the BLASTn results, where seven out of eight *sambong* HMPs were identified to be *B. balsamifera*. BB4 was substituted with *Oryza glaberrima*. Substitution occurs when barcodes of other species, other than the target species, are sequenced in the analysis, following the definition of Newmaster et al. (2013). For *tsaang-gubat* HMPs, five out of six derived products (EM1, EM2, EM3, EM4, and EMP2) were identified to be authentic, containing *E. microphylla* using ITS gene region with an average e-value of 0.0, and *E. laevis* identification using *trnH-psbA* with an average e-value of 2.6e-136. The discrepancy in the BLASTn identification could be attributed to the amounts of published sequences in the NCBI database. *Tsaang-gubat* HMP

EMP1 was also identified as a form of substitution, with the plant component identified as *Cymbopogon citratus*.

All 16 and 12 newly generated sequences from *sambong* and *tsaang-gubat*-derived HMPs, respectively, were incorporated into the established SRM herbal barcode libraries presented in Figure 3. Authentic *sambong* and *tsaang-gubat*-derived products should nest within their respective monophyletic clades. All codes in Figures 2.A and 2.B, in red, are the products tested in this study. As seen in the Figure 2A, product codes BB1, BB2, BB3, BMP1, BMP2, BMP3, and BMP4 were well nested within the *B. balsamifera* clade (BS=89%). Product code BB4 was placed outside the outgroup *Ligularia* sp. indicating its exclusion from family Asteraceae. While in figure 2B, product codes EM1, EM2, EM3, EM4, and EMP2 reside in the *E. microphylla* clade (BS=100%). Same with the product code BB4, product code EMP1 was placed outside the outgroup *Bourreria* sp., which indicates its exclusion from the family Boraginaceae.

Following both BLASTn and SRM Herbal Library criteria, it was concluded that out of the eight *sambong* and six *tsaang-gubat*-derived HMPs, one product code of each target HMP did not correspond to *B. balsamifera* and *E. microphylla*, respectively. The identification of different source plant materials in the two HMPs is evidence of product substitution. BB4 was identified to contain *Oryza glaberrima* as evidence of product fillers. African rice (*O. glaberrima*) has been classified as product filler in several commercial teas and capsules (Newmaster et al. 2013). On the other hand, the identification of *C. citratus* is an example of substitution wherein herbal plants may have been replaced with another raw material with similar benefits but cheaper production costs. Even though *C. citratus* has its own ethno-pharmaceutical properties, the use of this plant in the guise of another species is still a case of product

fraud which could pose health risks to unassuming consumers. The results of the study show the success and effectiveness of DNA Barcoding as a standard protocol for authenticating medicinal products.

Several studies showed that DNA barcoding is useful for HMPs' authentication. Olivar et al. (2016) revealed cases of adulterations in *Vitex negundo* (*lagundi*) HMPs by using BLAST and ML tree criterion of combined ITS-trnH-psbA datasets. BLAST unveiled only one of five samples was authentic while other samples contained a fungus and an uncultured eukaryote. Similarly, the study by Pedales et al. (2016) indicated that the BLAST results showed 4 of 19 ITS2-barcoded HMPs contained possible substitution or contamination. They further demonstrated that ITS2 can effectively discriminate among species but recommended an additional barcode to strengthen the resolution. Meanwhile, the work of Michel et al. (2016) proved that ITS is an effective marker in terms of species discrimination for the HMPs sold in New York City. ITS is regarded as a core DNA barcode in the identification of medicinal plants primarily due to its length, easy expansion, and high success rate (Yang et al. 2018). More importantly, the authentication in Apocynaceae HMPs proven the effectiveness of using both ITS and *trnH-psbA* (Lv et al. 2020). The strength of ITS and *trnH-psbA* as a marker for *sambong* and *tsaang-gubat* derived products' authentication was evidently seen as well in this study. Frigerio et al. (2021) also recommended the use of ITS and *trnH-psbA* for DNA-based herbal teas' authentication. Moreover, Alfeche et al. 2019 used SRM DNA barcode library for *Antidesma bunius* (*bignay*), where only three of 11 HMPs were authentic using combined dataset. Despite the strengths of DNA barcoding, it cannot determine the active compound available in the HMPs. Additionally, multiple species may have such compounds, and the actual plant material used may be adulterated.

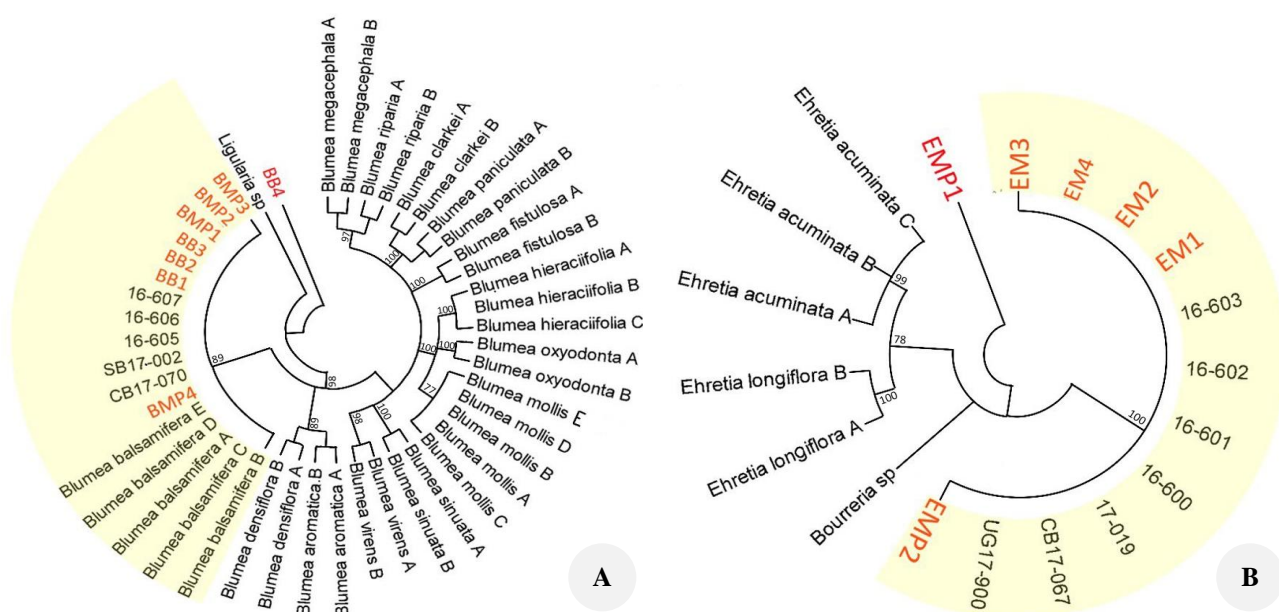


Figure 3. Maximum-Likelihood tree reconstruction for combined (ITS-*trnH-psbA*) barcode data of A. *Blumea* and B. *Ehretia* derived HMPs and wild samples

Establishment of the standard chromatographic profiles for *B. balsamifera* and *E. microphylla*

Currently, HPLC is the golden standard used for authenticating herbal medicines due to its efficiency in quality control. This method determines the active compound present in the HMPs. An active compound/bioactive compound is important since it is responsible for the product's effectiveness in treating or alleviating the effects of a certain illness/disease. Moreover, the long-term use of herbal products relies on maintaining the constant levels of such compounds (Zarsuelo et al. 2018). Qualitative solute identification via comparison of retention data of the active compound is done to screen the HMPs (Liang et al. 2004). Further, they stated that one or two pharmacologically active compounds can be used for screening the quality and authenticity of HMPs using HPLC.

B. balsamifera contains QN and *E. microphylla* includes RA. QN was determined as an active flavonoid in the leaves of *B. balsamifera* (Jirakitticharoen et al. 2022). It offers a wide range of functions such as anti-inflammatory and antioxidant properties. QN was also proven as an important active compound involved in the diuretic properties of *sambong* in treating patients with kidney stones (Rasonabe et al. 2023). *E. microphylla* leaves have RA which is attributed to its efficacy in controlling allergies (Shukla and Kaur 2018). Some of the HMPs' manufacturers also indicate the active compounds present in their products, as placed at the back of their boxes or labels just like QN and RA, respectively. Therefore, chromatograms for QN and RA showed great importance in authenticating *B. balsamifera* and *E. microphylla*, respectively. The retention time for QN in *B. balsamifera* was shown to be 20-30 minutes in the studies of Toralba et al. (2015) and Jirakitticharoen et al. (2022). Meanwhile, retention time for RA varies in different plant samples such as 52-54 min in *Ehretia tinifolia* (Monroy-Garcia et al. 2021) and 12.5-14.5 in selected Thai plants (Chaowuttikul 2020). The established chromatographic profiles were then used to authenticate HMPs (Figures 4.A-D). In this study, the peak for RA was consistently seen at a retention time of 16-18 minutes. Upon comparing the chromatographic profiles of the active compounds to the generated HPLC profiles of wild *B. balsamifera* and *E. microphylla*, similar distinct peaks can be found. This indicates that QN and RA are indeed present in *B. balsamifera* and *E. microphylla* extracts, respectively.

Authentication of *B. balsamifera* and *E. microphylla* derived HMPs following HPLC analysis

The HPLC chromatograms of the eight *B. balsamifera* HMPs are shown in Figure 5A-5H, and a comparison with the t_R of the standard QN (25-27 min) indicates the presence of QN in five of the eight HMPs not including BB2, BB4, and BMP3. The absence of QN in BB4 corroborated with the results of the DNA Barcoding, indicating that BB4 does not contain *B. balsamifera*. DNA Barcoding has identified BB2 and BMP3 as *B. balsamifera*; however, QN was not detected based on the HPLC. This bioactive compound may

have been destroyed or deactivated after the manufacturing process. With this said, HPLC must be used since it determines the presence of the active compound and must be corroborated with another authentication technique.

Comparison of the t_R of the standard RA (16-18 min) with those of the *E. microphylla* HMPs (Figures 6.A-F) demonstrate the presence of RAs except in EMP1. This corroborates with the results of the DNA Barcoding, indicating EMP1 is *Cymbopogon citratus*, and not *E. microphylla*.

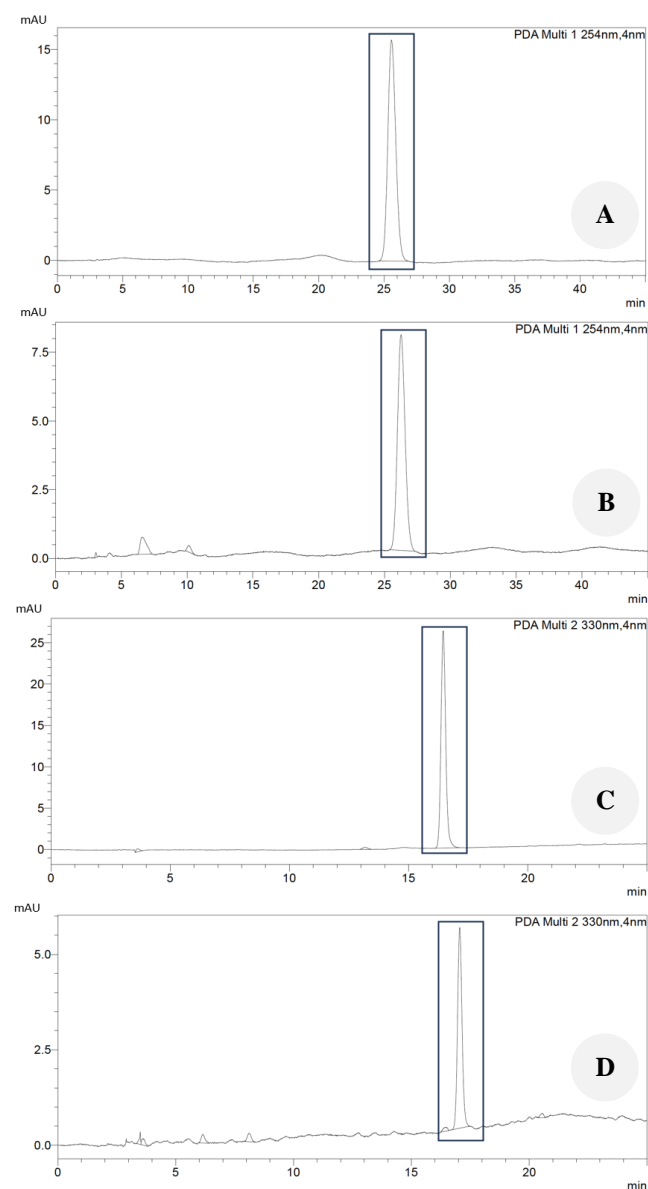


Figure 4. Chromatogram of A. Standard QN; B. Wild *B. balsamifera* (CB17-070); C. Standard RA; D. Wild *E. microphylla* (CB17-067)

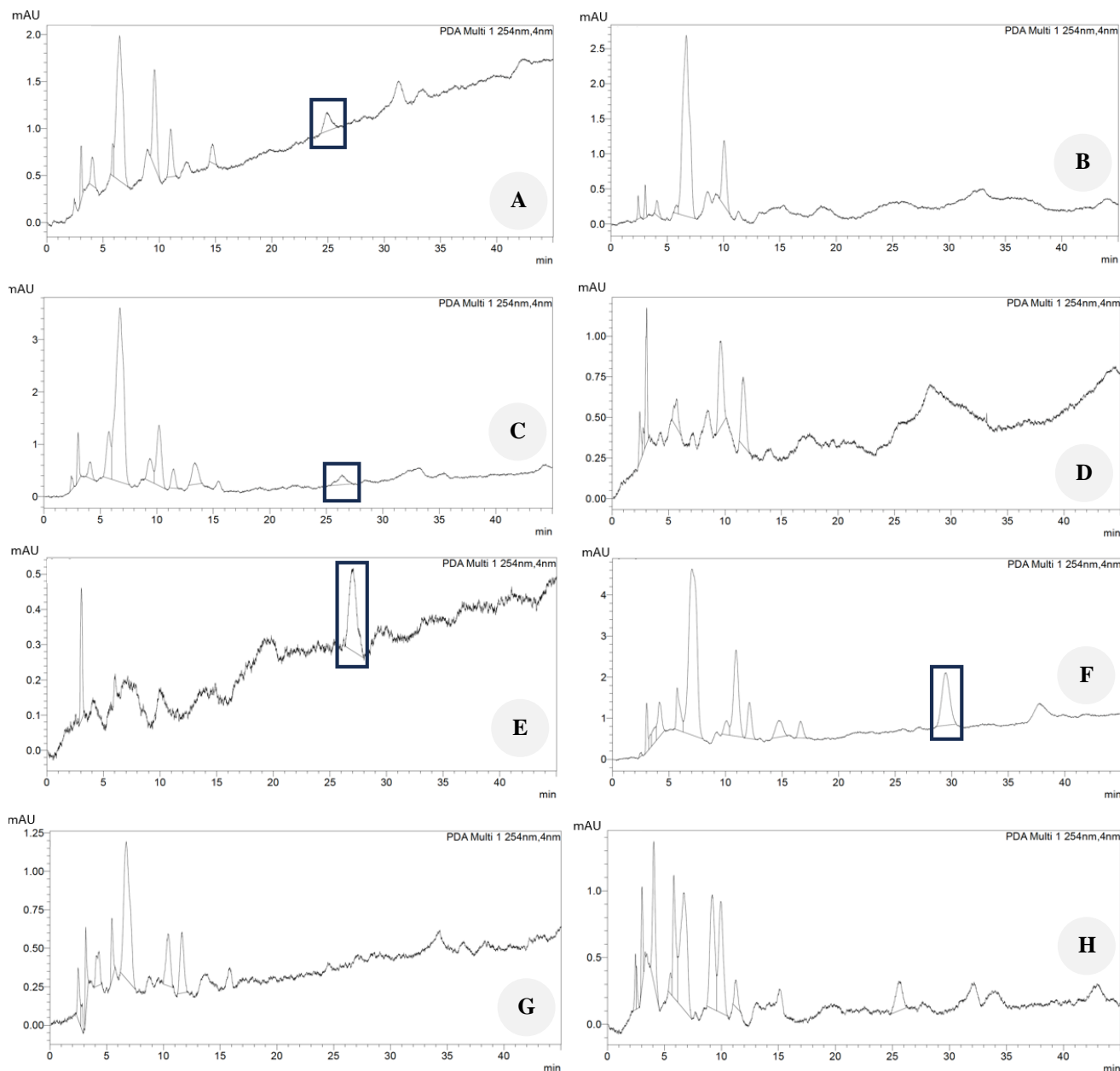


Figure 5. HPLC chromatogram of the eight *B. balsamifera* derived HMPs: A. BB1; B. BB2; C. BB3; D. BB4; E. BMP1; F. BMP2; G. BMP3; H. BMP4

Several reports displayed the importance of HPLC in the authentication of HMPs. Orman et al. (2022) showed the use of HPLC in assessing the quality of South African HMPs. The study showed that using flavonoids for rapid scanning could be performed when the active compound is unknown. Moreover, Xu et al. (2021) utilized HPLC for seven bioactive components of the “*Arnebiae Radix*” HMPs. The results of the study indicated that HPLC allows the simultaneous discrimination of the seven main naphthoquinones. Meanwhile, the study of Custers et al. (2017) emphasized that chromatographic fingerprinting is capable of detecting target plants in illegal herbal products even in complex samples.

However, there are limitations to HPLC due to the lack of long-term reproducibility and false results caused by

deliberate adulteration of the illegal addition of a marker compound to a medicinal product (Hassan 2012). In this study, results showed the success of HPLC in authenticating the presence of a single active compound. However, it must be noted that alternative medicine is effective due to the presence of the actual plant extract and its active compound/s. Therefore, another method should accompany HPLC to test the authenticity of the plant source material.

DNA barcoding and HPLC as golden standards in the authentication of herbal medicinal products

Results obtained from DNA Barcoding showed incongruencies with the results obtained from the chromatographic profiles of HPLC. Therefore, the combination of DNA barcoding and HPLC is necessary. DNA Barcoding

is only limited to genotypic identification whereas HPLC is only limited to the determination and isolation of chemical constituents.

Several studies revealed that DNA Barcoding and HPLC must be used together to authenticate HMPs. Xu et al. (2021) utilized both DNA barcoding and HPLC in the authentication of traditional Chinese medicine. They showed that ITS was also a good marker for checking the authenticity of the HMPs. Moreover, their study demonstrated that HPLC chromatograms could not differentiate the available species in the HMPs but only the presence of the active compounds. Furthermore, HPLC results unveiled that even though the actual plant material was present, the expected active compound in some of the samples was not detected (Abubakar et al. 2018). Similar results were identified in the present study wherein QN and RA were not detected in some of the HMPs. Meanwhile, HPLC determined the effective chemical constituents. In their study, Ghorbani et al. (2020) conducted the authentication of *Ruscus hyrcanus* using *trnH-psbA* barcoding and HPLC-PDA analysis. Their study illustrated the effectiveness of *trnH-psbA*, similar to its use in the barcoding of *B. balsamifera* and *E. microphylla*. Moreover, they used two saponin compounds for HPLC. Anantha Narayana and Johnson (2019) stated

that DNA Barcoding can detect raw materials from HMPs, but it cannot check chemical constituents or plant parts used. To identify the actual material and ingredients of the HMPs, it must be complemented with other techniques. However, HPLC cannot give a complete picture of the true plant present from the herbal products. If the safety of HMPs relies on specific bioactive compounds, absence of toxins, allergens, and admixed pharmaceuticals, then HPLC is more relevant than DNA-based analysis. To ascertain species substitution or adulteration, DNA Barcoding provides better resolution than HPLC (Techen et al. 2014; Palhares et al. 2015). Additionally, the review paper of Abubakar et al. (2017) suggested the combination of DNA barcoding and chromatographic fingerprinting since DNA barcoding can identify the herbal plants used, and HPLC can check the quality of the products. Another study by Zhang et al. (2016) stated that DNA barcoding and HPLC can discriminate between species in Chinese HMPs; however, DNA barcoding is more useful in terms of confirming the identities of medicinal materials from various sources. With the amount of available data that support the use of these competing techniques, DNA barcoding coupled with HPLC is hereby highly recommended for the authentication of Philippine herbal products.

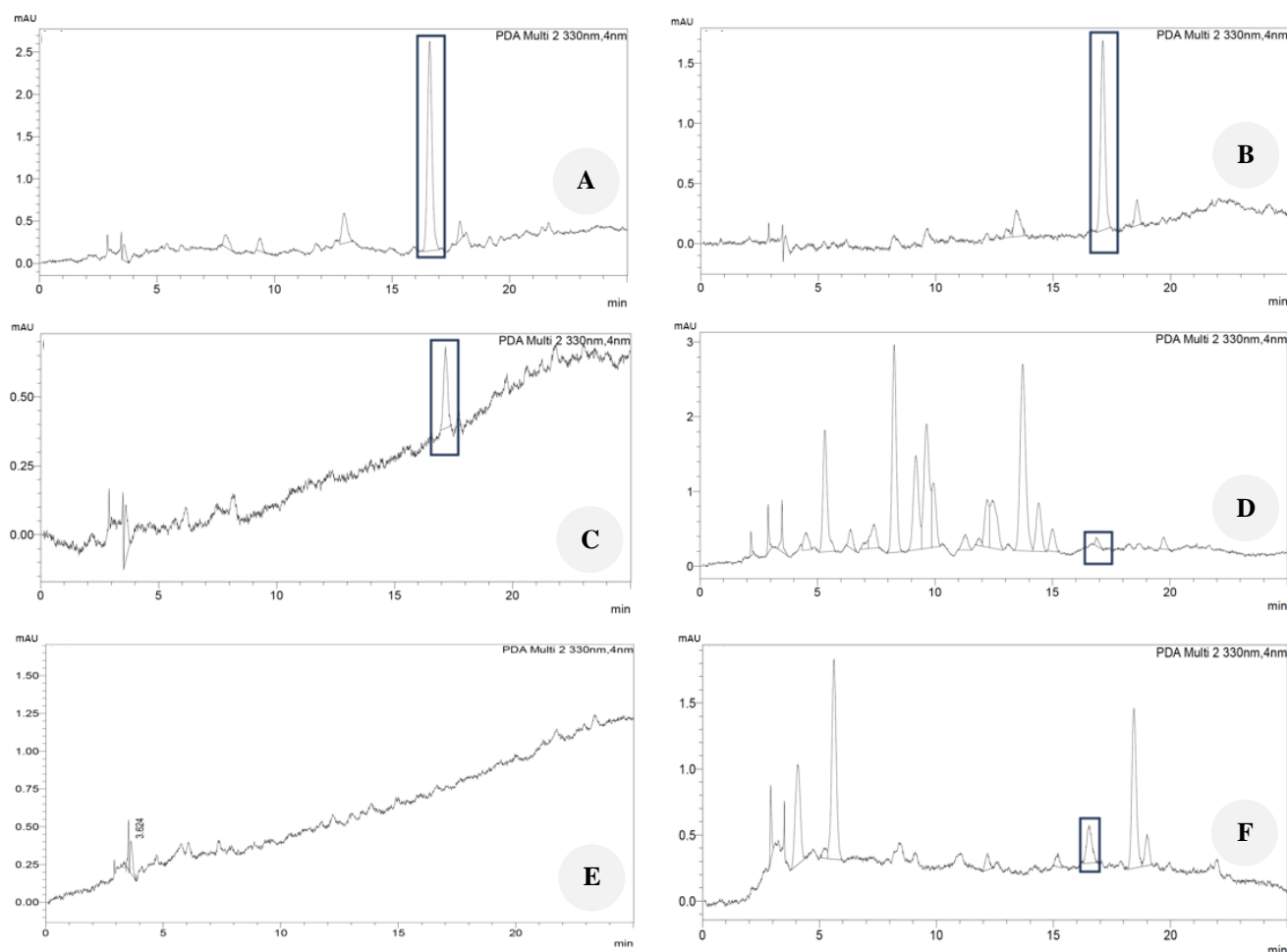


Figure 6. HPLC chromatogram of the six *E. microphylla* derived HMPs: A. EM1; B. EM2; C. EM3; D. EM4; E. EMP1; F. EMP2

Possible reasons, such as clear mistake in vernacular names between indigenous systems of medicine and local dialects, similarity in morphological features of other species of plants, and absence of authentic raw plant material can be attributed to product adulteration (Srirama et al. 2017). It is also possible that product substitution will occur given that there is no regulatory tool in place or commercial test for product authentication. Bearing in mind the consequences of adulteration in the drug trade, labors have been prepared to precisely identify and classify medicinal plants in commercially available herbal products (Kool et al. 2012). Besides conventional approaches, such as morpho-taxonomic keys, wood anatomy, histological, and histochemical techniques, DNA-based techniques have just been certified as simple, and reliable tools to authenticate the medicinal plant material used in natural health products (Seethapathy et al. 2014). The practice of substituting the original active crude drug in part, or in whole with other ingredients, is known as adulteration. Adulteration can be free form or inferior in therapeutic and chemical properties to that of the original drug replaced with the purpose of increasing revenues. Owing to having inexpensive prices of herbal products and their accessibility, this became the topic of adulteration (Seethapathy et al. 2014). The main topic in standardizing these herbal products is the authentication of raw botanical materials. It is disparagingly vital to authenticate a plant product and detect any adulteration throughout the manufacturing process for adulteration can result in an unsuccessful and ineffective product, or worse, can even harm the consumer. To properly identify plant source material of these HMPs, modern techniques such as DNA barcoding and HPLC can be utilized effectively in identification of medicinally important plant species. Thus, this study can serve as a benchmark for other HMP's evaluation for possible product adulteration.

The Philippines, being a third-world country, has economic systems that require advancement. This concern trickles down even in the process of acquiring quality healthcare products. This encourages the masses to lean towards cost-effective healthcare, such as cheap traditional and alternative medicines. The increase in awareness of the use of HMPs happened during the campaign done by the country's Department of Health (DOH) through "Traditional Health Program" that encourages the use of 10 medicinal plants which includes *sambong* and *tsaang-gubat* (Catublas 2016). Although the use of modern medicine may happen at the same time, HMPs have maintained their popularity for cultural and historical reasons (Tolentino et al. 2019). In fact, the Philippines has one of the largest numbers of registered herbal drugs (>2,000) worldwide (Sahoo et al. 2010). Moreover, the Department of Trade and Industry (DTI) in the country revealed that the export market value of natural health products, mostly HMPs, is an estimated 153 million USD as of 2011 (Zarsuelo et al. 2018). However, substitutions, contaminants, and adulterations of alternative medicines were determined. The studies of Alfeche et al. (2019), Olivar et al. (2016), and Pedales et al. (2016) proved that there is a need for a standard protocol in quality control of medicinal products as they unmasked the rampant adulteration in the Philippine herbal market. The

Philippine Food and Drug Administration (FDA) 2018 also stated that there is an increasing number of violations regarding the manufacturing, sales, and distributions of HMPs. With these said, the success of this study reiterates that a standard protocol for herbal product authentication must be applied. Should this be implemented, quality control protocols must take note of the most efficient barcoding loci and the most active chemical component of the target plant before conducting authentication methods. The use of a concatenated barcoding approach (using at least two loci) is highly recommended to elucidate species identity. Therefore, the Traditional and Alternative Medicine Act (TAMA) should integrate both techniques in herbal product authentication of *sambong* and *tsaang-gubat* and other high-valued herbal medicinal products for the safety of all its consumers.

In conclusion, this is the first study that used both DNA Barcoding and High-Performance Liquid Chromatography (HPLC) in the authentication of Philippine HMPs, specifically on *B. balsamifera* and *E. microphylla*. DNA barcoding using ITS-*trnH-psbA* datasets authenticated the plant source material while HPLC confirmed the presence of active compounds, quercetin and rosmarinic acid from the HMPs. Both analyses revealed cases of substitution. These HMP samples deviated from the standard DNA barcoding and HPLC profiles of both the wild samples and the other tested HMPs. The use of genomic data is direct; it determines the plant component of a sample. Therefore, HMPs subjected to DNA barcoding protocols are expected to match that of their marketed labels. The presence of a different species after BLAST search and SRM analysis would support the possibility of adulteration of the marketed HMPs.

HPLC detects the presence of active compounds from wild samples and HMPs. This further presented the possible degradation of active components due to manufacturing processes and environmental conditions. The determination of active compounds is necessary in HMPs since it is responsible for the product's effectiveness. However, multiple species can have the same compounds, and the actual plant material utilized may be adulterated. The coupling of DNA barcoding and HPLC, therefore, is highly recommended to ensure the safety of consumer health. To promote the safety and well-being of Filipino consumers, it is necessary to amend the Traditional and Alternative Medicine Act (TAMA) and integrate provisions on DNA barcoding and HPLC analysis protocols for the authentication of herbal products.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the DOST Philippine Council for Health, Research and Development (DOST-PCHRD) for the GD Alejandro research fund, and the DOST-Science Education Institute for the Accelerated Science and Technology Human Resource Development (DOST-SEI-ASTHRD) for the scholarships of AA Lola, SA Binag, and NG Alfeche. The authors would also like to acknowledge the private individuals who provided them with the wild samples used for this study. Lastly, the

researchers are grateful to the Bureau of Plant and Industry (BPI), Manila, Philippines for providing samples from their collections.

REFERENCES

- Abubakar BM, Salleh FM, Omar MSS, Wagiran A. 2017. DNA barcoding and chromatography fingerprints for the authentication of botanicals in herbal medicinal products. *Evid-Based Complement Alternat Med* 2017: 1352948. DOI: 10.1155/2017/1352948.
- Abubakar BM, Salleh FM, Shamsir Omar MS, Wagiran A. 2018. Assessing product adulteration of *Eurycoma longifolia* (tongkat ali) herbal medicinal product using DNA barcoding and HPLC analysis. *Pharm Biol* 56 (1): 368-377. DOI: 10.1080/13880209.2018.1479869.
- Alfeche NK, Binag SD, Medecilo MM, Alejandro GJ. 2019. Standard reference material (SRM) DNA barcode library approach for authenticating *Antidesma bunius* (L.) Spreng. (bignay) derived herbal medicinal products. *Food Addit Contam Part A Chem Anal Control Expo Risk Assess* 36 (12): 1777-1786. DOI: 10.1080/19440049.2019.1670868.
- Anantha Narayana DB, Johnson ST. 2019. DNA barcoding in authentication of herbal raw materials, extracts and dietary supplements: A perspective. *Plant Biotechnol Rep* 13 (3): 201-210. DOI: 10.1007/s11816-019-00538-z.
- Buddhachat K, Osathanunkul M, Madesis P, Chomdej S, Ongchai S. 2015. Authenticity analyses of *Phyllanthus amarus* using barcoding coupled with HRM analysis to control its quality for medicinal plant product. *Gene* 573 (1): 84-90. DOI: 10.1016/j.gene.2015.07.046.
- Cafino EJV, Lirazan MB, Marfori EC. 2016. A simple HPLC method for the analysis of [6]-gingerol produced by multiple shoot culture of ginger (*Zingiber officinale*). *Intl J Pharmacogn Phytochem Res* 8 (1): 38-42.
- Catublas HAL. 2016. Knowledge, attitudes and practices in the use of herbal medicine: The case of urban and rural mothers in the Philippines. *Mahidol Univ J Pharm Sci* 43 (1): 1-16. DOI: 10.14456/mujps.2016.1.
- Chaowuttikul C, Palanuvej C, Ruangrunsi N. 2020. Quantification of chlorogenic acid, rosmarinic acid, and caffeic acid contents in selected Thai medicinal plants using RP-HPLC-DAD. *Braz J Pharm Sci* 56: e17547. DOI: 10.1590/s2175-97902019000317547.
- Custers D, Van Praag N, Courselle P, Apers S, Deconinck E. 2017. Chromatographic fingerprinting as a strategy to identify regulated plants in illegal herbal supplements. *Talanta* 164: 490-502. DOI: 10.1016/j.talanta.2016.12.008.
- de Guzman CC. 2013. Country status report on medicinal and aromatic plants in the Philippines. In: Paroda R, Dasgupta S, Mal B, Ghosh SP, Pareek SK (eds). *Expert Consultation on Promotion of Medicinal and Aromatic Plants in the Asia-Pacific Region*. Asia-Pacific Association of Agricultural Research Institutions (APAARI), Bangkok.
- Fay MF, Swensen SM, Chase MW. 1997. Taxonomic affinities of *Medusagyne oppositifolia* (Medusagynaceae). *Kew Bull* 52: 111-120. DOI: 10.2307/4117844.
- FDA [Food and Drug Administration Philippines]. 2018. Manufacture, sale and distribution of traditional and alternative medicines. <https://ww2.fda.gov.ph/index.php/issuances-2/pharm-1/pharm-fda-circular/161090-fda-circular-no-2014-015>.
- Frigerio J, Agostinetto G, Mezzasalma V, De Mattia F, Labra M, Bruno A. 2021. DNA-based herbal teas' authentication: An ITS2 and *psbA-trnH* multi-marker DNA metabarcoding approach. *Plants* 10 (10): 2120. DOI: 10.3390/plants10102120.
- Galimberti A, Casiraghi M, Bruni I, Guzzetti L, Cortis P, Berterame NM, Labra M. 2019. From DNA barcoding to personalized nutrition: The evolution of food traceability. *Curr Opin Food Sci* 28: 41-48. DOI: 10.1016/j.cofs.2019.07.008.
- Ghorbani S, Sonboli A, Ebrahimi SN, Mirjalili MH. 2020. Molecular authentication and phytochemical assessment of *Ruscus hyrcanus* Woron. (Asparagaceae) based on *trnH-psbA* barcoding and HPLC-PDA analysis. *Biocatal Agric Biotechnol* 25: 101585. DOI: 10.1016/j.bcab.2020.101585.
- Hassan B. 2012. HPLC uses and importance in the pharmaceutical and industrial field. *Pharm Anal Acta* 3: 9. DOI: 10.4172/2153-2435.1000E133.
- Hebert PD, Cywinska A, Ball SL, deDewaraard JR. 2003. Biological identifications through DNA barcodes. *Proc R Soc Lond B Biol Sci* B: *Biol Sci* 270 (1512): 313-321. DOI: 10.1098/rspb.2002.2218.
- Hollingsworth PM, Graham SW, Little DP. 2011. Choosing and using a plant DNA barcode. *PLoS One* 6 (5): e19254. DOI: 10.1371/journal.pone.0019254.
- Ichim MC. 2019. The DNA-based authentication of commercial herbal products reveals their globally widespread adulteration. *Front Pharmacol* 10: 1227. DOI: 10.3389/fphar.2019.01227.
- Jirakitticharoen S, Wisuitiprot W, Jitareerat P, Wongs-Aree C. 2022. Phenolics, antioxidant and antibacterial activities of immature and mature *Blumea balsamifera* leaf extracts eluted with different solvents. *J Trop Med* 2022: 7794227. DOI: 10.1155/2022/7794227.
- Kool A, De Boer HJ, Kruger A, Rydberg A, Abbad A, Bjork L, Martin G. 2012. Molecular identification of commercialized medicinal plants in southern Morocco. *PLoS One* 7 (6): e39459. DOI: 10.1371/journal.pone.0039459.
- Kumar S, Stetcher G, Tamura K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Mol Biol Evol* 33 (7): 1870-1874. DOI: 10.1093/molbev/msw054.
- Kuzmina ML, Johnson KL, Barron HR, Hebert PD. 2012. Identification of the vascular plants of Churchill, Manitoba, using a DNA barcode library. *BMC Ecol* 12: 25. DOI: 10.1186/1472-6785-12-25.
- Legaspi CLB, Bagaosan DMA. 2020. *Ehretia microphylla* tablet formulation for biliary and gastrointestinal colic: A review of its phytochemical constituents, pharmacologic activities and clinical researches. *Acta Med Philipp* 54 (1): 80-85. DOI: 10.47895/amp.v54i1.1108.
- Liang YZ, Xie P, Chan K. 2004. Quality control of herbal medicines. *J Chromatogr B Analyt Technol Biomed Life Sci* 812 (1-2): 53-70. DOI: 10.1016/j.jchromb.2004.08.041.
- Lv YN, Yang CY, Shi LC, Zhang ZL, Xu AS, Zhang LX, Li XL, Li HT. 2020. Identification of medicinal plants within the Apocynaceae family using ITS2 and *psbA-trnH* barcodes. *Chin J Nat Med* 18 (8): 594-605. DOI: 10.1016/S1875-5364(20)30071-6.
- Mageswari S, Karpagam S. 2015. Evaluation of antimicrobial studies on root of *Carmona retusa* (Vahl.) Masam. *Intl J Curr Pharm Res* 7 (3): 60-63.
- Makino T, Furuta Y, Wakushima H, Fujii H, Saito KI, Kano Y. 2003. Anti-allergic effect of *Perilla frutescens* and its active constituents. *Phytother Res* 17: 240-243. DOI: 10.1002/ptr.1115.
- Maramba-Lazarte CC, Cavinta LL, Sara, MCL. 2020. Antibacterial activity of guyabano, ulasimang bato, sambong, and tsaang gubat leaf extracts against common drug-resistant bacteria. *Acta Med Philipp* 54 (1): 17-21. DOI: 10.47895/amp.v54i1.1087.
- Michel CI, Meyer RS, Taveras Y, Molina J. 2016. The nuclear internal transcribed spacer (ITS2) as a practical plant DNA barcode for herbal medicines. *J Appl Res Med Aromat Plants* 3 (3): 94-100. DOI: 10.1016/j.jarmap.2016.02.002.
- Mishra P, Kumar A, Nagireddy A, Mani DN, Shukla AK, Tiwari R, Sundaresan V. 2016. DNA barcoding: An efficient tool to overcome authentication challenges in the herbal market. *Plant Biotechnol J* 14 (1): 8-21. DOI: 10.1111/pbi.12419.
- Monroy-Garcia IN, Carranza-Torres IE, Carranza-Rosales P, Oyón-ardoiz M, García-Estévez I, Ayala-zavala JF, Viveros-valdez E. 2021. Phenolic profiles and biological activities of extracts from edible wild fruits *Ehretia tinifolia* and *Sideroxylon lanuginosum*. *Foods* 10 (11): 2710. DOI: 10.3390/foods10112710.
- Montealegre CM, De Leon RL. 2016. *Blumea balsamifera* (sambong) tea as a therapeutic drink for calcium oxalate stones. *MATEC Web Conf* 62: 02002. DOI: 10.1051/mateconf/20166202002.
- Nessa F, Ismail Z, Karupiah S, Mohamed N. 2005. RP-HPLC method for the quantitative analysis of naturally occurring flavonoids in leaves of *Blumea balsamifera* DC. *J Chromatogr Sci* 43 (8): 416-420. DOI: 10.1093/chromsci/43.8.416.
- Newmaster SG, Grguric M, Shanmughanandhan D, Ramalingam S, Ragupathy S. 2013. DNA barcoding detects contamination and substitution in North American herbal products. *BMC Med* 11 (1): 222. DOI: 10.1186/s12916-024-03504-x.
- Olivar J, Alaba J, Atienza J, Tan J, Umali IV M, Alejandro GJ. 2016. Establishment of a Standard Reference Material (SRM) herbal DNA barcode library of *Vitex negundo* L. (*lagundi*) for quality control measures. *Food Addit Contam: Part A* 33 (5): 741-748. DOI: 10.1080/19440049.2016.1166525.
- Orman E, Bekoe SO, Jato J, Spiegler V, Asare-Nkansah S, Agyare C, Hensel A, Bekoe EO. 2022. Quality assessment of African herbal

- medicine: A systematic review and the way forward. *Fitoterapia* 162: 105287. DOI: 10.1016/j.fitote.2022.105287.
- Palhares RM, Goncalves M, dos Santos B, Pereira G, das Graças Lins Brandão M, Oliveira G. 2015. Medicinal plants recommended by the world health organization: DNA barcode identification associated with chemical analyses guarantees their quality. *PLoS One* 10 (5): e0127866. DOI: 10.1371/journal.pone.0127866.
- Pedales R, Damatac A, Limbo C, Marquez C, Navarro A, Molina J. 2016. DNA barcoding of Philippine herbal medicinal products. *J AOAC Intl* 99 (6): 1479-1489. DOI: 10.5740/jaoacint.16-0185.
- Pencicilla GL, Magno CP. 2011. Antibacterial activity of extracts of twelve common medicinal plants from the Philippines. *J Med Plants Res* 5 (16): 3975-3981. DOI: 10.5897/JMPR.9000122.
- Rasonabe ZMP, Cruz JD, Tiausas CG, Areza F. 2023. Chemical composition and antimicrobial activity of the extracts and essential oil of *Blumea balsamifera* from the Philippines. *Intl J Herb Med* 11 (1): 6-14. DOI: 10.22271/flora.2023.v11i1a.847.
- Republic Act No. 8423. 1997. Traditional and Alternative Medicine Act (TAMA). The LAWPHIL Project. http://www.lawphil.net/statutes/repacts/ra1997/ra_8423_1997.html
- Sahoo N, Manchikanti P, Dey S. 2010. Herbal drugs: Standards and regulation. *Fitoterapia* 81: 462-471. DOI: 10.1016/j.fitote.2010.02.001.
- Seethapathy GS, Balasubramani SP, Venkatasubramanian P. 2014. nrDNA ITS sequence based SCAR marker to authenticate *Aconitum heterophyllum* and *Cyperus rotundus* in Ayurvedic raw drug source and prepared herbal products. *Food Chem* 145: 1015-1020. DOI: 10.1016/j.foodchem.2013.09.027.
- Semagn K. 2014. Leaf tissue sampling and DNA extraction protocols. *Methods Mol Biol* 1115: 53-67. DOI: 10.1007/978-1-62703-767-9_3.
- Shukla A, Kaur A. 2018. A systematic review of traditional uses bioactive phytoconstituents of genus *Ehretia*. *Asian J Pharm Clin Res* 11 (6): 88-100. DOI: 10.22159/ajpcr.2018.v11i6.25178.
- Srirama R, Santhosh Kumar JU, Seethapathy GS, Newmaster SG, Ragupathy S, Ganeshaiah KN, Shaanker RU, Ravikanth G. 2017. Species adulteration in the herbal trade: causes, consequences and mitigation. *Drug Saf* 40: 651-661. DOI: 10.1007/s40264-017-0527-0.
- Tarmizi AAA, Wagiran A, Mohd Salleh F, Chua LS, Abdullah FI, Hasham R, Binte Mostafiz S. 2021. Integrated approach for species identification and quality analysis for *Labisia pumila* using DNA barcoding and HPLC. *Plants* 10: 717. DOI: 10.3390/plants10040717.
- Techen N, Parveen I, Pan Z, Khan IA. 2014. DNA barcoding of medicinal plant material for identification. *Curr Opin Biotechnol* 25: 103-110. DOI: 10.1016/j.copbio.2013.09.010.
- Tolentino RD, Tomas VCB, Travezonda JC, Magnaye BP. 2019. Herbal medicine utilization among Batangueños. *Asian Pac J Educ Arts Sci* 6 (1): 9-22.
- Toralba JV, Quiming NS, Palacpac JS. 2015. RP-HPLC analysis of quercetin in the extract of *sambong* (*Blumea balsamifera* (L.) DC.) leaves. *Sci Diliman* 27 (1): 48-63.
- Tupas GD, Gido LJFJ. 2021. Perspective on herbal medicine in the Philippines, economic demands, quality control, and regulation. In: Egbuna C, Mishra AP, Goyal MR (eds). *Preparation of Phytopharmaceuticals for the Management of Disorders*. Academic Press, London. DOI: 10.1016/B978-0-12-820284-5.00009-5.
- Umali FAC, Chua AH. 2017. *Ehretia microphylla* (*Tsaang gubat*) versus Loratadine as treatment for allergic rhinitis: A randomized controlled trial. *Philipp J Otolaryngol Head Neck Surg* 32 (2): 6-10. DOI: 10.32412/pjohns.v32i2.57.
- Upton R, David B, Gafner S, Glasl S. 2020. Botanical ingredient identification and quality assessment: Strengths and limitations of analytical techniques. *Phytochem Rev* 19: 1157-1177. DOI: 10.1007/s11101-019-09625-z.
- Vassou SL, Kusuma G, Parani M. 2015. DNA barcoding for species identification from dried and powdered parts: A case study with authentication of the raw drug market samples of *Sida cordifolia*. *Gene* 559 (1): 86-93. DOI: 10.1016/j.gene.2015.01.025.
- Wang H, Provan GJ, Helliwell K. 2003. Determination of rosmarinic acid and caffeic acid in aromatic herbs by HPLC. *Food Chem* 87 (2): 307-311. DOI: 10.1016/j.foodchem.2003.12.029.
- Xu H, Li P, Ren G, Wang Y, Jiang D, Liu C. 2021. Authentication of three source spices of *Arnebiae radix* using DNA barcoding and HPLC. *Front Pharmacol* 12: 677014. DOI: 10.3389/fphar.2021.677014.
- Yang J, Dong LL, Wei GF, Hu HY, Zhu GW, Zhang J, Chen SL. 2018. Identification and quality analysis of *Panax notoginseng* and *Panax vietnamensis* var. *fuscidicus* through integrated DNA barcoding and HPLC. *Chin Herb Med* 10: 177-183. DOI: 10.1016/j.chmed.2018.03.008.
- Zarsuelo MAM, Zordilla ZD, Anacio DB. 2018. Review of regulatory policies on and benefits of herbal medicine in the Philippines. *Acta Med Philipp* 52 (5): 473-479. DOI: 10.47895/amp.v52i5.334.
- Zhang Z, Zhang Y, Zhang Z, Yao H, Liu H, Ben GZ, Liao Y. 2016. Comparative analysis of DNA barcoding and HPLC fingerprint to trace species of *Phellodendri Cortex*, an important traditional Chinese medicine from multiple sources. *Biol Pharm Bull* 39 (8): 1325-1330. DOI: 10.1248/bpb.b16-00210.