Korean Journal Of Agricultural Science

pISSN : 2466-2402
eISSN : 2466-2410

FOOD&CHEMISTRY

Evaluation of processing methods and harvest timings for the efficient extraction of *Syzygium formosum*

Jaeyoon Lim[1], Jaehan Kim[1], In-Seok Yeo[2,*], Jong-Tae Park[3,4,*]

[1]Department of Food and Nutrition, Chungnam National University, Daejeon 34134, Korea

[2]Graduate School of Analytical Science and Technology, Chungnam National University, Daejeon 34134, Korea

[3]Department of Food Science and Technology, Chungnam National University, Daejeon 34134, Korea

[4]CARBOEXPERT Inc., Daejeon 34134, Korea

[*]Corresponding authors: yisyis123@o.cnu.ac.kr, jtpark@cnu.ac.kr

Abstract

Syzygium formosum has been traditionally used in Vietnam as a folk medicine for treating skin and respiratory diseases. With the recent recognition of its beneficial effects such as anticancer, antioxidant, and anti-inflammatory properties, there is an increasing interest in industrial extraction of its bioactive compounds, such as triterpenoids and flavonoids. In this study, the extraction efficiencies of *S. formosum* were analyzed by extraction methods and harvest timings. Firstly, it was found that distilled water treatment substantially eliminated almost all flavonoids, suggesting that minimal pretreatment is important to preserve both triterpenoids and flavonoids. Secondly, extractions with 70% ethanol at both lab and pilot scales effectively yielded more than 70% of triterpenoids and flavonoids from *S. formosum*. Lastly, to investigate the seasonal variation in phytochemicals, extracts from the leaves of *S. formosum* harvested in April, August, October, and December in Vietnam were analyzed. It was revealed that seasons with lower temperatures led to higher concentrations of triterpenoids and flavonoids. Specifically, December was found to be optimal for obtaining high concentrations of triterpenoid and October for flavonoid. Consequently, this research provides a foundation for the industrial application of *S. formosum*, providing critical insights into maximizing the extraction efficiency and phytochemical contents through optimized processing methods and strategic harvesting.

Keywords: extraction, flavonoid, LC-MS/MS, *Syzygium formosum*, triterpenoid

Citation: Lim J, Kim J, Yeo IS, Park JT. 2024. Evaluation of processing methods and harvest timings for the efficient extraction of *Syzygium formosum*. Korean Journal of Agricultural Science 51:361-373. https://doi.org/10.7744/kjoas.510311

Received: July 29, 2024

Revised: August 14, 2024

Accepted: August 19, 2024

Introduction

Syzygium formosum (Wall.) Masam (SF) belongs to the myrtle family, *Myrtaceae*, and is widely distributed across Southeast Asian countries such as Malaysia and Vietnam (Uddin et al., 2022). Traditionally, in Vietnam, the leaves of this evergreen tree have been consumed as food or tea, or used as a folk medicine to relieve skin or respiratory-related symptoms such as skin

rashes, scabies, sore throats, and bronchitis (Loi, 2001). In addition, recent studies revealed additional functional properties of SF leaves, including antibacterial, anti-inflammatory, antioxidative, and anti-allergic activities (Lee et al., 2006; Phuong et al., 2006; Nguyen et al., 2018; Parul et al., 2020; Park et al., 2021). These significant effects are attributed to bioactive compounds such as flavonoids and triterpenoids found in SF leaves (Hoang et al., 2021).

Flavonoids are a diverse group of plant secondary metabolites with a polyphenolic structure found in almost all fruits and vegetables. In plants, their functions are related to growth and defence against pathogens (Havsteen, 2002). By inhibiting the formation of reactive oxygen species (ROS) through modulating enzymes such as xanthine oxidase, cyclooxygenase, lipoxygenase and phosphoinositide 3-kinase, they exhibit various biological activities, including antioxidant and anti-inflammatory effects (Metodiewa et al., 1997; Mansuri et al., 2014; Lin et al., 2015). Triterpenoids (C_{30} compounds), a class of chemical compounds composed of three terpene units, have diverse structures, but their biosynthetic pathways are highly conserved in plants (Dinday and Ghosh, 2023). Their precursors, isopentenyl diphosphate and its isomer dimethylallyl diphosphate, are derived from the cytosolic mevalonate pathway and they are diversified by oxidosqualene cyclases (Thimmappa et al., 2014; Ghosh, 2016). They also exhibit a wide range of biological activities, including anti-inflammatory, antiviral, and anticancer properties (Metodiewa et al., 1997; Martin et al., 2008; Ma et al., 2011; Andre et al., 2016; Misra et al., 2017; Sandeep et al., 2019). Collectively, these compounds contribute to the medicinal value of SF leaves, enhancing their potential application in various health-related industries (Table 1).

Table 1. Structures of phytochemicals analyzed in this study (Continued).

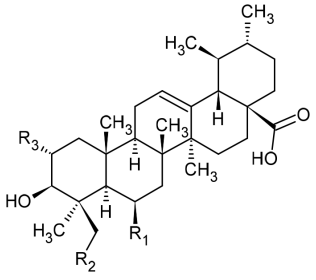
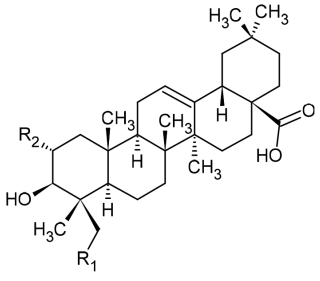
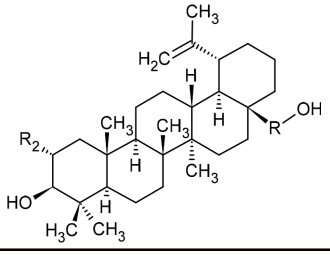
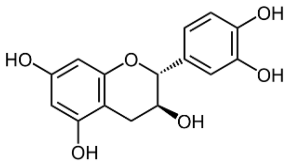
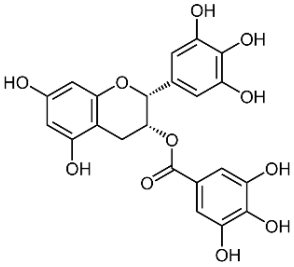
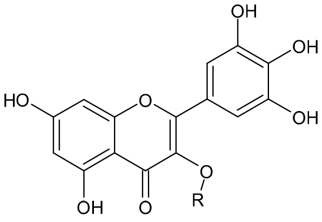
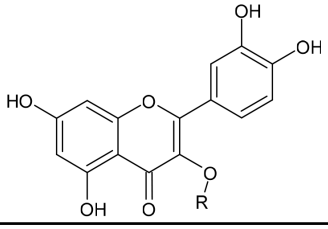
Structure	Compound name
Triterpenoids	
	Madecassic acid : R_1 = OH, R_2 = OH, R_3 = OH Asiatic acid : R_1 = H, R_2 = OH, R_3 = OH Corosolic acid : R_1 = H, R_2 = H, R_3 = OH Ursolic acid : R_1 = H, R_2 = H, R_3 = H
	Hederagenin : R_1 = OH, R_2 = H Maslinic acid : R_1 = H, R_2 = OH Oleanolic acid : R_1 = H, R_2 = H
	Bertulin : R = CH_2 Betulinic acid : R = CO

Table 1. Structures of phytochemicals analyzed in this study.

Structure	Compound name
Flavonoids	
	Catechin
	Epigallocatechin gallate
	Myricetin glucoside : R = Glucose Myricetin rhamnoside : R = Rhamnose
	Quercetin : R = H Quercetin glucoside : R = Glucose Quercetin arabinoside : R = Arabinose Quercetin rhamnoside : R = Rhamnose

For the effective utilization of SF leaf extracts (SFLEs), it is crucial to establish efficient extraction methods at both laboratory and industrial scales and determine the optimal harvest times for maximizing SFLEs yields. Therefore, in this study, we examined the efficiency of processing methods and quality control of SFLEs from three perspectives. First, we determined the effect of pretreatment with distilled water (DW) on the SFLEs of freeze-dried samples. Next, we compared the extraction efficiency of SFLEs using 70% ethanol at both laboratory and pilot scales. Finally, we evaluated the variation of the SFLEs based on the harvest season.

Materials and Methods

Chemicals and reagents

Analytical standard of madecassic acid (> 97%) was purchased from Toronto Research Chemicals Inc. (Canada). Maslinic acid (> 98%) was purchased from Chengdu Biopurify Phytochemicals Ltd. (China). Asiatic acid (> 97%), corosolic acid (≥ 85%), betulinic acid (≥ 98%), betulin (≥ 98%), and oleanolic acid (≥ 98%) were purchased from Sigma-Aldrich (USA). Ursolic acid (> 90.0%) and hederagenin (> 98%) were purchased from Tokyo Chemical

Industry Co., Ltd. (Japan). Catechin (> 98%), epigallocatechin gallate (> 98%), myricetin glucoside (> 98%), quercetin glucoside (> 98%) and quercetin rhamnoside (> 98%) were purchased from Extrasynthese SAS (France). Quercetin arabinoside (> 98%) and quercetin (> 98%) were acquired from ChemFaces (China). The chemical structures of triterpenoids and flavonoids are shown in Fig. 1. Solvent and reagent for analysis were purchased HPLC grade. Methanol (> 99.9%) was purchased from Sigma-Aldrich (USA). Acetonitrile (> 99.9%) and 2-propanol (> 99.7%) were purchased from J.T.Baker (USA). Ammonium formate (> 99.0%) and formic acid (> 98.0%) were purchased from Honeywell Fluka (USA). Distilled water was prepared using Direct-Q 5 UV Ultrapure water purification system (Merck Millipore, USA).

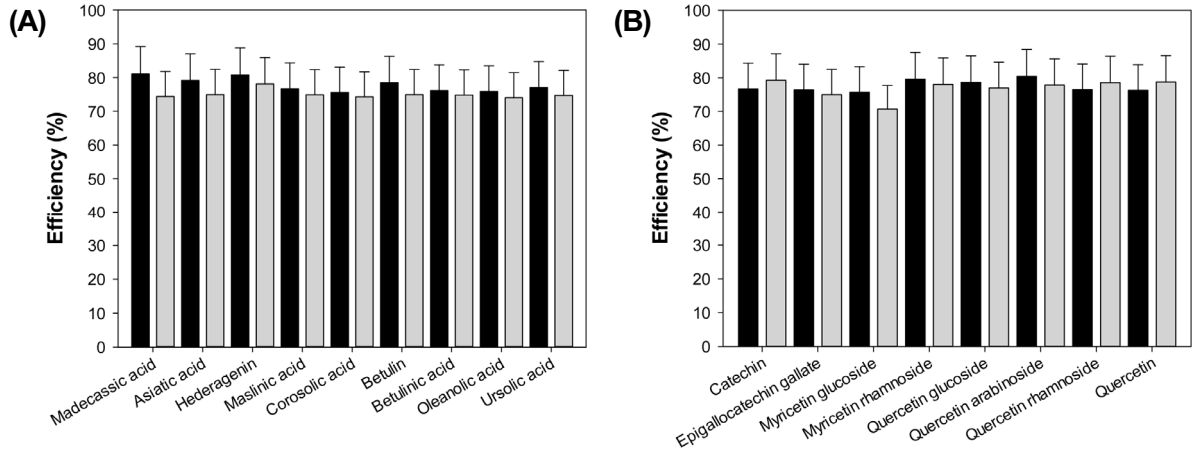


Fig. 1. The extraction efficiencies of (A) triterpenoids and (B) flavonoids using 70% ethanol. The black and gray bars represent the lab-scale and pilot-scale, respectively. Error bars represent the standard deviation.

Preparation of samples and standards

Triterpenoids

For liquid samples, 1 mL of the ethanolic extracts were completely dried through a SpeedVac evaporator (Modulspin 40, Hanil Science Medical Co., Ltd., Korea) and dissolved in 1 mL of 60% methanol. For freeze-dried powders, 10 mg of powder was dissolved in 1 mL of 60% methanol. And the samples in 60% methanol were subjected to solid phase extraction (SPE). SPE was performed through C18 cartridge (200 mg, 3 mL, Waters). The cartridge was activated with 6 mL of methanol and conditioned with 6 mL of 60% methanol. After loading 200 μL of sample, desalting was done with 6 mL of 30% methanol, followed by elution with 6 mL of methanol. The eluted samples were completely dried through a SpeedVac evaporator and then reconstitute in methanol for analysis. Triterpenoid standards in powder form were prepared by weighing 10 mg and dissolving in 1 mL of methanol. Standards in solution form were diluted to concentrations of 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1 $\mu g \cdot mL^{-1}$ for analysis.

Flavonoids

The preparation methods were similar to those for triterpenoids except for SPE. The SPE cartridge was activated with 6 mL of acetonitrile and conditioned with 6 mL of DW. After loading 200 μL of sample, desalting was done with 6 mL of DW, followed by elution with 6 mL of 80% acetonitrile. The eluted samples were completely dried through

a SpeedVac evaporator and then reconstitute in methanol for analysis. Flavonoid standards in powder form were prepared by weighing 10 mg and dissolving in 1 mL of methanol. Standards in solution form were diluted to concentrations of 0.05, 0.1, 0.25, 0.5, 1, 2.5, 5, and 10 $\mu g \cdot mL^{-1}$ for analysis.

Evaluation of the impact of DW treatment on SFLEs

Before the extraction, DW was added to the freeze-dried powder at a solid-to-solvent ratio of 1 : 20 (w/v). Then, the mixture was vortexed and centrifuged. The washing process was repeated twice, and the supernatant was collected for the evaluation of SFLEs loss.

Evaluation of extraction efficiency by purification scale

Dried leaves were collected and extracted at both the lab and pilot scales for the comparison of extraction efficiency. The leaves were grounded into powder for extraction. For the lab-scale extraction, 50 g of powder was used, while for the pilot-scale extraction, a quantity of powder equivalent to 1,000 times more than that of the lab-scale was utilized. The dried powder was extracted with ethanol at a solid-to-solvent ratio of 1 : 12 (w/v). The extraction process comprised two steps: first with 70% ethanol and second with 95% ethanol. Each extraction step contained mixing the ethanol with the dried powder at 50℃ for 12 h at 250 rpm. The extracts were concentrated by evaporator, freeze-dried, and powdered.

Evaluation of the variation in SFLEs with seasons

To determine seasonal variations in phytochemical contents, leaves collected from Thạch Thất district, Hanoi, Vietnam in April, August, October, and December of 2019 were analyzed. The leaves collected in April were designated as the spring batch, those collected in August as the summer batch, those collected in October as the autumn batch, and those collected in December as the winter batch. The collected leaves were extracted with 70% ethanol, freeze-dried to form powders, and then subjected to analysis.

LC-MS/MS analysis

The triterpenoids and flavonoids of SF were analyzed using the analytical methods established in our previous studies (Hoang et al., 2021). The analysis of triterpenoids was performed on an Agilent 1290 Infinity UHPLC system with 6470 triple quadrupole mass spectrometry (Agilent Technologies, Inc., USA). The chromatographic separation was performed on an Agilent ZORBAX RRHD Eclipse Plus C18 (2.1 × 100 mm, 1.8 μm, Agilent Technologies, Inc., USA) column and temperature was kept at 40℃. The injection volume was 2 μL and flow rate was 0.2 $mL \cdot min^{-1}$. Mobile phase were consisted of 5 mM ammonium formate in DW (solvent A) and 5 mM ammonium formate with 5% 2-propanol in methanol (solvent B). Gradient elution method was applied for efficient separation. Mass spectrometric parameters were as follows: Ionization method, electrospray ionization; analytical ion mode, positive mode; gas temp., 270℃; gas flow, 10 $mL \cdot min^{-1}$; nebulizer, 40 psi; sheath gas temp., 300℃; sheath gas flow, 11 $L \cdot min^{-1}$; capillary voltage, 3.5 kV; QQQ scan type, multiple reaction monitoring (MRM) mode.

The analysis of flavonoids was performed on an Agilent 1290 Infinity UHPLC system with 6460 triple quadrupole

mass spectrometry (Agilent Technologies, Inc., USA). The chromatographic separation was performed on an Agilent ZORBAX RRHD Eclipse Plus C18 (2.1 × 50 mm, 1.8 μm, Agilent Technologies, Inc., USA) column and temperature was kept at 40℃. The injection volume was 2 μL and flow rate was 0.3 mL·min^{-1}. Mobile phase were consisted of DW (solvent A) and acetonitrile (solvent B) with 0.1% formic acid. Gradient elution method was applied for efficient separation. Mass spectrometric parameters were as follows: Ionization method, electrospray ionization; analytical ion mode, positive mode; gas temp., 275℃; gas flow, 8 mL·min^{-1}; nebulizer, 45 psi; sheath gas temp., 350℃; sheath gas flow, 12 L·min^{-1}; capillary voltage, 3.5 kV; QQQ scan type, MRM mode. MRM parameters for the analysis of phytochemicals were shown in Table S1.

Results and Discussion

Evaluation of extraction efficiency by pretreatment

Extraction methods were evaluated for the establishment of efficient extraction process of SFLEs. To determine the effect of washing process with DW before the extraction of freeze-dried SF powders, the SFLEs contents were analyzed before and after washing. Moreover, the washing process was repeated twice, and the changes in SFLEs contents during the washing process were also observed.

The phytochemicals in DW were analyzed to quantify the loss due to the treatment (Table 2). Despite the repeated

Table 2. Evaluation of SFLEs loss by DW treatment.

Analytes	Before treatment (mg·g^{-1})	1st DW treatment		2nd DW treatment	
		Loss amount (mg·g^{-1})	Loss (%)	Loss amount (mg·g^{-1})	Loss (%)
Triterpenoids					
Madecassic acid	0.68	0.00	0.15	0.00	0.41
Asiatic acid	12.20	0.02	0.19	0.05	0.39
Hederagenin	0.16	0.00	0.06	0.00	0.16
Maslinic acid	4.70	0.01	0.18	0.01	0.24
Corosolic acid	11.22	0.02	0.22	0.02	0.17
Betulin	0.30	0.00	0.30	0.00	0.12
Betulinic acid	13.48	0.01	0.11	0.02	0.14
Oleanolic acid	0.77	0.00	0.06	0.00	0.11
Ursolic acid	2.10	0.00	0.06	0.00	0.03
Flavonoids					
Catechin	3.88	5.23	134.79	0.55	14.18
Epigallocatechin gallate	0.20	0.21	105.23	0.03	15.04
Myricetin glucoside	0.16	0.16	100.74	0.01	6.25
Myricetin rhamnoside	9.52	11.20	117.65	1.17	12.29
Quercetin glucoside	0.20	0.22	110.29	0.02	10.63
Quercetin arabinoside	3.22	3.26	101.24	0.39	12.11
Quercetin rhamnoside	0.53	0.67	126.42	0.07	13.21
Quercetin	0.22	0.15	68.18	0.02	9.09

SFLEs, *Syzygium formosum* (Wall.) Masam leaf extracts; DW, distilled water.

processing of DW, minimal losses of triterpenoids were observed (first treatment: 0.06 - 0.30%, second treatment: 0.03 - 0.41%). In contrast, nearly all flavonoids were lost after a single DW treatment, with losses ranging from 68.18% to 134.79%. In particular, catechin and myricetin rhamnoside exhibited the highest losses of 5.23 $mg \cdot g^{-1}$ and 11.20 $mg \cdot g^{-1}$, respectively. These findings suggest that DW treatment to SF powder for washing should be minimized for optimal extraction of SFLEs. Additionally, the results indicate that DW is more effective than ethanol for flavonoid extraction, and that DW treatment can facilitate the separation of flavonoids from triterpenoids.

Evaluation of extraction efficiency at different scales

Next, the extraction efficiencies at both laboratory and pilot scales were compared. Typically, 50 - 80% methanol or ethanol is used for extracting phytochemicals, especially flavonoids and triterpenoids (Altemimi et al., 2017; Cai et al., 2019; Bitwell et al., 2023). In this study, extractions with 70% ethanol were performed at both the lab and pilot scales using 50 g and 50 kg of powder, respectively.

The extractions were performed by mixing the ethanol with the dried powder at 50℃ for 12 h at 250 rpm. The detailed results are found in Table S2. The pilot-scale extraction yielded 16.03 $mg \cdot g^{-1}$ of triterpenoids, which is 1.87 times higher than the lab-scale extraction yield of 8.57 $mg \cdot g^{-1}$ (Fig. S1). Asiatic acid showed the largest increase, with 2.75 times more extracted at the pilot-scale, followed by corosolic acid with 2.26 times more extracted. However, flavonoids were more efficiently extracted at the lab-scale (2.77 $mg \cdot g^{-1}$) compared to the pilot-scale (2.58 $mg \cdot g^{-1}$). Specifically, catechin was extracted 2.08 times more efficient at the lab-scale, while myricetin rhamnoside was extracted 1.33 times more efficient at the pilot-scale.

Notably, the efficiency of extraction using 70% ethanol was investigated by comparing the relative concentrations of phytochemicals obtained from the extraction with 70% ethanol to those obtained from a subsequent extraction using 95% ethanol (Fig. 1). The results showed that extractions at both the lab and pilot scales could extract more than 70% of triterpenoids and flavonoids of SF powders using 70% ethanol, which indicates that the effective extraction was possible even when scaling up.

Evaluation of the variation in SFLEs with seasons

Phytochemicals in plants are known to be influenced by various environmental changes such as temperature, sunlight, soil fertility, and salinity (Berini et al., 2018; Yang et al., 2018). Therefore, to evaluate the seasonal variation in SFLEs contents, extractions were conducted using SF leaves harvested in April, August, October, and December of 2019. In 2019, the average temperatures in Hanoi, Vietnam, where the leaves were collected, were 29℃ in April, 30.4℃ in August, 26.4℃ in October, and 20.4℃ in December (Fig. S2). These correspond to the typical temperatures of spring, summer, autumn, and winter in Vietnam, respectively.

Triterpenoids showed a tendency to increase as temperatures decreased. Similarly, flavonoid contents also increased with lower temperatures, with the highest yield observed in October (Fig. 2). The detailed observations are found in Table 3.

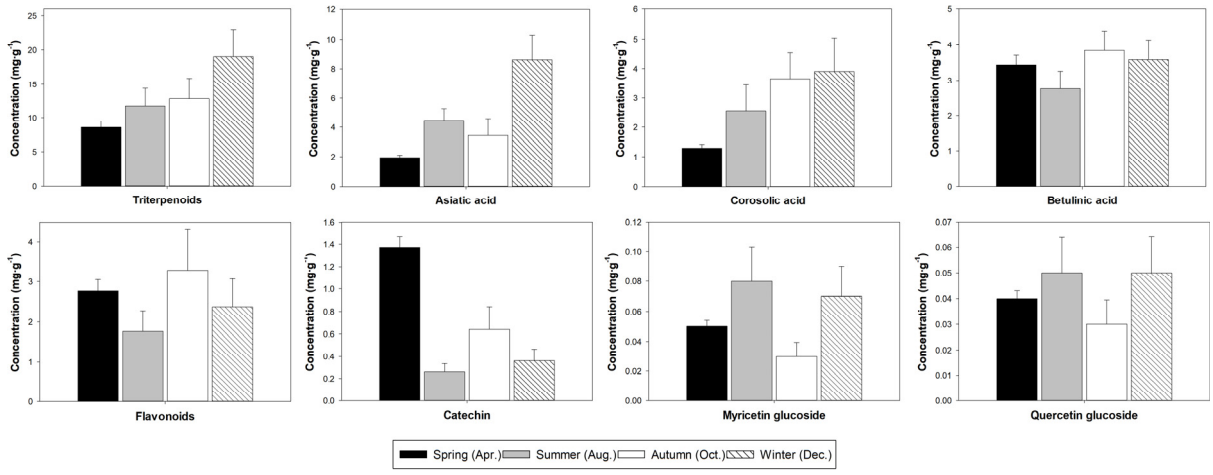


Fig. 2. Comparison of SFLEs concentrations across seasons. Error bars indicate the standard deviation. SFLEs, *Syzygium formosum* (Wall.) Masam leaf extracts.

Table 3. Evaluation of changes in the amount of SFLEs with seasons.

Analytes	Apr. batch (spring)		Aug. batch (summer)		Oct. batch (autumn)		Dec. batch (winter)	
	Conc. ($mg \cdot g^{-1}$)	Relative conc. (%)	Conc. ($mg \cdot g^{-1}$)	Relative conc. (%)	Conc. ($mg \cdot g^{-1}$)	Relative conc. (%)	Conc. ($mg \cdot g^{-1}$)	Relative conc. (%)
Triterpenoids								
Madecassic acid	0.25	2.92	0.36	3.06	0.08	0.59	0.41	2.18
Asiatic acid	1.95	22.75	4.45	37.83	3.44	26.83	8.59	45.18
Hederagenin	0.02	0.23	0.03	0.22	0.02	0.19	0.05	0.28
Maslinic acid	0.68	7.93	0.83	7.06	0.99	7.69	1.26	6.65
Corosolic acid	1.29	19.25	2.56	21.78	3.66	28.53	3.91	20.56
Betulin	0.06	0.70	0.11	0.90	0.09	0.67	0.15	0.79
Betulinic acid	3.44	40.14	2.76	23.43	3.84	29.98	3.58	18.85
Oleanolic acid	0.13	1.52	0.17	1.45	0.17	1.36	0.27	1.42
Ursolic acid	0.39	4.55	0.50	4.27	0.53	4.17	0.78	4.10
Flavonoids								
Catechin	1.37	49.46	0.26	14.92	0.64	19.35	0.36	15.03
Epigallocatechin gallate	0.04	1.44	0.01	0.68	0.04	1.34	0.02	0.68
Myricetin glucoside	0.05	1.81	0.08	4.63	0.03	0.79	0.07	2.87
Myricetin rhamnoside	1.02	36.82	1.05	59.21	1.88	57.33	1.30	54.81
Quercetin glucoside	0.04	1.44	0.05	2.60	0.03	1.03	0.05	2.03
Quercetin arabinoside	0.21	7.58	0.27	15.14	0.54	16.37	0.47	19.68
Quercetin rhamnoside	0.03	1.08	0.04	2.26	0.09	2.68	0.05	2.28
Quercetin	0.01	0.36	0.01	0.56	0.04	1.10	0.06	2.62

SFLEs, *Syzygium formosum* (Wall.) Masam leaf extracts; Conc., concentration.

Among the triterpenoids, asiatic acid had the highest concentration in December, 8.59 $mg \cdot g^{-1}$, which was 4.4 times higher than the lowest concentration in April (1.95 $mg \cdot g^{-1}$). Corosolic acid in December was 2.37 times higher (3.91

mg·g^{-1}) than the lowest in April (1.65 mg·g^{-1}). Betulinic acid peaked in October at 3.84 mg·g^{-1}, 1.39 times higher than in August (2.76 mg·g^{-1}).

For flavonoids, catechin had the most noticeable change, with the concentration in April (1.37 mg·g^{-1}) being 5.27 times higher than in August (0.26 mg·g^{-1}). Interestingly, it was observed that the glucose derivatives of myricetin and quercetin showed the lowest concentrations in October.

From the above results, it is concluded that the concentrations of triterpenoids and flavonoids exhibited a tendency to increase as temperatures decreased. Hence, SF should be harvested in December if high concentrations of triterpenoids were desired and in October if high concentrations of flavonoids were needed.

Temperature can serve as a significant abiotic stress factor that substantially influences the biosynthesis of secondary metabolites (Shi et al., 2015). Consequently, studies have investigated the effects of temperature on the phytochemical content of plants. For instance, it has been observed that the levels of phenolic acids and flavonols in red Chinese cabbages (*Brassica rapa* L. ssp. *pekinensis*) increase when harvested in the fall (Lee et al., 2018). In *Camellia japonica* L., transcriptomic analysis of cold-adapted cultivars compared to control groups revealed up-regulation of the unsaturated fatty acid biosynthesis pathway and the jasmonic acid biosynthesis pathway under low-temperature conditions, leading to elevated levels of unsaturated fatty acids and jasmonic acid in the leaves (Li et al., 2016). Additionally, in *Ginkgo biloba* L., exposure to low temperatures (15℃, daytime; 5℃, nighttime) combined with reduced soil moisture (30 - 35% of field capacity) resulted in increased activities of phenylalanine ammonia-lyase, cinnamate-4-hydroxylase, and *p*-coumarate CoA ligase, along with a corresponding rise in flavonoid content (Wang et al., 2014). Therefore, to elucidate the precise mechanisms underlying the observed increase in triterpenoids and flavonoids in SF leaves under low-temperature conditions, further research utilizing transcriptomics and proteomics approaches is needed.

Conclusion

Throughout the study, the extraction efficiency of SFLEs was investigated at different aspects: processing methods and seasonal variation. Firstly, DW pretreatment significantly reduced the flavonoid content, suggesting that pretreatment with DW should be minimized to extract SFLEs with undiminished triterpenoids and flavonoids. Furthermore, extraction using 70% ethanol yielded more than 70% of triterpenoids and flavonoids in both laboratory and pilot scales. Seasonal analysis showed that triterpenoids and flavonoids contents increased with decreasing temperature. Specifically, in Vietnam, December was found to be optimal for triterpenoid extraction and October was ideal for flavonoid. These findings therefore provide a basis for optimizing extraction and harvesting timing.

Conflict of Interests

No potential conflict of interest relevant to this article was reported.

Acknowledgements

This work was supported by the research fund of Chungnam National University.

Authors Information

Jaehan Kim, https://orcid.org/0000-0001-9719-9957

In-Seok Yeo, https://orcid.org/0000-0002-9687-7282

Jong-Tae Park, https://orcid.org/0000-0002-0650-6948

References

Altemimi A, Lakhssassi N, Baharlouei A, Watson DG, Lightfoot DA. 2017. Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. Plants 6:42.

Andre CM, Legay S, Deleruelle A, Nieuwenhuizen N, Punter M, Brendolise C, Cooney JM, Lateur M, Hausman JF, Larondelle Y, et al. 2016. Multifunctional oxidosqualene cyclases and cytochrome P450 involved in the biosynthesis of apple fruit triterpenic acids. New Phytologist 211:1279-1294.

Berini JL, Brockman SA, Hegeman AD, Reich PB, Muthukrishnan R, Montgomery RA, Forester JD. 2018. Combinations of abiotic factors differentially alter production of plant secondary metabolites in five woody plant species in the boreal-temperate transition zone. Frontiers in Plant Science 9:1257.

Bitwell C, Indra Sen S, Luke C, Kakoma MK. 2023. A review of modern and conventional extraction techniques and their applications for extracting phytochemicals from plants. Scientific African 19:e01585.

Cai C, Ma J, Han C, Jin Y, Zhao G, He X. 2019. Extraction and antioxidant activity of total triterpenoids in the mycelium of a medicinal fungus, *Sanghuangporus sanghuang*. Scientific Reports 9:7418.

Dinday S, Ghosh S. 2023. Recent advances in triterpenoid pathway elucidation and engineering. Biotechnology Advances 68:108214.

Ghosh S. 2016. Biosynthesis of structurally diverse triterpenes in plants: The role of oxidosqualene cyclases. Proceedings of the Indian National Science Academy 82:1189-1210.

Havsteen BH. 2002. The biochemistry and medical significance of the flavonoids. Pharmacology & Therapeutics 96: 67-202.

Hoang KHT, Lim J, Nguyen MTT, Lee NY, Lee CK, Nguyen VD, Kim HL, Park JT, Kim J. 2021. Comparative phytochemical analysis of *Syzygium formosum* (Wall.) Masam leaf and its biological activities. Applied Science 11:10552.

Lee H, Oh IN, Kim J, Jung DH, Cuong NP, Kim Y, Lee J, Kwon O, Park SU, Lim Y, et al. 2018. Phenolic compound profiles and their seasonal variations in new red-phenotype head-forming Chinese cabbages. LWT 90:433-439.

Lee MH, Jiang CB, Juan SH, Lin RD, Hou WC. 2006. Antioxidant and heme oxygenase-1 (HO-1)-induced effects of selected Taiwanese plants. Fitoterapia 77:109-115.

Li Q, Lei S, Du K, Li L, Pang X, Wang Z, Wei M, Fu S, Hu L, Xu L. 2016. RNA-seq based transcriptomic analysis uncovers α-linolenic acid and jasmonic acid biosynthesis pathways respond to cold acclimation in *Camellia japonica*. Scientific Reports 6:36463.

Lin S, Zhang G, Liao Y, Pan J, Gong D. 2015. Dietary flavonoids as xanthine oxidase inhibitors: Structure-affinity and structure-activity relationships. Journal of Agricultural and Food Chemistry 63:7784-7794.

Loi DT. 2001. Medicinal Plants and Remedies of Vietnam. p. 826. Medical Publishing House, Hanoi, Vietnam. [in Vietnamese]

Ma B, Ren W, Zhou Y, Ma J, Ruan Y, Wen CN. 2011. Triterpenoids from the spores of *Ganoderma lucidum*. North American Journal of Medical Sciences 3:495-498.

Mansuri ML, Parihar P, Solanki I, Parihar MS. 2014. Flavonoids in modulation of cell survival signalling pathways. Genes & Nutrition 9:400.

Martin DE, Salzwedel K, Allaway GP. 2008. Bevirimat: A novel maturation inhibitor for the treatment of HIV-1 infection. Antiviral Chemistry and Chemotherapy 19:107-113.

Metodiewa D, Kochman A, Karolczak S. 1997. Evidence for antiradical and antioxidant properties of four biologically active N,N-diethylaminoethyl ethers of flavanone oximes: A comparison with natural polyphenolic flavonoid

(rutin) action. Biochemistry and Molecular Biology International 41:1067-1075.
Misra RC, Sharma S, Sandeep, Garg A, Chanotiya CS, Ghosh, S. 2017. Two CYP716A subfamily cytochrome P450 monooxygenases of sweet basil play similar but nonredundant roles in ursane- and oleanane-type pentacyclic triterpene biosynthesis. New Phytologist 214:706-720.
Nguyen TMN, Lomunova M, Vu TPD, Le BV, Kim YH, Kang JS, Hwang, I. 2018. Anti-allergic effects of the ethanol extract of *Syzygium formosum* (Wall.) Masam leaves and its immunoregulatory mechanisms. Journal of Ethnopharmacology 211:171-179.
Park HA, Kim MY, Lee NY, Lim J, Park KB, Lee CK, Nguyen VD, Kim J, Park JT, Park JI. 2021. Variation of triterpenic acids in 12 wild *Syzygium formosum* and anti-inflammation activity on human keratinocyte HaCaT. Plants 10:2428.
Parul R, Das AK, Rana MS. 2020. Comparative pharmacological evaluation in respect to non-polar and polar solvent extracts of the leaves of *Syzygium balsameum* & *Syzygium formosum*. Journal of Pharmacognosy and Phytochemistry 9:45-52.
Phuong TT, Na M, Nguyen HD, Tran MH, Pham TK, Van Thanh T, Nguyen HN, Nguyen DT, Sok D, Bae, K. 2006. Antioxidant activities of Vietnamese medicinal plants. Natural Product Science 12:29-37.
Sandeep, Misra RC, Chanotiya CS, Mukhopadhyay P, Ghosh, S. 2019. Oxidosqualene cyclase and CYP716 enzymes contribute to triterpene structural diversity in the medicinal tree banaba. New Phytologist 222:408-424.
Shi Y, Ding Y, Yang, S. 2015. Cold signal transduction and its interplay with phytohormones during cold acclimation. Plant & Cell Physiology 56:7-15.
Thimmappa R, Geisler K, Louveau T, O'Maille P, Osbourn, A. 2014. Triterpene biosynthesis in plants. Annual Review of Plant Biology 65:225-257.
Uddin A, Hossain F, Reza A, Nasrin MS, Alam, A. 2022. Traditional uses, pharmacological activities, and phytochemical constituents of the genus *Syzygium*: A review. Food Science & Nutrition 10:1789-1819.
Wang GB, Cao FL, Chang L, Guo XQ, Wang, J. 2014. Temperature has more effects than soil moisture on biosynthesis of flavonoids in Ginkgo (*Ginkgo biloba* L.) leaves. New Forests 45:797-812.
Yang L, Wen KS, Ruan X, Zhao YX, Wei F, Wang, Q. 2018. Response of plant secondary metabolites to environmental factors. Molecules 23:762.

Table S1. MRM prameters for the analysis of triterpenoids and flavonoids.

Analytes	Retention time (min)	MRM transition (m/z)		Fragmentor	Collision energy (eV)	Ionization mode
		Precursor ion	Product ion			
Triterpenoids						
Madecassic acid	3.68	469.3	189.1	140	27	ESI+
Asiatic acid	4.61	453.3	201.1	130	25	
Hederagenin	7.08	455.3	189.1	120	25	
Maslinic acid	8.53	455.3	203.1	120	25	
Corosolic acid	8.93	455.3	205.1	120	20	
Betulin	11.71	425.3	191.1	130	25	
Betulinic acid	12.66	439.3	137.1	130	25	
Oleanolic acid	13.67	439.3	203.1	130	25	
Ursolic acid	13.98	439.3	203.1	130	25	
Flavonoids						
Catechin	1.32	291.1	139.0	90	7	ESI+
Epigallocatechin gallate	2.46	459.0	139.0	90	18	
Myricetin glucoside	4.14	481.1	319.1	90	7	
Myricetin rhamnoside	5.03	465.1	319.1	80	5	
Quercetin glucoside	5.64	465.1	303.1	100	7	
Quercetin arabinoside	6.50	435.1	303.1	90	2	
Quercetin rhamnoside	6.72	449.0	303.1	80	5	
Quercetin	8.13	303.1	153.2	170	30	

MRM, multiple reaction monitoring; ESI+, electrospray ionization.

Table S2. Extraction efficiency of SFLEs by purification scales.

Analytes	Laboratory scale				Pilot scale			
	1st extract		2nd extract		1st extract		2nd extract	
	Conc. ($mg \cdot g^{-1}$)	Efficiency (%)	Conc. ($mg \cdot g^{-1}$)	Efficiency (%)	Conc. ($mg \cdot g^{-1}$)	Efficiency (%)	Conc. ($mg \cdot g^{-1}$)	Efficiency (%)
Triterpenoids								
Madecassic acid	0.20	81.06	0.05	18.94	0.24	74.40	0.08	25.60
Asiatic acid	1.54	79.11	0.41	20.89	4.02	74.93	1.34	25.07
Hederagenin	0.02	80.73	0.00	19.27	0.03	78.11	0.01	21.89
Maslinic acid	0.52	76.67	0.16	23.33	0.90	74.87	0.30	25.13
Corosolic acid	1.25	75.53	0.40	24.47	2.77	74.26	0.96	25.74
Betulin	0.05	78.44	0.01	21.56	0.10	74.92	0.03	25.08
Betulinic acid	2.62	76.13	0.82	23.87	3.18	74.81	1.07	25.19
Oleanolic acid	0.10	75.89	0.03	24.11	0.18	74.03	0.06	25.97
Ursolic acid	0.30	77.03	0.09	22.97	0.57	74.69	0.19	25.31
Flavonoids								
Catechin	1.05	76.66	0.32	23.34	0.52	79.18	0.14	20.82
Epigallocatechin gallate	0.03	76.34	0.01	23.66	0.03	74.99	0.01	25.01
Myricetin glucoside	0.04	75.68	0.01	24.32	0.05	70.64	0.02	29.36
Myricetin rhamnoside	0.81	79.57	0.21	20.43	1.06	78.04	0.30	21.96
Quercetin glucoside	0.03	78.62	0.01	21.38	0.03	76.94	0.01	23.06
Quercetin arabinoside	0.17	80.38	0.04	19.62	0.24	77.84	0.07	22.16
Quercetin rhamnoside	0.02	76.43	0.01	23.57	0.04	78.53	0.01	21.47
Quercetin	0.01	76.25	0.00	23.75	0.04	78.71	0.01	21.29

SFLEs, *Syzygium formosum* (Wall.) Masam leaf extracts; Conc., concentration.

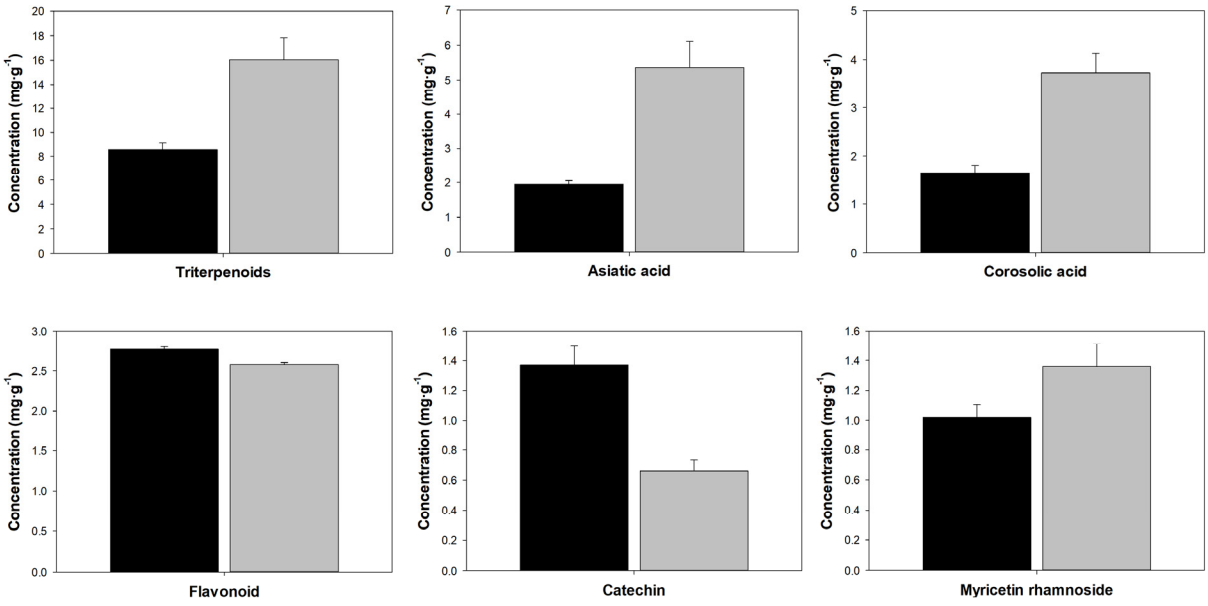


Fig. S1. Comparison of the triterpenoids and flavonoids acquired at different scales. The black and gray bars represent the lab-scale and pilot-scale, respectively. Error bars represent the standard deviation.

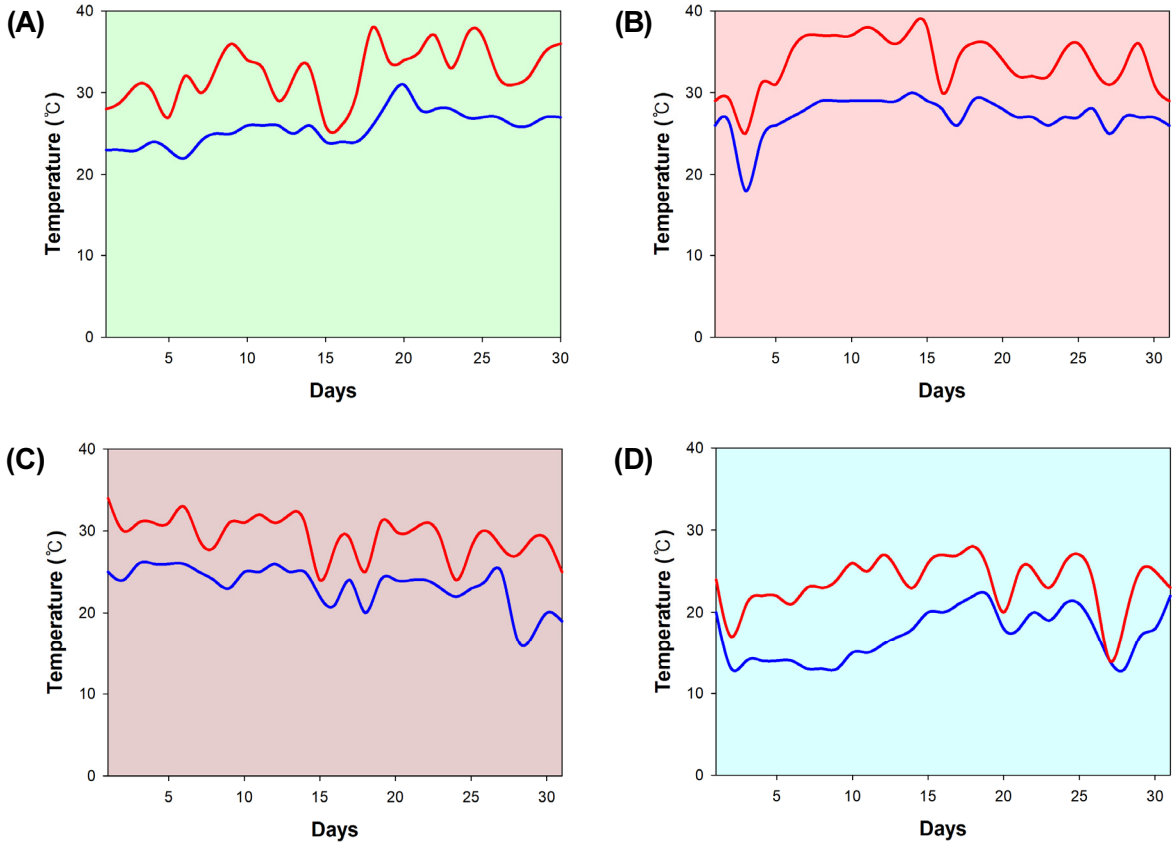


Fig. S2. The highest and lowest temperatures recorded in Hanoi, Vietnam, during (A) April, (B) August, (C) October, and (D) December of 2019 are shown. The red line represents the highest temperature, while the blue line represents the lowest temperature of the day.