Lipidomics of herbal tea revealed their potential lipid nutrients including novel fatty acid esters of hydroxy fatty acids

Rachana M. Gangadhara[a], Siddabasave Gowda B. Gowda[a,b*], Kota Yanagimoto[c], Divyavani Gowda[b], Hitoshi Chiba[d], and Shu-Ping Hui [b]

[a]Graduate School of Global Food Resources, Hokkaido University, Kita-9, Nishi-9, Kita-Ku, Sapporo 060-0809, Japan.

[b]Faculty of Health Sciences, Hokkaido University, Kita-12, Nishi-5, Kita-ku, Sapporo 060-0812, Japan.

[c]Graduate School of Health Sciences, Hokkaido University, Kita-12, Nishi-5, Kita-ku, Sapporo 060-0812, Japan.

[d]Department of Nutrition, Sapporo University of Health Sciences, Nakanuma, Nishi-4-3-1-15, Higashi-ku, Sapporo 007-0894, Japan.

Correspondences:

1. Siddabasave Gowda B. Gowda*
Faculty of Health Sciences, Hokkaido University,
Kita-12, Nishi-5, Kita-ku, Sapporo 060-0812, Japan.
E-mail: gowda@gfr.hokudai.ac.jp
Tel: +81-706-3576

2. Shu-Ping Hui
Faculty of Health Sciences, Hokkaido University,
Kita-12, Nishi-5, Kita-ku, Sapporo 060-0812, Japan.
E-mail: keino@hs.hokudai.ac.jp
Tel: +81-11-706-3693.

Abstract

Herbal tea is considered natural and harmless. With a long history of use, herbal tea acts as a healing remedy in various traditional medicines and has become a popular beverage worldwide. However, detailed lipid nutrient data have not been thoroughly investigated. This study applied lipidomics strategy using liquid chromatography/mass spectrometry to profile the lipid nutrients in ten types of herbal tea. Comprehensive lipidome analysis of herbal tea led to the identification of 299 lipid molecular species. Multivariate analysis revealed unique lipid compositions among different types of herbal tea. Five major lipid classes were characterized and semi-quantified including fatty acyls, sphingolipids, glycerophospholipids, glycerolipids, and sterols. Fatty acyls are the major lipids with polyunsaturated fatty acids (PUFA) are abundant in lemon balm, whereas saturated fatty acids (SFA) in lavender. Evaluation of the PUFA to SFA ratio for ten herbal teas showed that lemon balm and blue mallow have the highest values of 1.57 and 1.21 respectively. Furthermore, we uncovered seven isomeric fatty acid esters of hydroxy fatty acids (FAHFAs) in these herbal teas for the first time using high-resolution mass spectrometry and tandem mass spectrometry analyses. Hierarchical heatmap analyses revealed an abundance of phytoceramides in blue mallow and lysophospholipids in lemon balms. Triacylglycerols are unchanged in all the herbal teas analyzed. This extensive investigation of the lipid composition of herbal tea will help assess their nutritional benefits and potential uses in the food and health industries.

Keywords: herbal tea, lipid nutrients, fatty acid esters of hydroxy fatty acids, liquid chromatography, mass spectrometry

1. Introduction

Herbal teas, often referred to as herb extracts, are made from plants such as leaves, flowers, seeds, roots, and fruits [1]. Herbal plant extracts may contain various chemical compounds, which include polyphenols, flavonoids, terpenes, and catechins. These diverse elements contribute to tea flavors and aromas and potentially offer therapeutic and antioxidant properties [2,3]. Healthy food stores offer an array of teas derived from different botanical sources. Herein, the backgrounds of the ten herbal teas selected for this study are summarized. Lemongrass (*Cymbopogon citrates*), which belongs to the *Cymbopogon* genus, consists of approximately 180 grass species in the Poaceae family and is known for its antioxidant properties [4]. Lemon grass predominantly consists of citral (on average, 65–80%). Its leaves are commonly used in teas to add lemon flavor and in various popular beverages [5,6]. Furthermore, lemongrass has been used to treat digestive disorders, fever, menstrual issues, rheumatism, and joint pains [7]. Lamiaceae plants are cultivated worldwide for their medicinal uses [8]. Lemon balm (*Melissa officinalis*), peppermint (*Mentha piperita*), and lavender (*Lavandula angustifolia*) belong to the Lamiaceae family and are extensively grown to produce teas and essential oils for pharmaceuticals [8–10]. These herbs exhibit potent antibacterial, antiviral, anti-inflammatory, and antioxidant properties. In addition, peppermint and lemon balm have shown potential for treating gastrointestinal relaxation and nervous system abnormalities.

Lavender species serve as potential substitutes for certain synthetic materials in dentistry. All these numerous beneficial effects are attributed to flavonoids, phenolic acids, triterpenoids, and tannins [11–14]. Nettle (*Urtica dioica L.*) is a member of the Urticaceae family and contains essential vitamins (B, C, D, E, and P), inositols, and sesquiterpene lactones, which are key biologically active components. In addition, the leaves have greater Fe and Ca concentrations and a higher β-carotene concentration. Clinical studies have recommended that nettle leaf extract is effective against rheumatoid arthritis, osteoarthritis, and urinary tract infections [15,16]. The ginkgo (*Ginkgo biloba*) tree is the only surviving member of the Ginkgoaceae family and is native to China, Korea, and Japan [17,18]. This plant is rich in bioactive compounds, including flavonoids, ginkgolides, and phenolic compounds, so it has been the subject of extensive research. These bioactive ingredients offer several health advantages, including cardioprotective,

anti-inflammatory, antioxidant, and neuroprotective effects [17,19,20]. Globally, ginkgo is extensively used in the acupuncture, cosmetic, meal, and pharmaceutical sectors [21]. Rooibos (*Aspalathus linearis*) tea is a well-known herbal tea from South Africa that has spread worldwide. It is sold in over 37 countries, including Japan [22]. Unlike other teas, Rooibos contains no caffeine, no alkaloids, and a low content of tannins [23]. The primary flavonoids found in rooibos tea are aspalathin and nothofagin, which have more potent antioxidant properties than other flavonoids [24]. It has anti-inflammatory, antitumor, and antiapoptotic properties and is helpful in treating diabetic conditions [25].

German chamomile (*Matricaria recutita* L.) is an annual herbaceous flowering plant that originated in Europe [26]. Chamomile flowers are infused with water to produce herbal tea [27]. It has analgesic, anti-inflammatory, antibacterial, anti-allergic, and antioxidant properties and is used externally to treat chicken pox, cracked nipples, and ear and eye infections [28,29]. Blue mallow (*Malva sylvestris* L.) is the main species found in Southwest Asia, North Africa, and Europe [30]. The leaf and flower extracts are used to treat infections and inflammatory diseases. The plant also contains beneficial compounds such as unsaturated fatty acids, carbohydrates, and effective antioxidants [31,32]. Tannins, flavonoids, phenolic chemicals, and ascorbic acids present in *M. sylvestris* have been used to treat various cancers and improve wound healing [33].

Despite extensive research on the pharmacological properties and chemical composition of all selected herbal teas, no data have yet been published on comprehensive lipidomics. As lipids are macronutrients that play an important role in human health [34], it is important to study the lipid nutrients of herbal tea. The existing literature on the selected herbal tea indicates that targeted experiments have been conducted focusing on identifying fatty acids using gas chromatography, flame ionization detectors, and mass spectrometry techniques [35–42]. **Table 1** summarizes the general analytical techniques used for lipid analysis in tea plants its medicinal applications. However, our research aimed to investigate the lipid profiles of herbal teas to provide a broader understanding of their lipid composition at the molecular species level. Hence, we used high-performance liquid chromatography with linear ion trap-orbitrap mass spectrometry (HPLC/LTQ−Orbitrap−MS) for the nontargeted lipid analysis. The lipid compositions of the ten selected herbal teas were compared and correlated to determine their nutritional significance. This is the first study to identify and confirm the fatty acid esters of hydroxy

fatty acids (FAHFAs) in these herbal teas, a recently discovered class of bioactive lipids that show great promise for treating diabetes and inflammatory diseases [43]. The nutritional significance of the herbal tea in terms of lipid composition is discussed.

	English name	Scientific name	Bioactivities	Lipid analysis technique	Reference
1	Lemongrass, Nettle, Ginkgo	*Cymbopogon citratus, U. dioica, G. biloba*	antioxidant properties, effective against arthritis, urinary tract infections and anti-inflammatory effects.	GC−FID	[4,15,19, 35,37,38,]
2	Lemon balm	*M. officinalis*	Antioxidant, antimicrobial, anti-stress, anti-inflammatory, anticancer, and anti-cardiovascular.	GC/MS	[36]
3	Clary sage, Hyssop, Mint, lavender, Fennel, Thyme	*Salvia sclarea, Hyssopus officinalis L, Mentha piperita, Lavandula officinalis, Foeniculum vulgare, Thymus vulgaris*	Antibacterial, antispasmodic, antimicrobial activity.	GC−FID	[44]
4	Heart leaf, Japanese bamboo, horsetail, first wormwood	*Houttuynia cordata, Sasa vieitichi, Equisetum arvense, Artemisia princeps*	antioxidant, antibacterial, anti-obesity, anticancer, antimicrobial, antidiabetic activity.	LC/MS	[45]
5	Fennel, Ginger, juniper, Lemon peel, Orange peel, Rosehip	*Foeniculum vulgare Mill., Zingiber officinale, Juniperus communis, Citrus limon, Citrus*	antispasmodic, antiseptic, antioxidant, analgesic, hepatoprotective, antibacterial, anti-stress,	LC−MS	[46]

		aurantum, Rosa canina			
6	Green tea, black tea, oolong tea	C. sinensis var.	antioxidant and anti-inflammatory properties have been associated with the treatment of obesity, diabetes, and cancer diseases.	LC/MS,	[47,48]

Table 1: Summary of general analytical techniques used for lipid analysis in tea plants and their reported bioactivities.

2. Materials and Methods

2.1. Materials

LC/MS-grade solvents, such as methanol, isopropanol, and chloroform were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Ammonium acetate solution (1M) was obtained from Sigma-Aldrich (St. Louis, Missouri, USA). The internal standards, oleic acid-d9 and EquiSPLASH LIPIDOMIX quantitative standards were purchased from Avanti Polar Lipids (Alabaster, AL, USA). Bulk zirconium ceramic oxide beads (1.4 mm, Fisherbrand) were obtained from Thermo Fisher Scientific (Tokyo, Japan).

2.2. Sample collection

The ten selected herbal teas, namely lemon grass (LG)-*Cymbopogon citratus*, lemon balm (LB) -*M. officinalis*, peppermint (PM)-*M. x piperita*, nettle (NL)-*U. dioica*, ginkgo (GL)-*G. biloba*, rooibos red (RR)-*A. linearis*, rooibos green (RG)-*A. linearis*, lavender (LV)-*L. angustifolia*, German chamomile (GC)- *M. recutita* L., and blue mallow (BM)-*M. sylvestris L.* (**Fig. 1**) were purchased from an online store (onlineshop.treeoflife.co.jp). **Table 2** presents the common name, scientific name, family, percentage of total lipids, and extracted parts of the herbal tea used for lipid analysis. Among ten selected herbal

samples for the study, flower extract showed a higher percentage of lipid content compared to leaf extract.

	English name	Scientific name	Family name	Total lipid (%)	Extracted parts
a	Lemongrass (LG)	*Cymbopogon citratus*	Poaceae	0.32±0.08	Leaves
b	Lemon balm (LB)	*M. officinalis*	Lamiaceae	0.33±0.10	
c	Peppermint (PM)	*M. x piperita*	Lamiaceae	1.84±0.42	
d	Nettle (NL)	*U. dioica*	Urticaceae	0.61±0.27	
e	Ginkgo (GL)	*G. biloba*	Ginkgoaceae	0.63±0.42	
f	Rooibos Red (RR)	*A. linearis*	Fabaceae	0.24±0.10	
g	Rooibos Green (RG)	*A. linearis*	Fabaceae	0.96±0.27	
h	Lavender (LV)	*L. angustifolia*	Lamiaceae	5.08±1.24	Flowers
i	German Chamomile (GC)	*M. recutita* L.	Asteraceae	2.50±0.65	
j	Blue Mallow (BM)	*M. sylvestris* L.	Malvaceae	3.85	

Table 2: Details of the herbal tea samples used in this study and their total lipid amounts (n=3 for a-j whereas n=1 for j. The values are expressed in mean ± SD).

2.3. Lipid extraction

Total lipids were extracted from the herbal tea using the Bligh and Dyer method, with minor modifications [49,50]. The dried herb (2 g) was weighed into a 50-mL conical centrifuge tube, and 20 mL of methanol was added. In contrast, 40 mL of methanol was added to BM and GC. The mixture was then vortexed and incubated overnight at room temperature. On the next day, mixtures were vortexed for 1 min and centrifuged at 15000 rpm for 10 min at 4°C. Powdered activated charcoal was used to filter the mixture. After filtration, the extracts were evaporated in a centrifuge evaporator set at 4°C to 2 mL. Moreover, 100 µL of the pre-mixed internal standard in methanol was added to a fresh 1.5 mL vial (n = 5) containing 100 µL of the 2 mL extract (10 ng of each of the following: lysophosphatidylethanolamine (18:1(d7)), lysophosphatidylcholine (LPC) (18:1(d7)), sphingomyelin (d18:1/18:0(d9)), ceramide(Cer) (d18:1/15:0 (d7)), phosphatidylserine (15:0–18:1(d7)), phosphatidylinositol (PI) (15:0–18:1(d7)), phosphatidylethanolamine (15:0–18:1(d7)), phosphatidyl-glycerol (PG) (15:0–18:1(d7)), phosphatidylcholine (PC) (15:0–18:1(d7)), triacylglycerol (15:0–18:1(d7)–15:0), diacylglycerol (15:0–18:1(d7)), monoacylglycerol (18:1(d7)), and cholesterol ester (18:1(d7)), and 100 ng of oleic acid (d9)) and vortexed for 10 s. After adding 20 µL of Milli-Q water and 100 µL of $CHCl_3$, the mixture was vortexed for 10 min at 3500 rpm and centrifuged at 15000 rpm at 4°C. The centrifuge was transferred to a new vial and dried using a centrifuge evaporator. The dried residues were re-dissolved in 100 µL of methanol, vortexed for 1 min, and centrifuged at 15000 rpm at 4°C for 10 min. The centrifugate was transferred to a liquid chromatography (LC) vial for injection. Blank and quality control samples were prepared simultaneously for the analysis. An additional set of experiments were performed to determine the total lipid percentage in the selected herbal tea by using the Bligh and Dyer method and the results are shown in **Table 2**.

2.4. Lipid analysis by using HPLC/LTQ-Orbitrap-MS

A comprehensive lipid analysis was conducted using an HPLC/LTQ-Orbitrap-MS system. The setup includes an Orbitrap LTQ XL mass spectrometer (Thermo Fisher Scientific Inc., San Jose, CA) and LC-20AD HPLC (Shimadzu Corp., Kyoto, Japan) with separations conductions on an Atlantis T3 C18 column (2.1 × 150 mm, 3 µm, Waters, Milford, MA). The mobile phase comprised A: aqueous 10 mM CH_3COONH_4, B: isopropanol, and C: methanol with a flow rate of 0.2 mL/min at 40°C. Separation of lipids

was achieved by using a linear gradient of 30% of mobile phase B and 35% of mobile phase C for 1 min, 75% B and 15% C for 9 min followed by an increase of B to 82.5% and 95% and decrease of C to 15% and 5% in 21 min and 22.5 min respectively. Finally, the gradient was brought back to initial conditions from 25–26 min with an additional pre-conditioning for 4 min. MS analysis was performed using both negative and positive electrospray ionization by the method established earlier in our laboratory [51]. The following parameters were established for electron spray ionization: the capillary temperature was 330°C, nitrogen-auxiliary gas was 20 units, and nitrogen-sheath gas flow was 50 units. For the negative mode, the scan range was *m/z* 150–1700, and the source and capillary voltages were set to 3 kV and 10 V, respectively, whereas for the positive mode, the scan range was *m/z* 250–1600, and the source and capillary voltages were 4 kV and 25 V, respectively. The MS1 scan range was set at *m/z* 160–1900 for the negative mode and 100–1750 for the positive mode in the Fourier transform mode with a 60,000 resolving power to obtain the MS spectra for high-resolution masses. Low-resolution MS/MS spectra were obtained at 40 V collision energy in the ion trap mode. Using MS-DIAL (version 4.9) and Xcalibur 2.2 software, the raw data were processed, aligned, and integrated with a mass tolerance of 5.0 ppm. Lipid concentration levels were calculated by multiplying the peak area ratios of the analyte with the concentration of internal standard added and normalized by the weight of the sample.

2.5 Statistical analysis

The results are presented as the mean ± standard deviation (analysis of each herb, n = 5) using Microsoft Excel 2021 and GraphPad Prism 8 software (San Diego, CA, USA). Partial least squares discriminant and cluster correlation analyses were performed using MetaboAnalyst (version 5.0 (https://www.metaboanalyst.ca/) accessed on July 9, 2022.

3. Results and Discussion

3.1. Distribution of the amount of lipids in herbal tea based on lipid classes

We comprehensively analyzed herb profiles using HPLC/LTQ-Orbitrap-MS in both negative and positive ionization modes. A total of 299 lipid molecular species were identified based on their exact masses and MS/MS behavior using the MS-DIAL software.

The detailed information about each lipid species and their concentrations were provided in supporting information **Table S1.** The lipid elution profile of the herbal tea (**Fig. 2A**) obtained in the negative ionization mode primarily showed an abundance of free fatty acids, followed by glycerophospholipids and sphingolipids (SLs). The positive ionization mode data revealed the presence of triacylglycerols (TGs) and sterols. The percentage distribution of major lipid class (based on the relative amount) across the 10 different herbal teas illustrated in **Fig. 2B** and was categorized according to the LIPID MAPS guidelines. The results demonstrate the abundance of fatty acyls in most herbal tea predominantly in Rooibos red whereas sterols are most abundant in nettle mug. The partial least squares discriminant analysis score plots exhibited a unique grouping and visible separation between the herb groups (**Fig. 2C**). Components 1 and 2 explained 54.4% of the total variance, with component 1 contributing the most significantly (36.6%). Among the ten different groups of herbal tea, BM, LV, and LB displayed unique lipid compositions compared with the other seven groups. Larger positive and negative loading scores imply that a variable significantly affects its components. Lipids such as FA 18:3 and FA 16:0 had large negative loading scores and contributed mainly to group separation. These findings indicate that specific lipids contributed to the differentiation observed in the two-dimensional sparse partial least squares discriminant analysis plot.

3.2. Herbal tea free fatty acid compositions, nutritional indices, and hierarchical analysis

A total of 66 fatty acyls were identified and classified as saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs) based on the number of double bonds. **Fig. 3A** shows the distribution of total SFAs, MUFAs, and PUFAs in the 10 different types of herbal tea. LB and LV had the highest SFA values, whereas RG and RR had the lowest SFA levels. The herb with the highest content of health-beneficial PUFAs was LB, followed by LV, BM, and GL. The remaining herbal tea had relatively lower levels of PUFAs. Furthermore, MUFA levels were higher in the LB and BM species. The most commonly used index for evaluating the nutritional content of foods is the PUFA: SFA (P:S) ratio. Due to the vulnerability of PUFAs to lipid peroxidation, a diet rich in P:S may increase oxidative stress. The study from Kangal et al., suggests that maintaining a P:S ratio of 1.0–1.5 in the diet falls within a beneficial

range for reducing the risk of cardiovascular disease [52,53]. Our results suggest that LB and BM have a value close to this range, as shown in **Fig. 3B**. Therefore, the P:S value of LB and BM examined in this study will be useful for determining its potential nutritional significance. The heat map representing the abundance of fatty acyls in the 10 herbal teas is shown in **Fig. 3C**. Positive associations with high levels of certain fatty acids are indicated by red in the analysis. Negative associations at low levels are indicated in blue font. The results showed that SFAs were high in the LV, and PUFAs were high in the LB. This is the first study to identify one of the bioactive lipids FAHFAs in herbal tea. The heat map shows that FAHFAs are abundant in BM and LB species. Previous studies by Barros et al. identified some SFAs in the BM [31]; however, in our study, along with SFAs, we detected MUFAs, PUFAs, and novel FAHFAs.

3.3. Identification of FAHFAs in herbal tea

FAHFAs are a group of fatty acyls produced endogenously or consumed through the diet with reported biological benefits [54]. They play important roles as signaling molecules and structural components of lipid biofilms in the human body [55]. Adipose tissue produces endogenous compounds that are transported to various organs through the circulatory system [56]. Recently, there has been increased attention on FAHFAs due to their notable association with human insulin sensitivity. It has been reported that FAHFAs improved glucose tolerance and insulin sensitivity by promoting insulin secretion and enhancing glucose uptake in peripheral tissues. FAHFAs also exhibit anti-inflammatory properties by reducing the accumulation of pro-inflammatory macrophages and modulating inflammatory pathways[57–59]. The FAHFAs such as palmitic acid esters of hydroxy stearic acid and docosahexaenoic acid esters of hydroxy linoleic acid decreased the gene expression of proinflammatory interleukin (IL)-6, tumor necrosis factor-α (TNF-α) in macrophages [59]. Recent studies have focused on the metabolic pathways for FAHFAs, emphasizing their endogenous synthesis and control by dietary components [60]. Thus, the presence of FAHFAs in herbal teas may add to their overall health advantages. Furthermore, the interaction between FAHFAs and other bioactive chemicals in teas, such as polyphenols, may further boost their physiological benefits. A previous study by Zhu et al. identified FAHFAs in different types of tea, namely green, white,

oolong, and black teas, prepared from *Camellia sinensis* var. sinensis and *C. sinensis* var. assamica, respectively [61]. However, there is no existing literature on the lipids in the herbal tea used in this study, and this is the first study to report the identification and characterization of FAHFAs in this herbal tea, which are frequently consumed as herbal tea.

In this study, we identified seven molecular species of FAHFAs with the aid of HPLC/LTQ-Orbitrap-MS. The presence of these lipids was confirmed by acquiring high-resolution masses from the MS^1 spectra and studying their fragmentation behavior in low-resolution MS/MS spectra. Orbitrap-MS as a good sensitivity and high-resolution mass spectra will be helpful for finding the isomeric lipids by obtaining MS^n data for structural characterization. The application of orbitrap-MS for lipid identification was widely demonstrated in our previous studies [50,62]. **Fig. 4** depicts all seven FAHFA molecular species detected in the herbal samples, together with their extracted ion chromatograms and MS and MS/MS spectra. The heatmap in **Fig. 3C** shows the relative amounts of FAHFAs in each herbal species. The primary FAHFA fragment ions comprise the precursor, fatty acid fragment, and hydroxyl fatty acid fragment ions, along with its dehydration product [63]. The detected FAHFAs in this investigation were ionized in negative mode, generating $[M-H]^-$ ions as precursor ions. The compound, which elutes with a retention time of 13.71 min with an *m/z* value of 509.4562 ($[M-H]^-$, calculated *m/z*: 509.4575, error: −2.55 ppm), ionizes to generate *m/z* 271.3 as $[HPA-H]^-$ ion. Furthermore, a subsequent neutral loss of water results in a peak at *m/z* 253.3. Moreover, the peaks at *m/z* 255.2 [PA-H] in the MS/MS spectra suggested that the identified compound was a palmitic acid ester of hydroxy palmitic acid (PAHPA 16:0/16:0; O) [64]. Similarly, the other FAHFAs MS spectra are represented in **Fig. 4**. The fragmentation patterns of each molecular ion were closely aligned with a consistent pattern. The mass spectral behavior of the FAHFA characterized in this study is similar to that previously reported by Ma et al. **Table 3** provides a list of all seven FAHFA lipids that have been detected in the 10 herbal teas.

No.	Name	RT (min)	Observed *m/z*	Theoretical *m/z*	Mass error (ppm)

1	PAHPA 16:0/16:0;O	13.71	509.4562	509.4575	−2.55
2	PAHALA 16:0/18:3;O	13.52	531.4415	531.4419	−0.75
3	LAHOA 18:2/18:1;O	13.75	559.4731	559.4732	−0.17
4	LAHLA 18:2/18:2;O	13.41	557.4565	557.4575	−1.79
5	ALAHLA 18:3/18:2;O	13.16	555.4413	555.4419	−1.08
6	LAHALA 18:2/18:3;O	13.03	555.4410	555.4419	−1.62
7	ALAHALA 18:3/18:3;O	12.79	553.4250	553.4262	−2.16

Table 3: List of seven identified FAHFA lipids in ten herbal teas. PAHPA, palmitic acid esters of hydroxy palmitic acid; PAHALA, palmitic acid esters of alpha-linolenic acid; LAHOA, linoleic acid esters of hydroxy oleic acid; LAHLA, linoleic acid esters of hydroxy linoleic acid; ALAHLA, alpha-linolenic acid esters of hydroxy linoleic acid; LAHALA, linoleic acid esters of hydroxy alpha-linolenic acid; ALAHALA, alpha-linolenic acid esters of hydroxy alpha-linolenic acid.

3.4. Hierarchical correlation analysis of sphingolipids, glycerophospholipids, and lysophospholipids in herbal tea

Hierarchical heat maps showing the concentrations of lipids detected in the herbal tea. The concentrations of sphingolipids (SLs), such as ceramides (Cer), hexosylceramides (HexCer), sulfatide hexosyl ceramides (SHexCer), and acyl sterol glycosides (ASG) are shown in **Fig. 5A**. SLs are one of the most important components of eukaryotic cells, and Cer are bioactive lipids of the SL family that are involved in numerous cell signaling pathways [65,66]. Cer are potent biomarkers of insulin resistance, diabetes, and cardiovascular diseases and are measured clinically as disease risk

indicators [67]. Additionally, it is widely recognized that Cer plays a crucial role in organizing and sustaining the skin's water permeability barrier function [68]. We observed that the concentrations of Cer, HexCer, and SHexCer were the highest in the BM, LV, LG, and LB, emphasizing their significant importance. Phytoceramides are abundant in the BM species. In addition, we identified four ASG molecules in the lipids of LB and LG (**Fig. 5A**). ASG lipids belong to the sterol lipid family and actively contribute to lowering cholesterol levels and reducing the risk of cardiovascular diseases. These beneficial dietary components are predominantly present in plants [50].

The glycerophospholipid concentrations are shown in **Fig. 5B**. These included phosphatidic acid (PA), phosphatidylcholine (PC), phosphatidylglycerol (PG), phosphatidylinositol (PI), hemibis mono acyl glycerophosphate (HBMP) and lysophospholipids, including lysophosphatidic acid (LPA), lysophosphatidylcholines (LPC), lysophosphatidylglycerol (LPG), and lysophosphatidylinositol (LPI). In phospholipids, hydrophilic head groups and hydrophobic acyl chains are connected to alcohol components. The presence of a broad range of phospholipids results from the diversity of the head groups, aliphatic chains, and alcohols [69]. **Fig. 5B** illustrates the findings of our investigation, which showed that LB contained greater amounts of PI and PG species. In the RG, increased concentrations of PG, PC, HBMP, and PA were observed, whereas the LV displayed greater amounts of PA. PAs serve as central intermediates for synthesizing membranes and storing lipids, influencing diverse cellular processes. Their effects are integral to the survival, proliferation, and reproduction [70]. PCs are neutral lipids with both positive and negative charges and constitute a fundamental element of cell bilayer membranes that compartmentalize the cell [71]. The significance of PIs has been highlighted in the domains of lipid signaling, cell signaling, and membrane trafficking, whereas PGs have been identified as important constituents of biological membranes [72]. Lysophospholipids serve as intermediate precursors in cellular lipid biosynthesis. Among them, LPA stands out as the most extensively studied signaling lysophospholipid, and LPC has been identified as a crucial contributor to the biosynthesis of neuronal membranes [73]. Our findings showed that bioactive lysophospholipids were more abundant in the LB species.

3.5. Hierarchical correlation analysis of sterols and glycerolipids in herbal tea

The concentrations of sterols and glycerolipids in the herbal samples are shown in **Fig. 6A** and **6B**, respectively. These lipids undergo ionization in the positive mode. Sterols such as stigmasterol, sitosterol, and sterol ester were relatively high in the BM and LB species. Sterols are considered membrane reinforcers because they maintain the domain structure of cell membranes and control biological processes. Although cholesterol is the main sterol in vertebrates, plants exhibit a more complex sterol composition [74,75]. Sterols are recognized as valuable markers of geological samples containing organic matter [76]. Glycerolipids, including monoacylglycerol (MG), diacylglycerol (DG), digalactosyldiacylglycerol (DGDG), monogalactosyldiacylglycerol (MGDG), and sulfoquinovosyl diacylglycerol (SQDG), were abundant in LB, LV, and RB. While TGs are high in LB, BM is followed by LV. Triacylglycerols (TGs) are essential components of chylomicrons and very low-density lipoproteins that facilitate the transport of fat and energy throughout metabolic processes [77]. Additionally, TGs represent the principal components of human body fat, with twice the energy density of carbohydrates and proteins [78]. In addition, SQDG, MGDG, and DGDG have been shown to have positive effects on health and can block DNA polymerases, preventing the formation of tumors [79,80]. This finding implies that a diet that includes herbal tea may encourage a way of life that relies less on glucose. Despite the wide range of bioactive components in herbal teas, including polyphenols, tannins, and flavonoids, multiple factors can alter lipid digestion and metabolism. These chemicals could prevent digestive enzymes such as lipase, decrease lipid absorption, alter the gut microbiota, and lower lipid metabolic efficiency. The composition of herbal tea is complex, so their possible impacts on lipid metabolism and further on health outcomes have to be carefully considered [81,82]. Although this study conducted a comprehensive analysis of the lipidome of herbal samples, it has the following limitations: (1) the origin and conditions under which the herbal tea is grown may significantly affect the lipid composition, which has not been addressed; (2) the calculated lipid concentrations are relative based on the amount of internal standard added. Further studies are essential to quantify the lipids in this herbal tea to determine their absolute levels and include them as value-added food sources for nutraceutical development.

4. **Conclusions**

Our global lipid analysis study of ten herbal samples revealed diverse lipid compositions, showing variations in bioactive lipids such as PUFAs and FAHFAs. LB and BM was determined to have an optimal P:S ratio, suggesting its potential health benefits. This study identified FAHFAs in the selected herbal tea for the first time, offering promising implications for creating enriched foods for controlling diabetes and inflammation. Correlation analysis revealed unique lipid profiles of LB, LV, and BM. These findings deepen the understanding of the nutritional benefits of herbal tea and their potential applications in the food industry. This study highlights the significance of lipidomics in evaluating the health-promoting qualities of herbal tea, offering insightful information at the lipid molecular species level.

Credit authorship contribution statement:

R.M.G.: Formal analysis; methodology; visualization; writing-review and editing.
S.G.B.G.: Conceptualization; validation; investigation; supervision; project administration; writing-original draft; funding acquisition.

K.Y.: Formal analysis; writing-review and editing.

D.G.: Data curation; methodology; writing-review and editing.

H.C.: Supervision; resources; writing-review and editing.

S.P.H.: Supervision; resources; writing-review and editing.

Acknowledgements:

This work is supported by the Japanese Society for the Promotion of Science KAKENHI Grants (21 K1481201 and 22 K1485002).

Conflict of interest

The authors declare no conflict of interest.

Ethical approval

This study includes no human or animal experiments.

Informed consent

Informed consent was acquired from all the co-authors listed in the manuscript.

Data Availability Statement

The data will be available from the corresponding author upon a reasonable request.

References

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Figure 1

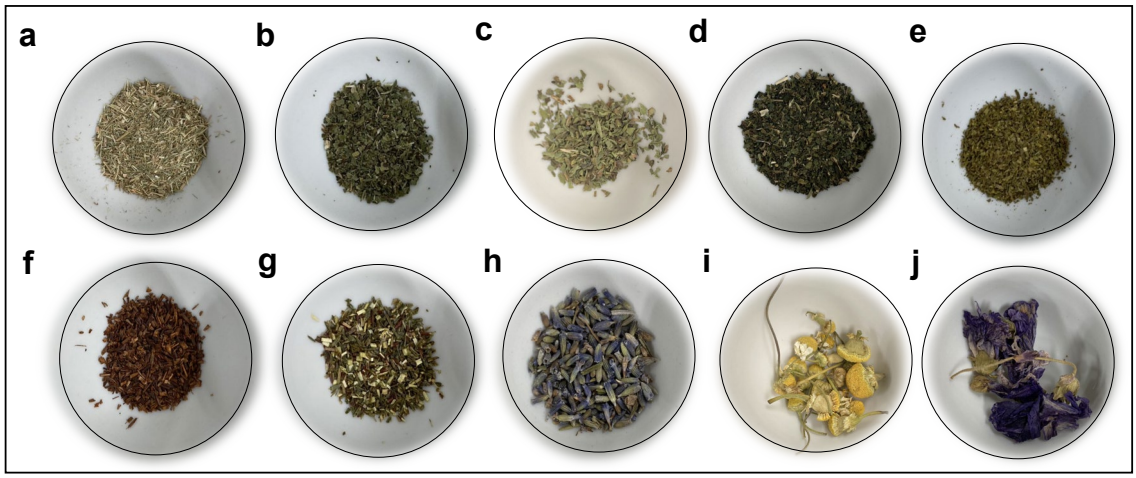


Fig. 1 Pictorial representation of the selected ten herbal tea used for lipidomics (**a.** Lemon grass, **b.** Lemon balm, **c.** Peppermint, **d.** Nettle, **e.** Ginkgo, **f.** Rooibos Red, **g.** Rooibos Green, **h.** Lavender, **i.** German Chamomile, **j.** Blue Mallow).

Figure 2

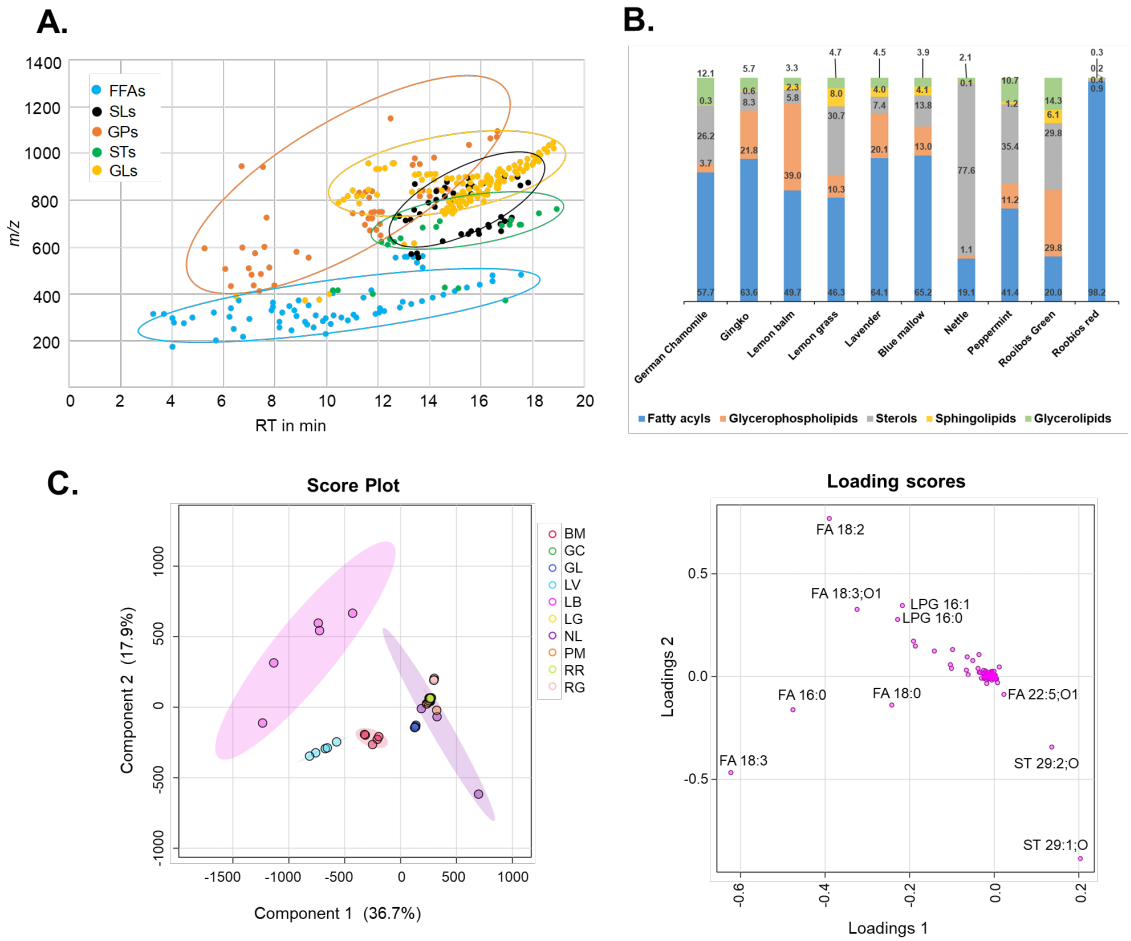
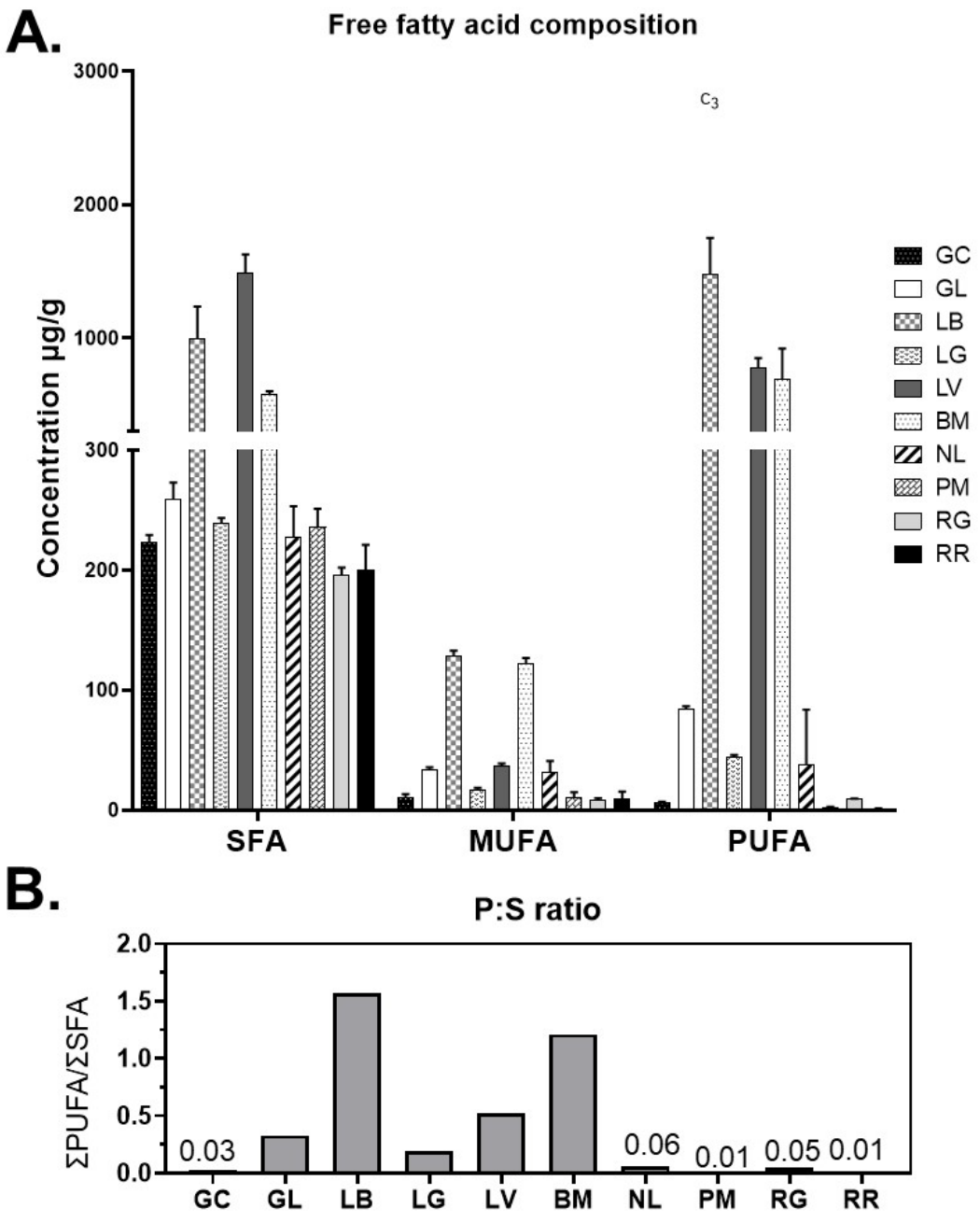


Fig. 2 Multivariate analysis of ten different herbal teas. **A.** Elution profiles of lipid matrices extracted from herbal tea. **B.** Percentage distribution of five major lipid classes in ten types of herbal tea based on their relative amount (FFAs, free fatty acids; GPs, glycerophospholipids; SLs, sphingolipids; STs, sterols; GLs, glycerolipids). **C.** Partial least squares discriminant analysis (PLSDA) score and loadings plots of lipid metabolites. BM, blue mallow; GC, German chamomile; GL, ginkgo; LV, lavender; LB, lemon balm; LG, lemon grass; NL, nettle; PM, peppermint; RR, rooibos red; RG, rooibos green.

Figure 3.


A.
Free fatty acid composition
C3
Concentration µg/g
3000
2000
1000
300
200
100
0
GC
GL
LB
LG
LV
BM
NL
PM
RG
RR
SFA
MUFA
PUFA
B.
P:S ratio
ΣPUFA/ΣSFA
2.0
1.5
1.0
0.5
0.0
0.03
0.06
0.01
0.05
0.01
GC
GL
LB
LG
LV
BM
NL
PM
RG
RR

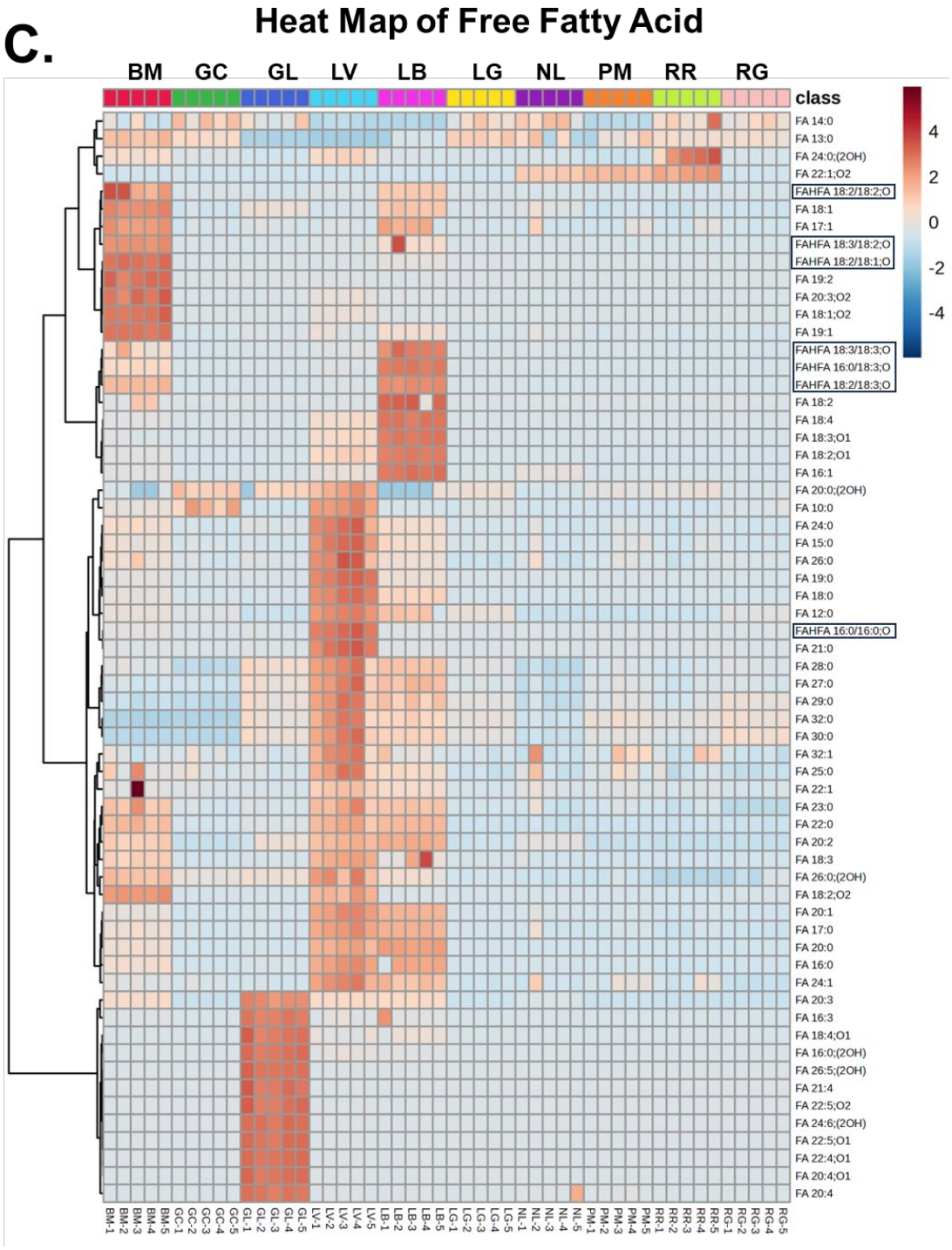


Fig. 3A. Free fatty acid composition based on unsaturation in ten herbal teas ($p < 0.05$, is considered statistically significant). **B**. PUFA to SFA (P:S) ratio. **C.** Hierarchical cluster correlation analysis of fatty acyls. GC, German chamomile; GL, ginkgo; LB, lemon balm; LG, lemon grass; LV, lavender; BM, blue mallow; NL, nettle; PM, peppermint; RG, rooibos green; RR, rooibos red

Figure 4

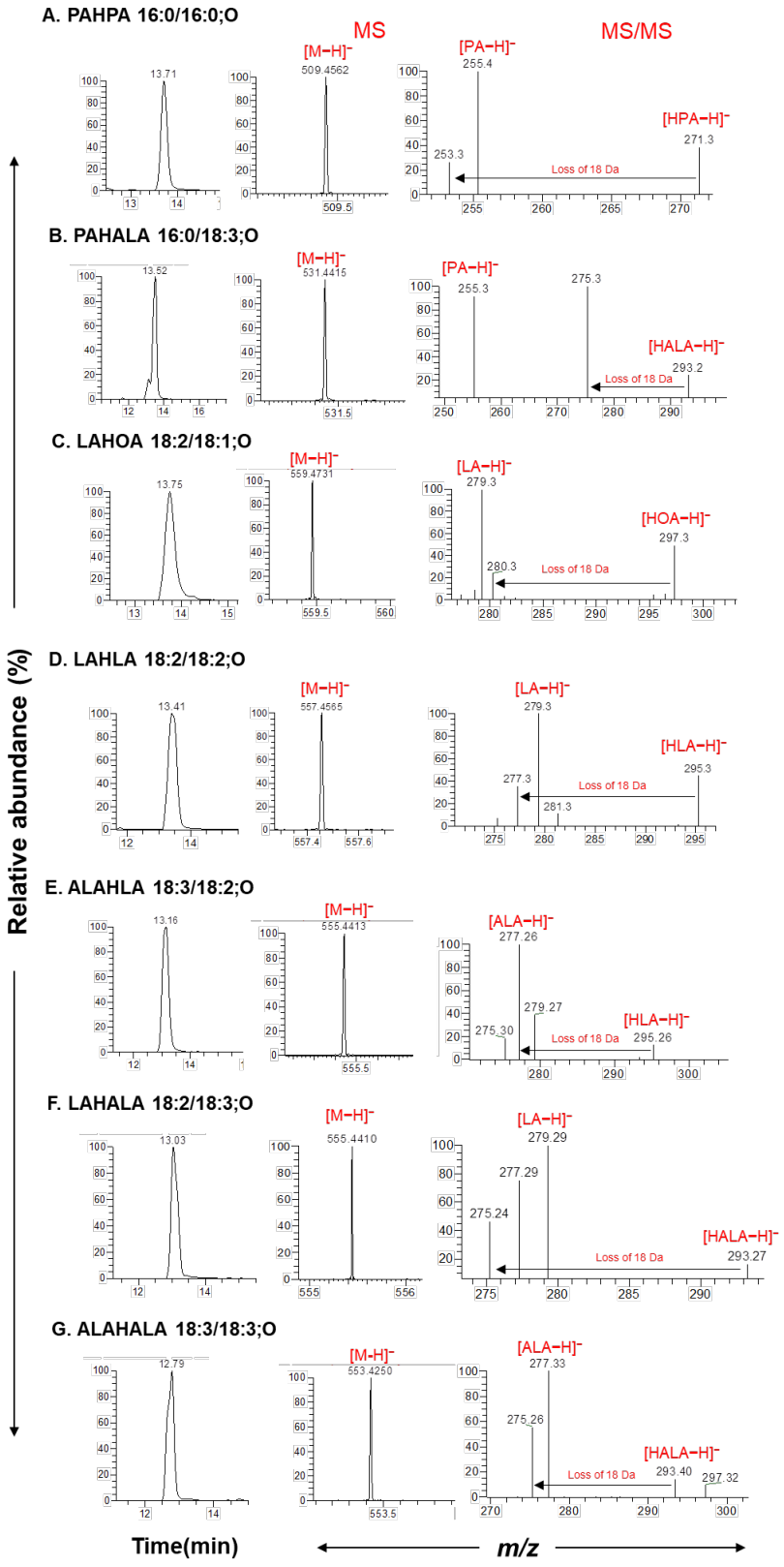
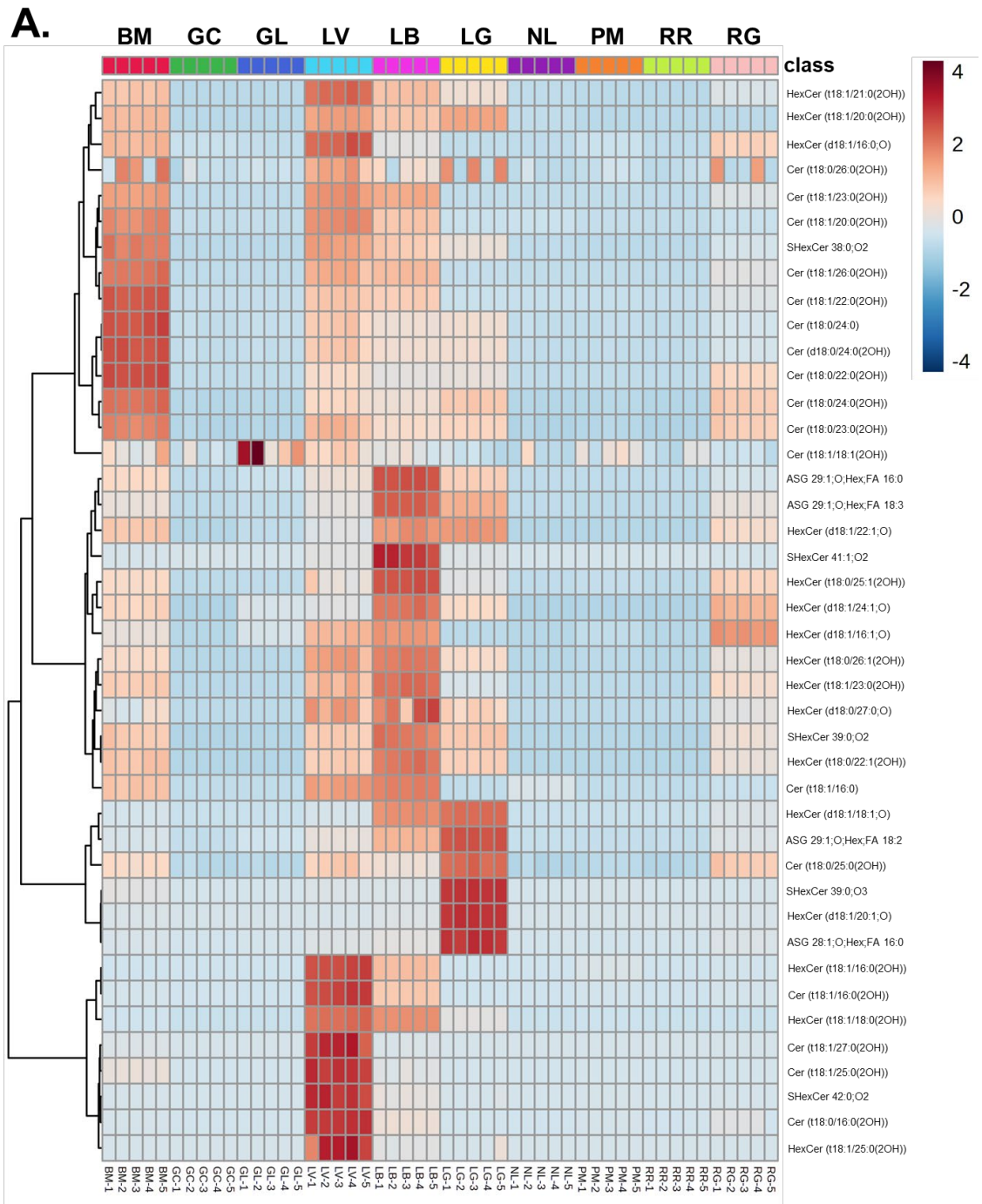

A. PAHPA 16:0/16:0;O
MS
MS/MS
[M−H]⁻
509.4562
13.71
[PA−H]⁻
255.4
[HPA−H]⁻
271.3
253.3
Loss of 18 Da
B. PAHALA 16:0/18:3;O
13.52
[M−H]⁻
531.4415
[PA−H]⁻
255.3
275.3
[HALA−H]⁻
293.2
Loss of 18 Da
C. LAHOA 18:2/18:1;O
13.75
[M−H]⁻
559.4731
[LA−H]⁻
279.3
[HOA−H]⁻
297.3
280.3
Loss of 18 Da
D. LAHLA 18:2/18:2;O
13.41
[M−H]⁻
557.4565
[LA−H]⁻
279.3
[HLA−H]⁻
295.3
277.3
281.3
Loss of 18 Da
E. ALAHLA 18:3/18:2;O
13.16
[M−H]⁻
555.4413
[ALA−H]⁻
277.26
279.27
275.30
[HLA−H]⁻
295.26
Loss of 18 Da
F. LAHALA 18:2/18:3;O
13.03
[M−H]⁻
555.4410
[LA−H]⁻
279.29
277.29
275.24
[HALA−H]⁻
293.27
Loss of 18 Da
G. ALAHALA 18:3/18:3;O
12.79
[M−H]⁻
553.4250
[ALA−H]⁻
277.33
275.26
[HALA−H]⁻
293.40
297.32
Loss of 18 Da
Relative abundance (%)
Time(min)
m/z

Fig. 4 Extracted ion chromatogram, MS, and MS/MS spectra of FAHFAs detected in ten different herbal tea. (**A.** PAHPA, palmitic acid esters of hydroxy palmitic acid; **B.** PAHALA, palmitic acid esters of alpha-linolenic acid; **C.** LAHOA, linoleic acid esters of hydroxy oleic acid; **D.** LAHLA, linoleic acid esters of hydroxy linoleic acid; **E.** ALAHLA, alpha-linolenic acid esters of hydroxy linoleic acid; **F.** LAHALA, linoleic acid esters of hydroxy alpha-linolenic acid; **G.** ALAHALA, alpha-linolenic acid esters of hydroxy alpha-linolenic acid).

Figure 5


A.
BM
GC
GL
LV
LB
LG
NL
PM
RR
RG
class
4
2
0
-2
-4
HexCer (t18:1/21:0(2OH))
HexCer (t18:1/20:0(2OH))
HexCer (d18:1/16:0;O)
Cer (t18:0/26:0(2OH))
Cer (t18:1/23:0(2OH))
Cer (t18:1/20:0(2OH))
SHexCer 38:0;O2
Cer (t18:1/26:0(2OH))
Cer (t18:1/22:0(2OH))
Cer (t18:0/24:0)
Cer (d18:0/24:0(2OH))
Cer (t18:0/22:0(2OH))
Cer (t18:0/24:0(2OH))
Cer (t18:0/23:0(2OH))
Cer (t18:1/18:1(2OH))
ASG 29:1;O;Hex;FA 16:0
ASG 29:1;O;Hex;FA 18:3
HexCer (d18:1/22:1;O)
SHexCer 41:1;O2
HexCer (t18:0/25:1(2OH))
HexCer (d18:1/24:1;O)
HexCer (d18:1/16:1;O)
HexCer (t18:0/26:1(2OH))
HexCer (t18:1/23:0(2OH))
HexCer (d18:0/27:0;O)
SHexCer 39:0;O2
HexCer (t18:0/22:1(2OH))
Cer (t18:1/16:0)
HexCer (d18:1/18:1;O)
ASG 29:1;O;Hex;FA 18:2
Cer (t18:0/25:0(2OH))
SHexCer 39:0;O3
HexCer (d18:1/20:1;O)
ASG 28:1;O;Hex;FA 16:0
HexCer (t18:1/16:0(2OH))
Cer (t18:1/16:0(2OH))
HexCer (t18:1/18:0(2OH))
Cer (t18:1/27:0(2OH))
Cer (t18:1/25:0(2OH))
SHexCer 42:0;O2
Cer (t18:0/16:0(2OH))
HexCer (t18:1/25:0(2OH))
BM-1
BM-2
BM-3
BM-4
BM-5
GC-1
GC-2
GC-3
GC-4
GC-5
GL-1
GL-2
GL-3
GL-4
GL-5
LV-1
LV-2
LV-3
LV-4
LV-5
LB-1
LB-2
LB-3
LB-4
LB-5
LG-1
LG-2
LG-3
LG-4
LG-5
NL-1
NL-2
NL-3
NL-4
NL-5
PM-1
PM-2
PM-3
PM-4
PM-5
RR-1
RR-2
RR-3
RR-4
RR-5
RG-1
RG-2
RG-3
RG-4
RG-5

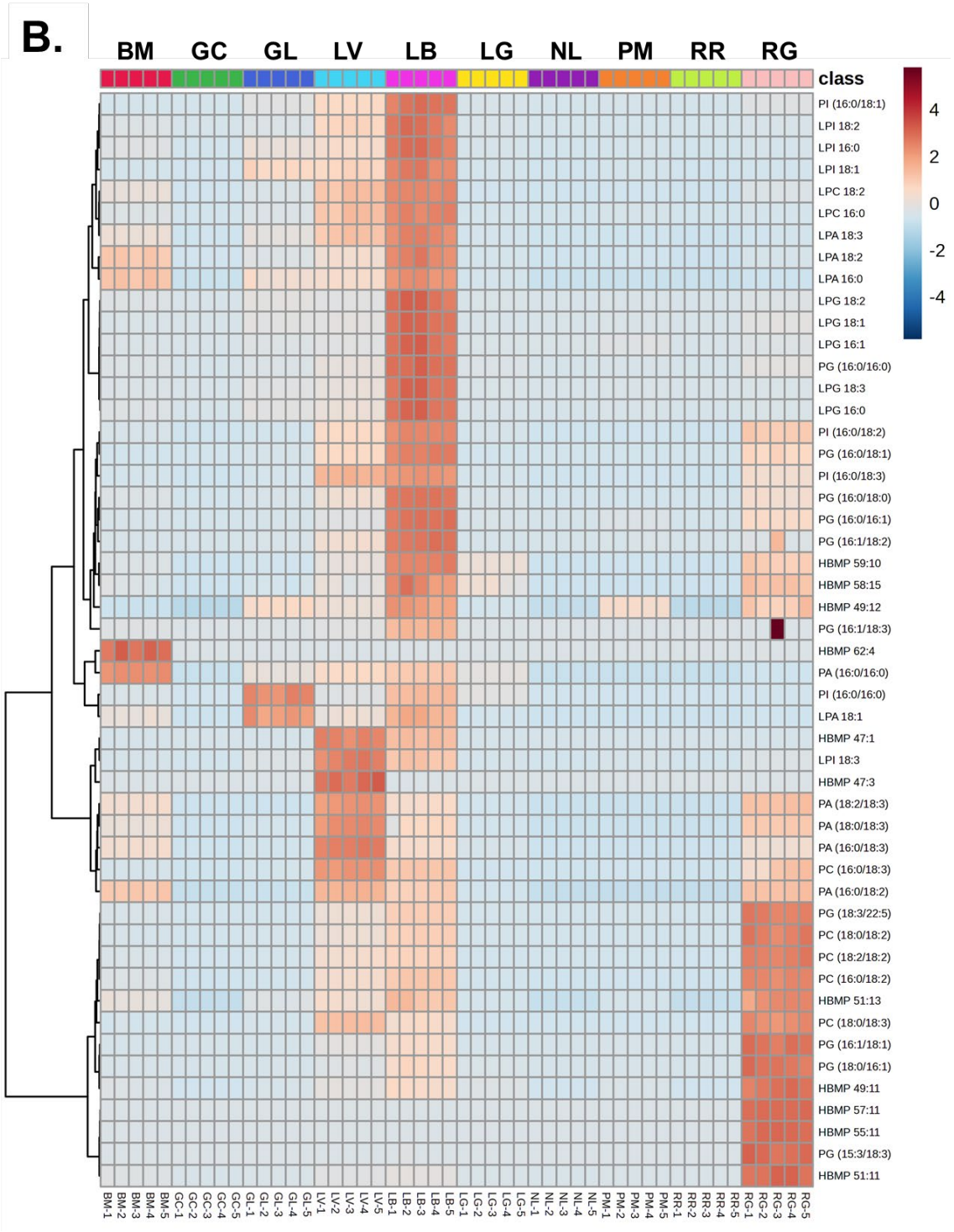
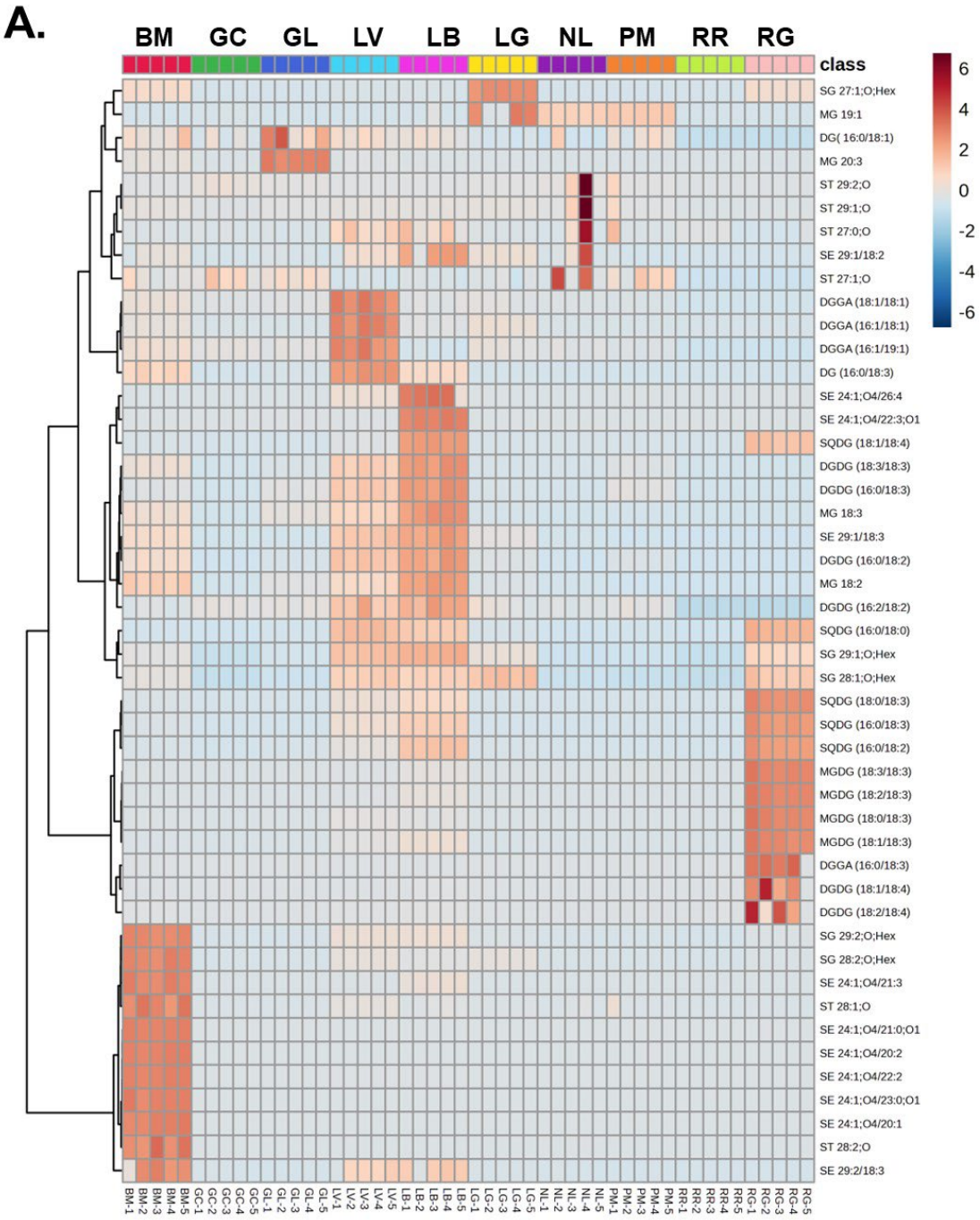

B.
BM
GC
GL
LV
LB
LG
NL
PM
RR
RG
class
PI (16:0/18:1)
LPI 18:2
LPI 16:0
LPI 18:1
LPC 18:2
LPC 16:0
LPA 18:3
LPA 18:2
LPA 16:0
LPG 18:2
LPG 18:1
LPG 16:1
PG (16:0/16:0)
LPG 18:3
LPG 16:0
PI (16:0/18:2)
PG (16:0/18:1)
PI (16:0/18:3)
PG (16:0/18:0)
PG (16:0/16:1)
PG (16:1/18:2)
HBMP 59:10
HBMP 58:15
HBMP 49:12
PG (16:1/18:3)
HBMP 62:4
PA (16:0/16:0)
PI (16:0/16:0)
LPA 18:1
HBMP 47:1
LPI 18:3
HBMP 47:3
PA (18:2/18:3)
PA (18:0/18:3)
PA (16:0/18:3)
PC (16:0/18:3)
PA (16:0/18:2)
PG (18:3/22:5)
PC (18:0/18:2)
PC (18:2/18:2)
PC (16:0/18:2)
HBMP 51:13
PC (18:0/18:3)
PG (16:1/18:1)
PG (18:0/16:1)
HBMP 49:11
HBMP 57:11
HBMP 55:11
PG (15:3/18:3)
HBMP 51:11
4
2
0
-2
-4
BM-1 BM-2 BM-3 BM-4 BM-5 GC-1 GC-2 GC-3 GC-4 GC-5 GL-1 GL-2 GL-3 GL-4 GL-5 LV-1 LV-2 LV-3 LV-4 LV-5 LB-1 LB-2 LB-3 LB-4 LB-5 LG-1 LG-2 LG-3 LG-4 LG-5 NL-1 NL-2 NL-3 NL-4 NL-5 PM-1 PM-2 PM-3 PM-4 PM-5 RR-1 RR-2 RR-3 RR-4 RR-5 RG-1 RG-2 RG-3 RG-4 RG-5

Fig. 5 Cluster correlations of **A.** sphingolipids including ceramides (Cer), hexosylceramides (HexCer), sulfatide hexosyl ceramides (SHexCer), and acyl sterol glycosides (ASG) **B.** glycerophospholipids: phosphatidic acid (PA), phosphatidylcholine (PC), phosphatidylglycerol (PG), phosphatidylinositol (PI), and hemibismonoacyl glycerophosphate (HBMP) and lysophospholipids: lysophosphatidic acid (LPA), lysophosphatidylcholines (LPC), lysophosphatidylglycerol (LPG), and lysophosphatidylinositol (LPI) in ten different herbs and their hierarchical cluster analysis. (clustering method: complete, distance measure: Euclidean). GC, German chamomile; GL, ginkgo; LB, lemon balm; LG, lemon grass; LV, lavender; BM, blue mallow; NL, nettle; PM, peppermint; RG, rooibos green; RR, rooibos red.

Figure 6


A.
BM
GC
GL
LV
LB
LG
NL
PM
RR
RG
class
6
4
2
0
-2
-4
-6
SG 27:1;O;Hex
MG 19:1
DG(16:0/18:1)
MG 20:3
ST 29:2;O
ST 29:1;O
ST 27:0;O
SE 29:1/18:2
ST 27:1;O
DGGA (18:1/18:1)
DGGA (16:1/18:1)
DGGA (16:1/19:1)
DG (16:0/18:3)
SE 24:1;O4/26:4
SE 24:1;O4/22:3;O1
SQDG (18:1/18:4)
DGDG (18:3/18:3)
DGDG (16:0/18:3)
MG 18:3
SE 29:1/18:3
DGDG (16:0/18:2)
MG 18:2
DGDG (16:2/18:2)
SQDG (16:0/18:0)
SG 29:1;O;Hex
SG 28:1;O;Hex
SQDG (18:0/18:3)
SQDG (16:0/18:3)
SQDG (16:0/18:2)
MGDG (18:3/18:3)
MGDG (18:2/18:3)
MGDG (18:0/18:3)
MGDG (18:1/18:3)
DGGA (16:0/18:3)
DGDG (18:1/18:4)
DGDG (18:2/18:4)
SG 29:2;O;Hex
SG 28:2;O;Hex
SE 24:1;O4/21:3
ST 28:1;O
SE 24:1;O4/21:0;O1
SE 24:1;O4/20:2
SE 24:1;O4/22:2
SE 24:1;O4/23:0;O1
SE 24:1;O4/20:1
ST 28:2;O
SE 29:2/18:3
BM-1
BM-2
BM-3
BM-4
BM-5
GC-1
GC-2
GC-3
GC-4
GC-5
GL-1
GL-2
GL-3
GL-4
GL-5
LV-1
LV-2
LV-3
LV-4
LV-5
LB-1
LB-2
LB-3
LB-4
LB-5
LG-1
LG-2
LG-3
LG-4
LG-5
NL-1
NL-2
NL-3
NL-4
NL-5
PM-1
PM-2
PM-3
PM-4
PM-5
RR-1
RR-2
RR-3
RR-4
RR-5
RG-1
RG-2
RG-3
RG-4
RG-5

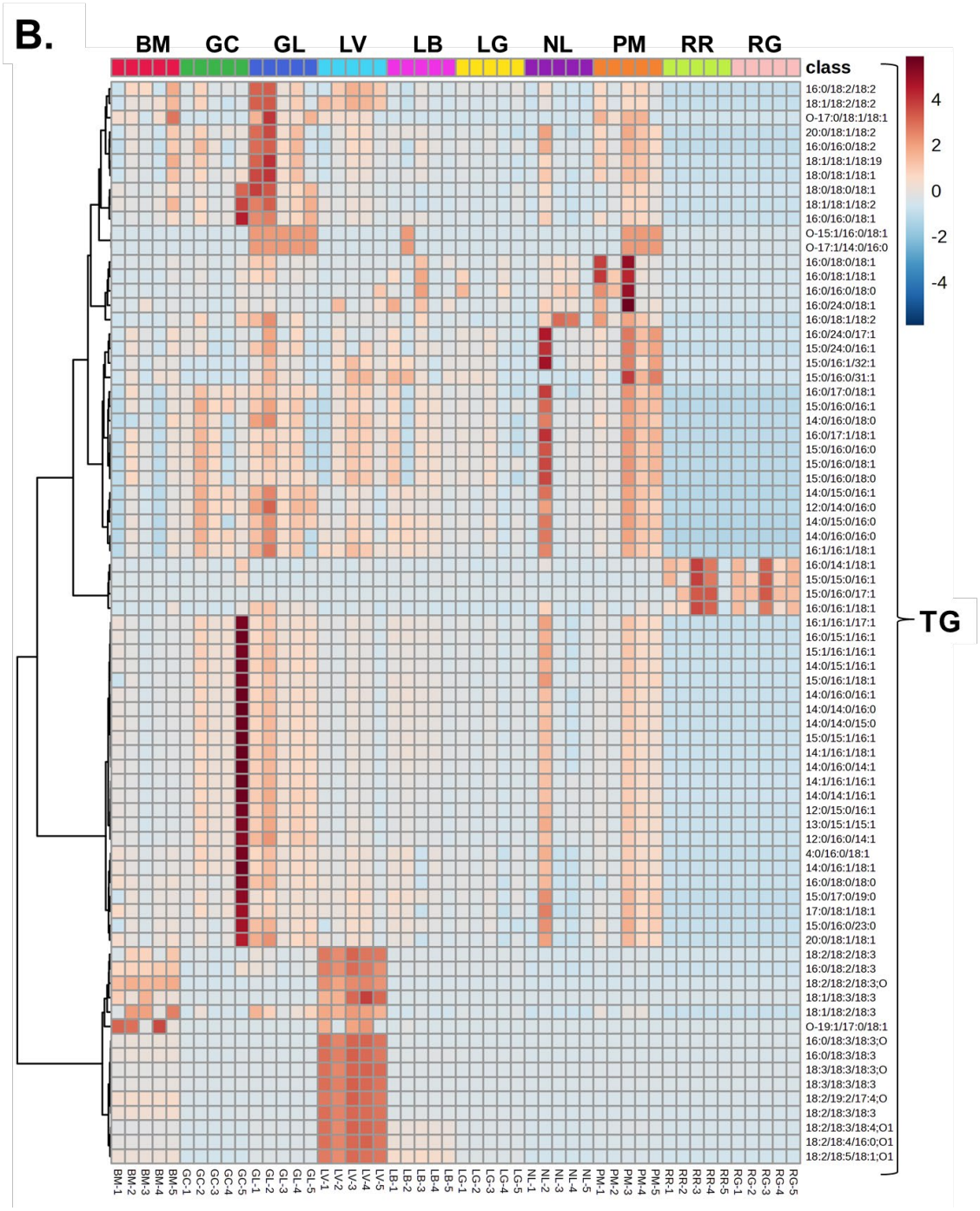

B.
BM
GC
GL
LV
LB
LG
NL
PM
RR
RG
class
4
2
0
-2
-4
16:0/18:2/18:2
18:1/18:2/18:2
O-17:0/18:1/18:1
20:0/18:1/18:2
16:0/16:0/18:2
18:1/18:1/18:19
18:0/18:1/18:1
18:0/18:0/18:1
18:1/18:1/18:2
16:0/16:0/18:1
O-15:1/16:0/18:1
O-17:1/14:0/16:0
16:0/18:0/18:1
16:0/18:1/18:1
16:0/16:0/18:0
16:0/24:0/18:1
16:0/18:1/18:2
16:0/24:0/17:1
15:0/24:0/16:1
15:0/16:1/32:1
15:0/16:0/31:1
16:0/17:0/18:1
15:0/16:0/16:1
14:0/16:0/18:0
16:0/17:1/18:1
15:0/16:0/16:0
15:0/16:0/18:1
15:0/16:0/18:0
14:0/15:0/16:1
12:0/14:0/16:0
14:0/15:0/16:0
14:0/16:0/16:0
16:1/16:1/18:1
16:0/14:1/18:1
15:0/15:0/16:1
15:0/16:0/17:1
16:0/16:1/18:1
16:1/16:1/17:1
16:0/15:1/16:1
15:1/16:1/16:1
14:0/15:1/16:1
15:0/16:1/18:1
14:0/16:0/16:1
14:0/14:0/16:0
14:0/14:0/15:0
15:0/15:1/16:1
14:1/16:1/18:1
14:0/16:0/14:1
14:1/16:1/16:1
14:0/14:1/16:1
12:0/15:0/16:1
13:0/15:1/15:1
12:0/16:0/14:1
4:0/16:0/18:1
14:0/16:1/18:1
16:0/18:0/18:0
15:0/17:0/19:0
17:0/18:1/18:1
15:0/16:0/23:0
20:0/18:1/18:1
18:2/18:2/18:3
16:0/18:2/18:3
18:2/18:2/18:3;O
18:1/18:3/18:3
18:1/18:2/18:3
O-19:1/17:0/18:1
16:0/18:3/18:3;O
16:0/18:3/18:3
18:3/18:3/18:3;O
18:3/18:3/18:3
18:2/19:2/17:4;O
18:2/18:3/18:3
18:2/18:3/18:4;O1
18:2/18:4/16:0;O1
18:2/18:5/18:1;O1
TG
BM-1
BM-2
BM-3
BM-4
BM-5
GC-1
GC-2
GC-3
GC-4
GC-5
GL-1
GL-2
GL-3
GL-4
GL-5
LV-1
LV-2
LV-3
LV-4
LV-5
LB-1
LB-2
LB-3
LB-4
LB-5
LG-1
LG-2
LG-3
LG-4
LG-5
NL-1
NL-2
NL-3
NL-4
NL-5
PM-1
PM-2
PM-3
PM-4
PM-5
RR-1
RR-2
RR-3
RR-4
RR-5
RG-1
RG-2
RG-3
RG-4
RG-5

Fig. 6 Cluster correlations of **A.** Sterols (SG (stigmasterol), ST (sitosterol), and SE (sterol ester)), and glycerolipids: in ten different herbal tea and their hierarchical cluster analysis. (clustering method: complete, distance measure: Euclidean). MG (monoacylglycerol), DG (diacylglycerol), DGDG (digalactosyldiacylglycerol), MGDG (monogalactosyldiacylglycerol), SQDG (sulfoquinovosyl diacylglycerol). **B.** TG (triacylglycerol). GC, German chamomile; GL, ginkgo; LB, lemon balm; LG, lemon grass; LV, lavender; BM, blue mallow; NL, nettle; PM, peppermint; RG, rooibos green; RR, rooibos red.

Graphical abstract