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Original Research

Plasma Metabolite Profiles of Healthy Volunteers after Administration of a Thai Herbal Formula for Dizziness

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Abstract

The Thai herbal Yahom 20 formula (YHF20), is traditionally used for dizziness and fainting and off-label use for sleep aid, with inadequate substantial evidence afterward. This study's primary objective is to employ metabolomics to investigate YHF20's effects, comparing it with lorazepam and a placebo in healthy volunteers. Phytochemical and metabolite profiling were performed using liquid chromatography-tandem mass spectrometry (LC-MS/MS) and LC/MS Q-ToF, respectively, on plasma samples from 90 healthy participants aged 20 to 60 years. These participants were randomized into three groups: YHF20 (n=30), Lorazepam (n=30), and Placebo (n=30). Principal Component Analysis (PCA) and Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) were then conducted to identify differential metabolites and pathways. Six phytochemicals, including ellagic acid, glycyrrhizic acid, (E)-ferulic acid hexacosyl ester, 6-aldehydo-7-methoxy-isoophiopogonone B, melianol, and myristic acid were identified in YHF20. Despite PCA showing no significant overall metabolite profile differences among the groups, OPLS-DA pinpointed eight YHF20-associated metabolites, such as DHA ethyl ester, α-linolenic acid, (9Z)-9-octadecenamide, ricinoleic acid methyl ester, idazoxan, 13-HPODE, 12,13-DiHODE, and myristoleic acid, implying at anti-inflammatory pathway involvement, especially in α-linoleic and linoleic acid metabolism. No direct impact on sleep-related metabolites was found, the anti-inflammatory effects suggested by YHF20 could indirectly improve sleep quality by mitigating inflammation, a common sleep disruptor. These results highlight YHF20's potential for enhancing life quality through anti-inflammatory mechanisms. They offer a scientific basis for its traditional and anecdotal uses and suggest a novel approach to sleep quality improvement not previously documented.

Keywords: Thai herbal Yahom 20 formula; Herbal medicine; Sleep quality; Metabolomics

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Introduction

Insomnia is a pervasive condition that spans all age groups, notably impacting the working age and elderly populations. Studies estimate that chronic insomnia affects about 30% of the general populace [1], leading to significant detriments in concentration, memory retention, learning capabilities, and causing long-term adverse health outcomes, including reduced life expectancy [2-4]. Conventional treatments for insomnia typically involve a combination of promoting healthy sleep habits, cognitive behavioral therapy (CBT), and pharmacological interventions. Commonly prescribed medications include benzodiazepines, tricyclic antidepressants, and melatonin-receptor agonists, which, while generally effective, are associated with risks of drug resistance, addiction, and various adverse effects ranging from dizziness and confusion to gastrointestinal disturbances [5].

In this context, the Thai herbal Yahom 20 formula (YHF20), a concoction used in Applied Thai Traditional Medicine (ATM) for over three decades, emerges as an intriguing alternative. Comprising 25 ingredients (Supplementary Materials, Table S1), YHF20 is traditionally employed to mitigate symptoms such as dizziness, fainting, and general weakness. Notably, ATM practitioners have observed that YHF20 also appears to enhance sleep quality among patients aged 20–60, a benefit yet to be substantiated by scientific evidence. Preliminary research suggests that certain YHF20 constituents might modulate neurotransmitter levels, potentially explaining its observed effects on sleep. For instance, *Conioselinum anthriscoides* (H. Boissieu) Pimenov & Kljuykov has been reported to elevate central nervous system levels of 5-hydroxytryptamine [6]; while *Glycyrrhiza glabra* L. has shown potential in reducing brain enzyme levels in hypoxic conditions [7]. However, there is inadequate scientific evidence to support the use of YHF20 as a sleep aid.

Despite these promising indications, the scientific community lacks comprehensive evidence validating YHF20's efficacy as a sleep aid. This gap underscores the need for advanced analytical techniques capable of capturing the multifaceted impacts of traditional Thai herbal medicine (TTM). Untargeted metabolomics analysis, by facilitating the profiling of a wide array of plasma metabolites, offers a powerful tool to unearth the biochemical pathways influenced by such herbal interventions. Hundreds of metabolites can be detected by plasma metabolite profiling. This information can be useful to identify the pathways involved in the efficacy of a drug [8]. This approach not only promises to unveil the mechanistic underpinnings of YHF20's effects but also aligns with the holistic ethos of TTM, which seeks to address the root causes of ailments by targeting multiple physiological pathways.

Aimed at bridging this knowledge gap, the present study employs metabolomics to investigate the plasma metabolite profiles of healthy volunteers following administration of YHF20, in comparison to those receiving lorazepam or a placebo. Through this comparative analysis, we seek to elucidate the pharmacological effects and potential therapeutic benefits of YHF20, particularly in relation to sleep enhancement. By scientifically substantiating the anecdotal sleep-related benefits of YHF20, this research endeavors to contribute to the validation of traditional herbal remedies and explore their potential as safer alternatives to conventional insomnia treatments.

Materials and Methods

Ethics and Consent

This study has provided ethical approval by The Siriraj Institutional Review Board (SIRB) of the Faculty of Medicine Siriraj Hospital, Mahidol University (COA no. Si 680/2019), and confirmation that informed consent was obtained. The trial is registered at thaiclinicaltrials.org, number TCTR20240704003, on July 4, 2024.

Study design and participants

The sample size calculation was based on a previous study and conducted using the nQuery Advisor program, aiming for a 95% confidence level and 90% power, resulting in 25 subjects per group. Accounting for an estimated 15% loss to follow-up, a total of 90 participants were required, divided into three groups with 30 participants each. Healthy volunteers aged 20 – 60 years old with proficiency in Thai, including reading and writing skills from Siriraj Institute of Clinical Research, Faculty of Medicine Siriraj Hospital, Mahidol University. Health practitioners screened participants using the Thai Pittsburgh Sleep Quality Index (T-PSQI), Likert scale, and The World Health Organization Quality of Life (WHOQoL)-BREF. Researchers then employed stratified block randomization by T-PSQI scores to assign participants into three groups: YHF20, placebo, and lorazepam. All health practitioners and participants were blinded throughout the process [9]. The Siriraj Institutional Review Board (SIRB) of the Faculty of Medicine Siriraj Hospital, Mahidol University, provided ethical approval (COA no. Si 680/2019). A flow diagram, depicted in Figure 1, outlines the study procedure, including blood sample collection before the initial treatment dose (Day 0) and two weeks post-administration (Day 15), with subsequent storage at -80°C. On Day 0, participants were evaluated with the T-PSQI and WHOQOL-BREF by health practitioners, underwent physical examination, and 15 ml of blood collection for complete blood count, kidney function, and liver function assessment. On Day 15, participants were re-evaluated with the

T-PSQI and WHOQOL-BREF, underwent physical examination, and 15 ml of blood collection by health practitioners for complete blood count, kidney function, and liver function assessment. Data from sleep diaries and actigraphs were also collected [9].

Intervention

The Ayurved Siriraj Manufacturing Unit of Herbal Medicines and Products, under PIC/S GMP standards, produced YHF20 and placebo capsules. Ingredients, sourced within Thailand, underwent authentication by two expert TTM practitioners. The formulation process involved cleaning, weighing, grinding, and encapsulating 250 mg of YHF20 powder per capsule. Placebo capsules, filled with starch and herbally scented to resemble YHF20, alongside lorazepam capsules (0.5 mg each), constituted the study's interventions. Participants in the lorazepam group were instructed to consume one lorazepam capsule and two placebo capsules; while the other groups took three capsules daily before bedtime from Day 8 to Day 14.

Sample preparation for LC-MS/MS analysis

Plasma samples

Plasma samples were thawed on ice. Next, 200 µL of each plasma sample was mixed with 50 µL of 0.25 ng/µL internal standards [caffeine (3-methyl-13C,99%), L-phenylalanine (1-13C, 99%) from Sigma-Aldrich (Missouri, USA) and cholic acid (2,2,4,4-D4, 98%) from IsoSciences (Pennsylvania, USA)]. The samples were extracted by treating them with 600 µL cold methanol (Optima™ LC-MS grade) from Fisher Chemical (Loughborough, UK). The samples were subjected to vortexing and centrifuging at 15800 x g for 15 min at 4 °C, and the supernatant was collected and transferred 300 µL into a microtube and also pipetted 50 µL for TQC into a conical tube 50 ml and mixed before aliquoting 300 µL of TQC into a microtube. After that, set SpeedVac at 30 °C, heater off and cold trap for 300 – 420 minutes until completely dry. Store the dry samples at -80 °C until analysis.

YHF20, lorazepam, and placebo samples

YHF20, lorazepam, and placebo powder were accurately weighed as 250, 2.5, and 2.5 mg, respectively, and mixed with 2 mL methanol under 10 min vortexing and 60 min sonication. Then the mixtures were centrifuged at 12,000 rpm for 10 min at 4 ºC and filtered through a PVDF syringe filter 0.2 µm. Then, the supernatants were diluted with methanol to achieve the desired concentrations. The eventual volume was 1

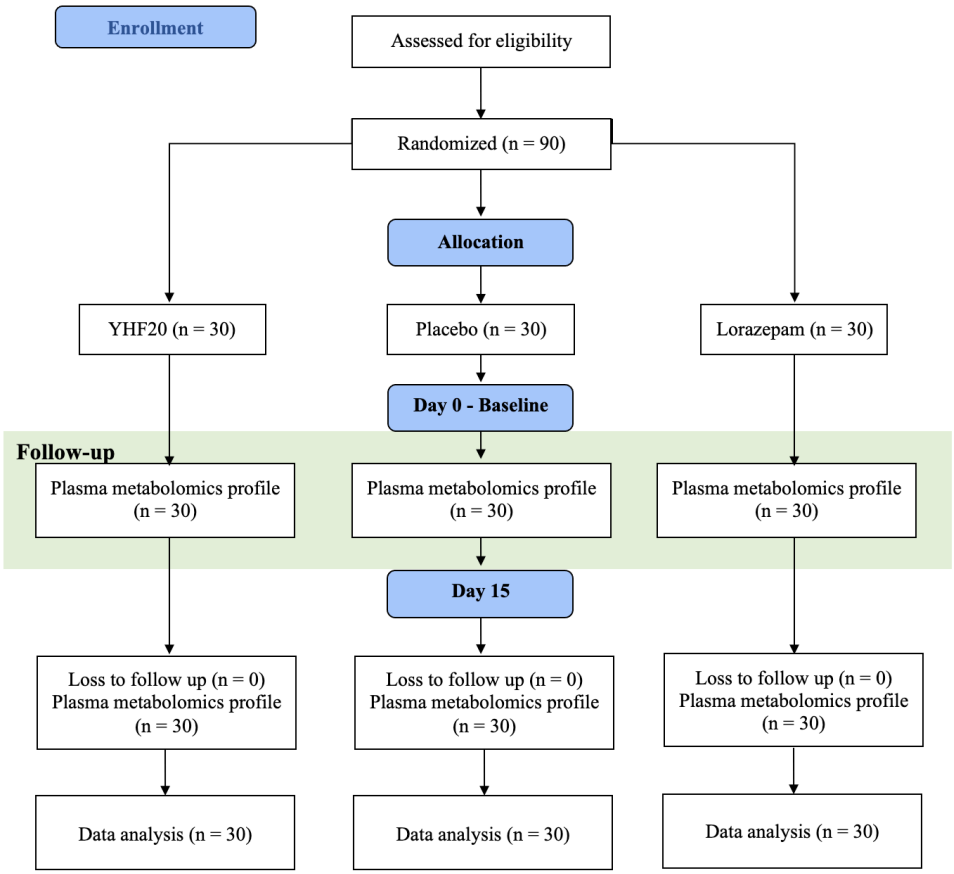

Figure 1. Study flow diagram

mL, including 0.25 mg/μL internal standard solution.

Ultra-high-performance liquid-chromatography with quadrupole time-of-flight tandem mass spectrometry (LCMS-QTOF) analysis

The chromatography technique was performed using a Waters Acquity UPLC® system (Waters Corporation, Milford, USA). The LCMS-QTOF protocol was derived from a previous study [10]. The stationary phase was provided by a Waters ACQUITY HSS T3 (100 mm × 2.1 mm, 1.8 μm) column set at 40 ºC. The auto sampler was maintained at 4 ºC. The mobile phase was combined with 0.1% formic acid in water (A) and 0.1% formic acid in absolute methanol (B). The separation was performed with gradient elution (0 to 100%B in 20 min) and 0.4 mL/min of flow rate.

Analysis of the nontargeted plasma metabolomics profile was performed using a Waters® SYNAPT G2-Si mass spectrometer (Waters Corporation, Milford, USA) with an electrospray ionization (ESI) source in both the positive (ESI+) and negative (ESI-) analysis modes. The MS^E mode was set up as the full scan mode. The MS mass range was determined at 50–1,200 m/z and operated in the continuum mode with a scan time of 0.5 seconds. In the resolution mode, the source conditions were set as follows: capillary voltage at 3 kV, sample cone at 40 V, source offset at 80 V, source temperature at 150°C, desolvation temperature at 500°C, cone gas flow rate at 50 L/h, and N_2 flow rate at 1000 L/h. During data acquisition, 200 pg/mL of leucine enkephalin was continuously infused at 5 μL/min via a lock spray interface to ensure mass accuracy and consistency.

For calibration, 5 mM sodium formate was injected at 20 μL/min. The calibration criteria were: RMS residual mass < 0.5 ppm, 95% confidence band < 0.5 ppm with a threshold < 1 ppm, and all 13 peaks matched in the mass range 50–1200 Da in the resolution mode.

Outcomes - Data analysis

MassLynx™ V4.1 software and UNIFI 1.8.0 (Waters, Manchester, UK) were used for the data acquisition and processing. The potential markers from the UNIFI software were identified using Waters Traditional Chinese medicine library and various online databases, including NIST, KEGG, ChemSpider, and Pubchem.

Multivariate analysis (MVA) was used to investigate the untargeted metabolomics by using the MS^E raw data from UNIFI software transferred to EZinfo software (Waters Corp., MA, USA). Principle component analysis (PCA) and orthogonal projections to the latent structures discriminant analysis (OPLS-DA) were used for analyzing the metabolic profiles in this study. S-plots were used to differentiate selected metabolites between groups of intervention. The criteria for the significantly selected metabolites were as follows: a responding compound > 50000 counts and variable importance in projection (VIP) value > 1, and p-value < 0.05.

The relationship between the metabolites discovered after YHF20 administration related to sleep factors and the metabolic pathways were explored using the MetaboAnalyst database. The pathways were analyzed by considering two major factors: the p value and impact value. MetaboAnalyst calculated the p-values from every study in which each metabolite compound was involved, reported in a -log10(p) form that is inverse to the p value, where a higher -log10(p) means higher reliability.

Results

Plasma metabolomics

The demographic data of the healthy volunteers are shown in table 1. No harm occurred after the administration of all intervention groups.

Tentative identified compounds in YHF20

The various constituents in YHF20 were characterized based on their fragmentation behaviors, accurate mass, and retention times. The total percentage of 90 chemical constituents in YHF20 was 30.4 % in the positive mode (table S2) and 21.7% in the negative mode (Table S3). The majority of the 90 identified constituents belonged to triterpenoids (18%), lipids (15%), and phenols (11%) groups in the positive mode. While the three highest ranked groups in the negative mode were lipids (16%), triterpenoids (15%), and steroids (8%).

PCA analysis of the plasma samples

According to the PCA score plots, the plasma metabolites after the intakes of YHF20, lorazepam, and placebo could not clearly be separated from each intervention in both the negative and positive modes (Figure 2).

OPLS-DA analysis of the plasma samples

The OPLS-DA score plots showed that the plasma metabolites after the intakes of YHF20, lorazepam, and placebo were different before (D0) and after (D15) administration (Figure 3).

Comparing the OPLS-DA score plots between interventions at day 0 and day 15, the OPLS-DA score plots showed that the plasma metabolites were different, while the S-plots showed a small amount of significantly selected metabolites. For more in-depth investigation, the dominant elements or Dhātu Chao Ruean in Thai traditional medicine theory were categorized in the subgroup analysis in the YHF20 group (Figure 4), lorazepam group (Supplementary Materials, Figure S1), and placebo (Figure S2).

Metabolite biomarkers identification

Table 1. Demographic data of the participant

Characteristic data	n (%)		
	YHF20 (n = 30)	Lorazepam (n = 30)	Placebo (n = 30)
Sex			
male	3 (10%)	11 (36.7%)	10 (33.3%)
female	27 (90%)	19 (63.3%)	20 (66.7%)
Age			
20–40	23 (76.7%)	26 (86.7%)	26 (86.7%)
41–60	7 (23.3%)	4 (13.3%)	4 (13.3%)
Dominant element*			
Earth	9 (30%)	11 (36.7%)	10 (33.3%)
Water	5 (16.7%)	6 (20%)	6 (20%)
Wind	9 (30%)	3 (10%)	3 (10%)
Fire	7 (23.3%)	10 (33.3%)	11 (36.7%)

*Dominant elements or Dhātu Chao Ruean in Thai traditional medicine were determined using the participant's month of birth; the Fire group covers December, January, and February; the Wind group covers March, April, and May; the Water group covers June, July, and August; and the Earth group covers September, October, and November [11].

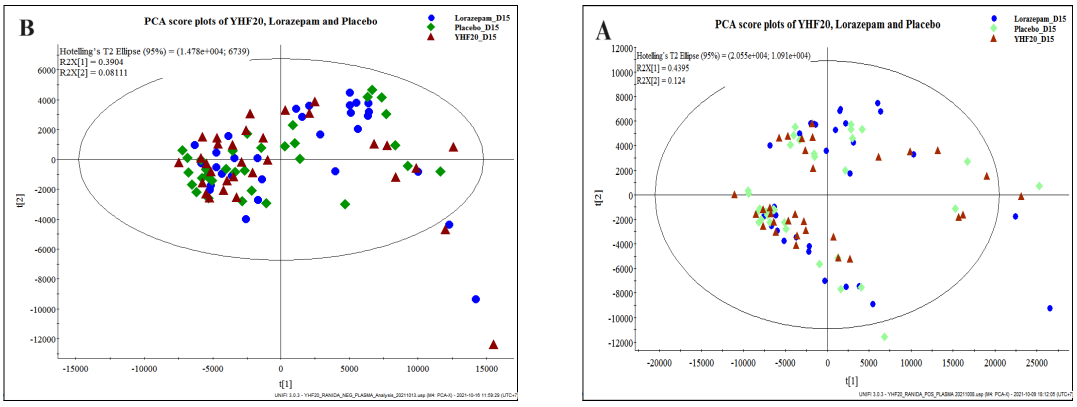

Figure 2. Score plots from PCA analysis of the plasma samples from the YHF20 group (▲), Lorazepam group (●), and placebo group (◆) at Day 15 in the positive mode (A) and negative mode (B).

Overall, 997 metabolites from YHF20 group were qualified by searching the Human Metabolome Database (HMDB), Kyoto Encyclopedia of Genes and Genomes (KEGG), Massbank, and NIST databases. After excluding the repeated tentatively identified compounds in both the positive and negative modes, 8 potential metabolites were identified (Table 2).

Pathway analysis

The results with the highest to lowest impact values were linoleic acid metabolism, α-linoleic acid metabolism, fatty acid biosynthesis, the biosynthesis of unsaturated fatty acids, and fatty acid degradation, respectively (Figure 5).

Discussion

Chemical profiling of Thai herbal Yahom 20 formula (YHF20)

LC-MS/MS analysis of the Thai herbal Yahom 20 formula (YHF20) tentatively identified 90 markers across phenols, triterpenoids, steroids, and lipids. These compounds are known for their anti-inflammatory and antioxidant properties [12-23], corroborating the therapeutic potential of YHF20. Notably (Tables S2 and S3), substances such as (E)-ferulic acid hexacosyl ester and glycyrrhizic acid, found in recognized medicinal plants like *Dracaena cochinchinensis* and *Glycyrrhiza glabra*, underscore the formula's rich phytochemical composition.

Notably (Tables S2 and S3), substances such as (E)-ferulic acid hexacosyl ester, 6-aldehydo-7-methoxy-isoophiopogonone B, ellagic acid, glycyrrhizic acid, melianol, and myristic acid, found in recognized medicinal plants like *Dracaena cochinchinensis* (Lour.) S.C.Chen, *Glycyrrhiza glabra* L., *Terminalia chebula* Retz., *Aquilaria crassna* Pierre ex Lecomte, and *Myristica fragrans* Houtt. *G.glabra*,

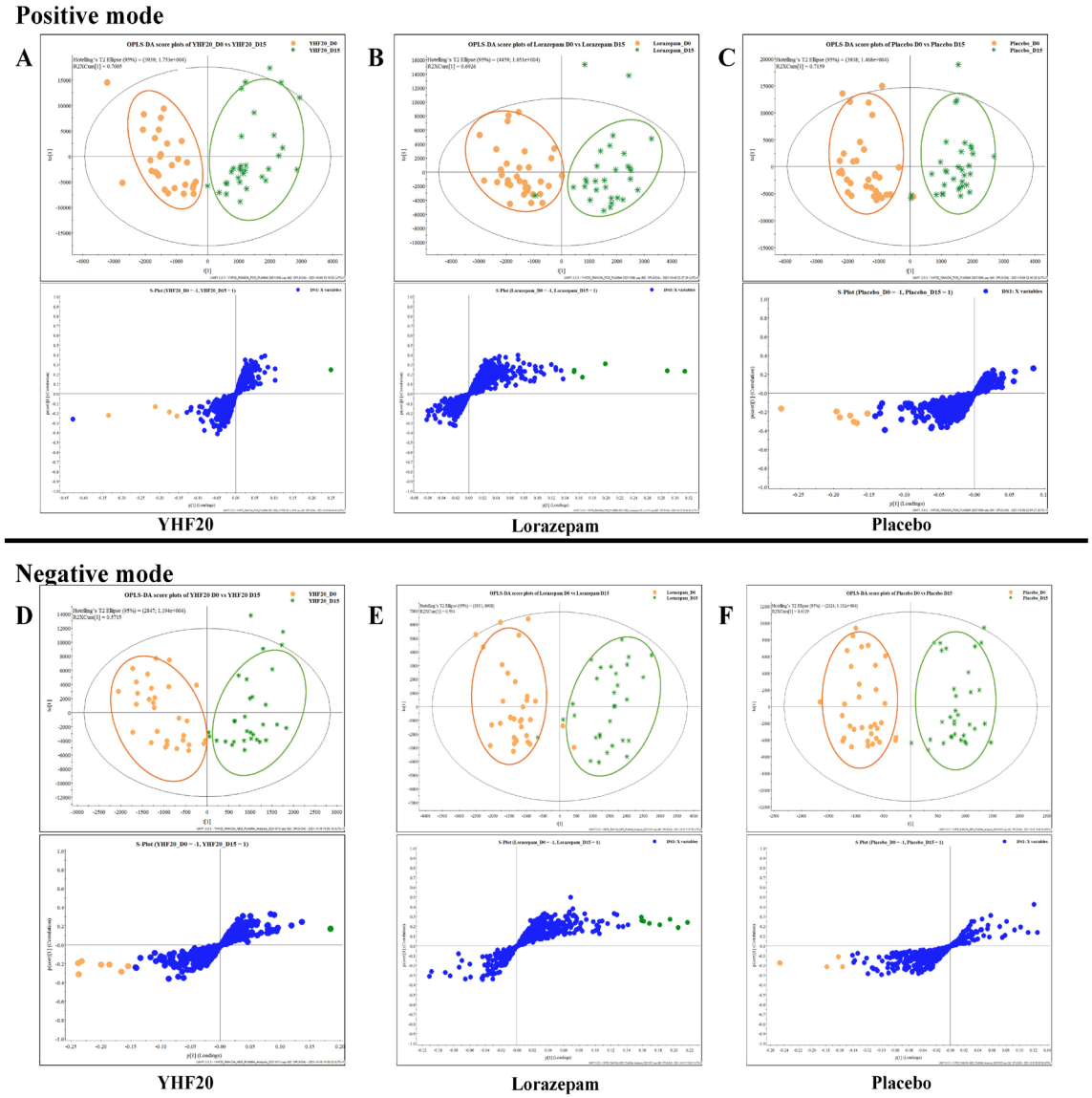

Figure 3. OPLS-DA analysis and the corresponding S-plots of the plasma samples between before (●) and after (∗) the administration of (A) YHF20 treatment in the positive mode, (B) Lorazepam treatment in the positive mode, (C) Placebo treatment in the positive mode, (D) YHF20 treatment in the negative mode, (E) Lorazepam treatment in the negative mode, (F) Placebo treatment in the negative mode.

underscore the formula's rich phytochemical composition.

Relationship between YHF20 phytochemicals and plasma metabolic profiles of the healthy volunteers after YHF20 administration

Upon administration of YHF20 to healthy volunteers, eight metabolites associated with sleep and mood regulation were identified, including (9Z)-9-octadecenamide, known for its sedative effects [24] such as drowsiness or sleep and reduced psychological excitement or anxiety, and ethyl docosahexaenoate (DHA ethyl ester), with pronounced anti-inflammatory benefits and positive impact on cardiovascular health. [25]. These findings suggest a complex interaction between YHF20's phytochemicals and the body's metabolic pathways, influencing sleep, mood, and inflammation. Nisinic acid (α-linolenic acid), an omega-3 fatty acid, has been reported to inhibit prostaglandin synthesis, potentially leading to reduced inflammation. These inflammatory substances tend to increase in people who suffering from insomnia or poor sleep quality, as exhibited by elevated systemic markers of inflammation, such as C-reactive protein and interleukin-6 [44]. Furthermore, with advancing age, there is an increase in inflammatory substances. Some of these elevated

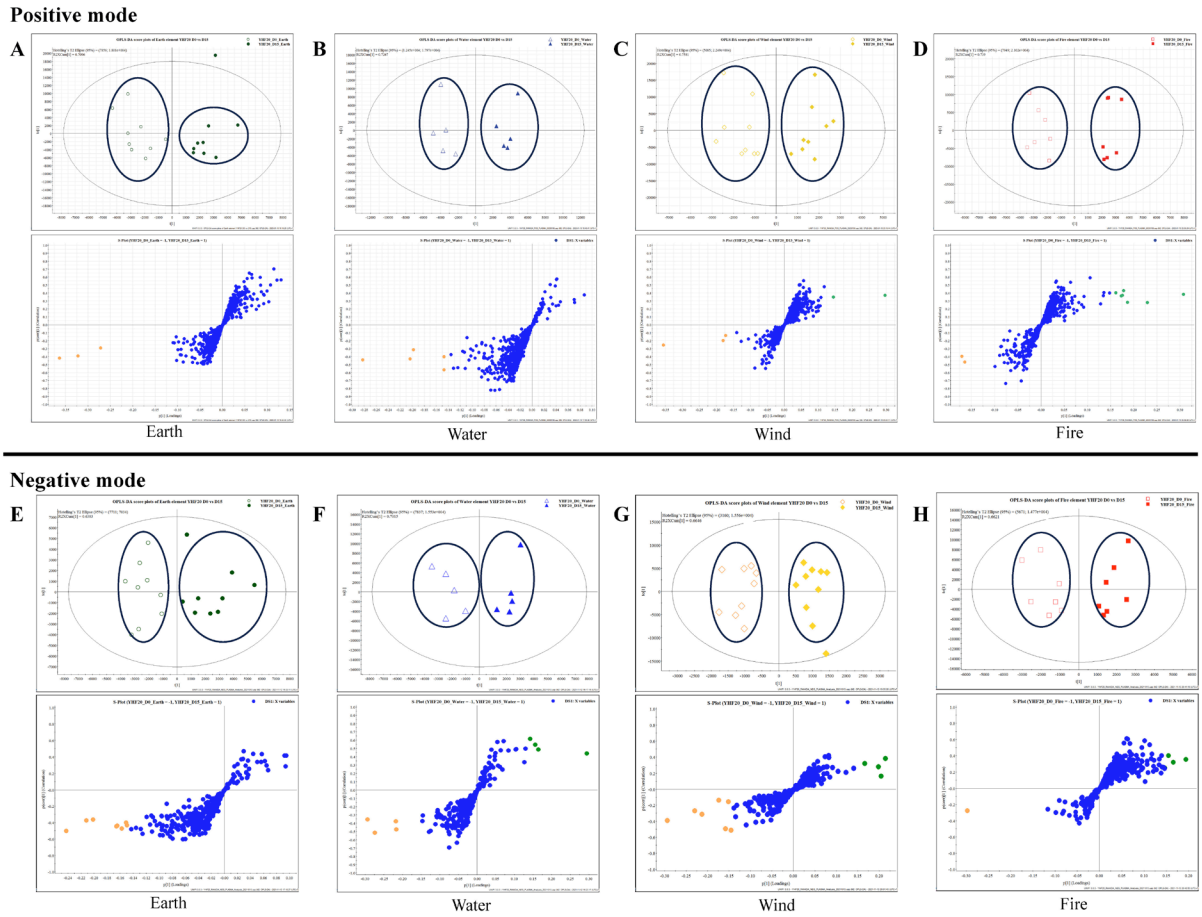

Figure 4. OPLS-DA score plots and S-plots of plasma samples from healthy volunteers with different dominant elements before and after the intake of YHF20. (A) Earth element in the positive mode, (B) Water element in the positive mode, (C) Wind element in the positive mode, (D) Fire element in the positive mode, (E) Earth element in the negative mode, (F) Water element in the negative mode, (G) Wind element in the negative mode, (H) Fire element in the negative mode.

inflammatory markers can significantly impact health, potentially leading to conditions like heart disease or depression. However, sufficient and high-quality sleep can decrease these inflammatory markers, help prevent sleep disturbance, and prolong sleep duration [26,41]. Nisinic acid can also balance neurotransmitter levels, such as serotonin [42], which have a role in regulating arousal and phasic events in the REM sleep cycle [43].

Linoleic acid, an omega-6 fatty acid, has been reported to regulate neuropharmacological effects. Adequate quantities and appropriate ratios of α-linolenic to linoleic acid can improve sleep quality [42].

Ricinoleic acid methyl ester, which serves as a substrate for the synthesis of conjugated linoleic acids, plays a role in the formation of specific fatty acids [27]. Idazoxan was investigated as an antidepressant and as an adjunctive treatment for schizophrenia. Its potential effects on enhancing dopamine neurotransmission in the prefrontal cortex of the brain make it of interest in these contexts [28]. 13-HPODE (13-hydroperoxyoctadecadienoic acid) and 12,13-DiHODE (12,13-dihydroxyoctadecadienoic acid) were derivatives of linoleic acid. Linoleic acid is an essential omega-6 fatty acid found in various dietary sources. These derivatives may have specific roles in biological processes and signaling pathways [29]. Myristoleic acid (9-tetradecenoic acid) is an omega-5 fatty acid, which exhibited various physiological effects and potential health benefits [30].

All these eight metabolites can be categorized into many groups. Lipids are one of those interesting results, and we found some of the components in YHF20 that were not only related to mood and sleep disorders, such as α-linoleic and linoleic fatty acid [31,32], but also played a crucial role in psychiatric health, the pathophysiology of neurodegenerative disorders, such as Alzheimer's disease, and contribute to the protection and maintenance of the neuron membrane.

Myristoleic acid was found in plasma and originates from the *Myristicaceae* plant family. It is a marker substance present in the YHF20 recipe which can be

Table 2. List of potential identified metabolites in plasma before and after YHF20 administration
Biosynthesis of unsaturated fatty acids

No.	m/z	Retention time (min)	Elemental composition	i-FIT Confidence (%)	Common name	Before	After
1	203.0825	4.90	$C_{11}H_{12}N_2O_2$	99.68	Idazoxan	✓	✓
2	357.2791	14.80	$C_{24}H_{36}O_2$	85.12	Ethyl docosahexaenoate (DHA ethyl ester)	✓	✓
3	357.2791	14.80	$C_{24}H_{36}O_2$	85.12	Nisinic acid (**α**-Linolenic acid)	✓	✓
4	282.2792	15.98	$C_{18}H_{35}NO$	100.00	(9Z)-9-Octadecenamide	✓	✓
5	313.2739	16.00	$C_{19}H_{36}O_3$	99.99	MFCD00046712 (Ricinoleic acid methyl ester)	✓	✓
6	311.2220	14.75	$C_{18}H_{32}O_4$	94.81	13-HPODE	✓	✓
7	311.2220	14.75	$C_{18}H_{32}O_4$	94.81	12,13-DiHODE	✓	✓
8	225.1860	15.22	$C_{14}H_{26}O_2$	100.00	Myristoleic acid (9-tetradecenoic acid)	✓	✓

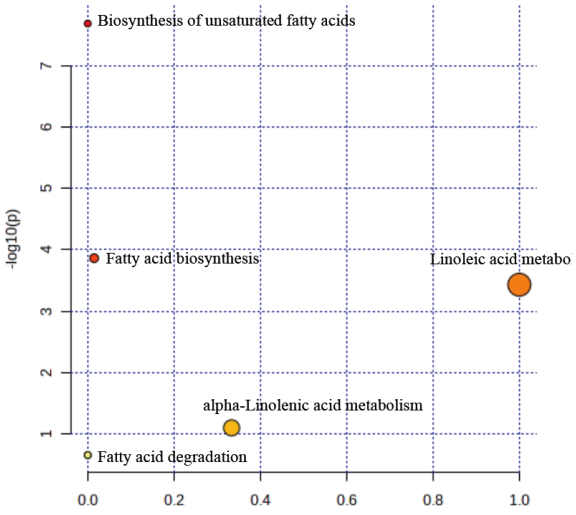

Figure 5. Summary of the pathway analysis results obtained using MetaboAnalyst

derived from *Myristica fragrans* Houtt. and can also be categorized as a secondary ingredient in the recipe [33,34]. The presence of myristoleic acid in plasma suggests that the human body is capable of biosynthesizing it, from precursor compounds such as myristic acid. This correlation highlights the intricate relationships between dietary components, herbal medicine, biosynthesis, and their impact on the metabolic profile. α-Linoleic acid in most potential target pathways (Figure 5) is one of the omega-3 polyunsaturated fatty acids (PUFAs) that have been shown to have multiple beneficial effects in cardiovascular disease, anti-inflammatory and antithrombotic properties, neuroprotective effects, improved endothelial dysfunction, and can positively affect the resting heart rate (HR), HR variability, heart rhythm, and cardiac remodeling [35,36].

Besides, omega-3 fatty acids (α-linoleic acid) not only play a role in preventing the production of pro-inflammatory factors, but also in inhibiting the formation of omega-6 fatty acids. Omega-6 fatty acids are potent precursors for inflammatory mediators, even though they are still constituent components of membrane phospholipids [37,38]. These inflammatory substances are increased in people who suffer from insomnia or poor sleep quality, by increasing the systemic markers of inflammation, such as C-reactive protein and interleukin-6 [39]. Further, with a rising age, there is an increase in inflammatory substances. Some of these elevated inflammatory markers can have significant impacts on health, potentially leading to conditions like heart disease or depression. However, sufficient and high-quality sleep has the potential to decrease these inflammatory markers. Also, sleeping behavioral adjustments can not only help in alleviating insomnia symptoms but also contribute to reducing the inflammatory processes associated with aging, ultimately enhancing the overall quality of life [40].

According to the similarities among all groups from PCA analysis, this implied that YHF20 did not interfere metabolites of healthy volunteers. However, since our study was performed in disease absent subjects, the effects of the treatment to metabolites that are abnormal only in patients might be marginal. Therefore, we suggest that further studies should be performed.

Conclusion

The administration of YHF20 showed no direct mod-

ulation of plasma metabolites directly associated with sleep regulation. Nevertheless, its influence on anti-inflammatory pathways suggests potential benefits in addressing inflammation related to poor sleep quality (Figure 6). Future research should delve into the clinical efficacy of YHF20 as a sleep aid, focusing on its anti-inflammatory mechanisms, to validate its application in managing insomnia.

Funding

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Supplementary Materials

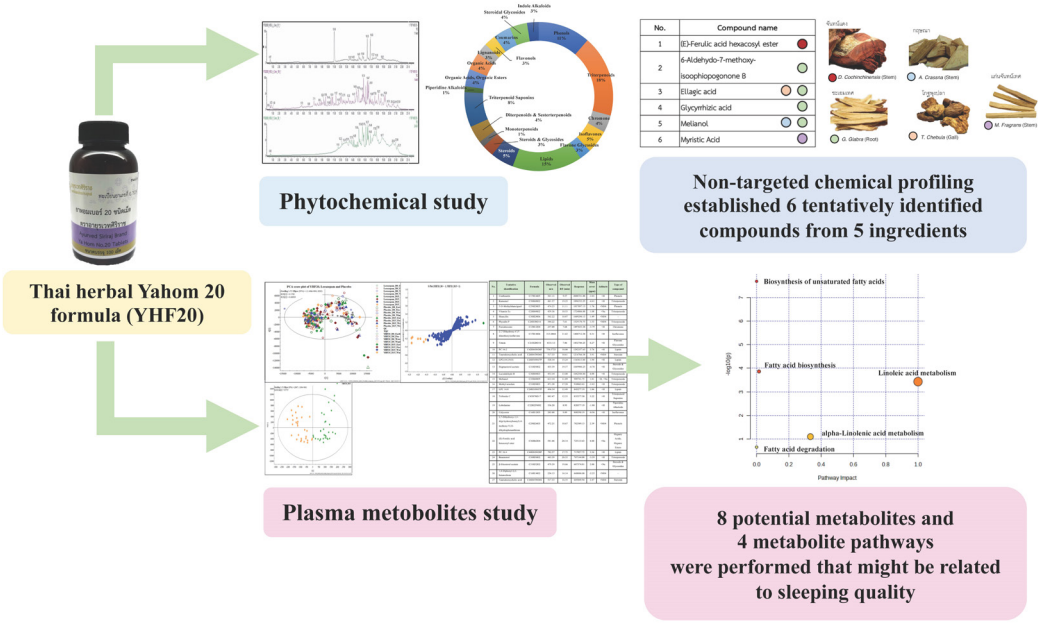


Figure 6. Summary of the study: This study investigated the phytochemical profiles of YHF20 and plasma metabolites of healthy volunteers after the intake of YHF20.

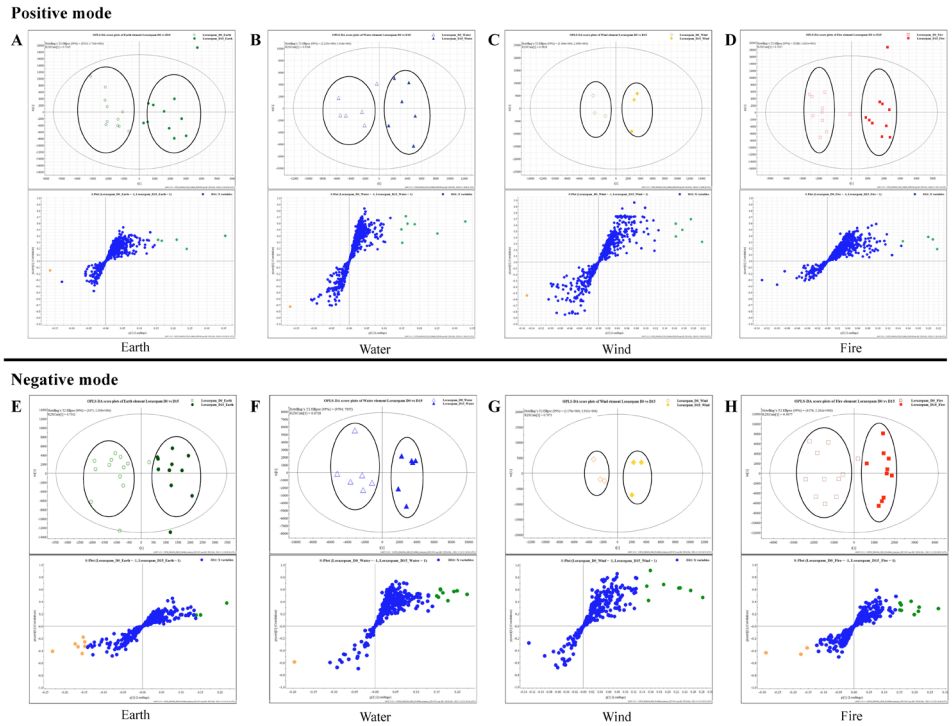


Figure S1. OPLS-DA score plots and S-plots of plasma samples from healthy volunteers with different dominant elements before and after the intake of lorazepam. (A) Earth element in the positive mode, (B) Water element in the positive mode, (C) Wind element in the positive mode, (D) Fire element in the positive mode, (E) Earth element in the negative mode, (F) Water element in the negative mode, (G) Wind element in the negative mode, (H) Fire element in the negative mode.

Table S1. Ingredients of YHF20 and information on their constituents and pharmacological effects

Name	Part of usage	Constituents	Pharmacological effects
		Plant-derived materials	
Euphorbia antiquorum L.	Stem	Terpenoids, coumarins, flavonoids, polyphenols	-
Wurfbainia testacea (Ridl.) Škorničk. & A.D.Poulsen (Synonym - *Amomum testaceum* Ridl)	Fruit	Alkaloid, Flavonoids, Terpenoids	Antimicrobial effect, Antioxidant effect
Aquilaria crassna Pierre ex Lecomte	Stem	β-caryophyllene	Antimicrobial activity, Anti-inflammatory effect, Anti-ischemic effect
Syzygium aromaticum (L.) Merr. & L.M. Perry	Flower	Eugenol, flavonoids, tannins, terpenoids, polyphenols	Hypoglycemic effects, Antioxidant effect, Increasing sexual behavior, Effect of kidney function, Gastroprotective activity, Effect of learning and memory, Cardiac effect, Antihyperlipidemic effect, Immunomodulatory activity, Central nervous system effects, Anticancer activities
Artemisia pallens Wall. ex DC.	Aerial part	Cirsimaritin, Davana acid, Davanon	-
Angelica sinensis (Oliv.) Diels	Root	Alkylphthalides, terpenoids, phenylpropanoids, coumarins, sulfonamide	Antioxidant activity, Hematopoietic activity, Anti-aortic tension,
Terminalia chebula Retz.	Gall	Tannins, phenolics, triterpenoids, flavonoids, polyphenols, saponins	Antiviral activity, Antioxidant activity, Anti-inflammatory activity, Anti-aging
Conioselinum anthriscoides (H. Boissieu) Pimenov & Kljuykov (Synonym - *Ligusticum sinense* Oliv.)	Rhizome	Cnidium lactone, cnidic acid, phthalide, ferulic acid, ligustiphenol, Alkaloids, Polyphenols	Vasorelaxation activity, Antioxidant effect, Anti-inflammatory activity, Progestogenic activity, Anti-atherosclerotic activity
Mimusops elengi L.	Natural fungal infected wood	Terpenoids, saponin, alkaloids, tannins, flavonoids	Antioxidant effect, Wound healing activity, Anti-ulcer activity, Diuretic activity, Hypotensive activity, Antihyperlipidemic activity, Analgesic activity, Antipyretic activity
Dracaena cochinchinensis (Lour.) S.C.Chen.	Stem	Homoisoflavanones, retrodihydrochalcones, Stilbenes, flavonoids, terpenoids	Antimalarial activity, Antinociceptive activities, Antipyretic activity, Estrogenic activity, Anti-HIV-1 integrase activity, Anti-inflammatory effect

Myristica fragrans Houtt.	Fruit and Stem	Resorcinols, dihydroguaiaretic acid, aroma glycosides, lignans, neolignans, terpenes, macelignan, neolignan(myrislignan),	Antibacterial activity, Antihyperglycemic activity, Anticonvulsant activity, Antidepressant-like activity
Alyxia reinwardtii Blume	Bark	Coumarins, iridoiddiglucoside, macrolactone glycoside	Antifungal activity
Glycyrrhiza glabra L.	Root	Triterpene Saponins, Flavonoids, Isoflavonoids, Coumarins, Stilbenes, gums and wax	Effects on GABAA receptors Cerebroprotective effect, Anticonvulsant activity, Anti-inflammatory activity, Antiallergic effects, Antioxidant activity, Analgesic, uterine relaxant effects, Antidepressant-like activity, Effect of gastrointestinal motility
Nelumbo nucifera Gaertn.	Stamen	Flavonoids, Alkaloid, β-sitosterol glucopyranoside, adenine, myo-inositol, arbutin	Sleep-promoting activity Antioxidant effects, Anti-inflammatory effects, Anti-allergic effect
Mesua ferrea L.	Flower	Coumarins, Flavonoids, mesuol, mesuone	Antibacterial effect, Central nervous system effects, Anti-inflammatory effect, Anticonvulsant effect
Chrysopogon zizanioides (L.) Roberty	Root	Essential oil	Anti-inflammatory activity, Antimycobacterial activity, Antioxidant activity, Anticonvulsant activity,
Mimusops elengi L.	Flower	Volatile oil, Sugar, Phytosterols	Antioxidant effect, Diuretic effect, Cardiovascular effect
Urceola rosea (Hook. & Arn.) D.J.Middleton	Stem	Ecdysantherin, 20-Epi-Kibataline, 3β, 14β, 20-trihydroxy-18oic (18→20) lactone pregnen-5, 5-O-caffeoylquinic derivers, scopoletin, tartaric acid, malic acid, phytosterol, triterpenoid, saponine, D-friedours-14-en-11α, 12α-epoxy-3β-yl palmitate	Central nervous system depressant effect
Cinnamomum bejolghota (Buch.-Ham.) Sweet	Bark	1,8-cineole, α-terpineol, linalool, α-pinene, β-pinene	Antibacterial activity, Antihyperglycemic effect
Mammea siamensis (Miq.) T.Anderson	Flower	β-sitosterol, stigmasterol, Triterpenoids, Coumarins, 3,4-dihydrobenzoic acid, gallic acid	Antimicrobial effect, Antioxidant activity, Anticancer activity

Cinnamomum verum J.S. Presl.	Bark	Essential oil, Benzaldehyde, Benzylalcohol, Benzoic acid, α-phellandrene, linalool, linalylacetate, benzyl cinnamate	Antibacterial activity, Antifungal activity, Antiparasitic activity, Antioxidant activity, Anti-inflammatory, Anti-arthritic activity, Anti-asthma activity, Anti-hypertensive effect, Anticoagulant effect, Anti-secretagogue, Anti-ulcer effects, Wound Healing effect, Chemopreventive effects, Anti-diabetic effect
Cinnamomum loureirii Nees.	Bark	Tannins, cinnamic acid, cinnamaldehyde, cinnamyl alcohol	Analgesic, Anti-inflammation activity, Antihyperglycemia activity, Collagen biosynthesis
		Compounds	
Sodium Tetraborate	-	Sodium Tetraborate	-
Borneol camphor	-	1,7,7-Trimethyl- bicyclo[2.2.1] heptan-2-one	Antihypertensive effect, Antioxidant effect, Advanced Drug Delivery Systems

Table S2. Tentatively identified compounds of YHF20 in Positive mode

No.	RT (min)	Formula	Adducts	Calculate Mass (Da)	Mass Error (ppm)	Response	Identification	Phytochemical group
1	9.57	$C_{17}H_{16}O_5$	^+H	301.11	-3.01	2088721.88	Confusarin	Phenols
2	19.25	$C_{30}H_{48}O_2$	^+H	441.37	-4.41	1894332.25	Kansenol	Triterpenoids
3	11.11	$C_{29}H_{28}O_5$	$^+NH_4$	474.23	1.76	1857897.13	5-O-Methylshanciguol	Phenols
4	18.55	$C_{28}H_{48}O_2$	^+Na	439.36	1.08	1724806.88	Vitamin Eγ	Triterpenoids
5	10.87	$C_{30}H_{28}O_6$	$^+NH_4$	502.22	1.49	1609290.13	Shancilin	-
6	7.61	$C_{28}H_{30}O_{10}$	$^+NH_4$	544.22	1.53	1529170.75	Physalin P	Triterpenoids
7	7.68	$C_{15}H_{12}O_4$	^+H	257.08	-3.79	1497655.38	Furoaloesone	Chromone
8	11.65	$C_{17}H_{14}O_6$	^+H	315.0864	0.31	1488712.38	2′,7-Dihydroxy-4′,5′-dimethoxyisoflavone	Isoflavones
9	7.86	$C_{21}H_{20}O_{10}$	^+H	433.113	0.27	1452744.25	Tetuin	Flavone Glycosides
10	18.00	$C_{42}H_{80}NO_8P$	^+H	758.5723	3.76	1292357.63	PC 34:2	Lipids
11	18.61	$C_{26}H_{45}NO_6S$	$^+NH_4$	517.33	3.91	1216766.38	Taurodeoxycholic acid	Steroids
12	15.24	$C_{26}H_{50}NO_7P$	^+H	520.34	1.94	1163613.00	LPC(18:2/0:0)	Lipids
13	19.37	$C_{31}H_{50}O_2$	^+H	455.39	-4.70	1049980.25	Stigmasterol acetate	Steroids & Glycosides

14	13.00	$C_{30}H_{44}O_3$	^+H	453.34	0.88	1042544.44	Lucialdehyde B	Triterpenoids
15	11.89	$C_{35}H_{48}O_9$	^+H, ^+Na	613.34	1.81	989741.75	Melianol	Triterpenoids
16	17.58	$C_{31}H_{50}O_3$	^+H	471.38	-3.43	910063.63	Methyl ursolate	Triterpenoids
17	15.49	$C_{24}H_{50}NO_7P$	^+H	496.34	1.06	845277.19	LPC 16:0	Lipids
18	12.33	$C_{45}H_{70}O_{17}$	^+H	883.47	3.22	835377.50	Trifoside C	Triterpenoid Saponins
19	8.95	$C_{22}H_{25}NO_2$	^+H	336.20	-1.88	820577.19	Lobelanine	Piperidine Alkaloids
20	9.89	$C_{16}H_{12}O_5$	^+H	285.08	-0.96	808390.19	Calycosin	Isoflavones
21	10.67	$C_{29}H_{26}O_5$	$^+NH_4$	472.21	2.39	782389.13	2,7-Dihydroxy-1,3-di(p-hydroxybenzyl)-4-methoxy-9,10-dihydrophenanthrene	Phenols
22	20.14	$C_{36}H_{62}O_4$	^+Na	581.46	4.00	725113.63	(E)-Ferulic acid hexacosyl ester	Organic Acids, Organic Esters
23	17.75	$C_{44}H_{80}NO_8P$	^+H	782.57	3.36	717857.75	PC 36:4	Lipids
24	20.35	$C_{30}H_{50}O_2$	^+H	443.39	-3.59	707144.88	Bauenenol	Triterpenoids
25	19.66	$C_{31}H_{52}O_2$	^+Na	479.39	2.00	687374.81	β-Sitosterol acetate	Steroids & Glycosides
26	16.14	$C_{16}H_{14}O_2$	$^+NH_4$	256.13	-2.25	668006.00	1,4-Diphenyl-2,3-butanedione	-
27	16.35	$C_{26}H_{45}NO_6S$	$^+NH_4$	517.33	2.47	605849.94	Taurodeoxycholic acid	Steroids
28	10.57	$C_{15}H_{14}O_3$	^+H	243.10	-3.24	596081.00	3,5-Dihydroxy-4'-methoxystilbene	Phenols
29	12.46	$C_{16}H_{16}O_3$	^+H	257.12	-1.86	593780.94	Orchinol	Phenols
30	11.09	$C_{16}H_{12}O_6$	^+H	301.07	-1.20	498700.19	Hydroxygenkwanin	Flavones
31	13.20	$C_{21}H_{22}O_4$	^+H	339.16	-3.06	495117.56	Anhydronotoptol	Monoterpenoids
32	19.66	$C_{35}H_{68}O_5$	+Na	591.50	2.76	468241.69	1,2-Dipalmitoyl-SN-Glycerol	Lipids
33	17.28	$C_{33}H_{34}N_4O_3$	^+H	535.27	4.70	441465.09	Pyrophaeophorbide A	Organic Acids
34	20.55	$C_{30}H_{54}O_2$	+Na	469.40	1.42	441121.94	Tamarixinol	Phenols
35	5.70	$C_{16}H_{18}O_9$	^+H	355.10	-2.30	413998.97	4-O-Caffeoylquinic acid	Organic Acids
36	18.41	$C_{42}H_{82}NO_8P$	^+H	760.59	2.51	401192.53	PC 34:1	Lipids
37	15.66	$C_{26}H_{52}NO_7P$	^+H	522.36	1.79	400008.72	LPC 18:1	Lipids
38	8.56	$C_{14}H_{12}O_3$	^+H	229.08	-4.78	393200.28	2,4,7-Trihydroxy-9,10-dihydrophenanthrene	Phenols
39	12.74	$C_{17}H_{14}O_2$	^+H	251.11	-2.90	382469.84	2-(2-Phenylethyl) chromone	Chromone

40	16.87	$C_{40}H_{64}O_{12}$	^{+}H	737.44	-4.90	378486.88	Hederagenin-3-O-β-D-xylopyrano-	Triterpenoid Saponins
41	12.05	$C_{20}H_{22}O_4$	^{+}H	327.16	-3.51	365361.25	Dehydrodiisoeugenol	Lignanoids
42	8.44	$C_{15}H_{10}O_7$	^{+}H	303.05	-1.69	363522.34	Herbacetin	Flavonols
43	9.07	$C_{15}H_{10}O_6$	^{+}H	287.05	-2.21	363519.44	5,7,2',5'-Tetrahydroxy-flavone	Triterpenoids
44	12.48	$C_{45}H_{70}O_{17}$	^{+}H	883.47	2.98	357290.09	Trifoside C	Triterpenoid Saponins
45	10.70	$C_{16}H_{14}O_5$	^{+}H	287.09	-2.05	346846.81	3,4-Dihydro-5-hydroxy-4-(4′-hydroxy-phenyl)-7-methoxycoumarin	Coumarins
46	5.54	$C_{16}H_{18}O_9$	^{+}H	355.10	-2.62	338208.81	4-O-Caffeoylquinic acid	Organic Acids
47	13.00	$C_{36}H_{54}O_{10}$	^{+}H	647.38	1.73	337565.50	Abrusoside A	Steroidal Glycosides
48	11.16	$C_{18}H_{18}O_5$	^{+}H	315.12	-2.27	335098.28	(E)-3-(2,3,4-Trimetho-xyphenyl) acrylic acid	-
49	8.31	$C_{15}H_{10}O_7$	^{+}H	303.05	-1.50	334913.72	Herbacetin	Flavonols
50	9.18	$C_{15}H_{12}O_4$	^{+}H	257.08	-3.63	330160.66	Furoaloesone	Chromone
51	16.12	$C_{25}H_{24}O_5$	^{+}H	405.17	2.31	319847.31	Puerarol	Coumarins
52	11.45	$C_{30}H_{30}O_5$	$^{+}NH_4$	488.24	1.56	319176.16	2,6-Bis(4-hydroxyphenyl)-3′,5-dimethoxy-3-hydroxybibenzyl	Phenols
53	16.55	$C_{27}H_{36}O_6$	^{+}H, ^{+}Na	457.26	0.32	308903.31	Ganolactone	Triterpenoids
54	18.63	$C_{44}H_{84}NO_8P$	^{+}H	786.60	3.14	304662.09	PC 36:2	Lipids
55	18.10	$C_{44}H_{82}NO_8P$	^{+}H	784.59	1.98	303892.91	PC 36:3	Lipids
56	8.78	$C_{30}H_{26}N_4O_6$	$^{+}NH_4$	556.22	-0.09	301001.38	Picrasidine R	Indole Alkaloids
57	19.05	$C_{29}H_{48}O$	^{+}H, ^{+}Na	413.38	0.05	292886.44	β-Sitosterone	Triterpenoids
58	7.93	$C_{26}H_{28}O_{10}$	$^{+}NH_4$	518.20	1.21	291261.72	12-α-Hydroxyevodol	Diterpenoids & Sesterterpenoids
59	16.97	$C_{30}H_{46}O_4$	^{+}Na, ^{+}H	493.33	1.98	287656.22	16,23-Epoxy-alisol B	Steroids
60	9.11	$C_{16}H_{12}O_4$	^{+}H	269.08	-3.23	287042.44	Formononetin	Isoflavones
61	18.33	$C_{34}H_{58}O_4$	^{+}H	531.44	-3.14	284479.66	Lignoceryl ferulate	Organic Acids, Organic Esters
62	17.31	$C_{40}H_{64}O_{12}$	^{+}H	737.44	-4.45	276454.22	Hederagenin-3-O-β-D-xylopyrano-	Triterpenoid Saponins
63	10.97	$C_{15}H_{10}O_5$	^{+}H	271.06	0.86	269887.25	1,3-Dihydroxy-2-methoxy-anthraquinone	Quinonoids

64	19.66	$C_{29}H_{50}O_2$	^{+}Na	453.37	0.69	268870.28	Vitamin Eα	Triterpenoids
65	15.65	$C_{22}H_{28}O_6$	^{+}H, ^{+}Na	389.20	-0.06	268266.38	Quassin	Diterpenoids & Sesterterpenoids
66	12.83	$C_{18}H_{16}O_7$	^{+}H	345.10	-0.47	262375.53	5,4'-Dihydroxy-6,7,8-trimethoxyflavone	Flavones
67	8.39	$C_{27}H_{30}O_{14}$	^{+}H	579.17	4.97	258908.14	Daidzein-4′,7-diglucoside	Isoflavone Glycosides
68	8.21	$C_{21}H_{18}O_9$	^{+}H	415.10	1.24	240956.50	Pinnatifinoside A	Flavone Glycosides
69	9.25	$C_{16}H_{14}O_4$	^{+}H	271.10	-3.67	224664.80	2,5-Dihydroxy-4,9-dimethoxyphenanthrene	Phenols
70	17.71	$C_{42}H_{78}NO_8P$	^{+}H	756.56	1.85	218609.95	PC 34:3	Lipids
71	8.75	$C_{28}H_{30}O_{10}$	$^{+}NH_4$	544.22	1.84	215339.03	Physalin P	Triterpenoids
72	13.00	$C_{30}H_{46}O_4$	^{+}H	471.35	-0.63	214477.47	16,23-Epoxy-alisol B	Steroids
73	18.78	$C_{40}H_{64}O_{11}$	^{+}H	721.45	-2.43	207169.89	Progenin III	Steroidal Glycosides
74	12.66	$C_{30}H_{44}O_4$	^{+}H	469.33	-0.31	206346.13	Ganoderic acid DM	Triterpenoids
75	8.84	$C_{16}H_{12}O_4$	^{+}H	269.08	-3.29	201032.20	Formononetin	Isoflavones
76	12.35	$C_{50}H_{80}O_{21}$	^{+}H	1017.53	1.87	199549.84	Pariphyllin B	Steroidal Glycosides
77	19.33	$C_{31}H_{50}O_3$	^{+}H	471.38	-4.07	196715.33	Methyl ursolate	Triterpenoids, Shijunzi
78	7.25	$C_{15}H_{14}O_2$	$^{+}NH_4$	244.13	-3.84	190433.91	Benzyl phenylacetate	Organic Acids, Organic Esters
79	13.02	$C_{20}H_{16}O_5$	^{+}H	337.11	0.23	189412.52	Isopsoralidin	Coumarins
80	18.90	$C_{39}H_{81}N_2O_6P$	^{+}Na	727.57	-3.71	185258.13	C16DH Sphingomyelin	Lipids
81	8.30	$C_{27}H_{30}O_{14}$	^{+}H	579.17	4.66	183775.67	Daidzein-4′,7-diglucoside	Isoflavone Glycosides
82	9.11	$C_{22}H_{22}O_9$	^{+}H	431.13	0.09	183239.80	Ononin	-
83	13.98	$C_{42}H_{64}O_{16}$	^{+}H	825.43	2.29	181345.33	3-O-β-D-Galactopyra-no-	Triterpenoid Saponins
84	16.11	$C_{26}H_{54}NO_7P$	^{+}H	524.37	0.81	180136.34	LPC 18:0	Lipids
85	17.36	$C_{31}H_{46}O_4$	^{+}H	483.35	1.36	179801.28	Poricoic acid C	Triterpenoids
86	10.59	$C_{20}H_{20}O_6$	^{+}H	357.13	0.12	179054.89	Fibleucin	Diterpenoids & Sesterterpenoids
87	14.22	$C_{30}H_{37}N_5O_5$	$^{+}NH_4$	565.32	4.86	176815.14	Ergosine	Indole Alkaloids
88	15.65	$C_{18}H_{18}O_5$	^{+}H	315.12	-0.61	176657.25	(E)-3-(2,3,4-Trimetho-xyphenyl) acrylic acid	-
89	10.36	$C_{22}H_{24}O_8$	^{+}H	417.15	1.00	176481.06	(+)-Acetoxypinoresinol	Lignanoids
90	13.00	$C_{42}H_{62}O_{16}$	^{+}H	823.41	2.45	174692.58	Glycyrrhizic acid	Triterpenoid Saponins

Supplementary Materials

Table S3. Tentative identified compounds of YHF20 in Negative mode

No.	RT (min)	Formula	Adducts	Calculate Mass (Da)	Mass Error (ppm)	Response	Identification	Phytochemical group
1	15.69	$C_{22}H_{28}O_6$	^-H	387.18	-0.77	5245221.00	Pseudolaric acid A	Organic Acids, Esters & Glycosides
2	16.59	$C_{27}H_{36}O_6$	^-H	455.24	-0.40	4978464.50	Ganolactone	Triterpenoids
3	11.28	$C_{17}H_{16}O_6$	^-H	315.09	-0.17	3281323.75	5,7-Dihydroxy-6-methyl-3-(2′,4′-dihydroxybenzyl) chroman-4-one	Homoflavonoids
4	13.02	$C_{42}H_{62}O_{16}$	^-H	821.40	-0.64	3111380.50	Glycyrrhizic acid	Triterpenoid Saponins
5	16.47	$C_{26}H_{34}O_6$	^-H	441.23	-0.47	2746255.25	Cinobufagin	Steroids
6	9.61	$C_{17}H_{16}O_5$	^-H	299.09	-1.72	2679676.75	Naringenin-4′,7-dimethyl ether	Flavanones
7	19.32	$C_{34}H_{67}O_{10}P$	^-H	665.44	2.16	2497910.00	PG 28:0	Lipids
8	7.75	$C_{26}H_{30}O_{13}$	^-H	549.1608498	-0.94	2447509.75	Liquiritin apioside	Flavanone Glycosides
9	8.80	$C_{14}H_6O_8$	^-H	300.9992271	0.79	2435786.50	Ellagic acid	Polyphenols (Tannin)
10	15.50	$C_{21}H_{26}O_6$	^-H	373.1653365	-0.87	2297288.50	Biondinin A	Lignanoids
11	16.77	$C_{27}H_{36}O_5$	^-H	439.25	-1.03	2222112.75	(25R)-Spirostan-4-ene-3,6,12-trione	Steroids
12	16.36	$C_{26}H_{34}O_6$	^-H	441.23	-1.15	2221271.25	Cinobufagin	Steroids
13	7.70	$C_{21}H_{22}O_9$	^-H	417.12	-1.00	2060733.50	1,3-Dihydroxy-6,7-dimethylxanthone-1-β-D-glucoside	Quinonoids
14	9.29	$C_{16}H_{16}O_5$	^-H	287.09	-1.08	1930912.50	4,7,2′-Trihydroxy-4′-methoxy-isoflavanol	Isoflavonones
15	7.89	$C_{21}H_{20}O_{10}$	^-H	431.10	-0.21	1906262.50	Genistin_1	Isoflavone Glycosides
16	17.60	$C_{24}H_{48}O_3$	^-H	383.35	1.05	1891858.50	α-Hydroxy tetracosanic acid	Organic Acids
17	10.41	$C_{17}H_{18}O_5$	^-H	301.11	-1.68	1820481.38	4’-O-Methylbrazilin	Phenols
18	8.24	$C_{21}H_{20}O_{10}$	^-H	431.10	-0.36	1755480.13	Physcion-8-O-β-D-glucoside	-
19	14.67	$C_{44}H_{62}O_{14}$	^+HCOO	859.42	5.00	1698074.00	Tenacissimoside B	Steroidal Glycosides
20	13.32	$C_{35}H_{48}O_9$	^-H	611.32	-1.21	1689336.00	Melianol	Triterpenoids
21	18.18	$C_{20}H_{22}O_3$	$^+CH_3COO$, ^+HCOO	369.17	0.42	1597883.13	Yakuchinone B	Phenols

22	10.74	$C_{16}H_{14}O_5$	^-H	285.08	-2.17	1584385.75	3,7-Dihydroxy-6-methoxyflacanonol	Flavanonols
23	9.44	$C_{16}H_{14}O_6$	^-H	301.07	-0.50	1495278.75	(3R)-4′-Methoxy-2′,3′,7-trihydroxy-isoflavanone	Isoflavonones
24	16.14	$C_{18}H_{32}O_2$	^-H	279.23	0.80	1491363.63	Linoleate	Lipids
25	20.44	$C_{35}H_{69}O_8P$	^+HCOO	693.47	2.08	1487668.88	PA 32:0	Lipids
26	15.88	$C_{22}H_{28}O_5$	^-H	371.19	-1.86	1381903.50	Galgravin	Lignanoids
27	11.99	$C_{18}H_{20}O_5$	^-H	315.12	0.23	1362339.88	2′-Hydroxy-4,4′,6′-trimethoxy-dihydrochalcone	Dihydrochalcones
28	16.50	$C_{29}H_{38}O_7$	^-H	497.25	-2.25	1288881.50	Scutellone H	Diterpenoids & Sesterterpenoids
29	13.14	$C_{42}H_{62}O_{16}$	^-H	821.40	-0.45	1225128.25	Atratoglaucoside B	Steroidal Glycosides
30	9.10	$C_{21}H_{20}O_{11}$	^-H	447.09	-1.54	1209982.75	Kaempferol-3-O-β-D-glucopyranoside	-
31	20.63	$C_{27}H_{54}$	^+HCOO	423.42	0.64	1171627.13	9-Heptacosylene	-
32	19.40	$C_{34}H_{67}O_{10}P$	^-H	665.44	2.05	1124768.88	PG 28:0	Lipids
33	17.16	$C_{20}H_{40}O$	$^+CH_3COO$	355.32	1.46	1081728.50	Isophytol	Diterpenoids & Sesterterpenoids
34	11.68	$C_{17}H_{14}O_6$	^-H	313.07	0.26	1077629.50	3′,5-Dihydroxy-7,4’-dimethoxy flavone	Flavones
35	10.83	$C_{17}H_{18}O_5$	^-H	301.11	-1.32	1073577.25	2-Methoxybenzyl-2,6-dimethoxybenzoate	Organic Acids, Esters & Glycosides
36	13.67	$C_{36}H_{58}O_{12}S$	^-H	713.36	-0.69	981678.38	Eclalbasaponin V	Triterpenoids
37	8.92	$C_{17}H_{16}O_6$	^-H	315.09	0.38	962916.44	Persicogenin	Flavanones
38	12.16	$C_{30}H_{48}O_6$	^-H	503.34	-1.17	908582.75	Esculentagenic acid	Triterpenoids
39	20.67	$C_{38}H_{73}O_{10}P$	^-H	719.49	2.75	883244.81	PG 32:1	Lipids
40	16.49	$C_{18}H_{34}O_2$	^-H	281.25	0.83	842901.00	9e-Octadecenoic Acid	Lipids
41	12.52	$C_{19}H_{16}O_4$	^+HCOO	353.10	0.49	838693.00	Moracin G	Phenols
42	15.82	$C_{18}H_{30}O_2$	^-H	277.22	0.75	834141.25	9z 12z 15z-Octadecatrienoic Acid	Lipids
43	8.48	$C_{21}H_{20}O_{12}$	^-H	463.09	-0.57	816908.88	6-Hydroxykaempferol-3-O-glucoside	Flavonol Glycosides
44	17.36	$C_{21}H_{42}O$	$^+CH_3COO$	369.34	1.16	811834.69	n-Henicosanal	-
45	8.93	$C_{21}H_{20}O_{11}$	^-H	447.09	-0.74	768862.50	Cimicifugic acid B	Triterpenoid Saponins

46	10.61	$C_{15}H_{14}O_3$	^-H	241.09	0.11	751124.81	Flavanthrinin_1	-
47	15.76	$C_{14}H_{28}O_2$	^-H	227.20	0.14	733878.38	Myristic Acid	Lipids
48	20.57	$C_{35}H_{69}O_8P$	^+HCOO	693.47	2.96	729403.81	PA 32:0	Lipids
49	16.08	$C_{30}H_{48}O_3$	^-H	455.35	-1.18	721914.19	Apocynin D	Triterpenoids
50	17.98	$C_{32}H_{52}O$	$^+CH_3COO$	511.42	-0.30	678895.81	24(E)-Ethylidenecycloartanone	Triterpenoids
51	8.60	$C_{14}H_{12}O_3$	^-H	227.07	-0.23	660587.94	Angenomalin	Coumarins
52	19.11	$C_{48}H_{93}NO_{10}$	^+HCOO, ^-H	888.68	0.08	652793.13	Momor-cerebrosideI	Glycosides
53	18.46	$C_{24}H_{48}O_2$	^-H	367.36	0.39	640857.81	Tetracosanoic Acid	Lipids
54	7.57	$C_9H_6O_3$	^-H	161.02	-1.04	639424.81	Umbelliferone	-
55	16.37	$C_{16}H_{32}O_2$	^-H	255.23	0.33	629644.06	Palmitate	Lipids
56	17.10	$C_{23}H_{46}O_2$	^+HCOO	399.35	0.19	610001.44	n-Tricosanoic acid	Organic Acids
57	15.98	$C_{25}H_{26}O_5$	^-H	405.17	-0.22	607913.25	Lupinifolin	Flavanones
58	14.60	$C_{18}H_{32}O_3$	^-H	295.23	0.38	595985.31	Coronaric acid	Organic Acids
59	10.74	$C_9H_8O_4$	^-H	179.03	-1.68	594743.38	Caffeate	Phenols
60	15.73	$C_{21}H_{28}O_4$	^-H	343.19	-0.69	584574.88	Neotussilagolactone	Sesquiterpenoids
61	0.99	$C_{12}H_{22}O_{11}$	^-H	341.11	-1.63	580837.19	D-(+)-Trehalose	Polar Metabolites, Mono- and Disaccharides
62	18.28	$C_{30}H_{50}O_2$	^+HCOO	487.38	0.37	572021.06	Olean-12-ene-3β,24-diol	Triterpenoids
63	13.38	$C_{20}H_{16}O_6$	^-H	351.09	-2.09	566483.13	Bavacoumestan A	Coumarins
64	14.55	$C_{22}H_{20}O_4$	$^+CH_3COO$	407.15	1.18	544211.19	2,7-Dihydroxy-1-(p-hydroxybenzyl)-4-methoxy-9,10-dihydrophenanthrene	Phenols
65	8.58	$C_{20}H_{28}O_9$	^+HCOO	457.17	-2.19	541436.06	Bruceine E_1	Diterpenoids & Sesterterpenoids
66	9.07	$C_{25}H_{28}O_{11}$	^+HCOO	549.16	-1.17	532678.94	Scroneoside A	Organic Acids, Organic Esters & Glycosides
67	10.34	$C_{15}H_{10}O_6$	^-H	285.04	-0.32	526379.88	5,7,2',5'-Tetrahydroxy-flavone	Triterpenoids
68	12.75	$C_{37}H_{50}O_{12}$	$^+CH_3COO$	745.35	3.85	504233.63	Nimbolidin C	Diterpenoids & Sesterterpenoids
69	9.20	$C_{21}H_{22}O_9$	^-H	417.12	-1.48	502487.06	Neoisoliquiritin	Chalcone Glycosides

70	15.23	$C_{21}H_{24}O_5$	$^-$H, $^+$CH-$_3$COO	355.16	1.82	492318.56	Kadsurenin F	Lignanoids
71	8.66	$C_{25}H_{24}O_{12}$	$^-$H	515.12	-0.34	487930.06	3,5-Dicaffeoylquinic acid	Organic Acids
72	14.75	$C_{17}H_{30}O_2$	$^+$HCOO	311.22	1.17	479364.94	Cireneol G	-
73	14.28	$C_{35}H_{44}O_8$	$^-$H	591.30	-0.92	467818.41	Marstenacigenin B	Steroids
74	12.19	$C_{18}H_{18}O_6$	$^-$H	329.10	0.04	463050.38	Acetylalkannin	Quinonoids
75	14.61	$C_{32}H_{40}O_8$	$^-$H	551.27	0.00	462880.41	6,17-Epoxylathyrol-5,15-diacetate-3-phenylacetate	Triterpenoids
76	16.17	$C_{22}H_{28}O_4$	$^+$CH-$_3$COO	415.21	-0.31	461849.41	Crocetin dimethyl ester	Diterpenoids & Sesterterpenoids
77	11.13	$C_{16}H_{12}O_6$	$^-$H	299.06	0.52	444153.50	1,6-Dihydroxy-2,4-dimethoxyanthraquinone	Quinonoids
78	12.15	$C_{30}H_{18}O_{10}$	$^-$H	537.08	-0.76	443119.44	Robustaflavone	Biflavonones
79	5.49	$C_{14}H_{16}O_9$	$^+$HCOO	373.08	-0.54	438826.94	Bergenin	Coumarins
80	5.72	$C_{16}H_{18}O_9$	$^-$H	353.09	-0.97	436095.81	Scopolin	Coumarin Glycosides
81	14.83	$C_{36}H_{58}O_{12}S$	$^-$H	713.36	-0.44	430075.81	Eclalbasaponin V	Triterpenoids
82	5.56	$C_{16}H_{18}O_9$	$^-$H	353.09	-1.18	419344.72	Chlorogenic acid	-
83	20.80	$C_{38}H_{73}O_{10}P$	$^-$H	719.49	2.60	416592.06	PG 32:1	Lipids
84	13.76	$C_{30}H_{48}O_5$	$^-$H	487.34	-1.36	414884.28	Madasiatic acid	Triterpenoids
85	12.01	$C_{18}H_{34}O_5$	$^-$H	329.23	-0.07	411272.06	Sanleng acid	Organic Acids, Esters & Glycosides
86	10.59	$C_{30}H_{22}O_{10}$	$^-$H	541.11	-0.11	410291.28	Mahuannin G	Flavan-3-ols
87	15.11	$C_{35}H_{44}O_8$	$^-$H	591.30	0.06	392869.88	Marstenacigenin B	Steroids
88	9.02	$C_{21}H_{18}O_{12}$	$^-$H	461.07	0.98	392667.78	Kaempferol-3-O-β-D-glucuronide	Flavonol Glycosides
89	14.81	$C_{26}H_{36}O_6$	$^+$HCOO	489.25	-1.99	392275.19	Bufotalin	Steroids
90	13.85	$C_{50}H_{80}O_{23}$	$^-$H	1047.50	1.02	380080.53	Anemarsaponin G	Steroidal Glycosides

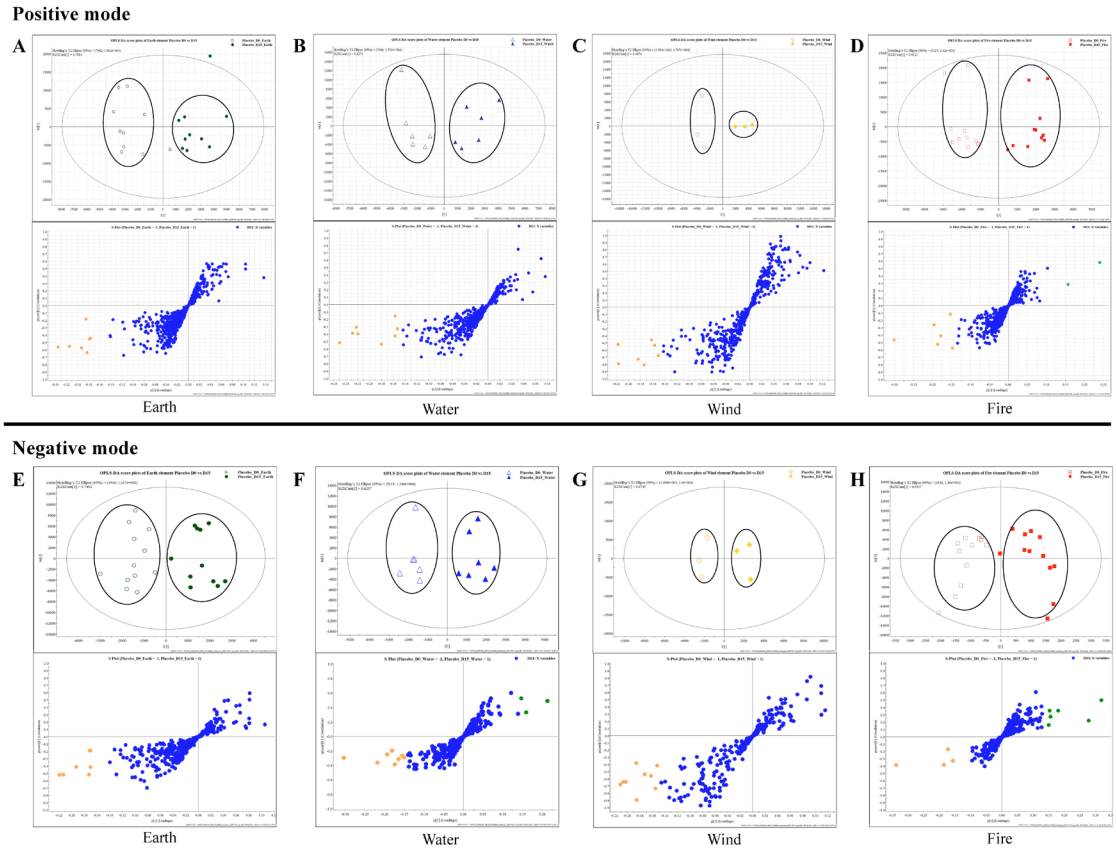
Table S3. Tentative identified compounds of YHF20 in Negative mode

No.	RT (min)	Formula	Adducts	Calculate Mass (Da)	Mass Error (ppm)	Response	Identification	Phytochemical group
1	15.69	$C_{22}H_{28}O_6$	^{-}H	387.18	-0.77	5245221.00	Pseudolaric acid A	Organic Acids, Esters & Glycosides
2	16.59	$C_{27}H_{36}O_6$	^{-}H	455.24	-0.40	4978464.50	Ganolactone	Triterpenoids
3	11.28	$C_{17}H_{16}O_6$	^{-}H	315.09	-0.17	3281323.75	5,7-Dihydroxy-6-methyl-3-(2′,4′-dihydroxybenzyl)chroman-4-one	Homoflavonoids
4	13.02	$C_{42}H_{62}O_{16}$	^{-}H	821.40	-0.64	3111380.50	Glycyrrhizic acid	Triterpenoid Saponins
5	16.47	$C_{26}H_{34}O_6$	^{-}H	441.23	-0.47	2746255.25	Cinobufagin	Steroids
6	9.61	$C_{17}H_{16}O_5$	^{-}H	299.09	-1.72	2679676.75	Naringenin-4′,7-dimethyl ether	Flavanones
7	19.32	$C_{34}H_{67}O_{10}P$	^{-}H	665.44	2.16	2497910.00	PG 28:0	Lipids
8	7.75	$C_{26}H_{30}O_{13}$	^{-}H	549.1608498	-0.94	2447509.75	Liquiritin apioside	Flavanone Glycosides
9	8.80	$C_{14}H_6O_8$	^{-}H	300.9992271	0.79	2435786.50	Ellagic acid	Polyphenols (Tannin)
10	15.50	$C_{21}H_{26}O_6$	^{-}H	373.1653365	-0.87	2297288.50	Biondinin A	Lignanoids
11	16.77	$C_{27}H_{36}O_5$	^{-}H	439.25	-1.03	2222112.75	(25R)-Spirostan-4-ene-3,6,12-trione	Steroids
12	16.36	$C_{26}H_{34}O_6$	^{-}H	441.23	-1.15	2221271.25	Cinobufagin	Steroids
13	7.70	$C_{21}H_{22}O_9$	^{-}H	417.12	-1.00	2060733.50	1,3-Dihydroxy-6,7-dimethylxanthone-1-β-D-glucoside	Quinonoids
14	9.29	$C_{16}H_{16}O_5$	^{-}H	287.09	-1.08	1930912.50	4,7,2′-Trihydroxy-4′-methoxyisoflavanol	Isoflavonones
15	7.89	$C_{21}H_{20}O_{10}$	^{-}H	431.10	-0.21	1906262.50	Genistin_1	Isoflavone Glycosides
16	17.60	$C_{24}H_{48}O_3$	^{-}H	383.35	1.05	1891858.50	α-Hydroxy tetracosanic acid	Organic Acids
17	10.41	$C_{17}H_{18}O_5$	^{-}H	301.11	-1.68	1820481.38	4’-O-Methylbrazilin	Phenols
18	8.24	$C_{21}H_{20}O_{10}$	^{-}H	431.10	-0.36	1755480.13	Physcion-8-O-β-D-glucoside	-
19	14.67	$C_{44}H_{62}O_{14}$	$^{+}HCOO$	859.42	5.00	1698074.00	Tenacissimoside B	Steroidal Glycosides
20	13.32	$C_{35}H_{48}O_9$	^{-}H	611.32	-1.21	1689336.00	Melianol	Triterpenoids
21	18.18	$C_{20}H_{22}O_3$	$^{+}CH_3COO$, $^{+}HCOO$	369.17	0.42	1597883.13	Yakuchinone B	Phenols
22	10.74	$C_{16}H_{14}O_5$	^{-}H	285.08	-2.17	1584385.75	3,7-Dihydroxy-6-methoxyflacanonol	Flavanonols
23	9.44	$C_{16}H_{14}O_6$	^{-}H	301.07	-0.50	1495278.75	(3R)-4′-Methoxy-2′,3′,7-trihydroxy-isoflavanone	Isoflavonones
24	16.14	$C_{18}H_{32}O_2$	^{-}H	279.23	0.80	1491363.63	Linoleate	Lipids

25	20.44	$C_{35}H_{69}O_8P$	^+HCOO	693.47	2.08	1487668.88	PA 32:0	Lipids
26	15.88	$C_{22}H_{28}O_5$	^-H	371.19	-1.86	1381903.50	Galgravin	Lignanoids
27	11.99	$C_{18}H_{20}O_5$	^-H	315.12	0.23	1362339.88	2′-Hydroxy-4,4′,6′-trimethoxydihydrochalcone	Dihydrochalcones
28	16.50	$C_{29}H_{38}O_7$	^-H	497.25	-2.25	1288881.50	Scutellone H	Diterpenoids & Sesterterpenoids
29	13.14	$C_{42}H_{62}O_{16}$	^-H	821.40	-0.45	1225128.25	Atratoglaucoside B	Steroidal Glycosides
30	9.10	$C_{21}H_{20}O_{11}$	^-H	447.09	-1.54	1209982.75	Kaempferol-3-O-β-D-glucopyranoside	-
31	20.63	$C_{27}H_{54}$	^+HCOO	423.42	0.64	1171627.13	9-Heptacosylene	-
32	19.40	$C_{34}H_{67}O_{10}P$	^-H	665.44	2.05	1124768.88	PG 28:0	Lipids
33	17.16	$C_{20}H_{40}O$	$^+CH_3COO$	355.32	1.46	1081728.50	Isophytol	Diterpenoids & Sesterterpenoids
34	11.68	$C_{17}H_{14}O_6$	^-H	313.07	0.26	1077629.50	3′,5-Dihydroxy-7,4’-dimethoxy flavone	Flavones
35	10.83	$C_{17}H_{18}O_5$	^-H	301.11	-1.32	1073577.25	2-Methoxybenzyl-2,6-dimethoxybenzoate	Organic Acids, Esters & Glycosides
36	13.67	$C_{36}H_{58}O_{12}S$	^-H	713.36	-0.69	981678.38	Eclalbasaponin V	Triterpenoids
37	8.92	$C_{17}H_{16}O_6$	^-H	315.09	0.38	962916.44	Persicogenin	Flavanones
38	12.16	$C_{30}H_{48}O_6$	^-H	503.34	-1.17	908582.75	Esculentagenic acid	Triterpenoids
39	20.67	$C_{38}H_{73}O_{10}P$	^-H	719.49	2.75	883244.81	PG 32:1	Lipids
40	16.49	$C_{18}H_{34}O_2$	^-H	281.25	0.83	842901.00	9e-Octadecenoic Acid	Lipids
41	12.52	$C_{19}H_{16}O_4$	^+HCOO	353.10	0.49	838693.00	Moracin G	Phenols
42	15.82	$C_{18}H_{30}O_2$	^-H	277.22	0.75	834141.25	9z 12z 15z-Octadecatrienoic Acid	Lipids
43	8.48	$C_{21}H_{20}O_{12}$	^-H	463.09	-0.57	816908.88	6-Hydroxykaempferol-3-O-glucoside	Flavonol Glycosides
44	17.36	$C_{21}H_{42}O$	$^+CH_3COO$	369.34	1.16	811834.69	n-Henicosanal	-
45	8.93	$C_{21}H_{20}O_{11}$	^-H	447.09	-0.74	768862.50	Cimicifugic acid B	Triterpenoid Saponins
46	10.61	$C_{15}H_{14}O_3$	^-H	241.09	0.11	751124.81	Flavanthrinin_1	-
47	15.76	$C_{14}H_{28}O_2$	^-H	227.20	0.14	733878.38	Myristic Acid	Lipids
48	20.57	$C_{35}H_{69}O_8P$	^+HCOO	693.47	2.96	729403.81	PA 32:0	Lipids
49	16.08	$C_{30}H_{48}O_3$	^-H	455.35	-1.18	721914.19	Apocynin D	Triterpenoids
50	17.98	$C_{32}H_{52}O$	$^+CH_3COO$	511.42	-0.30	678895.81	24(E)-Ethylidenecycloartanone	Triterpenoids
51	8.60	$C_{14}H_{12}O_3$	^-H	227.07	-0.23	660587.94	Angenomalin	Coumarins
52	19.11	$C_{48}H_{93}NO_{10}$	^+HCOO, ^-H	888.68	0.08	652793.13	Momor-cerebrosideI	Glycosides
53	18.46	$C_{24}H_{48}O_2$	^-H	367.36	0.39	640857.81	Tetracosanoic Acid	Lipids

54	7.57	$C_9H_6O_3$	$^-$H	161.02	-1.04	639424.81	Umbelliferone	-
55	16.37	$C_{16}H_{32}O_2$	$^-$H	255.23	0.33	629644.06	Palmitate	Lipids
56	17.10	$C_{23}H_{46}O_2$	$^+$HCOO	399.35	0.19	610001.44	n-Tricosanoic acid	Organic Acids
57	15.98	$C_{25}H_{26}O_5$	$^-$H	405.17	-0.22	607913.25	Lupinifolin	Flavanones
58	14.60	$C_{18}H_{32}O_3$	$^-$H	295.23	0.38	595985.31	Coronaric acid	Organic Acids
59	10.74	$C_9H_8O_4$	$^-$H	179.03	-1.68	594743.38	Caffeate	Phenols
60	15.73	$C_{21}H_{28}O_4$	$^-$H	343.19	-0.69	584574.88	Neotussilagolactone	Sesquiterpenoids
61	0.99	$C_{12}H_{22}O_{11}$	$^-$H	341.11	-1.63	580837.19	D-(+)-Trehalose	Polar Metabolites, Mono- and Disaccharides
62	18.28	$C_{30}H_{50}O_2$	$^+$HCOO	487.38	0.37	572021.06	Olean-12-ene-3β,24-diol	Triterpenoids
63	13.38	$C_{20}H_{16}O_6$	$^-$H	351.09	-2.09	566483.13	Bavacoumestan A	Coumarins
64	14.55	$C_{22}H_{20}O_4$	$^+$CH$_3$COO	407.15	1.18	544211.19	2,7-Dihydroxy-1-(p-hydroxybenzyl)-4-methoxy-9,10-dihydrophenanthrene	Phenols
65	8.58	$C_{20}H_{28}O_9$	$^+$HCOO	457.17	-2.19	541436.06	Bruceine E_1	Diterpenoids & Sesterterpenoids
66	9.07	$C_{25}H_{28}O_{11}$	$^+$HCOO	549.16	-1.17	532678.94	Scroneoside A	Organic Acids, Organic Esters & Glycosides
67	10.34	$C_{15}H_{10}O_6$	$^-$H	285.04	-0.32	526379.88	5,7,2',5'-Tetrahydroxy-flavone	Triterpenoids
68	12.75	$C_{37}H_{50}O_{12}$	$^+$CH$_3$COO	745.35	3.85	504233.63	Nimbolidin C	Diterpenoids & Sesterterpenoids
69	9.20	$C_{21}H_{22}O_9$	$^-$H	417.12	-1.48	502487.06	Neoisoliquiritin	Chalcone Glycosides
70	15.23	$C_{21}H_{24}O_5$	$^-$H, $^+$CH$_3$COO	355.16	1.82	492318.56	Kadsurenin F	Lignanoids
71	8.66	$C_{25}H_{24}O_{12}$	$^-$H	515.12	-0.34	487930.06	3,5-Dicaffeoylquinic acid	Organic Acids
72	14.75	$C_{17}H_{30}O_2$	$^+$HCOO	311.22	1.17	479364.94	Cireneol G	-
73	14.28	$C_{35}H_{44}O_8$	$^-$H	591.30	-0.92	467818.41	Marstenacigenin B	Steroids
74	12.19	$C_{18}H_{18}O_6$	$^-$H	329.10	0.04	463050.38	Acetylalkannin	Quinonoids
75	14.61	$C_{32}H_{40}O_8$	$^-$H	551.27	0.00	462880.41	6,17-Epoxylathyrol-5,15-diacetate-3-phenylacetate	Triterpenoids
76	16.17	$C_{22}H_{28}O_4$	$^+$CH$_3$COO	415.21	-0.31	461849.41	Crocetin dimethyl ester	Diterpenoids & Sesterterpenoids
77	11.13	$C_{16}H_{12}O_6$	$^-$H	299.06	0.52	444153.50	1,6-Dihydroxy-2,4-dimethoxyanthraquinone	Quinonoids
78	12.15	$C_{30}H_{18}O_{10}$	$^-$H	537.08	-0.76	443119.44	Robustaflavone	Biflavonones
79	5.49	$C_{14}H_{16}O_9$	$^+$HCOO	373.08	-0.54	438826.94	Bergenin	Coumarins
80	5.72	$C_{16}H_{18}O_9$	$^-$H	353.09	-0.97	436095.81	Scopolin	Coumarin Glycosides

81	14.83	$C_{36}H_{58}O_{12}S$	^{-}H	713.36	-0.44	430075.81	Eclalbasaponin V	Triterpenoids
82	5.56	$C_{16}H_{18}O_{9}$	^{-}H	353.09	-1.18	419344.72	Chlorogenic acid	-
83	20.80	$C_{38}H_{73}O_{10}P$	^{-}H	719.49	2.60	416592.06	PG 32:1	Lipids
84	13.76	$C_{30}H_{48}O_{5}$	^{-}H	487.34	-1.36	414884.28	Madasiatic acid	Triterpenoids
85	12.01	$C_{18}H_{34}O_{5}$	^{-}H	329.23	-0.07	411272.06	Sanleng acid	Organic Acids, Esters & Glycosides
86	10.59	$C_{30}H_{22}O_{10}$	^{-}H	541.11	-0.11	410291.28	Mahuannin G	Flavan-3-ols
87	15.11	$C_{35}H_{44}O_{8}$	^{-}H	591.30	0.06	392869.88	Marstenacigenin B	Steroids
88	9.02	$C_{21}H_{18}O_{12}$	^{-}H	461.07	0.98	392667.78	Kaempferol-3-O-β-D-glucuronide	Flavonol Glycosides
89	14.81	$C_{26}H_{36}O_{6}$	$^{+}HCOO$	489.25	-1.99	392275.19	Bufotalin	Steroids
90	13.85	$C_{50}H_{80}O_{23}$	^{-}H	1047.50	1.02	380080.53	Anemarsaponin G	Steroidal Glycosides



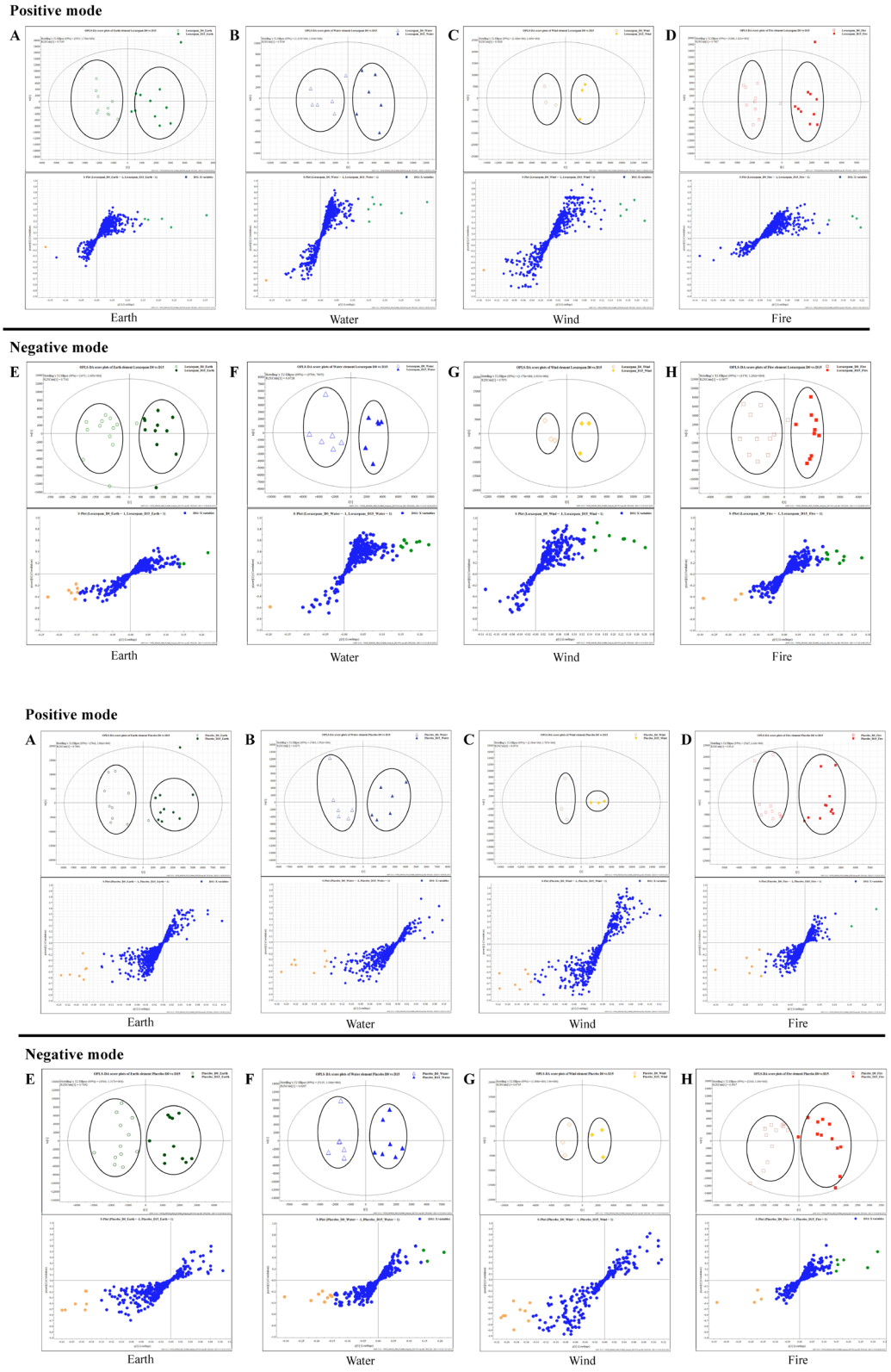


Figure S2. OPLS-DA score plots and S-plots of plasma samples from healthy volunteers with different dominant elements before and after the intake of placebo. (A) Earth element in the positive mode, (B) Water element in the positive mode, (C) Wind element in the positive mode, (D) Fire element in the positive mode, (E) Earth element in the negative mode, (F) Water element in the negative mode, (G) Wind element in the negative mode, (H) Fire element in the negative mode.

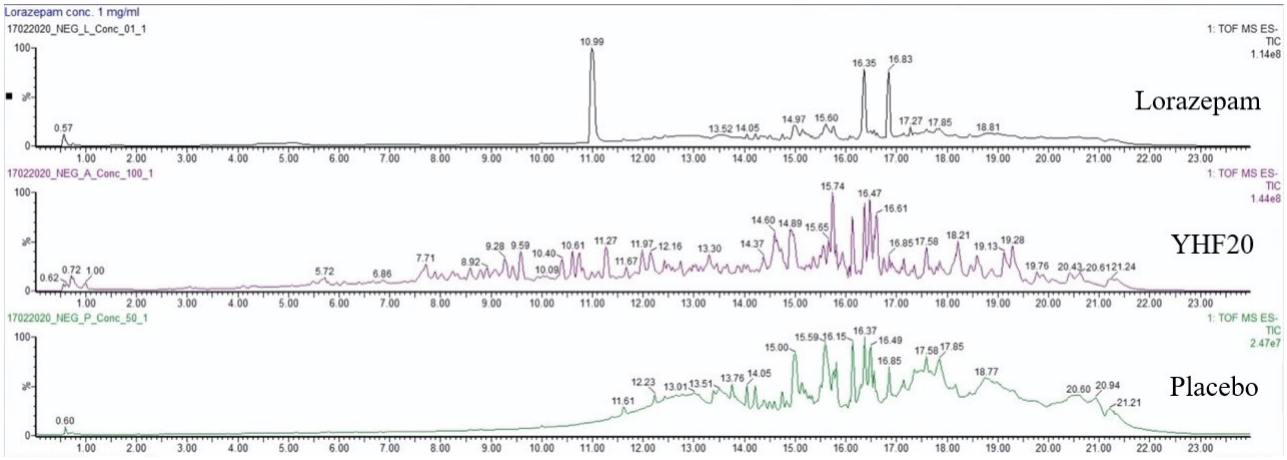


Figure S3. Chromatogram of Lorazepam, YHF20 and Placebo in Negative mode

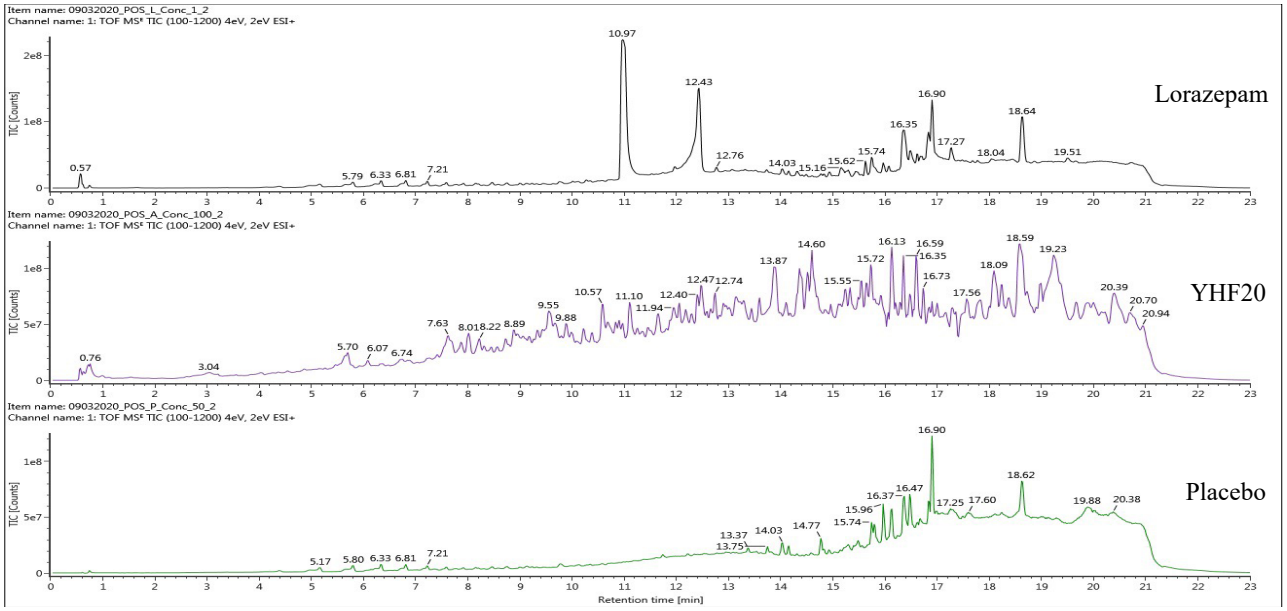


Figure S4. Chromatogram of Lorazepam, YHF20 and Placebo in Positive mode

Chemical constituents of YHF20

TIC of YHF20, lorazepam, and placebo

The total ion current (TIC) chromatogram displayed the combined intensity of all masses detected throughout the entire analysis. In these complex samples, the TIC chromatogram offered limited information because multiple analytes obscured the individual components within the injections, making it challenging to distinguish them. (Figure S3-S4)

Conflict of Interests

None.

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None.

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