Heliyon 10 (2024) e35974

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Metabolomics-based profiling of anti-inflammatory compounds from *Mentha spicata* in shanghe, China

Wenzhai Li [a], Peihai Li [a], Xiaobin Li [a], Hairong Hou [a], Houwen Lin [b], Meng Jin [a], Kechun Liu [a], Xuanming Zhang [a,*], Wenlong Sheng [a,**]

[a] *Engineering Research Center of Zebrafish Models for Human Diseases and Drug Screening, Biology Institute, Qilu University of Technology (Shandong Academy of Sciences), Jinan, 250103, China*

[b] *Research Center for Marine Drugs, State Key Laboratory of Oncogenes and Related Genes, Department of Pharmacy, School of Medicine, Shanghai Jiao Tong University, Shanghai, 200127, China*

ARTICLE INFO

Keywords:
Mentha spicata
Metabolomics
Inflammatory disease
Signaling pathway
Molecular interaction

ABSTRACT

Mentha spicata is a popular herb used in foods, cosmetics, and medicines. In the present study, liquid chromatography-mass spectrometry-based metabolomics analysis and the zebrafish model were used to investigate the potential biomarkers of *M. spicata* growing in Shanghe County (Shandong Province, China) and their anti-inflammatory properties. Network pharmacology and molecular docking were performed to screen the main targets of the characteristic compounds to understand their mechanisms of action. Nine potential markers including sugars (**1**,**2**), polyphenolic acids (**3**–**5**), and flavonoids (**6**–**9**) were identified from the species. The inhibitory effects on leukocyte migration confirmed that compounds **1** and **3**–**9** played a positive role in the protective effect of Shanghe *M. spicata* (SM) extract against inflammation. Akt (protein kinase B), EGFR (epidermal growth factor receptor), and MMP9 (matrix metalloproteinase 9) were the core target proteins of the identified compounds in the anti-inflammatory process. The most significant Gene Ontology and Kyoto Encyclopedia of Genes and Genomes enrichment terms were response to abiotic stimulus (Biological Process), carbohydrate derivative binding (Molecular Function), and pathways in cancer. In docking simulations, 3-p-coumaroylquinic acid (3-PC, **4**) and cirsimaritin (CN, **7**) exhibited the highest potential affinity to the active sites of Akt and EGFR proteins, respectively; additionally, 5-demethylsinensetin (5-DS, **9**) and luteolin (LN, **6**) were considered the most suitable ligands for the MMP9 protein. The present study highlighted the use of SM resources as functional products with health benefits.

1. Introduction

Inflammation, as part of the human immune defense system, is an evolutionarily conserved protection mechanism and a critical survival strategy. It is induced by various stimuli, including microbial invasion, chemical irritants, physical damage, and immune responses [1,2]. Inflammation induces the recruitment of different immune cells, and these innate immune cells produce proinflammatory cytokines and chemokines that trigger an adaptive immune response. Various inflammatory signaling cascades are

* Corresponding author.
** Corresponding author.
E-mail addresses: zhangmx@sdas.org (X. Zhang), shengwenlong@sdas.org (W. Sheng).

https://doi.org/10.1016/j.heliyon.2024.e35974
Received 8 March 2024; Received in revised form 3 August 2024; Accepted 7 August 2024
Available online 8 August 2024

involved in tissue damage, fibrosis, cell proliferation, etc., and these cascades lead to the development of several diseases such as atherosclerosis, neurodegenerative diseases, cancer, and diabetes [3]. These diseases are becoming increasingly common in the aging society. Given this background, an in-depth understanding of the pivotal role of inflammation can enable to develop and use new therapeutic methods and drugs.

Plants contain a wide range of biologically active molecules of medicinal value and are an important resource of compounds with therapeutic potential for humans [4]. *Mentha spicata* Linn., commonly called spearmint, is a perennial herb commercially grown worldwide. The essential oil extracted from its fresh and dried leaves produces a characteristic smell and is commercially used as an additive in chewing gum, toothpaste, and cosmetics. In the medical field, *M. spicata* is used to treat fever, cough, digestive disorders, asthma, and obesity [5,6]. There is currently a growing interest in the use of nonvolatile extracts of the *M. spicata* plant as sources of bioactive compounds and as pharmaceutical ingredients of high-quality medicinal products. In 2004, *M. spicata* was planted for the first time in Shanghe, China, and since then, its cultivated area has increased to more than 12000 mu. Because of their unique quality, the local *M. spicata* resources have become increasingly popular in domestic and international markets.

Plants show certain changes in their chemical composition depending on the different growth areas. Thus far, there have been no systematic studies on the chemical constituents of *M. spicata* species growing in Shanghe County, Shandong Province, China. In the present study, the metabolomic profile of Shanghe *M. spicata* (SM) was established using the liquid chromatography-tandem mass spectrometry (LC-MS/MS) technique to determine the anti-inflammatory compounds present in the plant extract. Network pharmacology and molecular docking were used to screen the main targets of the biomarkers, which could enable to understand the mechanisms of action of these natural products. Our findings could provide insights into nonvolatile compounds of SM species, which can serve as new functional ingredients for health-promoting products.

2. Materials and methods

2.1. Chemicals and reagents

Stachyose (ST), Sucrose (SU), Chlorogenic acid (CA), Luteolin (LN), Cirsimaritin (CN), Cirsilineol (CL), 5-demethylsinensetin (5-DS), and ibuprofen were purchased from Shanghai Yuanye Biotechnology Co., Ltd. The 3-p-coumaroylquinic acid (3-PC) and 4-p-coumaroylquinic acid (4-PC) were obtained from Baoji Herbest Bio-Tech Co., Ltd. $CuSO_4$ was supplied by Sinopharm Chemical Reagent Co., Ltd. MS-grade water and acetonitrile were acquired from Watsons Ltd. and Tedia Company Inc., respectively. *M. spicata* samples were obtained from Shanghe, Shangdong province (voucher number: swsys23-5) and Bozhou, Anhui province (voucher number: swsys23-7) and deposited at the Key Laboratory for Drug Screening Technology, Biology Institute, Qilu University of Technology (Shandong Academy of Sciences).

2.2. Sample preparation

Extracts were obtained from the dried Shanghe *M. spicata* (SM species) and Bozhou *M. spicata* (BM species) by adding 0.5 g of the samples (five batches for each cultivar) to 5 mL methanol and subjecting the solution to ultrasound treatment at 50 °C for 60 min. The solutions were filtered with 0.22 μm Millipore filters and concentrated to acquire the residues for further determination.

2.3. LC-MS-based metabolomics analysis

The sample solutions (2 mg/mL) were analyzed by the Agilent 1260 HPLC-6530 QTOF analyzer under the following conditions: Agilent Eclipse XDB-C_{18} column (4.6 × 250 mm, 5 μm); mobile phase A (water) and mobile phase B (acetonitrile) at 1 mL/min; elution conditions 10 %–50 % B for 0−15 min, 50 % B for 15−25 min, 50−100 % B for 25−40 min, and 100 % B for 40−45 min; optimized MS settings of 100–1500 *m/z* in negative and positive ionization modes; nebulizer pressure 30 psi, gas temperature 350 °C, drying gas flow rate 10 L/min, and capillary voltage 4000 V. A subset of the LC-MS data was exported into SIMCA-P software for PCA and OPLS-DA. [VIP] > 2 and $p < 0.05$ were used to filter multiple components for revealing the potential biomarkers in SM species. Qualitative and quantitative experiments of these characteristic compounds were further performed based on the comprehensive LC-MS/MS analysis.

2.4. Anti-inflammatory assay in transgenic zebrafish

Three day post-fertilization transgenic zebrafish (Tg: zlyz-EGFP) larvae were used to evaluate the anti-inflammatory activity of *M. spicata* extracts and the identified compounds. The experiment was performed using 10 larvae per well in 24-well culture plates and included control groups, model groups, positive groups (20 μM ibuprofen), and intervention groups (SM and BM extracts at 25, 50, and 100 μg/mL concentrations; ST, CA, 3-PC, 4-PC, LN, CN, CL, and 5-DS at 50 μM concentration). All treatments were applied in triplicate, and the plates were incubated at 28 °C for 3 h. Subsequently, the zebrafish larvae were treated with 20 μM $CuSO_4$ (except for the control group) for 1 h, and the inflammatory response was then observed. The number of fluorescent cells that accumulated at the zebrafish lateral line was counted under a fluorescence microscope (SZX16, Olympus, Tokyo, Japan) to evaluate the effects of each treatment.

2.5. Target prediction and pathway analysis

The molecular structures of ST, CA, 3-PC, 4-PC, LN, CN, CL, and 5-DS were downloaded from the PubChem database (http://pubchem.ncbi.nlm.nih.gov/). The potential targets of the eight compounds were searched using the Swiss Target Prediction Database (http://www.swisstargetprediction.ch/). The inflammatory disease targets were screened using the DisGeNET (http://www.disgenet.org/) and GeneCards (https://www.genecards.org/) databases with the term "Inflammation." After obtaining the intersections through a Venn diagram (http://bioinformatics.psb.ugent.be/webtools/Venn/), the candidate targets were inputted to the STRING database version 12.0 (https://cn.string-db.org/) to acquire the relevant information on protein interactions. A C-T interaction network was then constructed using Cytoscape 3.7.1. OmicShare Tools (https://www.omicshare.com/tools) was used for the GO and KEGG pathway enrichment analyses to determine the therapeutic mechanisms.

2.6. Molecular docking assay

Based on the results of the C-T network analysis, Akt, EGFR, and MMP9 proteins were regarded as candidate targets to predict the mechanisms of the compounds involved in the anti-inflammatory processes by using a molecular docking assay. The 3D structures of ST, CA, 3-PC, 4-PC, LN, CN, CL, and 5-DS were stored as Protein Data Bank (PDB) files, and the structural data for the human proteins Akt (0.98 Å, 1UNQ), EGFR (1.90 Å, 3W2S), and MMP9 (2.50 Å, 1L6J) were acquired from the PDB (https://www.rcsb.org/). AutoDock Tools was used in the docking run based on the genetic algorithm, and each ligand was docked into the active sites of the macromolecules by using default parameters [7]. The best binding modes for ligand-protein complexes were analyzed and visualized using PyMol package.

2.7. Statistical analysis

ANOVA tests for the biological assays were performed using an online tool (OmicShare Tools, https://www.omicshare.com/tools), and differences with a p-value of <0.05 were considered statistically significant.

3. Results

3.1. Metabolic profiling and biomarkers for M. spicata

To reveal the biomarkers of SM species, an LC-MS-based approach was used to capture the metabolomics profile of the plant. Figs. S1 and S2 show the LC-MS chromatograms of the samples. By using an R-based software package, 619 and 1527 primary

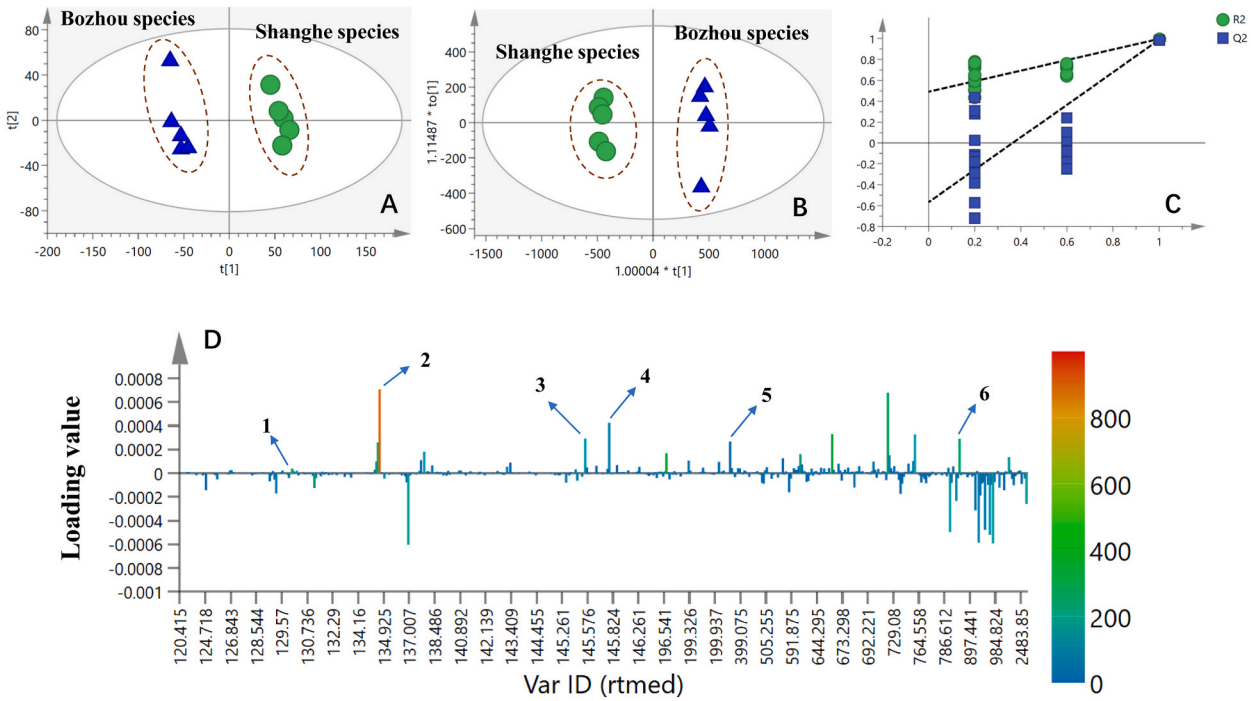

Fig. 1. Metabolic profiling of the LC-MS spectra of SM and BM extracts in negative modes. (A) PCA score plots, (B) OPLS-DA score plots, (C) validation plots of the model, and (D) coefficient-coded loading plots (6 potential biomarkers corresponding to the compounds in Table 1).

metabolites were detected in the negative and positive modes, respectively. In the principal component analysis model, the samples were divided into two blocks, which indicated the separation profile between the SM and BM groups (Fig. 1A and 2A). The R2X (Cum: 0.755 and 0.774) and Q2 (Cum: 0.495 and 0.618) values in the negative and positive modes, respectively, demonstrated the good description and prediction ability of the model. The orthogonal partial least squares-discriminant analysis (OPLS-DA) score plots confirmed a significant separation between the two groups (Fig. 1B and 2B). All the permuted R2 (Cum) and Q2 (Cum) values to the left were lower than the original point to the right, and all the regression lines of the Q2 (cum) points showed a negative intercept (Fig. 1C and 2C), thus signifying that the models were valid without overfitting [8]. Based on the overview of the loading plots (Fig. 1D and 2D), nine potential markers were found to contribute the most to cluster and discrimination in SM species. These characteristic compounds were identified as stachyose (ST, **1**), sucrose (SU, **2**), chlorogenic acid (CA, **3**), 3-p-coumaroylquinic acid (3-PC, **4**), 4-p-coumaroylquinic acid (4-PC, **5**), luteolin (LN, 6), cirsimaritin (CN, **7**), cirsilineol (CL, **8**), and 5-demethylsinensetin (5-DS, **9**) (Table 1 and Figs. S3–S12) [9–16]. All compound contents were determined using single-point calibration in the LC-MS analysis. In accordance with the preceding analysis, the compounds were predominantly accumulated within the SM extracts, exhibiting notably higher concentrations for ST (79.8 ± 3.8 μg/mg), SU (75.9 ± 3.0 μg/mg), CL (57.9 ± 3.1 μg/mg), and 5-DS (55.9 ± 1.6 μg/mg), respectively.

3.2. Anti-inflammatory activity of *M. spicata*

The immune system of zebrafish shows a high similarity to that of humans, which offers the possibility to study inflammatory responses in vivo and facilitates the development of anti-inflammatory agents [17]. Following neuronal damage under $CuSO_4$-induced conditions, the leukocytes were highly distributed in the zebrafish lateral line location, thus indicating the activation of inflammatory responses. The inflammatory cell migration was significantly suppressed by SM extracts at 50 and 100 μg/mL concentrations ($p < 0.01$), and a concentration-dependent decrease in the cell number was observed at the test doses (Fig. 3 and Table S1). Similarly, the inhibitory effects of cell migration were observed in the BM group at 50 and 100 μg/mL concentrations ($p < 0.01$). Compared to the SM group, the BM group showed a slightly higher level of inflammatory cell count in the right area. The results revealed that the nonvolatile compounds in *M. spicata* extracts possessed potent anti-inflammatory activity and that SM achieved better functional efficiency.

3.3. Anti-inflammatory activity of the characteristic compounds of SM

To determine the role of SM biomarkers in inflammation development, we studied their effects on leukocyte migration by using $CuSO_4$-treated transgenic zebrafish embryos. Considering the liability of potential toxicity [18], the concentration of 50 μM was chosen as the treatment dose for the compounds. As shown in Fig. 4 and Table S2, these compounds effectively inhibited the migration of inflammatory cells to the lateral line location. A remarkable finding was that the anti-inflammatory activity of 3-PC (**4**) was slightly

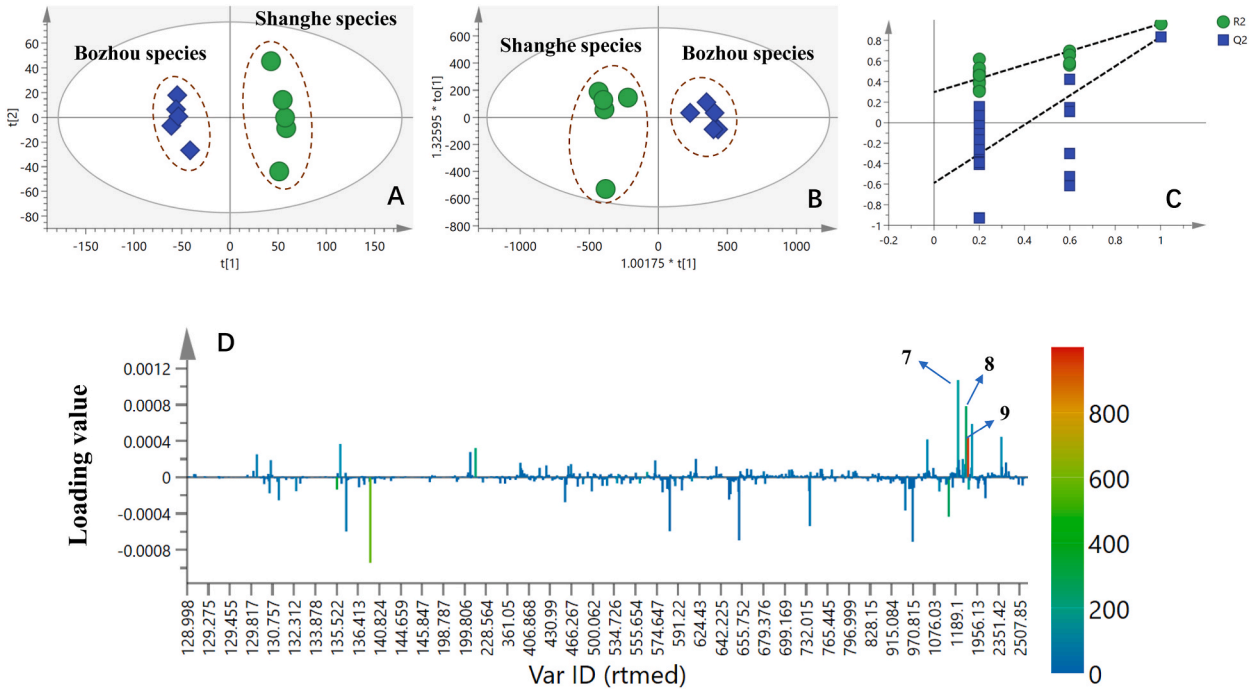

Fig. 2. Metabolic profiling of the LC-MS spectra of SM and BM extracts in positive modes. (A) PCA score plots, (B) OPLS-DA score plots, (C) validation plots of the model, and (D) coefficient-coded loading plots (3 potential biomarkers corresponding to the compounds in Table 1).

Table 1
Nine potential markers in the Shanghe *M. spicata* (SM).

Compounds	RT (min)	ESI-MS (*m/z*)	MS/MS (*m/z*)	VIP	p	Metabolites	Content in SM extract (μg/mg)	References
1	2.17	701.1862 $[M+Cl]^-$	665 $[M-H]^-$, 383, 341, 179	2.6	−0.07490	Stachyose	79.8 ± 3.8	[9]
2	2.25	377.0963 $[M+Cl]^-$	341 $[M-H]^-$, 179, 119, 113, 101	7.4	−0.32867	Sucrose	75.9 ± 3.0	[10]
3	2.42	353.0980 $[M-H]^-$	191 [quinic acid-H]$^-$, 173, 135	3.6	−0.15709	Chlorogenic acid	40.1 ± 2.1	[11]
4	2.43	337.1027 $[M-H]^-$	163 [p-coumaric acid-H]$^-$	5.3	−0.23156	3-p-Coumaroylquinic acid	22.3 ± 1.3	[12]
5	3.34	337.1027 $[M-H]^-$	173 [quinic acid–H_2O–H]$^-$	3.1	−0.13748	4-p-Coumaroylquinic acid	36.1 ± 2.4	[12]
6	14.30	285.0500 $[M-H]^-$	133	2.8	−0.12478	Luteolin	19.6 ± 0.7	[13]
7	19.84	315.0863 $[M+H]^+$	300, 272, 168	10.4	−0.29108	Cirsimaritin	47.4 ± 1.8	[14]
8	22.14	345.0967 $[M+H]^+$	330, 315, 312, 240	8.6	−0.23787	Cirsilineol	57.9 ± 3.1	[15]
9	24.93	359.1112 $[M+H]^+$	344, 326, 298	21.7	−0.52759	5-Demethylsinensetin	55.9 ± 1.6	[16]

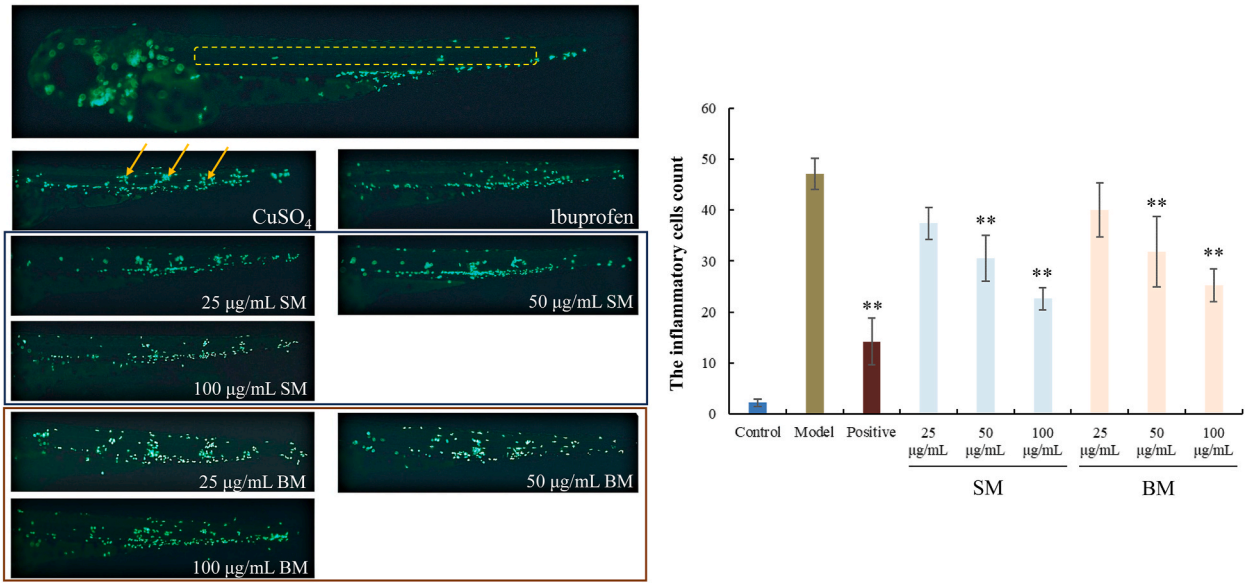

Fig. 3. SM and BM extracts inhibit $CuSO_4$-induced inflammatory responses in transgenic zebrafish (**$p < 0.01$, compared to the model group).

better than that of other compounds, because of the least number of migrated leukocytes. All the treatment groups showed significant results ($p < 0.01$), which suggested that the eight compounds from SM extracts played a positive role in alleviating inflammatory symptoms.

3.4. Compound-target interaction network analysis

Network pharmacology was used to reveal the possible mechanisms of the compounds. Inflammatory-related targets, screened using GeneCards and DisGeNET, were intersected with 190 protein targets of the characteristic compounds and then inputted to the STRING database version 12.0 (https://string-db.org/) to construct a ligand-protein interaction network. The network comprised 47 nodes and 365 edges with eight compounds and 39 targets. As shown in Fig. 5 and Table S3, AKT1 (Degree: 33), EGFR (Degree: 30), and MMP9 (Degree: 30) were the most highly connected targets, thus suggesting that they had an important role in the network functionality and were recognized as the hubs in the interaction network. Additionally, CL (**8**) and 5-DS (**9**) had the most therapeutic targets (Degree: 18), followed by 4-PC (**5**), LN (**6**), 3-PC (**4**), CN (**7**), CA (**3**), and ST (**1**). The results suggested that these compounds with different structural types provided multifunctional efficacy against the inflammatory process.

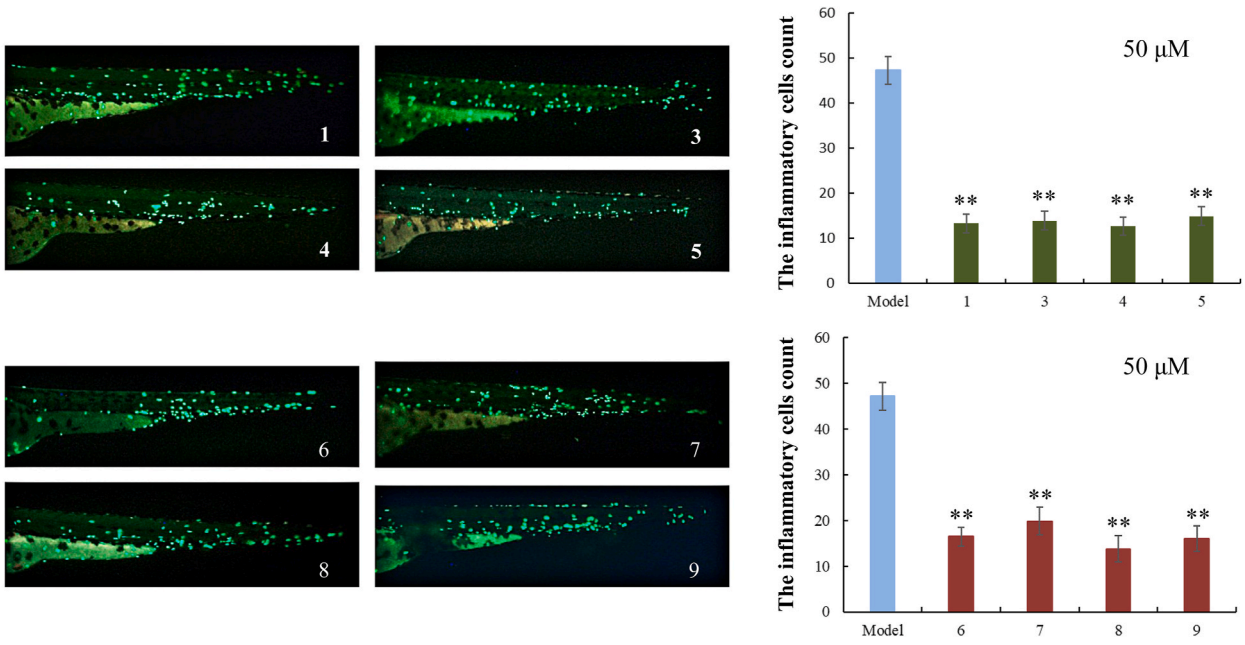

Fig. 4. Effects of the characteristic compounds on leukocyte migration in zebrafish larvae (**$p < 0.01$, compared to the model group).

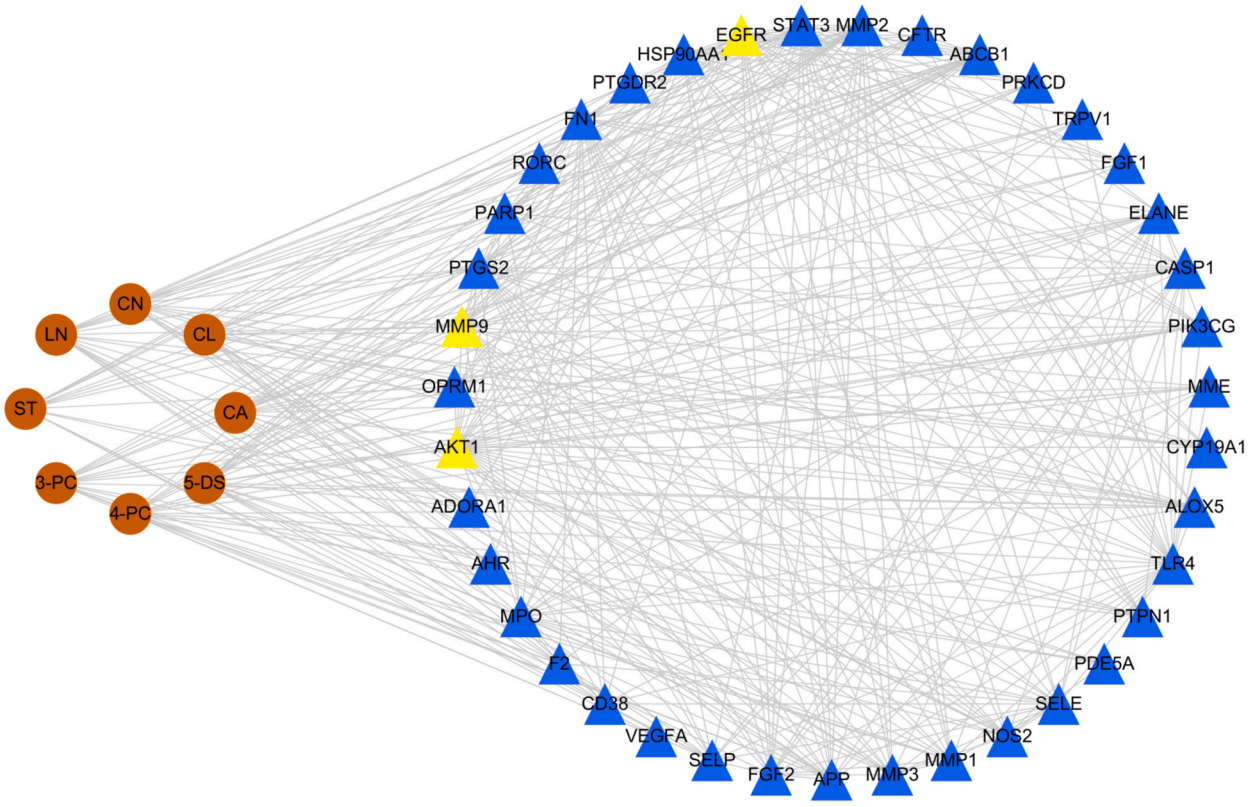

Fig. 5. Candidate-target network for the characteristic compounds against the inflammatory process.

3.5. Enrichment analysis of molecular targets

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were performed to further reveal the biological function of the targets (Fig. 6). The top enriched biological processes (BP) included the following categories: response to abiotic stimulus, response to oxygen-containing compound, positive regulation of cell communication, and immune system process. The top enriched molecular functions (MF) were carbohydrate derivative binding, heparin binding, glycosaminoglycan binding, identical protein binding, etc. Carbohydrate derivative binding, with the most significant *p*-value ($p = 7.10 \times 10^{-11}$), plays a role in the localization of leukocytes to the inflammatory regions, and the compounds that can inhibit the binding events are regarded as candidates for developing anti-inflammatory agents [19]. The results of the KEGG analysis indicated that the pathways in cancer (14 genes) and lipid and atherosclerosis (10 genes) were the main enriched pathways. Inflammation is a consequence of carcinogenesis and a driver of tumorigenesis and has been recognized as an important hallmark of cancer [20]. Recent studies have revealed that the inflammatory microenvironment plays a critical role in the development of many diseases. The drugs that suppress inflammation and induce microenvironmental remodeling are considered a promising agent with therapeutic and preventive effects [21]. The other KEGG pathways were associated with the AGE-RAGE signaling pathway in diabetic complications (seven genes), the relaxin signaling pathway (seven genes), the estrogen signaling pathway (seven genes), the HIF-1 signaling pathway (six genes), etc.

3.6. Molecular interaction studies

The principal compounds involved in SM species were used to dock with the predicted proteins for elucidating the ligand-receptor interactions. The output docking poses and scores are visualized and tabulated in Fig. 7 and S13and Tables S4–S6. CA (**3**), 3-PC (**4**), LN (**6**), and 5-DS (**9**) were found to interact strongly with the active pocket of the Akt receptor. The 3-PC (**4**) exhibited the lowest binding free energy (ΔG_b: 5.34 kcal/mol) and formed 10 hydrogen bonds (one with Asn-54, one with Asn-53, two with Lys-14, one with Arg-86, one with Arg-25, two with Arg-23, one with Glu-17, and one with Tyr-18), which indicated the highest potential affinity with the binding site. LN (**6**), 5-DS (**9**), and CA (**3**) were also docked deeply within the pocket region of Akt, and their binding free energies (−4.88, −4.53, and −4.38 kcal/mol, respectively) and hydrogen bonds (nine, six, and eight, respectively) were generated.

Based on the docking results, it was predicted that CN (**7**) was the most effective molecule with a strong binding affinity to the EGFR protein. This compound had the lowest binding energy value of −7.27 kcal/mol and yielded six hydrogen bond interactions (one with Gln-791, four with Met-793, and one with Asp-800). LN (**6**) and CL (**8**) also demonstrated high affinity toward EGFR with the binding energy values of −6.9 and −6.12 kcal/mol and six and three hydrogen bonds in the ligand-receptor complexes, respectively. In the MMP9 docking analysis, 5-DS (**9**) showed the binding energy value of −6.69 kcal/mol and five hydrogen bonds (one with Leu-243, one with Arg-249, two with Ala-189, and one with Leu-188), and LN (**6**) exhibited the binding energy value of −6.68 kcal/mol and nine hydrogen bonds (one with Tyr-245, one with Pro-246, one with Leu-188, two with Ala-189, one with His-236, one with His-226, and two with Ala-191). These compounds were considered the most suitable ligands for the protein against inflammatory diseases. CN (**7**) and 3-PC (**4**) were also found to penetrate deep into the protein domain target with the binding energy values ranging from −6.46 to −5.74 kcal/mol. The docking simulations revealed that 5-DS (**9**), LN (**6**), CN (**7**), and 3-PC (**4**) appeared to have good docking interactions with the active site of MMP9.

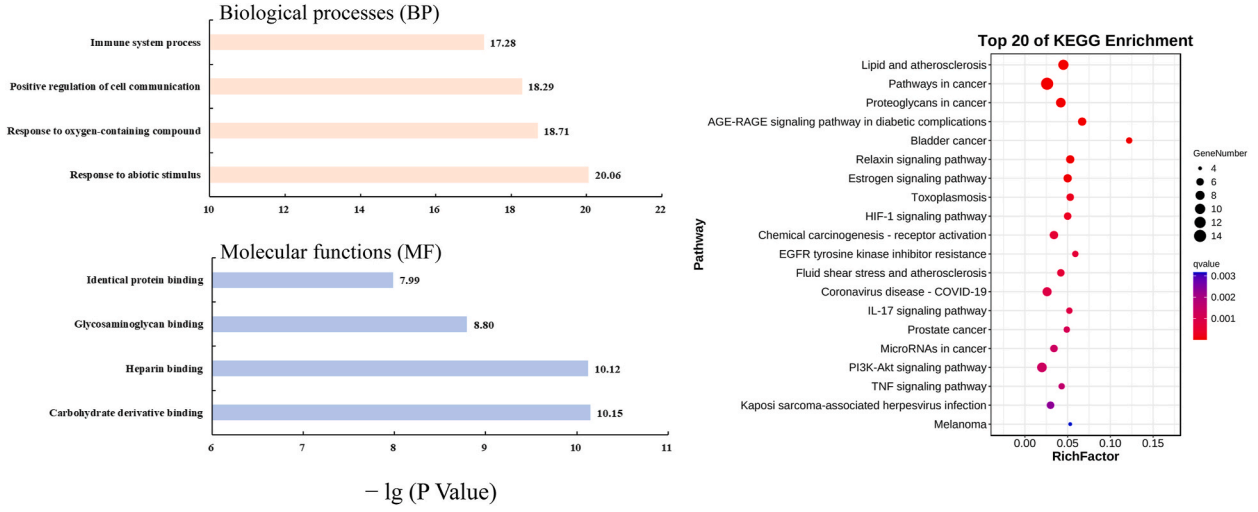

Fig. 6. GO and KEGG analyses reveal pathways associated with the targets from the candidate-target network.

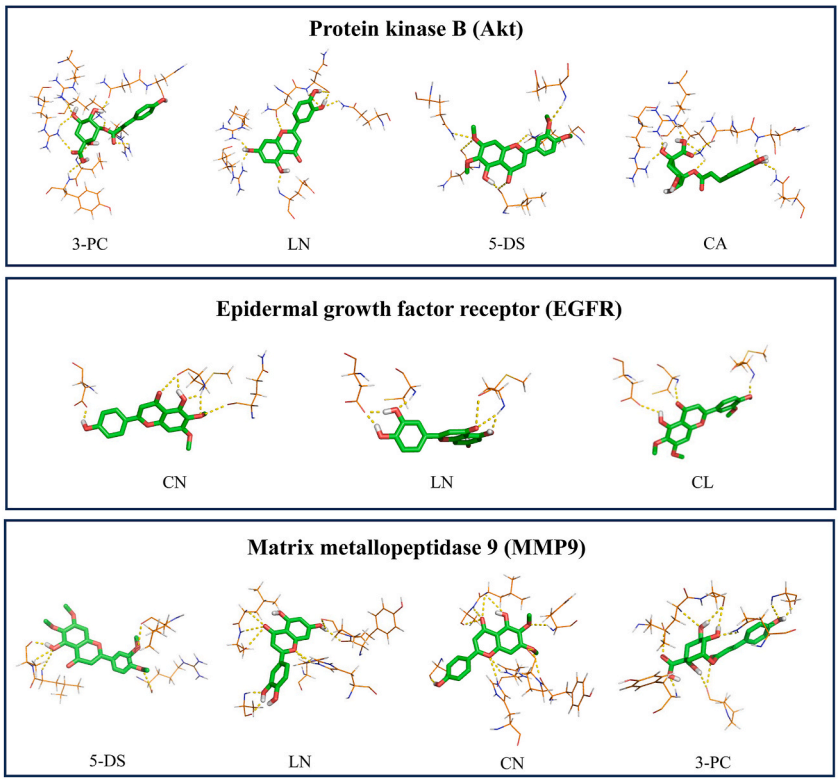

Fig. 7. Potential interactions between the ligands and receptors in docking simulations.

4. Discussion

Preliminary phytochemical analysis has shown that *M. spicata* had a rich content of volatile essential oils. Many of these volatile compounds, such as carvone and limonene, are widely used in foods, cosmetics, and health and pharmaceutical products. The nonvolatile fraction of *M. spicata* also contained many classes of components with beneficial effects on human health and could be used for medical purposes [22]. By using the LC-MS based metabolomics-driven approach, nine potential markers were discovered from Shanghe *M. spicata* (SM) and identified as ST (**1**), SU (**2**), CA (**3**), 3-PC (**4**), 4-PC (**5**), LN (**6**), CN (**7**), CL (**8**), and 5-DS (**9**). The present study highlighted the structural diversity of natural molecules in *M. spicata* and provided further motivation for their subsequent utilization.

Because of their anti-inflammatory properties, polyphenols and flavonoids are valued as crucial regulators of various diseases [23,24]. Stachyose can also function as an effective agent to prevent and treat inflammatory diseases [25]. In the present study, by using the in vivo zebrafish model, we confirmed that compounds **1** and **3**–**9** were the active constituents of SM extracts that inhibited leukocyte migration and played a role in inducing positive responses to alleviate the inflammatory symptoms. We attempted to elucidate the molecular mechanisms of the compounds involved in the anti-inflammatory process through network pharmacology and in-depth analyses of molecular docking. Carbohydrate derivative binding, heparin binding, glycosaminoglycan binding, etc. were annotated as the top GO MFs; moreover, pathways in cancer, lipid and atherosclerosis, the AGE-RAGE signaling pathway in diabetic complications, the relaxin signaling pathway, etc. were indicated as the top enriched KEGG pathways. Based on the accumulated evidence, these signaling pathways played a crucial role in modulating inflammatory microenvironments, thus preventing the occurrence and development of diseases [19,26].

Akt (protein kinase B), a serine-threonine kinase, functions as a key signaling node in MAPK, PI3K-Akt, T-cell receptor, and m-TOR signaling pathways. Once activated, Akt phosphorylates a variety of proteins, regulates innate immune responses, and induces an inflammatory response. Several studies have reported the critical role of Akt in immune cell differentiation, proliferation, and migration and suggested that various naturally occurring anti-inflammatory agents acted by suppressing the Akt signaling pathway [27,28]. EGFR (epidermal growth factor receptor) belongs to the tyrosine kinase receptor family, and the Akt pathway is one of its downstream effectors. The activated EGFR subsequently stimulates the Akt pathway, leading to inflammation initiation and progression. Following targeting and binding to EGFR, the inhibitors of EGFR decreased the levels of activated EGFR, reduced the production of inflammatory mediators, and contributed to the anti-inflammatory effect [29]. MMPs (Matrix metalloproteinases) are zinc-dependent endopeptidases that cleave extracellular matrix proteins, which can modulate the outcome of various physiological and pathological processes. MMP9 is a widely studied member of MMPs and is an important regulatory molecule in inflammation. Recent findings have indicated that MMP9 mediated leukocyte migration, and the use of its inhibitors led to the development of new

therapies for acute and chronic inflammation [30]. On the basis of these results, we speculate that our characteristic markers functioned as ligands with effective binding to Akt, EGFR, and MMP9. The interactions between the compounds and the proteins inactivated the signaling pathways involved in inflammation and induced the anti-inflammatory effect.

5. Conclusions

M. spicata is a medicinal plant widely cultivated worldwide. In the present study, the metabolomics-based profiling of the bioactive compounds of Shanghe *M. spicata* (SM) was performed with the LC-MS technique in both negative and positive modes. ST, SU, CA, 3-PC, 4-PC, LN, CN, CL, and 5-DS were identified as the nine biomarker components of the SM species. The compounds were also found to play a positive role in the protective effect of the SM extract against inflammation. The molecular target analysis revealed that the identified biomarkers effectively bound to Akt, EGFR, and MMP9 proteins to block the downstream signaling pathways related to inflammation, leading to the anti-inflammatory activity. Our findings provided insights into the utilization of the bioactive components of SM species for developing health-promoting products.

Funding

The study was financially supported by the Major Scientific and Technological Innovation Project of Shandong Province (grant number: 2022CXGC020515), the Key Innovation Project of Qilu University of Technology (Shandong Academy of Sciences) (grant number: 2022JBZ01-06), the Natural Science Foundation of Shandong Province (grant number: ZR2021MC055), and the Taishan Scholar Project from Shandong Province (grant number:ts20190950 and tsqn202312233).

Data availability statement

Data associated with the study has not been deposited into a publicly available repository. All data generated or analyzed during this study are included in the article/supplementary material.

Ethical approval

Ethics approval was not required for this research.

CRediT authorship contribution statement

Wenzhai Li: Writing – original draft, Software, Resources, Methodology, Investigation, Data curation, Conceptualization. **Peihai Li:** Validation, Investigation. **Xiaobin Li:** Software, Formal analysis. **Hairong Hou:** Visualization, Resources. **Houwen Lin:** Project administration. **Meng Jin:** Supervision, Resources. **Kechun Liu:** Supervision, Funding acquisition. **Xuanming Zhang:** Writing – review & editing, Visualization, Investigation. **Wenlong Sheng:** Writing – review & editing, Funding acquisition, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e35974.

References

[1] A.K. Dhingra, B. Chopra, Inflammation as a therapeutic target for various deadly disorders: a review, Curr. Drug Targets 21 (2020) 582–588, https://doi.org/10.2174/1389450120666191204154115.
[2] R. Brusini, M. Varna, P. Couvreur, Advanced nanomedicines for the treatment of inflammatory diseases, Adv. Drug Deliver. Rev. 157 (2020) 161–178, https://doi.org/10.1016/j.addr.2020.07.010.
[3] A. Leuti, D. Fazio, M. Fava, A. Piccoli, S. Oddi, A. Maccarrone, Bioactive lipids, inflammation and chronic diseases, Adv. Drug Deliver. Rev. 159 (2020) 133–169, https://doi.org/10.1016/j.addr.2020.06.028.
[4] C.D.R. Nunes, M. Barreto Arantes, S. Menezes de Faria Pereira, L. Leandro da Cruz, M. de Souza Passos, L. Pereira de Moraes, I.J.C. Vieira, D. Barros de Oliveira, Plants as sources of anti-inflammatory agents, Molecules 25 (2020) 3726, https://doi.org/10.3390/molecules25163726.
[5] G. Mahendran, S.K. Verma, L.U. Rahman, The traditional uses, phytochemistry and pharmacology of spearmint (*Mentha spicata* L.): a review, J. Ethnopharmacol. 278 (2021) 114266, https://doi.org/10.1016/j.jep.2021.114266.
[6] L.L. Zhang, Y. Chen, Z.J. Li, X. Li, G. Fan, Bioactive properties of the aromatic molecules of spearmint (*Mentha spicata* L.) essential oil: a review, Food Funct. 13 (2022) 3110–3132, https://doi.org/10.1039/d1fo04080d.

[7] X.M. Zhang, L.W. Han, P.H. Li, S.S. Zhang, M.Q. Zhang, X.B. Li, J. Chu, L.Z. Wang, P.F. Tu, Y. Zhang, K.C. Liu, Region-specific biomarkers and their mechanisms in the treatment of lung adenocarcinoma: a study of *Panax quinquefolius* from Wendeng, China, Molecules 26 (2021) 6829, https://doi.org/10.3390/molecules26226829.
[8] Y.F. Zhou, Y.Y. Hou, Q. Ban, M.L. Zhang, T. Huang, B. Ma, L. Shi, Q. Zhang, Metabolomics profiling of seminal plasma in obesity-induced asthenozoospermia, Andrology 11 (2023) 1303–1319, https://doi.org/10.1111/andr.13412.
[9] Y. Zhou, D.S. Xu, L. Liu, F.R. Qiu, J.L. Chen, G.L. Xu, A LC-MS/MS method for the determination of stachyose in rat plasma and its application to a pharmacokinetic study, J. Pharm. Biomed. Anal. 123 (2016) 24–30, https://doi.org/10.1016/j.jpba.2015.11.041.
[10] G.G.C. Kuhnle, A.M.C.P. Joosen, T.R. Wood, S.A. Runswick, J.L. Griffin, S.A. Bingham, Detection and quantification of sucrose as dietary biomarker using gas chromatography and liquid chromatography with mass spectrometry, Rapid Commun. Mass Sp. 22 (2008) 279–282, https://doi.org/10.1002/rcm.3355.
[11] J.X. Ye, W. Wei, L.H. Quan, C.Y. Liu, Q. Chang, Y.H. Liao, An LC-MS/MS method for the simultaneous determination of chlorogenic acid, forsythiaside A and baicalin in rat plasma and its application to pharmacokinetic study of shuang-huang-lian in rats, J. Pharmaceut. Biomed. 52 (2010) 625–630, https://doi.org/10.1016/j.jpba.2010.01.035.
[12] Y. Kiselova-Kaneva, B. Galunska, M. Nikolova, I. Dincheva, I. Badjakov, High resolution LC-MS/MS characterization of polyphenolic composition and evaluation of antioxidant activity of *Sambucus ebulus* fruit tea traditionally used in Bulgaria as a functional food, Food Chem. 367 (2022) 130759, https://doi.org/10.1016/j.foodchem.2021.130759.
[13] H.S. Cheruvu, N.K. Yadav, G.R. Valicherla, R.K. Arya, Z. Hussain, C. Sharma, K.R. Arya, R.K. Singh, D. Datta, J.R. Gayen, LC-MS/MS method for the simultaneous quantification of luteolin, wedelolactone and apigenin in mice plasma using hansen solubility parameters for liquid-liquid extraction: application to pharmacokinetics of *Eclipta alba* chloroform fraction, J. Chromatogr. B. 1081–1082 (2018) 76–86, https://doi.org/10.1016/j.jchromb.2018.01.035.
[14] W. Jesionek, B. Majer-Dziedzic, Györgyi Horváth, Á.M. Móricz, I.M. Choma, Screening of antibacterial compounds in *Salvia officinalis* L. tincture using thin-layer chromatography-direct bioautography and liquid chromatography-tandem mass spectrometry techniques, J. Planar Chromat. 30 (2017) 357–362, https://doi.org/10.1556/1006.2017.30.5.4.
[15] A.K. Rai, S. Khan, A. Kumar, B.K. Dubey, R.K. Lal, A. Tiwari, P.K. Trivedi, C.T. Elliott, R. Ch, Comprehensive metabolomic fingerprinting combined with chemometrics identifies species- and variety-specific variation of medicinal herbs: an Ocimum study, Metabolites 13 (2023) 122, https://doi.org/10.3390/metabo13010122.
[16] H.J. Zhang, G.F. Tian, C.Y. Zhao, Y.H. Han, C. DiMarco-Crook, C. Lu, Y. Bao, C.X. Li, H. Xiao, J.K. Zheng, Characterization of polymethoxyflavone demethylation during drying processes of citrus peels, Food Funct. 10 (2019) 5707–5717, https://doi.org/10.1039/c9fo01053j.
[17] Y.H. Gui, W.H. Jiao, M. Zhou, Y. Zhang, D.Q. Zeng, H.R. Zhu, K.C. Liu, F. Sun, H.F. Chen, H.W. Lin, A.-C. Septosones, In vivo anti-inflammatory meroterpenoids with rearranged carbon skeletons from the marine sponge *Dysidea septosa*, Org. Lett. 21 (2019) 767–770, https://doi.org/10.1021/acs.orglett.8b04019.
[18] J.R. Naidu, R. Ismail, S. Sasidharan, Acute oral toxicity and brine shrimp lethality of methanol extract of *Mentha Spicata* L (Lamiaceae), Trop. J. Pharm. Res. 13 (2014) 101–107, https://doi.org/10.4314/tjpr.v13i1.15.
[19] L.A. Lasky Selectins, Interpreters of cell-specific carbohydrate information during inflammation, Science 258 (1992) 964–969, https://doi.org/10.1126/science.1439808.
[20] D. Nikitovic, M. Tzardi, A. Berdiaki, A. Tsatsakis, G.N. Tzanakakis, Cancer microenvironment and inflammation: role of hyaluronan, Front. Immunol. 6 (2015) 169, https://doi.org/10.3389/fimmu.2015.00169.
[21] P. Libby, Inflammatory mechanisms: the molecular basis of inflammation and disease, Nutr. Rev. 65 (2007) S140–S146, https://doi.org/10.1111/j.1753-4887.2007.tb00352.x.
[22] S. Ćavar Zeljković, J. Šišková, K. Komzáková, N. De Diego, K. Kaffková, P. Tarkowski, Phenolic compounds and biological activity of selected Mentha Species, Plants 10 (2021) 550, https://doi.org/10.3390/plants10030550.
[23] Z.C. Gai, S.H. Hu, G.L. Gong, J.Q. Zhao, Recent advances in understanding dietary polyphenols protecting against hypertension, Trends Food Sci. Tech. 138 (2023) 685–696, https://doi.org/10.1016/j.tifs.2023.07.008.
[24] N. Duan, X. Hu, R. Zhou, Y. Li, W. Wu, N. Liu, A review on dietary flavonoids as modulators of the tumor microenvironment, Mol. Nutr. Food Res. 67 (2023) e2200435, https://doi.org/10.1002/mnfr.202200435.
[25] S. Jiang, Q. Li, S. Han, H. Wang, X. Tang, T. Wang, X. Meng, Study on the anti-inflammatory effect of stachyose by inhibiting TLR4/NF-κB signalling pathway in vitro and in vivo, Clin. Exp. Pharmacol. Physiol. 50 (2023) 573–582, https://doi.org/10.1111/1440-1681.13774.
[26] Y.K. Ju, X.J. Liu, X.Z. Ye, M.H. Dai, B.R. Fang, X.K. Shen, L.L. Liu, Nanozyme-based remodeling of disease microenvironments for disease prevention and treatment: a review, ACS Appl. Nano Mater. 6 (2023) 13792–13823, https://doi.org/10.1021/acsanm.3c02097.
[27] Q.D. Xia, Y. Xun, J.L. Lu, Y.C. Lu, Y.Y. Yang, P. Zhou, J. Hu, C. Li, S.G. Wang, Network pharmacology and molecular docking analyses on Lianhua Qingwen capsule indicate Akt1 is a potential target to treat and prevent COVID-19, Cell Prolif. 53 (2020) e12949, https://doi.org/10.1111/cpr.12949.
[28] Y.G. Lee, J. Lee, S.E. Byeon, D.S. Yoo, M.H. Kim, S.Y. Lee, J.Y. Cho, Functional role of Akt in macrophage-mediated innate immunity, Front. Biosci. 16 (2011) 517–530, https://doi.org/10.2741/3702.
[29] A. Elkamhawy, A.H.E. Hassan, S. Paik, Y. Sup Lee, H.H. Lee, J.S. Shin, K.T. Lee, E.J. Roh, EGFR inhibitors from cancer to inflammation: discovery of 4-fluoro-N-(4-(3-(trifluoromethyl)phenoxy)pyrimidin-5-yl)benzamide as a novel anti-inflammatory EGFR inhibitor, Bioorg. Chem. 86 (2019) 112–118, https://doi.org/10.1016/j.bioorg.2019.01.017.
[30] J. Hu, P.E. Van den Steen, Q.X. Sang, G. Opdenakker, Matrix metalloproteinase inhibitors as therapy for inflammatory and vascular diseases, Nat. Rev. Drug Discov. 6 (2007) 480–498, https://doi.org/10.1038/nrd2308.