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"Efficacy–Nature–Structure" Relationship of Traditional Chinese Medicine Based on Chemical Structural Data and Bioinformatics Analysis

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Cite This: *ACS Omega* 2021, 6, 33583–33598

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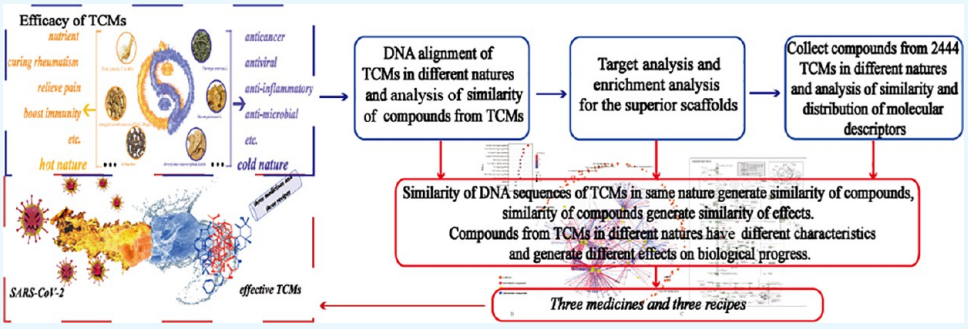

ABSTRACT: Traditional Chinese medicines (TCMs) have wide pharmacological activities, and the ingredients in individual TCMs determine their efficacies. To understand the "efficacy–nature–structure" relationship of TCM, compounds from 2444 kinds of herbs were collected, and the associations between family, structure, nature, and biological activities were mined and analyzed. Bernoulli Naïve Bayes profiling and a data analysis method were used to predict the targets of compounds. The results show that genetic material determined the representation of ingredients from herbs and the nature of TCMs and that the superior scaffolds of compounds of cold nature were 2-phenylochrotinone, anthraquinone, and coumarin, while the compounds of hot nature were cyclohexene. The results of the similarity analysis and distribution for molecular descriptors of compounds show that compounds associated with the same nature were similar and compounds associated with different natures occurred as a transition in part. As for integral compounds from 2-phenylochrotinone, anthraquinone, coumarin, and cyclohexene, the value of the shape index increased, indicating the transition of scaffolds from a spherical structure to a linear structure, with various molecular descriptors decreasing. Three medicines and three recipes prescribed based on "efficacy–nature–structure" had a higher survival rate in the clinic and provided powerful evidence for TCM principles. The research improves the understanding of the "efficacy–nature–structure" relationship and extends TCM applications.

INTRODUCTION

Traditional Chinese medicines (TCMs) have a wide range of pharmacological activities. Different TCMs show different pharmacological applications according to their different compositions and structures. The nature of the TCM is considered to be the link between the clinical application and pharmacological action of the TCM. The nature (siqi, e.g., cold nature, cool nature, warm nature, and hot nature, also simplified as hot and cold nature) is a generalization of the rules of TCM prescription and a generalization for pharmacological activities. The cold or hot nature, as well as the pharmacology, are an objective response when ingredients from a TCM act on an organism. The nature can also be characterized as a bridge between the clinical pharmacological activity and the component structure. In 2020, China created a custom approach for managing COVID-19 called "integrate Chinese and Western approaches, adopt individual treatment." China has adopted different treatments to develop better curative medicine. Regarding COVID-19, China has regarded "three medicines and three recipes" as the foremost approach under the theory of the nature of TCMs. Owing to its high effectiveness, the three medicines and three recipes have improved the survival rate and delayed disease progression, giving Chinese patients with typical

Received: August 16, 2021
Accepted: November 18, 2021
Published: December 1, 2021

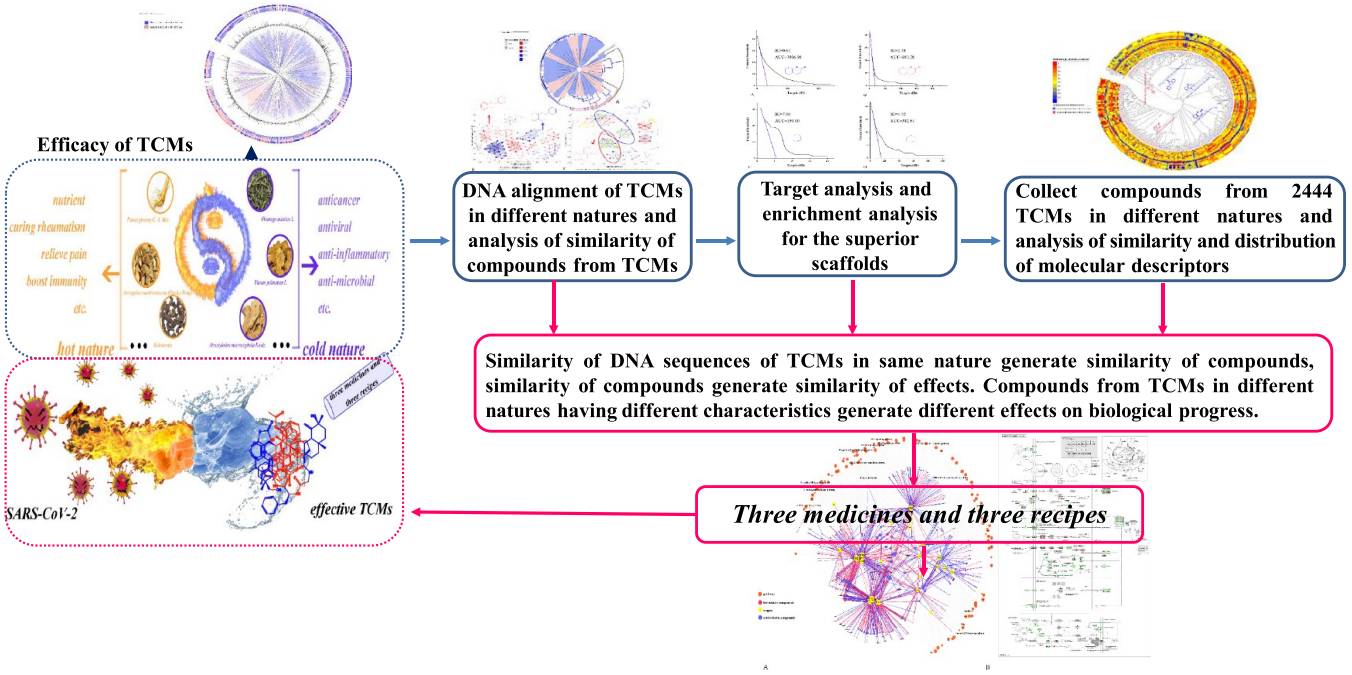

Figure 1. Flowchart of the process for prescribing three medicines and three recipes based on the nature and efficacy of TCMs.

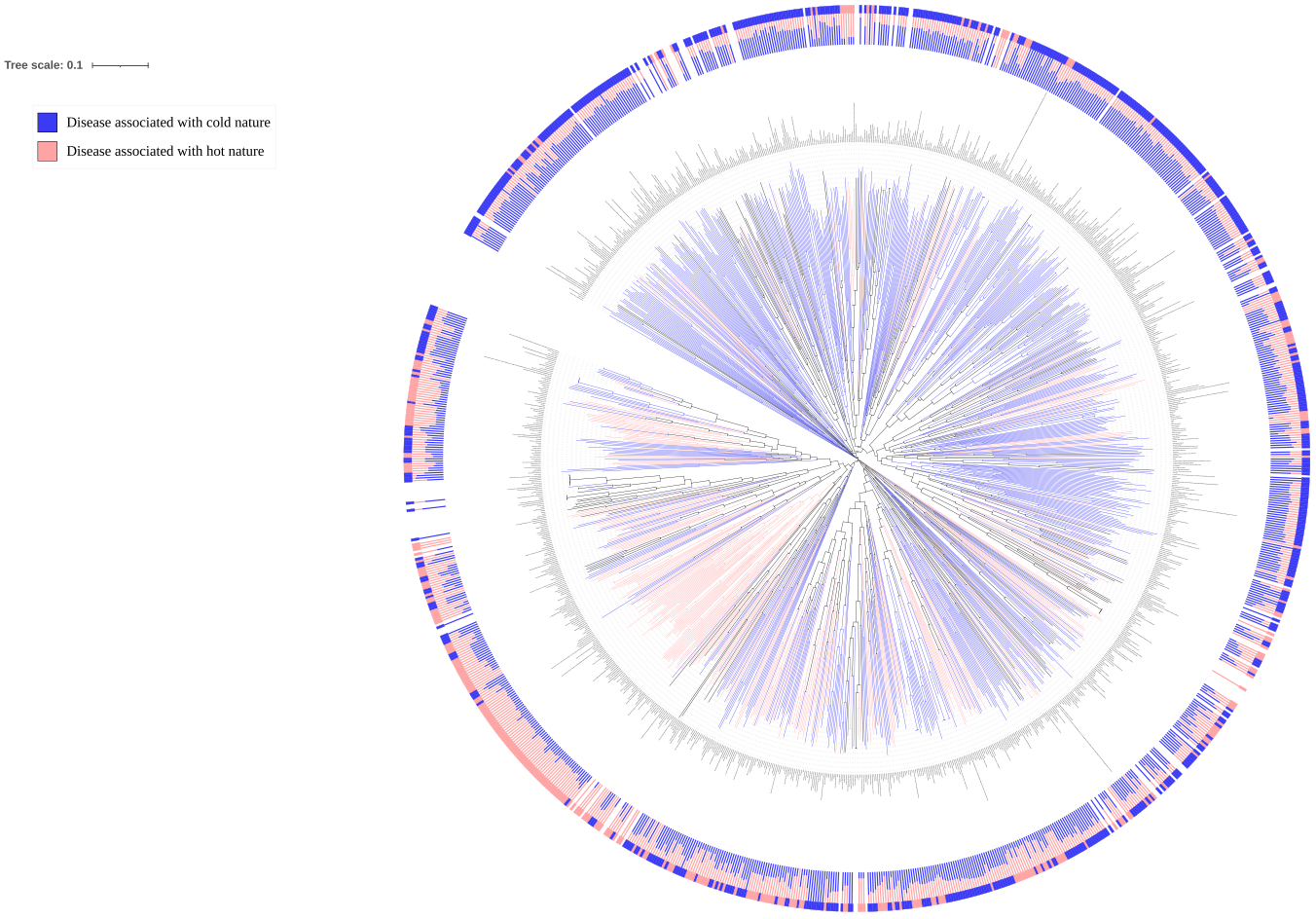

Figure 2. Diseases and coverage rate of compounds from different-nature TCMs.

syndromes of COVID-19 more time until they receive medical assistance.

Many researchers have examined this topic. For example, Wang et al.[1] focused on the nature of TCMs and found that the structure of compounds and the pharmacological effects from

https://doi.org/10.1021/acsomega.1c04440
ACS Omega 2021, 6, 33583−33598

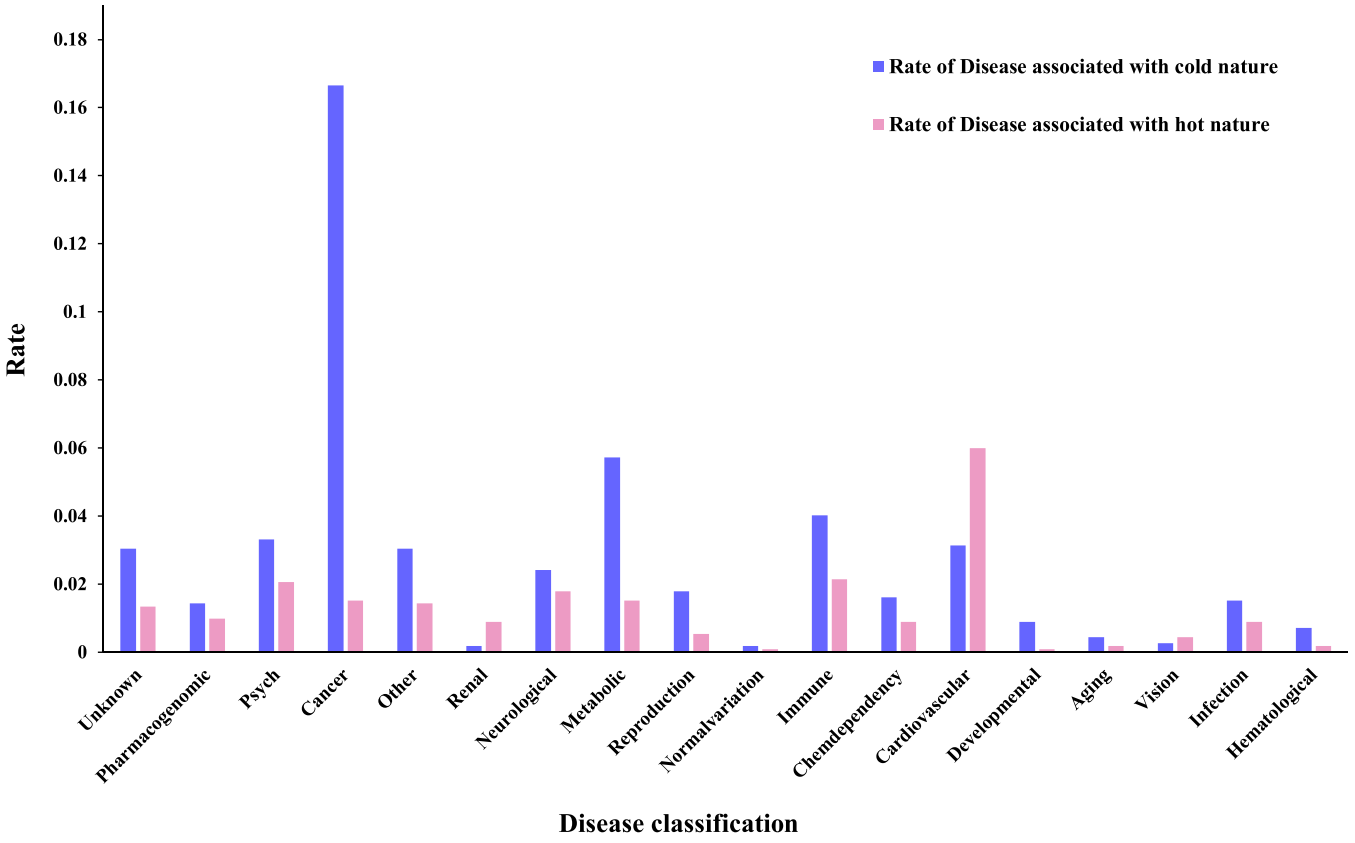

Figure 3. Disease classification and statistics associated with the nature of TCMs.

different TCMs were associated closely with the different natures of different TCMs. Fu et al.[2] found that traditional Chinese marine medicines (TCMMs) from different species were clustered closer with cold nature and demonstrated that TCMMs of cold nature have better anticancer activity. Meng-yu et al.[3] found that more compounds from cold-nature herbs, including 2-phenylochrotinone, anthraquinone, coumarin, and triterpenoid, could have antibacterial activity. Wei et al.[4] analyzed ultraviolet spectrum data extracted from TCMs and found that TCMs of a similar nature could generate a similar fingerprint owing to the similar structure of the TCMs. Guo et al.[5] aimed to take metabolites to judge the nature of TCMs and found that hot-nature herbs contained more nucleotides and cold-nature herbs contained more amino acids. Sui et al.[6] and Kong et al.[7] found that compounds from cold-nature herbs could downregulate TRPM8 and maintain clinical TCMs' balance of cold or hot in light of the regulation of transient receptor potential ion channel proteins (TRPs). Some researchers have also tried to illustrate the nature of TCMs based on an analysis of the structure or pharmacophore. For example, Fu et al.[8] explored the relationship between the characteristics for the component structure and nature of TCMs through a calculation of atomic environments. Zhang et al.[9] hypothesized that the relationship of "efficacy−structure" can be described with the pharmacophore theory.

However, the aforementioned approaches cannot reveal the association between pharmacological activities, the nature of TCMs, and component structure. They separate the efficacy and nature of TCMs from structures, which was inconsistent with the integral research that focused on the "efficacy−nature−structure" relationship in a macroscopic perspective or small sample study, and thus, they cannot offer the complete chain of evidence for illustrating the "efficacy−nature−structure" relationship. Therefore, this research focused on the association between the pharmacological activities of TCMs, their nature, and their structure in terms of DNA sequence alignment, similarity of compounds, and molecular descriptors (Figure 1).

RESULTS AND DISCUSSION

Analysis of Disease and Scaffolds Associated with the Natures of TCMs. After exploring the association between diseases and the natures of TCMs, our results show that the coverage rate of compounds from cold-nature TCMs was different from that of hot-nature TCMs. Overall, the compounds from TCMs of cold nature covered more diseases (563 kinds) than those from TCMs of hot nature (257 kinds) (Figure 2). After frequency statistics were classified according to the GAD disease classification,[10] we found that cold nature was associated more closely with cancer and metabolic, reproduction, developmental, hematological, and immune diseases. The hot nature was associated more closely with cardiovascular, renal, and vision diseases (Figure 3).

According to the distribution of compounds and their Murcko scaffolds, the results show that different compounds could cover different diseases (Figure 4). Compounds included the benzene ring, which was associated with many kinds of diseases. They also included 2-phenylchrotinone, anthraquinone, and coumarin, which were associated closely with cancer and metabolic and immune diseases. Compounds including cyclohexene were more closely associated with cardiovascular diseases. Compounds including alkaloids and lignans were associated more closely with psychological and neurological diseases.

DNA Sequence Alignment and Analysis. According to analysis of the association between diseases and TCMs as well as

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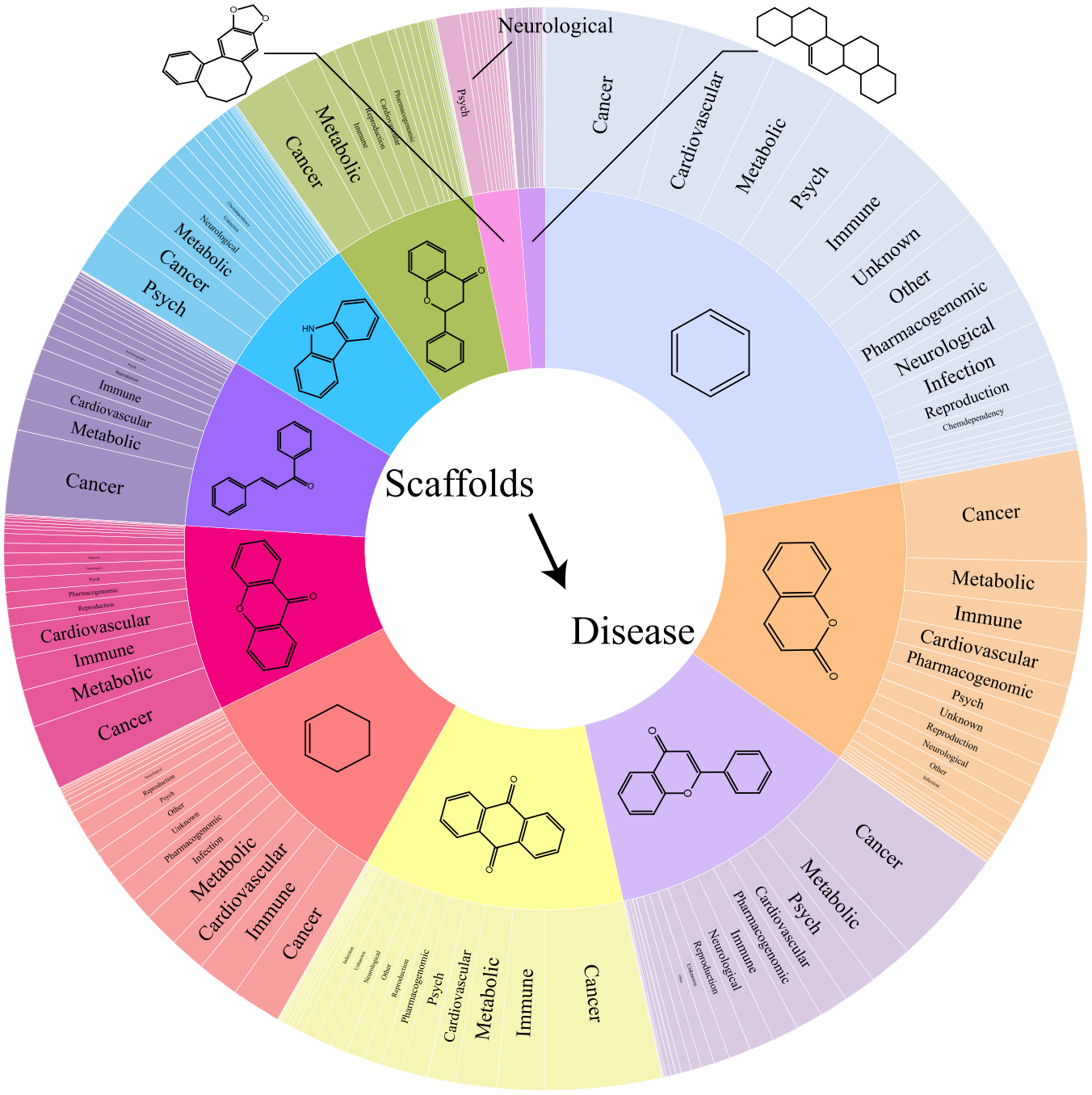

Figure 4. Scaffolds associated with diseases.

theory based on structure−activity relationship, certain similar substances had similar efficacies. However, the overwhelming majority of similar substances belonged to secondary metabolites and were associated more closely with the physiological activities of plants. To analyze the correlation between hereditary materials from herbs, we sorted out the herbs according to family and recorded only herbs belonging to one nature (hot or cold) to avoid interference from the sheer amount of data (Figure 5). The result shows that species from 14 families belonged only to hot nature (Figure 5A) and species from 22 families belonged only to cold nature (Figure 5B).

Chloroplasts of plants have self-governed DNA and encode and translate a large number of active proteins that are closely related to photosynthesis control and the production and conversion of secondary substances. We collected the DNA sequences of the above herbs belonging only to cold nature or hot nature and analyzed them. With the result of gene alignment, the law of the TCMs' nature and its influence on gene analysis was explored. Based on the results of chloroplast DNA alignment, we found that *Gnetum montanum* and *Gnetum parvifolium*, related to hot nature, had a higher similarity sequence because they derive from Gnetales (Figure 6A). We imported structures into DataWarrior and analyzed the similarity of ingredients between *Gnetum montanum* and *Gnetum parvifolium*. The two plants have many active ingredients, but they also have some distance, so the similarity of their ingredients is not rich and mainly focus on stilbenes (Figure

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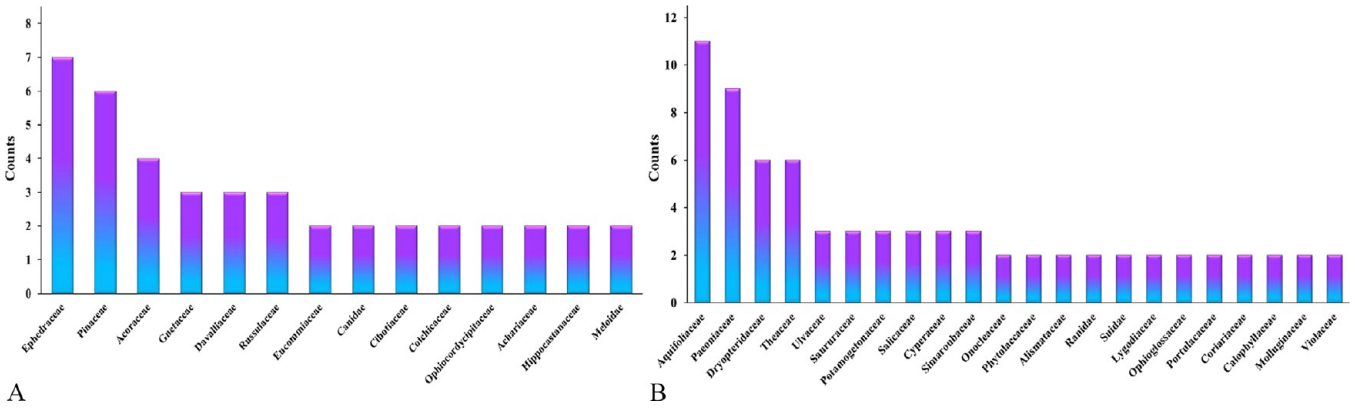

Figure 5. Frequency of families associated with only one nature. (A) Frequency of families associated only with hot nature. (B) Frequencies of families associated only with cold nature.

6B). As revealed by the results, the five plants derived from Gnetopsida were associated more closely with hot nature.

Based on alignment between *Reboulia hemisphaerica*, *Osmunda japonica*, *Lygodium japonicum*, *Cibotium barometz*, *Davallia solida*, *Matteuccia struthiopteris*, and *Cyrtomium fortune*, the result shows that *R. hemisphaerica* emerged earlier than the others, which aligns with the theory of evolution and seven plant sequence alignments (Figure 6A). Through further studies on the ingredients, we found that the compounds from the five herbs were similar to each other and focused on triterpenoids and flavonoids (Figure 6C). Combined with phylogeny, there was a closer generic relationship among them. Combined with the TCMs' nature, the herbs associated with hot and cold natures were crossed. Note that *C. barometz* and *D. solida* are also regarded as warm-nature (also regarded as a transition to hot-nature) instead of hot-nature according to *Chinese Pharmacopoeia* (2015).

The mitochondria of plants or animals have a handful of DNA and various enzyme systems; major enzyme systems include the tricarboxylic acid cycle (TAC), the fatty acid β-oxidase system, electron transport chain enzymes, oxidative phosphorylase, amino acid metabolism enzymes, and the protein- and nucleic acid-synthesizing enzyme system. These enzyme systems are closely associated not only with the normal function of mitochondria but also with the production and metabolism of secondary products; for example, lignans are easily influenced by the enzymes, and the structure will be transformed as the activity of enzyme changes.[11,12] Through the mitochondrial DNA analysis, we discovered that Bootstrap has >0.95 sources of TCMs focused on funguses, lower plants, and animals. As shown in the results, *Rana temporaria*, *Rana nigromaculata*, *Takifugu ocellatus*, and *Python molurus* had a closer generic relationship, and the TCM natures (hot and cold) were crossed. Therefore, *Takifugu ocellatus* was also defined as warm-nature instead of hot-nature, according to *Chinese Materia Medica*. In the aspect of fungi, *Ophiocordyceps sinensi*, *Tolypocladium ophioglossoides*, *Pleurotus ostreatus*, *Inonotus obliquus*, and *Lactarius piperatus* were associated with hot nature, and they were clustered in the same branch of phylogeny. As revealed in the DNA alignment of mitochondria (Figure 7), the TCM natures of funguses and animals can be associated with the DNA sequence of mitochondria and display a certain law. Interestingly enough, similar species in the same nature live in a similar habitat. This shows that the nature of TCMs may be defined with genetic material and living environment simultaneously and not merely defined with only genetic material.

Scaffold Analysis. Comparing scaffolds of compounds from herbs of cold and hot natures and combining the DNA alignment, the compounds of triterpenoids and flavonoids appeared approximately near the origin. Thus, the result of the scaffold analysis shows that the scaffolds of compounds were not entirely different in multiformity, but the main differences focused on quantity and substituents. The top three scaffolds of herbs of cold nature mainly included 2-phenylchrotinone and anthraquinone, and the top three scaffolds of herbs of hot nature mainly included coumarin and cyclohexene (Table 1).

Scaffolds Associated with Nature of TCMs Analysis. Next, 3539 compounds from herbs of cold nature and 3197 compounds from herbs of hot nature were obtained. We predicted the targets of the compounds in light of Bernoulli Naïve Bayes profiling and built a network. Later, we used the screen score (greater than 0.9) of the relative targets for analysis and discovery of 1935 targets, including 1763 targets related to cold nature and 1696 compounds related to hot nature. Moreover, 196 targets only belonged to cold nature, and 129 targets only belonged to hot nature.

Analysis of 2-Phenylochrotinone and Anthraquinone Associated with Cold Nature. Through a differential equation and observing the distribution of 2-phenylchrotinone and anthraquinone, we discovered that the $|K|$ of 2-phenylchrotinone covering targets related to cold nature was greater than that covering targets related to hot nature (Figure 8A), but the AUC related to cold nature was larger than that related to hot nature (Figure 8B). The larger AUC demonstrated that 2-phenylchrotinone is more relevant to cold nature because it covered more targets related to cold nature than hot nature. The larger $|K|$ shows that more compounds of 2-phenylchrotinone covered a few targets related to hot nature, and it demonstrates highly specific targets related to hot nature. Thus, 2-phenylchrotinone is on a spectrum of targets related to cold nature. Similarly, anthraquinone is more related to cold nature because it covered more targets related to cold nature (Figure 8C), although it had a lower value of $|K|$ (Figure 8D).

Analysis of Coumarin and Cyclohexene Associated with Hot Nature. Through previous calculations, we discovered that coumarin and cyclohexene were associated with hot nature. The AUC of coumarin covering targets related to cold nature was larger than hot nature, which indicates that coumarin is related to cold nature (Figure 9A,B). The chemoinformation analysis result of coumarin was different from that of the previous scaffold analysis (see "Scaffold analysis"). However, the AUC of cyclohexene covering targets

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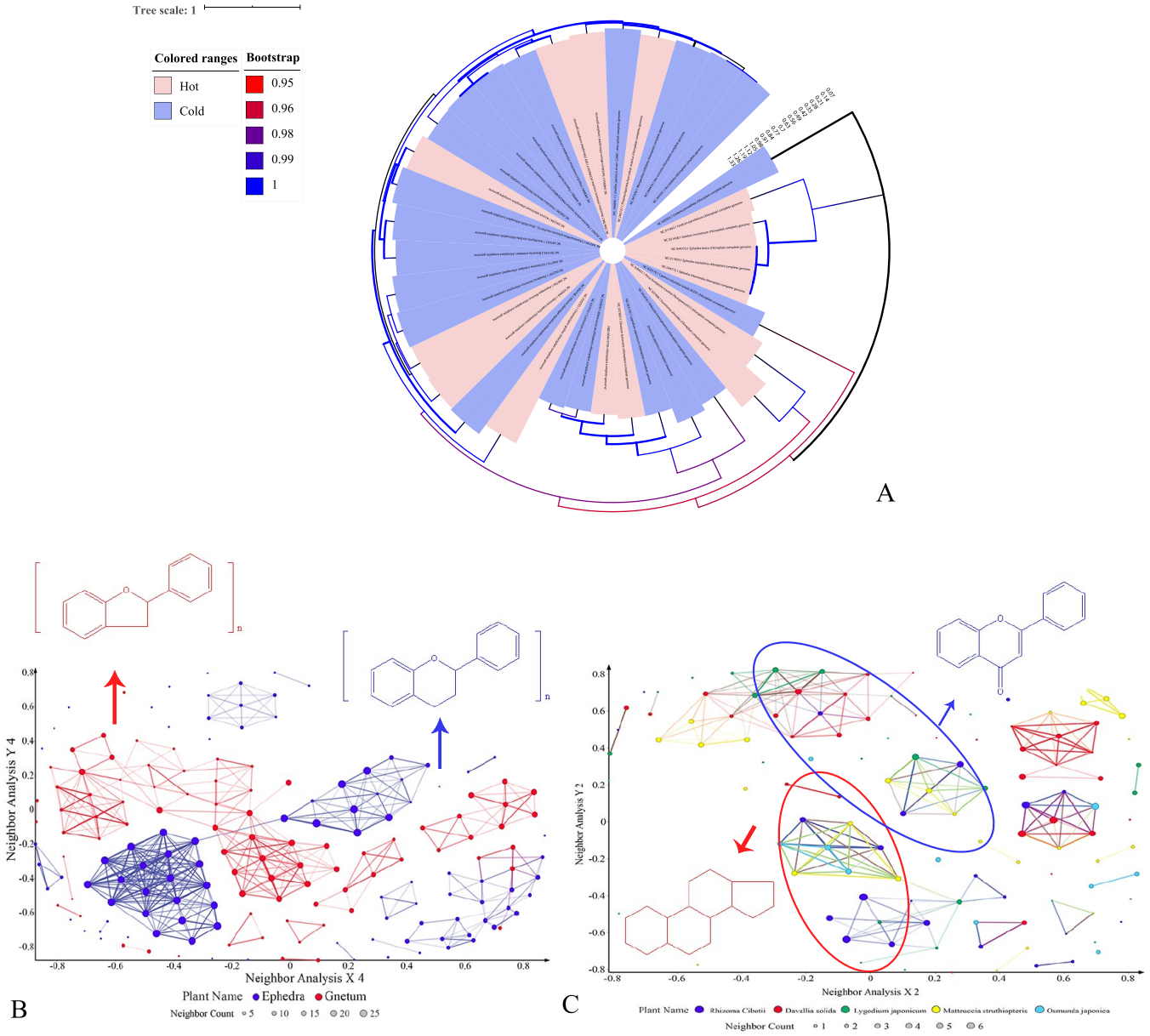

Figure 6. Sequence alignment for DNA and similarity analysis for compounds. (A) Self-governed DNA alignment and phylogeny of chloroplasts, and the names of the sequences were all annotated according to NCBI. (B) Compounds from *Ephedra* and *Gnetum* in a similarity analysis. (C) Compounds from *Rhizoma cibotii* (annotated as *C. barometz*), *D. solida* (annotated as HBG0044-0736), *L. japonicum*, *M. sruthiopteris*, and *O. japonica* in a similarity analysis.

related to hot nature was larger than that related to cold nature. The lower value of |*K*| shows that cyclohexene has high specificity to certain targets associated with cold nature (Figure 9C,D).

To further study the above concepts regarding coumarin, we analyzed the counts of coumarin associated with hot nature and its top 10 corresponding targets (Figure 9E). The result shows that the high specificity for Q16790, Q08499, P14270, and Q9Y616 are key factors that interfere with the results and cause contrary results. In addition to Q16790, Q08499, P14270, and Q9Y616, others in the top 10 targets associated with hot nature were related to more coumarins from herbs of cold nature. Although prior statistics for scaffolds of compounds revealed that the frequency of coumarin associated with hot nature was larger than that of cold nature, in terms of pharmacology research, compounds including coumarin from herbs of cold nature covered more targets. Understanding the nature of TCMs requires pharmacodynamic evaluation. Hence, coumarin should be related more closely to cold nature.

Molecular Descriptors and Similarity Analysis of Compounds. The similarities of compounds associated with different natures were crossed and had a transition between compounds associated with cold and hot nature (Figure 10). As for 2-phenylochrotinone and anthraquinone, the values of the compounds associated with hot nature were larger than those with cold nature on H-acceptors, total surface area, polar surface area, electronegative atoms, rotatable bonds, aromatic rings, aromatic atoms, and sp^3 atoms. As for coumarin, the values of the compounds associated with hot nature were larger than those associated with cold nature on polar surface area, aromatic rings, aromatic atoms, and sp^3 atoms. As for cyclohexene, the compounds associated with cold and hot nature were crossed

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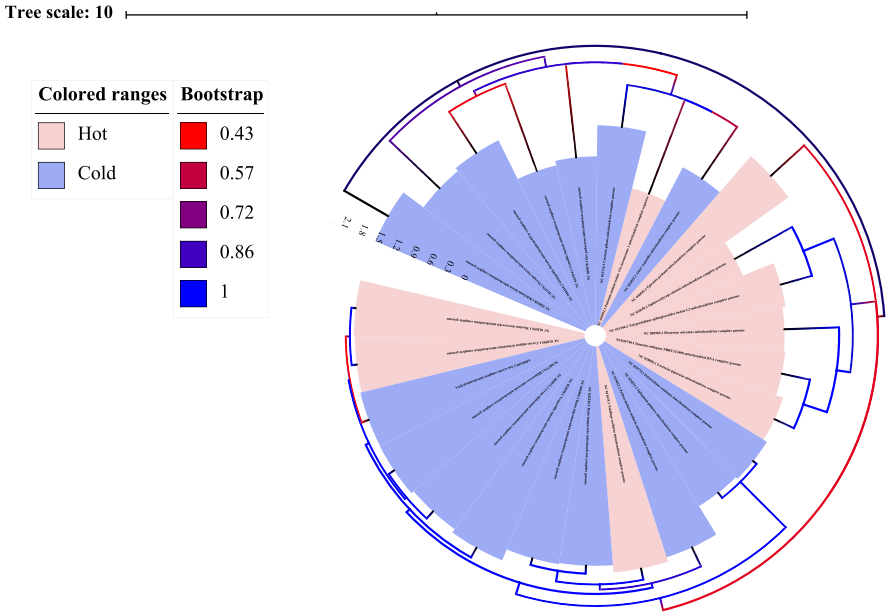


Figure 7. DNA alignment of mitochondria. The names of the sequences are all annotated according to NCBI.

and had high similarity. For integral compounds, from 2-phenylochrotinone, anthraquinone, coumarin to cyclohexene, the value of the shape index increased, indicating the transition of scaffolds from a spherical structure to a linear structure. Moreover, the value of molecular complexity, rotatable bonds, aromatic rings, aromatic atoms, total surface area, polar surface area, H-acceptors, and H-donors decreased, which indicates that the characteristics of compounds associated with hot nature are simpler.

As the above results revealed, the compounds from TCMs of different natures have different characteristics of compounds on molecular descriptors. Moreover, various kinds of compounds display a trend (decreasing or increasing) on molecular descriptors. Compounds from the same scaffold between different natures usually appeared to be crossing. Hence, 2-phenylochrotinone, anthraquinone, and coumarin represent TCMs associated with cold nature, and cyclohexene represent TCMs associated with hot nature. The two categories play different roles in biological activities.

Enrichment Analysis. As shown in the results of the enrichment analysis, 1396 biological functions were associated with cold nature and 1384 biological functions were associated with hot nature. Through comparison, we found that 109 biological functions were only associated with cold nature and 97 biological functions were only associated with hot nature.

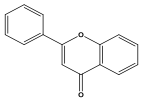
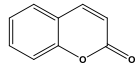
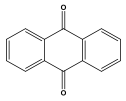
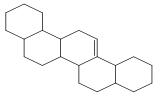
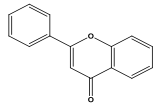
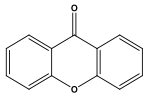
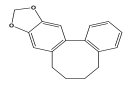
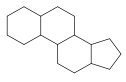
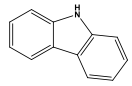
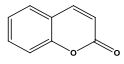
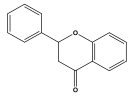
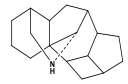
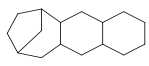
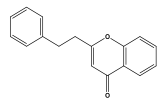
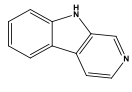
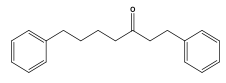
The enrichment analysis showed that functions divided into a focus on signal transmission, the motor system, cardiac condition, blood circulation, the immune system, energy metabolism, and the cell cycle. Functions associated with cold nature were contrary to those of hot nature, which were focused on signal transmission, the motor system, cardiac condition, and blood circulation. For example, targets associated with hot nature could generate positive regulation of cell−cell adhesion, but targets associated with cold nature generated negative regulation of cell−cell adhesion in organisms. In aspects of energy metabolism, cell cycle regulation, and immune regulation, targets associated with cold and hot natures could keep coordination. For example, in the energy metabolism of cells, targets of hot nature could positively regulate the cellular response to the insulin stimulus, positively regulate glucose import, and develop the digestive system, but targets associated with cold nature could prompt ATP generation from ADP (Table 2). Moreover, functions associated with cold nature and hot nature were in coordination with each other in energy metabolism, the cell cycle, and the immune system.

Analysis of Anti-COVID-19 Drugs. To analyze the prescription pattern of anti-COVID-19 TCMs to offer guidance on the utilization of TCMs, we collected the three medicines and three recipes to analyze their efficacies with VOSviewer in association with strength methods. The results show that TCMs involved in relieving cough and eliminating dampness, reducing phlegm, detumescence, relieving pain, and heat-clearing and detoxification were applied to combat the SARS-CoV-2 virus. As shown in the diagram, the cold nature was more closely associated with heat-clearing and detoxification than reducing phlegm. The hot nature was more closely associated with tonifying spleen, dispelling wind, and diaphoresis. However, both cold and hot natures were related to resolving phlegm (Figure 11).

We collected components of the three medicines and three recipes and predicted targets according to eq 1. Then, we conducted a gene ontology (GO) enrichment analysis of predicted targets and obtained the pathway from Kyoto Encyclopedia of Genes and Genomes (KEGG). We transferred effects of TCMs and the KEGG pathway into the matrix with its targets and adopted the paired group algorithm[13] to cluster (Figure 12). The result reveals that some effects of TCMs contained and maintained a large distance with more KEGG pathways, for example, clearing heat, cooling blood, and activating blood. However, relieving cough and relieving phlegm maintained a short distance with the regulation of lipolysis in adipocytes, the PPAR signaling pathway, and the Ras signaling pathway. Relieving pain, regulating qi, and relieving asthma maintained a short distance with the Rap1 signaling pathway, the Hedgehog signaling pathway, and arachidonic acid metabolism. Patients infected with SARS-CoV-2 usually experience diarrhea, inappetence, dyspepsia, and atony. TCMs from three medicines and three recipes could have an antidiarrheal effect when

https://doi.org/10.1021/acsomega.1c04440
ACS Omega 2021, 6, 33583−33598

Table 1. Top 10 Scaffolds of TCM in Cold/Hot Nature

NO	Cold			Hot		
	Scaffold	count	Fre.	Scaffold	count	Fre.
1	 C_6H_6	110	3.108	 C_6H_6	182	5.818
2	 $C_{15}H_{10}O_2$	62	1.952	 $C_9H_6O_2$	62	1.939
3	 $C_{14}H_8O_2$	49	1.328	 C_6H_{10}	38	1.189
4	 $C_{22}H_{34}$	49	1.328	 $C_{15}H_{10}O_2$	39	1.159
5	 $C_{13}H_8O_2$	45	1.292	 $C_{19}H_{16}O_2$	29	0.909
6	 $C_{19}H_{28}$	44	1.243	 $C_{12}H_9N$	28	0.896
9	 $C_9H_6O_2$	40	1.13	 C_6H_{12}	28	0.896
8	 $C_{15}H_{12}O_2$	38	1.094	 $C_{19}H_{25}N$	26	0.813
9	 $C_{16}H_{26}$	34	0.961	 $C_{19}H_{14}O_2$	19	0.594
10	 $C_{11}H_8N_2$	26	0.935	 $C_{19}H_{22}O$	19	0.594

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ACS Omega 2021, 6, 33583−33598

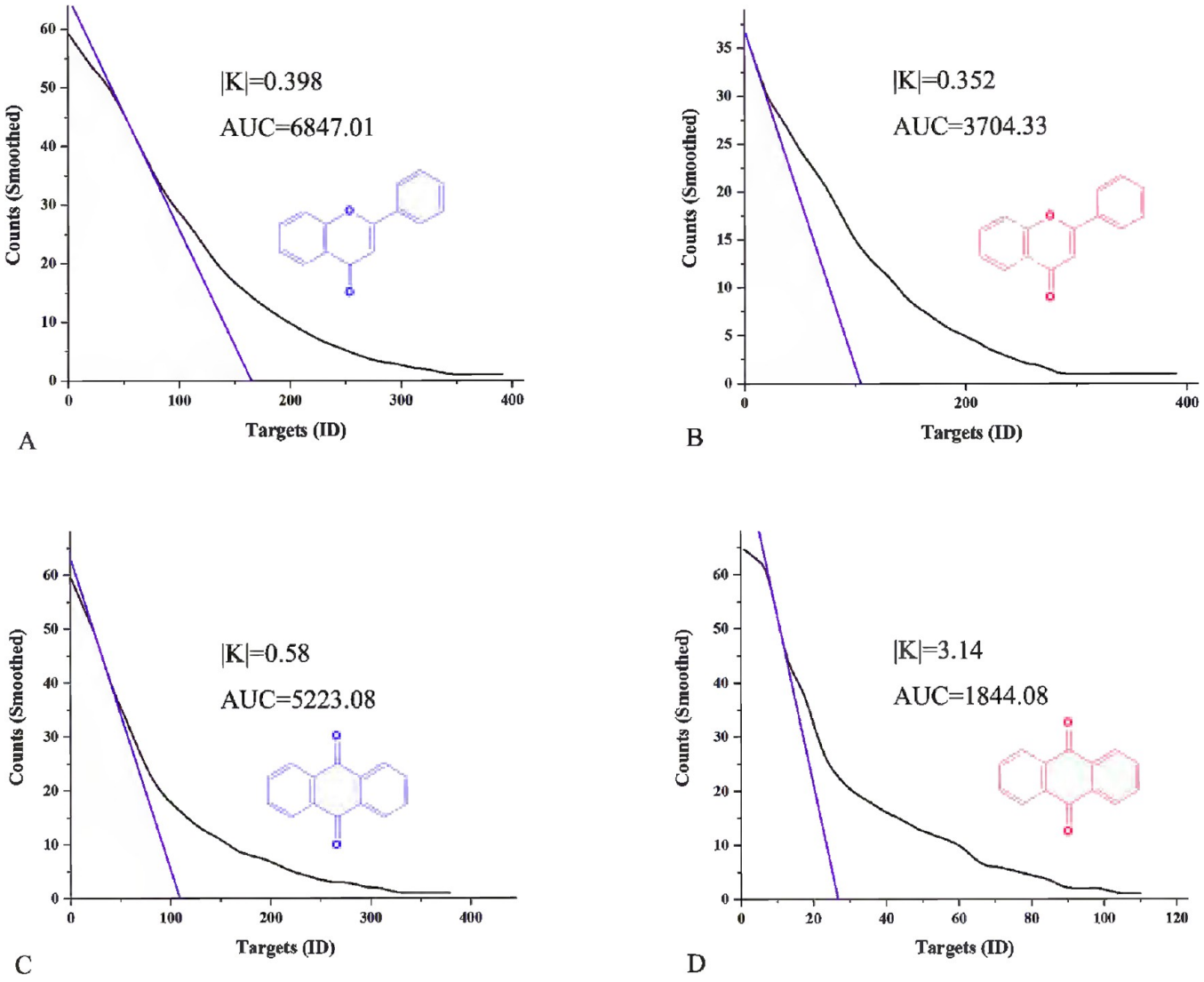

Figure 8. AUC and |*K*| analysis for compounds and their corresponding targets. (A) AUC and |*K*| of 2-phenylchrotinone covering targets associated with cold nature. (B) AUC and |*K*| of 2-phenylchrotinone covering targets associated with hot nature. (C) AUC and |*K*| of anthraquinone covering targets associated with cold nature. (D) AUC and |*K*| of anthraquinone covering targets associated with hot nature. The structures of the compounds marked in red color are associated with hot nature, and those in blue color are associated with cold nature.

expelling retained food, involving the pathway of the Gap junction and phagosomes. Tonifying spleen and tonifying qi could also be interpreted as boosting immunity and strengthening patients' physiques, which involves the pathway of tyrosine metabolism, the Wnt signaling pathway, the VEGF signaling pathway, and vascular smooth muscle contraction to avoid the occurrence rate of critical illness.

Through the statistical result of compounds from the three medicines and three recipes and associated targets, the result shows that each traditional effect of the TCMs covered many pathways of KEGG, they had more corresponding compounds, and these compounds played a role in the pathways of KEGG (Figure 12). Combining the molecular descriptors and similarity of the compounds (Figure 10), 2-phenylochrotinone, anthraquinone, and coumarin had high similarity, so compounds containing scaffolds of 2-phenylochrotinone, anthraquinone, and coumarin often took effect on the same targets and in the same pathway; for example, compounds containing the scaffolds of 2-phenylochrotinone, anthraquinone, and coumarin could take effect on the adipocytokine signaling pathway and GnRH signaling pathway. In addition, compounds containing different scaffolds often have trends and play certain effects. For example, cyclohexene and its compounds were similar to cyclohexene and played roles in neuroactive ligand-receptor interaction, inflammatory mediators, and natural killer cell-mediated cytotoxicity. Anthraquinones and their similar compounds could play a role in the neurotrophin signaling pathway, tyrosine metabolism, the B cell receptor signaling pathway, and bile secretion. Moreover, 2-phenylochrotinone and their similar compounds played a role in steroid biosynthesis.

We extracted targets according to the COVID-19 pathway from KEGG and constructed a network among compounds, targets, and pathways (Figure 13B). To explore the relationship between three medicines and three recipes and COVID-19, we did an enrichment analysis for targets we collected (Figure 13A,B), and the enrichment score ($P > 0.01$) shows that three medicines and three recipes could fight against the virus, mainly through the TNF signaling pathway, T cell receptor signaling pathway, Toll-like receptor signaling pathway, VEGF signaling pathway, and Fc gamma R-mediated phagocytosis (Figure 13C).

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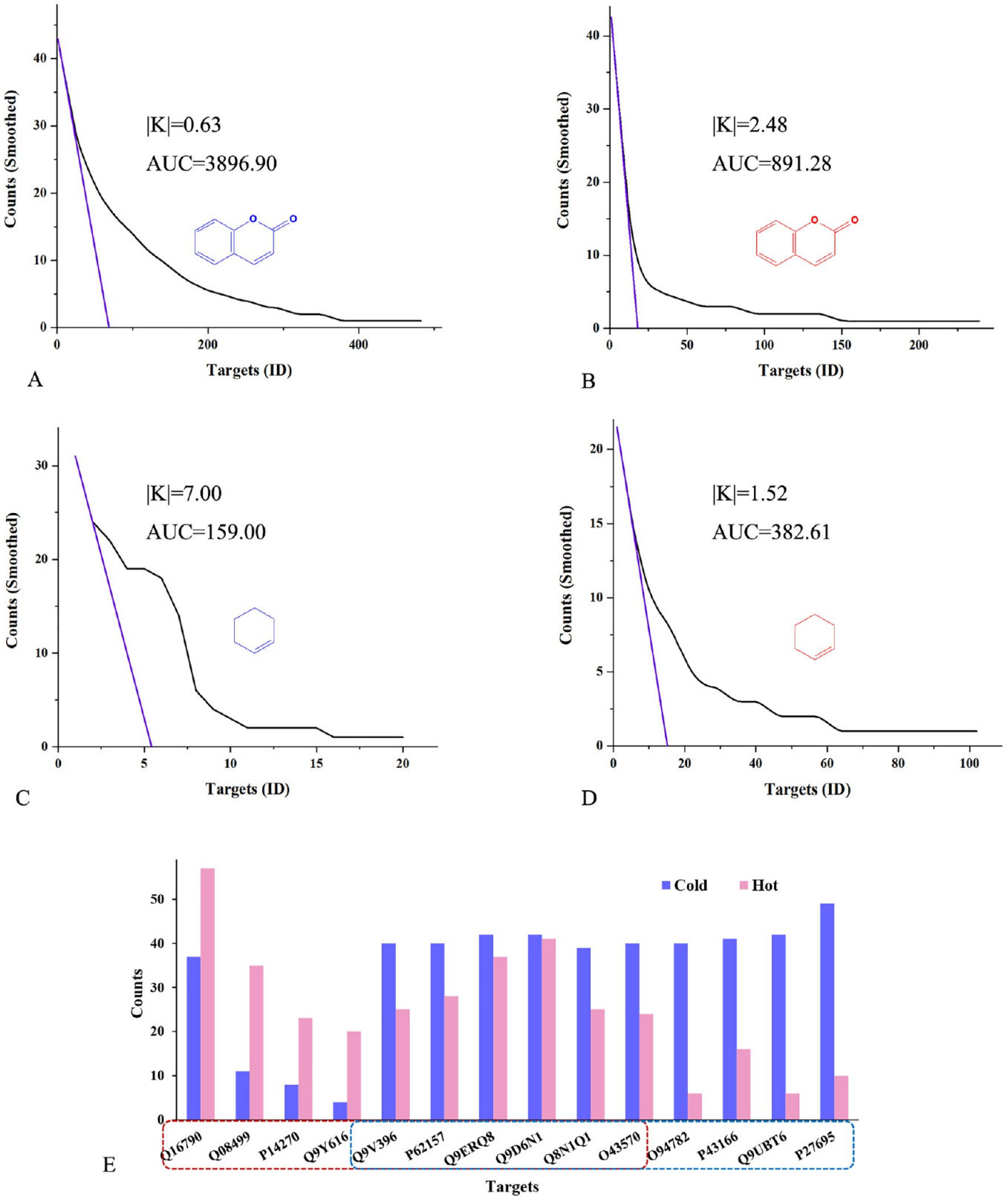

Figure 9. AUC and |*K*| analysis for compounds and its corresponding targets. (A) AUC and |*K*| of coumarin covering targets associated with cold nature. (B) AUC and |*K*| of coumarin covering targets associated with hot nature. (C) AUC and |*K*| of cyclohexene covering targets associated with cold nature. (D) AUC and |*K*| of cyclohexene covering targets associated with hot nature. (E) Counts of coumarin associated with different natures and their top 10 corresponding targets. The structures of the compounds marked in red color are associated with hot nature, and the structures of the compounds marked in blue color are associated with cold nature.

By establishing the gene signature profiles of 166 TCMs and performing high-throughput sequence-based screening, the 139 pathways related to virus infection, immunity, inflammation, metabolism, cell proliferation, apoptosis, and migration (such as

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ACS Omega 2021, 6, 33583−33598

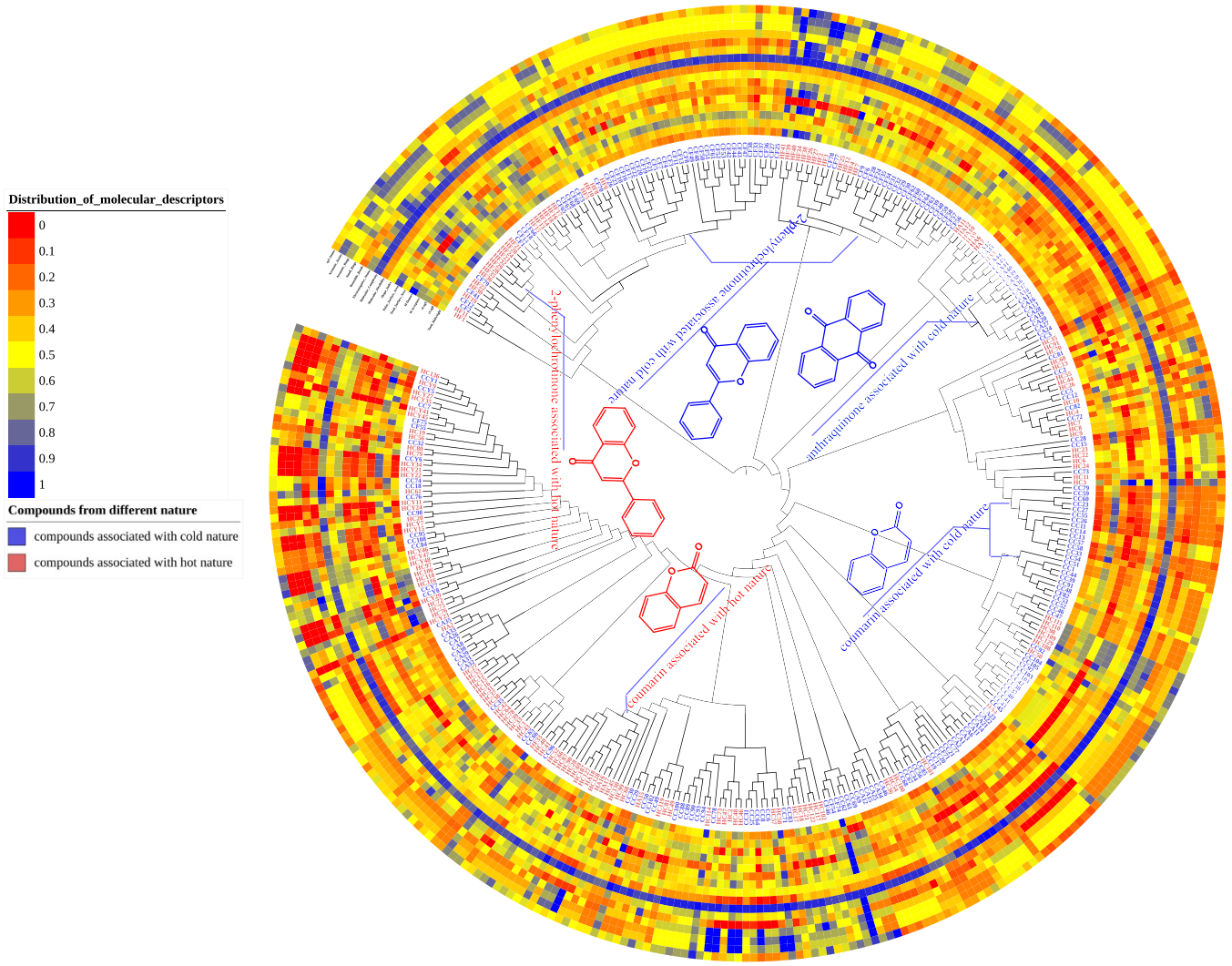

Figure 10. Similarity analysis and distribution of molecular descriptors for compounds. HF, 2-phenylchrotinone associated with hot nature; CF, 2-phenylchrotinone associated with cold nature; HA, anthraquinone associated with hot nature; CA, anthraquinone associated with cold nature; HC, coumarin associated with hot nature; CC, coumarin associated with cold nature; HCY, cyclohexene associated with hot nature; CCY, cyclohexene associated with cold nature. The structures of the compounds marked in red color are associated with hot nature, and those in blue color are associated with cold nature.

the Toll-like receptor signaling pathway, VEGF signaling pathway, NF-κB signaling pathway, and RIG-I-like receptor signaling pathway) were exposed.[14] In the clinic, the Lianhua Qingwen capsule[15] and the Xuebijing injection[16] could reduce proinflammatory cytokines of TNF-α (P01375). Owing to the limitation of the clinic study, more targets and mechanisms for the three medicines and three recipes were not verified, but for the current clinic studies, the three medicines and three recipes, under the guidance of "efficacy−nature−structure," obtained good clinical effects and demonstrated the scientific theory of "efficacy−nature−structure."

CONCLUSIONS

Over the 2000-year history of TCMs, the nature of TCMs has come to be known as the principle of empiricism and has its own scientific connotation. China has adopted TCMs to fight against COVID-19 and has gained more time for victory in 2021. The research showed the different natures and scaffolds covering different disease classifications. The sequence of DNA alignment and scaffold analysis showed that the natures (cold and hot) of TCMs were crossed and appeared to be in a transition between cold nature and hot nature. Based on the theory of "efficacy−nature−structure," the research uncovered many results covering pharmacology, structural chemistry, and biology. The results reveal that the compounds of cold nature included 2-phenylchrotinone, anthraquinone, and coumarin, which were associated more closely with cancer and metabolic and immune diseases. Compounds including cyclohexene, which is of hot nature, were more closely associated with cardiovascular diseases. The results can provide a reference for pharmacology and structural biology. These inherent laws of TCMs may also offer a reference for new drug screening and localization of foreign herbs as well as guide the application of TCM prescriptions.

METHODS

Data Collection and Standardization. We looked up and retrieved compounds from ChemSpider (http://chemspider.com), PubChem (https://pubchem.ncbi.nlm.nih.gov), ChemExper (http://chemexper.com), ChEMBL (https://ebi.ac.uk/

https://doi.org/10.1021/acsomega.1c04440
ACS Omega 2021, 6, 33583−33598

Table 2. Comparison of Main Functions between Enrichment Associated with Cold and Hot Nature

relationship	biological system	hot	cold
opposition	signal transmission	secretion by tissue	negative regulation of secretion by cell
		positive regulation of cell−cell adhesion	negative regulation of cell−cell adhesion
		anion transmembrane transport	regulation of cation channel activity
	motor system	positive regulation of muscle contraction	negative regulation of muscle contraction
	cardiac condition, blood circulation	cardiac muscle tissue growth	cardiac muscle cell apoptotic process
		positive regulation of blood coagulation	regulation of platelet aggregation
			positive regulation of blood vessel diameter
coordination	cell cycle	negative regulation of cell cycle G1/S phase transition	G2 DNA damage checkpoint
		DNA damage checkpoint	cell-cycle checkpoint
		chromosome organization	
		DNA damage response, signal transduction by p53 class mediator	
		DNA integrity checkpoint	
		positive regulation of cell cycle arrest	
	energy metabolism	response to food	cellular response to glucose stimulus
		regulation of digestive system process	ATP generation from ADP
		positive regulation of cellular response to insulin stimulus	
		negative regulation of insulin receptor signaling pathway	
		digestive tract development	
		positive regulation of glucose import	
		eating behavior	
	immune system	acute inflammatory response	cytokine secretion
		regulation of tumor necrosis factor production	response to interleukin-4
		positive regulation of inflammatory response	A−β T cell activation
		regulation of tumor necrosis factor superfamily cytokine production	T cell differentiation
		positive regulation of T cell activation	
		interferon γ-mediated signaling pathway	

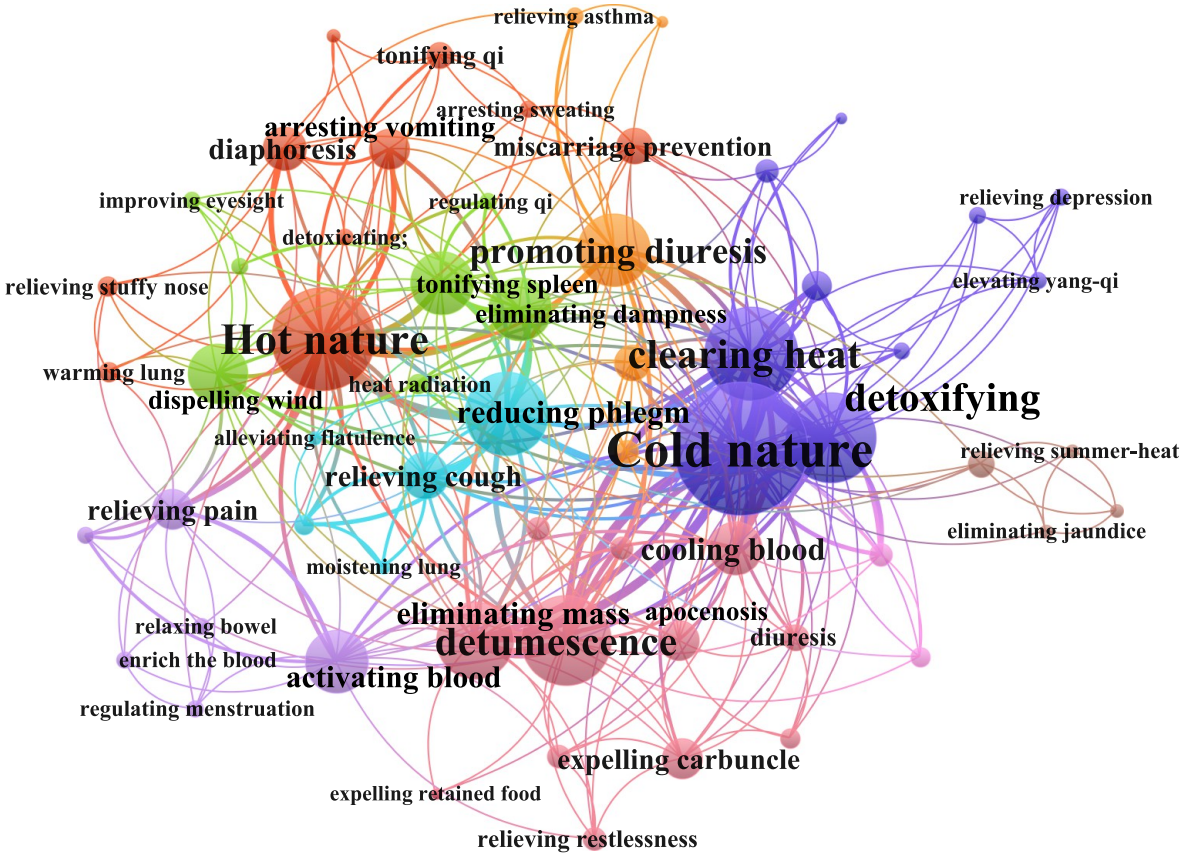

Figure 11. Effects of TCM clustering analysis.

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ACS Omega 2021, 6, 33583−33598

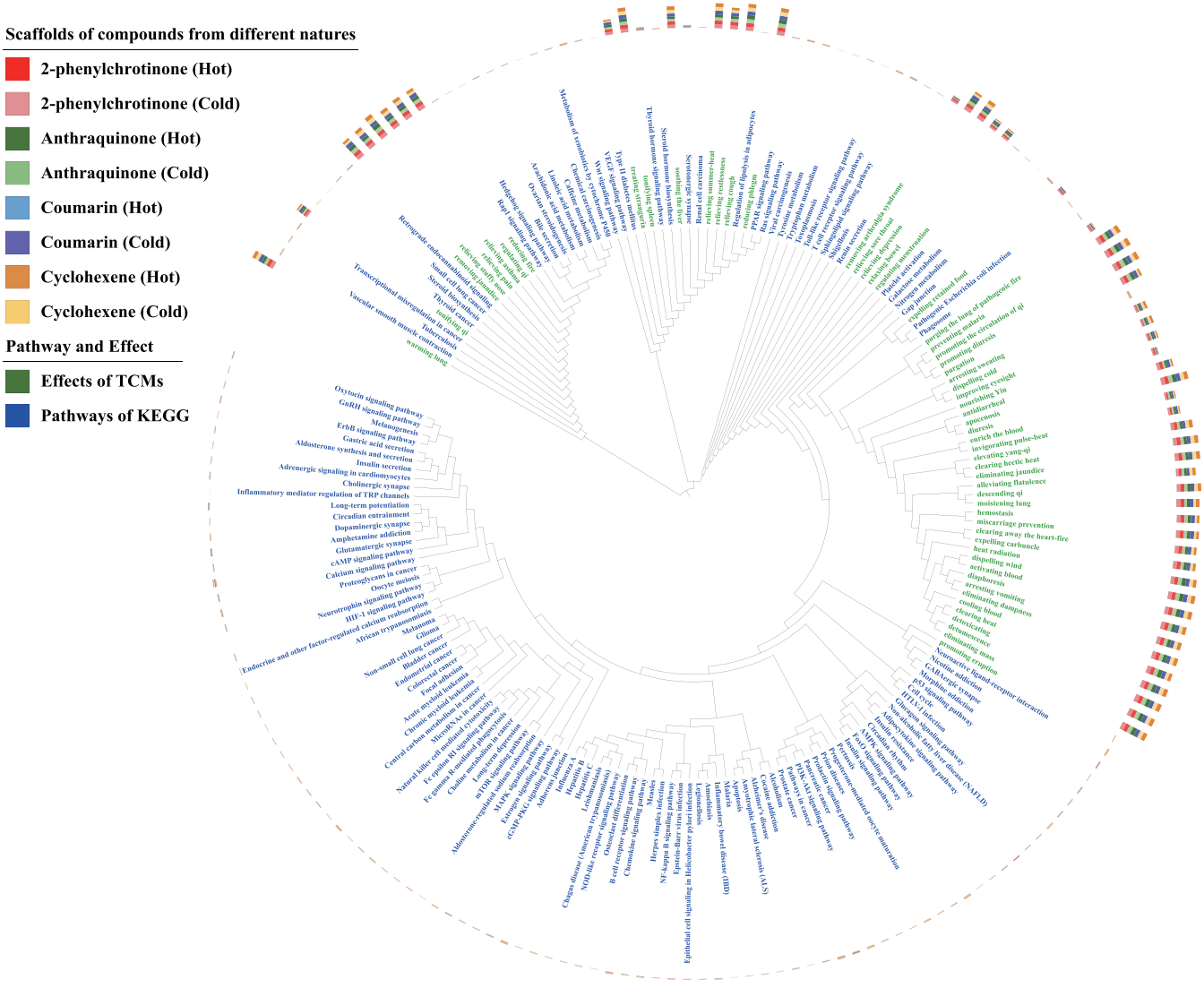

Figure 12. Clustering analysis of targets from effects of TCMs and the KEGG pathway.

chembldb/), and TCMD (version 2009), collecting 23,033 compounds from 2444 kinds of herbs. Later, we standardized the nature of ingredients according to the source of herbs based on *Pharmacopoeia of the People's Republic (2015)* and *Chinese Materia Medica*. We used Discovery Studio 4.5 to calculate ADMET descriptors to predict oral bioavailability, and the collected compounds' absorption level was defined as 0 (good) or 1 (moderate) for screening.[1] Ultimately, after screening and collecting, 3539 ingredients from herbs of cold nature and 3197 ingredients from herbs of hot nature were obtained.

Target Prediction and Enrichment Analysis. The compounds were subjected to a target prediction algorithm comprising Bernoulli Naïve Bayes profiling,[17,18] and the targets were named with their UniProt ID (http://beta.uniprot.org/). The model was constructed through the assimilation of over 195 million bioactivity data points deposited in the ChEMBL and PubChem repositories. We regarded the calculation score as an evaluation of the correlation between compounds and targets in light of eq 1, and considered a score higher than 0.9 as the default to collect relative targets and their corresponding compounds. Later, we imported relative targets into ClueGO[19] and set the *P* value to 0.01 to screen the pathway and molecular function.

$$\text{average ratio} = \frac{\frac{F(\text{random set1})}{F(\text{test set})} + \frac{F(\text{random set2})}{F(\text{test set})} + \ldots + \frac{F(\text{random set10,000})}{F(\text{test set})}}{10{,}000} \quad (1)$$

Diseases Associated with the Nature of TCMs. We added compounds associated with targets into the GAD Database[10] to obtain relevant diseases. Then, we classified the diseases according to GAD disease class. We built a matrix between targets and relevant diseases to analyze the coverage rate of compounds in different natures. To avoid disturbance, we excluded some low-correlation targets $\left(\frac{\chi_{\text{compounds in cold nature}}}{\chi_{\text{compounds in hot nature}}} = 0.6\right)$ according to eq 2 and obtained the coverage rate using eq 3 to explore the association between the nature of TCMs and disease.

$$\chi \le \left| 1 - \left| \frac{\chi_{\text{compounds in cold nature}}}{\chi_{\text{compounds in hot nature}}} \right| \right| \quad (2)$$

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ACS Omega 2021, 6, 33583−33598

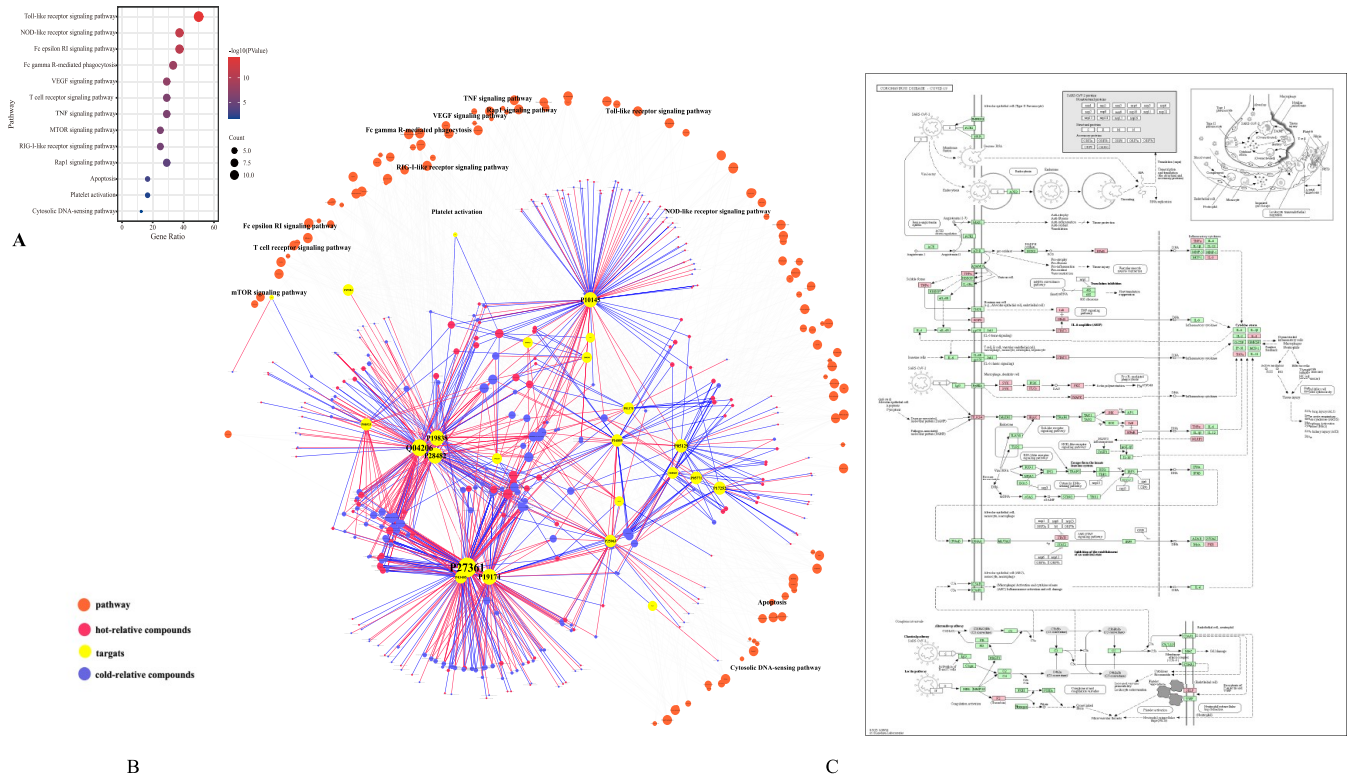

Figure 13. Biological analysis for the three medicines and three recipes. (A) Enrichment analysis for targets from the three medicines and three recipes. (B) Network of the compound−target−pathway analysis. (C) Enrichment analysis for targets in the pathway of COVID-19.

$$\text{coverage rate}_{\text{compounds in cold or hot}} = \frac{\chi_{\text{compounds in cold or hot}}}{\chi_{\text{compounds in cold}} + \chi_{\text{compounds in hot}}} \quad (3)$$

Analysis for Scaffold-Associated Diseases. To further study the relationship between compounds and disease, we calculated Murcko scaffolds of compounds and frequency, and collected compounds with frequencies of >27 to enrich associated diseases so as to get the result of the compound distribution.

Gene Alignment of Organelles between Cold-Relative and Hot-Relative Herbs. The mitochondria and chloroplasts of higher plants are known as semiautonomous organelles because they are controlled by two sets of genetic information and have self-governed DNA to maintain normal function. Mitochondria and chloroplasts are closely related to the biochemical reaction, production, and metabolism of a secondary substance and contain a great deal of active compounds that can be used to treat disease. Molecular hybridization has demonstrated that the same genus has a higher hybridizing rate; thus, the sequences have higher homology among the closely related species. To align the sequences to mine heredity laws between herbs of cold nature or hot nature, we downloaded relative reference sequence genes of species from NCBI (https://ncbi.nlm.nih.gov/), and imported them into the megaX[20] Clustal W to align sequences so that we could later compute the pairwise distance and build a phylogeny.

Scaffolds of Compounds Retrieval and Analysis. DataWarrior[21] is an interactive, chemistry-aware, multipurpose data visualization and analysis program that provides views to visualize data, discover correlations, and extract hidden knowledge from large data sets. We imported the SMILES of the compounds into DataWarrior to calculate Murcko Scaffolds.[22] Then, we collected ingredients to calculate the similarity and counted the kinds of scaffolds.

We combined the scaffolds with corresponding targets to analyze and distinguish predominant scaffolds from herbs associated with cold nature from scaffolds from herbs associated with hot nature. Then, we collected the compounds and corresponding targets and imported them into Origin 2018. Later, we used the LOWESS algorithm[23] in eq 4 to smooth and get $f(x)$. We integrated $f(x)$ into a differential equation to calculate $f(x)'$ in eq 5 and acquired an absolute value of maximum slope $|K|$ to describe the specificity of compounds. Then, we computed the integral of the area under curve (AUC) in eq 6 to describe the spectrum of compounds. Through the process of smoothing, we performed the calculations and obtained the results.

$$wi = \left(1 - \left|\frac{x - xi}{d(x)}\right|^3\right)^3 \quad (4)$$

$$f(x)' = \frac{dy}{dx} = \frac{d}{dx}f(x) \quad (5)$$

$$\text{AUC} = \int_a^b f(x)\mathrm{d}x = F(b) - F(a) = F(x)|_a^b \quad (6)$$

We analyzed the similarity of compounds based on the fragment-based similarity measure and built a similarity tree. Then, we calculated molecular descriptors (Total Molweight, cLogP, cLogS, H-Acceptors, H-Donors, Total Surface Area, Polar Surface Area, Shape Index, Molecular Flexibility,

https://doi.org/10.1021/acsomega.1c04440
ACS Omega 2021, 6, 33583−33598

Molecular Complexity, Electronegative Atoms, Rotatable Bonds, Small Rings, Aromatic Rings, Aromatic Atoms, and sp^3 Atoms) to standardize the characteristics of the compounds and normalize the datasets in linear normalization (eq 7) for comparison.

$$\chi_{norm} = \frac{\chi - \chi_{min}}{\chi_{max} - \chi_{min}} \quad (7)$$

Anti-COVID-19 TCM Analysis. The three medicines and three recipes are typical successful cases of application for TCM prescription exhibiting high efficacy rate in TCM clinics. The three medicines are the Jinhua Qinggan granule (73% effective),[24] the Lianhua Qingwen capsule (94.29% effective),[25] and the Xuebijing injection (60% effective).[26] The three recipes are the Qingfei Paidu decoction (>90% effective),[27] the Huashi Baidu decoction (74.7% effective),[28] and the Xuanfei Baidu decoction (72.3% effective).[29] Therefore, we collected the efficacy of TCMs from three medicines and three recipes and standardized them according to *Chinese Pharmacopoeia (2015)*. Later, we collected compounds from three medicines and three recipes and analyzed the targets with eq 1. Moreover, we adopted a correlation and cluster analysis between the efficacy and the targets. Ultimately, the biological mechanism of three medicines and three recipes was revealed by an enrichment analysis with DAVID (https://david.ncifcrf.gov/) and was visualized by Cytoscape 3.4.0.[30]

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors thank the Natural Science Foundation of China (NSFC, 81473369) for their financial support. This work was also supported by the National Key Research and Development Program of China (2017YFC1702703), the Key R&D Programs in Shandong (2016CYJS08A01−1), and the Shandong Taishan Scholar Climbing Project.

ABBREVIATIONS

AUC, area under the curve; Fre., frequency; K, slope; TCM, traditional Chinese medicine; TCMM, traditional Chinese marine medicine; TRP, transient receptor potential ion channel protein; |K|, absolute value of slope

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https://doi.org/10.1021/acsomega.1c04440
ACS Omega 2021, 6, 33583−33598