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The characterization of traditional Chinese medicine natures and flavors using network pharmacology integrated strategy

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ABSTRACT

Background and aim: Due to the complexity of TCM ingredients and medication compatibility, TCM cannot be used like chemical medicines. The theory of "Four Natures and five Flavors" provides a theoretical basis for the use of TCM. "Four Natures and five Flavors" are originated from pharmacological rules based on clinical practices. Whereas, How to describe and characterize "Natures"(Warm, Hot, Cold and Cool) and "Flavors" (Pungent, Sour, Sweet, Bitter and Salty) scientifically remain the issue that needs to be solved. The aim of this study is to establish the TCM characterization models based on the integrated pharmacology network strategy and provide a deeper understanding of TCM theory.

Experimental procedure: Five "Pungent-Neutral", nine "Sweet-Neutral and nine "Bitter-Neutral" TCMs were selected to characterize the "Flavors" (Pungent, Sweet and Bitter). Nine "Pungent-Warm" and nine "Bitter-Cold" TCMs were selected to characterize the "Natures" (Warm and Cold). The screened chemical ingredients were analyzed by classification and the screened characteristics targets were analyzed by GO and KEGG enrichment analysis.

Results and conclusion: In the "Pungent" group, flavonoids are the most. "Pungent" may have immune-regulatory effects and potential anticancer activity. In the "Sweet" group, isoflavones are the most. "Sweet" are related to effectively invigorate health. Fatty acids in the "Warm" group are the most. Flavonoids in the "Cold' group are far more than other components. "Warm" and "Cold" are both related to fatty acid and energy metabolism.

1. Introduction

Traditional Chinese Medicine (TCM) has a rich history and is widely recognized for its efficacy in China. Among the multidimensional attributes of TCM, "Natures and Flavors", "Tropisms", "Ascending-Descending-Floating-Sinking", "Toxic and Non-Toxic", "Natures and Flavors" belong to the core theory of them. Understanding and characterizing them is a crucial scientific challenge in TCM modernization study. The "Natures" include "Warm, Hot, Cold and Cool" called "four Natures". Some researchers have experimentally confirmed that some key players distinguishing Cold/Hot are affected by corresponding Cold/Hot ZHENG-oriented herbal treatments, respectively.[1] It can be summarized that TCMs which can alleviate or eliminate fever syndrome generally belong to chill "Natures", which can be divided into "Cold and Cool" in degree. On the other hand, The TCMs that can alleviate or eliminate chill syndrome generally belongs to thermal "Natures", which can be divided into "Hot and Warm" in degree.[2] The essence of the "Natures" is that they can regulate the body's energy production process.[3] Some TCMs do not express the "Natures" trend that belongs to the "Neutral" attribute. Some researchers have found that the correlations between volatile oil and "Warm", followed by "Cold" and alkaloids and organic acids, "Neutral" and saccharides and organic acids.[4] "Flavors" include "Pungent, Sour, Sweet, Bitter and Salty" called "five Flavors". The concepts of "Flavors" originated from people's taste perception of the TCM and gradually evolved into a high-level summary of TCM's efficacy. They are used to describe the pharmacological response of TCM to the body. The main ingredients of "Pungent" TCM include volatile oils, glycosides, and alkaloids.[5,6] "Sweet" TCM often contains nutrients such as saccharides, amino acids, vitamins, proteins sterols, and saponins, that maintain body metabolism.[7,8] Some studies have reported that "Bitter-Warm" TCM is mainly composed of volatile oil. "Bitter-Cold" is mainly composed of alkaloids and glycosides.[9] The pH value of pure"-Sour" TCM is mostly below five.[10] The ingredients of "Sour" TCM

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List of abbreviations

TCM	Traditional chinese medicine
TCMSP	Traditional chinese medicine systems pharmacology database and analysis platform
ETCM	The encyclopedia of traditional chinese medicine
PN	Pungent-neutral
SN	Sweet-neutral
BN	Bitter-neutral
BC	Bitter-cold
PW	Pungent-warm
PPI	Protein-protein interaction
MF	Molecular function
BP	Biological process
CC	Cell component
KEGG	Kyoto Encyclopedia Of Genes And Genomes

include organic acids, flavonoids, tannins, and volatile oils.[11,12] Most "Salty" TCMs are derived from animal sources and contain inorganic salts and minerals. A kind of TCM can belong to multiple "Flavors", but only belongs to one "Nature". The theoretical explanations of "Natures and Flavors" are primarily documented in ancient books, and their efficacy are expounded within the theoretical framework of TCM. How to understand the scientific underpinnings of TCM while preserving and leveraging its distinctive strengths is a challenging and valuable issue. Some scholars have proposed the methods based on the separability and composability of TCM "Natures and Flavors" can promote to solve the TCM research issue.[13]

Network pharmacology integrates systems biology, bioinformatics, network science, and other disciplines and analyzes the molecular relationship between drugs and treatment objects to reveal the systematic pharmacological mechanism of drugs from an overall perspective of the system level and biological network, thereby guiding the drug development and clinical diagnosis

and treatment.[14,15] The multi-level network models using network pharmacology technology to study TCM from a comprehensive perspective, have become novel strategies to elucidate the TCM effectiveness and scientific basis. At present, there are few studies on the theory of the "Natures and Flavors" by using network pharmacology technology. Jiang Miao proposed a new strategy to construct a classification model of "Cold and Hot" of TCM from pharmacological biological effects using network pharmacology.[16]

In our previous study, we characterized the "Natures" (Pungent, Sweet, Bitter) and "Flavors" (Warm, Cold) based on metabolomics.[17] In this study, We collected "Pungent-Neutral", "Sweet-Neutral, "Bitter--Neutral", "Pungent-Warm" and "Bitter-Cold" TCMs to established the "Natures and Flavors" -ingredients-targets network models to characterize "Flavors" (Pungent, Sweet and Bitter) and "Natures" (Warm and Cold) using integrated network pharmacology. The characteristics ingredients were classified and analyzed, and the characteristics targets were analyzed using GO and KEGG enrichment analysis to gain further understanding of the "Natures and Flavors" mechanism.

2. Database and tools

2.1. Database

TCMSP(old.tcmsp-e.com/tcmsp.php), contains 499 Chinese herbal medicines and approximately 29000 ingredients[18]; HERB(herb.ac.cn/), contains 263 Chinese herbal medicines and over 28000 related ingredients[19]; Shanghai Chemical Professional Database(www.organchem.csdb.cn/scdb/), contains over 22000 herbs and more than 19700 ingredients[20]; ETCM(www.tcmip.cn/ETCM/), contains more than 400 Chinese herbal medicines and over 7200 ingredients[21]; PubChem (pubchem.ncbi.nlm.nih.gov/), is the world's largest database for free access to chemical information.

2.2. Tools

SwissADME (http://www.swissadme.ch/), is an online tool that can calculate the physicochemical properties of compounds, and predict pharmacokinetic parameters and drug-like properties[22]; SwissTargetPredition (http://www.swisstargetprediction.ch/), is an online tool for the most likely macro-molecular targets for predicting small compounds. The prediction tool is based on the 2D and 3D structural similarity of over 3000 proteins and 3.7 million known active compounds from three different species, including humans[23]; Metascape(https://metascape.org/), is an integrated and user-friendly online tool that provides comprehensive gene list annotation and analysis resources, which can be annotated and enriched based on targets obtained from network pharmacology.[24] Cytoscape 3.9.1, is an open-source freeware tool that can be used to analyze, edit and visualize the network data of the protein-protein interactions.[25]

3. Method

3.1. Candidate tool TCMs

The "single-Flavor" TCMs were obtained and counted from the Chinese Pharmacopoeia (2020 edition). The appropriate TCMs were selected as the candidate tool TCMs for studying "Natures and Flavors" based on the statistical results. Finally, five "Pungent-Neutral", nine "Sweet-Neutral" and nine "Bitter-Neutral" plant TCMs were selected to study "Flavors" (Pungent, Sweet, and Bitter). Nine "Pungent-Warm" and nine "Bitter-Cold" plant TCMs were selected to study the "Natures' (Warm and Cold). The candidate TCMs divided into five groups as follows :

Pungent-Neutral: *Lobelia chinensis* Lour.(PN1), *Euphorbia humifusa* Willd.(PN2), *Selaginella tamariscina* (Beauv.) Spring(PN3), *Hibiscus mutabilis* L.(PN4), *Eupatorium fortunei* Turcz.(PN5)

Sweet-Neutral: *Codonopsis pilosula* (Franch.) Nannf.(SN1), *Glycyrrhiza uralensis* Fisch.(SN2), *Lycium barbarum* L.(SN3), *Gastrodia elata* Bl. (SN4), *Cannabis sativa* L.(SN5), *Dioscorea opposita* Thunb(SN6), *Glycine max* (L.).Merr.(SN7), *Crocus sativus* L.(SN8), *Sesamum indicum* L.(SN9)

Bitter-Neutral: *Nelumbo nucifera* Gaertn.(BN1), *Viscum coloratum* (Komar.) Nakai(BN2), *Dioscorea spongiosa* J. Q.Xi, M. Mizuno et W.L. Zhao(BN3), *Morus alba* L.(BN4), *Eupatorium lindleyanum* DC(BN5), *Prunus persica* (L.). Batsch(BN6), *Sargentodoxa cuneata* (Oliv.) Rehd. et Wils. (BN7), *Citrus reticulata* Blanco(BN8), *Vaccaria segetalis* (Neck.) Garcke (BN9)

Pungent-Warm: *Angelica dahurica* (Fisch.ex Hoffm.) Benth.et Hook. f.(PW1), *Ligusticum chuanxiong* Hort.(PW2), *Eugenia caryophyllata* Thunb.(PW3), *Eucommia ulmoides* Oliv.(PW4), *Lindera aggregata* (Sims) Kos-term.(PW5), *Sinapis alba* L.(PW6), *Carthamus tinctorius* L.(PW7), *Magnolia biondii* Pamp.(PW8), *Asarumsieboldii* Miq.(PW9)

Bitter-Cold: *Isatis indigotica* Fort.(BC1), *Iris tectorum* Maxim(BC2), *Belamcanda chinensis* (L.). DC(BC3), *Gardenia jasminoides* Ellis(BC4), *Scutellaria baicalensis* Georgi(BC5), *Sophora flavescens* Ait.(BC6), *Aloe barbadensis* Miller(BC7), *Rheum palmatum* L.(BC8), *Stephania tetrandra* S. Moore(BC9)

3.2. Collection of chemical ingredients

The ingredients obtained from various databases and literature sources. On the one hand, the pertinent chemical ingredients of each candidate TCM were gathered from TCM databases, such as TCMSP and HERB et al. Additionally, the ingredients mentioned the relevant activity in the published literature were added. All of them were searched and recorded through the PubChem database, which provided basic

information such as chemical names, formulas and CAS numbers. Theirs SDF structural files were downloaded simultaneously. For the ingredients were not included in the PubChem database, We drew their structures using the software ChemDraw 19.0. Finally, all ingredients of each TCM were collected together to remove duplicates to establish a chemical ingredients set for each TCM.

3.3. Target prediction and screening of the active ingredients

The TCMs mentioned above are all for oral use (not external use). All collected chemical ingredients were predicted their pharmacokinetics parameters in the body firstly using the SwissADME tool for ADME screening. The screening parameters were set to Gastrointestinal absorption = High, and the druglike parameters were at least two "Yes" were as the screening criteria. Gastrointestinal absorption is predicted with the BOILED-Egg model classification. The classification showed 10-fold cross-validation accuracy of 88 % for Gastrointestinal absorption. The Drug-likeness includes six rule-based methods include the Lipinski rule-of-five, the Ghose filter, the Veber filter, the Egan filter, the Muegge filter and the Bioavailability Score.[22] In addition, Some collected ingredients have been confirmed that their were pharmacological components through literature, which were regarded as the active ingredients without screening process. SwissTargetPrediction tool was used to predict the human targets for all screened active ingredients. The targets prediction are based on the similarity principle, in 2D and 3D, within a larger collection of 376342 compounds known to be experimentally active on an extended set of 3068 macromolecular targets. In validation, for 72 % molecules (total 500), at least one of the experimentally known targets can be found among the predicted top-15.[23]

Finally, ingredients with predicted targets and the corresponding targets were collected. The top 15 predicted targets of each ingredients with the probability greater than 0.1 were selected, as well the probability greater than 0.5. The probability values provided by SwitssTargetPrediction tool are calculated from the Combined-Scores of the most similar compounds to the query molecule (in 2D and 3D) known to be active on a given protein. Importantly, this value precisely illustrates the probability for a bioactive molecule to have a given protein as target, but not the probability of being bioactive.[23] The data sets for the active ingredients-targets fo each candidate TCMs were obtained.

3.4. Protein-protein interaction network and screening of "natures and flavors" tool TCMs

The predicted targets of each TCM were imported into the software Cytoscape 3.9.1. The GeneMANIA plugin tool in the software was used by setting the species as H.sapiens (Human) and performing protein-protein interaction (PPI) analysis to obtain the PPI network set of each TCM. The PPI network obtained for each TCM can be viewed as a compilation of all potential targets that the TCM can impact within the body, along with their corresponding interaction relationships. Except for the "Pungent-Neutral" group, which only contains five candidate TCMs, the other groups were compared and analyzed based on their collection information, including chemical composition, active ingredients and PPI network data of TCMs within their respective groups. Each groups was selected only six candidates as the tool TCMs.

3.5. Natures and flavors related targets and characteristic targets

The Merge function in Cytoscape 3.9.1 was used to merge and extract same targets from the PPI targets network of five "Pungent-Neutral" tool TCMs to construct a "Pungent-Neutral" common targets network. Under the same operation, the common targets of other groups were merged and extracted. The "Sweet-Neutral" common target network, "Bitter-Neutral" common target network, "Pungent-Warm" common target network and "Bitter-Cold" common target network have been acquired.

The schematic acquisition of Natures and Flavors targets are shown in Fig. 1. The "Pungent-Neutral", "Sweet-Neutral" and "Bitter-Neutral" common target networks were merged to eliminate the same targets of the three networks, and obtained their own exclusive target network regarded as the "Pungent" target network, "Sweet" target network, and "Bitter" target network respectively. The "Pungent-Warm" common targets were regarded as the "Warm" targets by eliminating the targets that same with the "Pungent-Neutral' common targets. The "Bitter-Cold" common targets were regarded as the "Cold" targets by eliminating the targets that sa with the "Bitter-Neutral' common targets. The Network Analyzer tool of Cytoscape 3.9.1was used to calculate the network topology parameters of the "Natures and Flavors" target networks. The core targets in each ""Natures and Flavors"" target network were screened by using the parameters (Degree, Betweenness, Closeness and other parameters) as the screening rules which were greater than the median or twice the median. The screened core targets of the "Pungent" targets network were regarded as "Pungent" characteristic targets. On the same operation, The characteristic targets of "Sweet", "Bitter", "Warm" and "Cold" were obtained, respectively.

3.6. "Natures and flavors" ingredient sets analysis

The chemical ingredients corresponding to the characteristic targets of their respective "Natures and Flavors" tool TCMs were collected and formed into "Natures and Flavors" ingredients sets, respectively. The "Pungent" ingredients set was formed from five "Pungent-Neutral" tool TCMs. During the same operation, The "Sweet", "Bitter", "Warm" and "Cold" ingredients sets were obtained, respectively. Afterward, the above mentioned ingredients sets were analyzed by classification.

3.7. GO and KEGG enrichment analysis

The online tool Metascape was used to conduct the GO gene functional analysis of the related targets and all characteristic targets, including molecular function (MF), biological process (BP), cell component (CC) analysis and KEGG pathway enrichment analysis. By setting the threshold at $p < 0.05$ and selecting the top enrichment results for visualization, and the mechanisms related to "Natures and Flavors" were discussed.

4. Results and discussion

4.1. Selection of candidate tool TCMs

"Natures" and "Flavors" are different attributes of TCM, While TCM have the characteristics of "single-Nature and multi-Flavors" generally and it is difficult to control variables in research. From the viewpoint that the "Neutral" TCM does not make the body heat or cold and only exerts efficacy through itself "Flavors".[26] It is appropriate to study the "Flavors" in relation to the "Neutral" TCM. Thus, We counted only the "single-flavor" TCMs to simplify complexity of the "multi-flavors". In our previous study[17] according to Table S1, we chose the

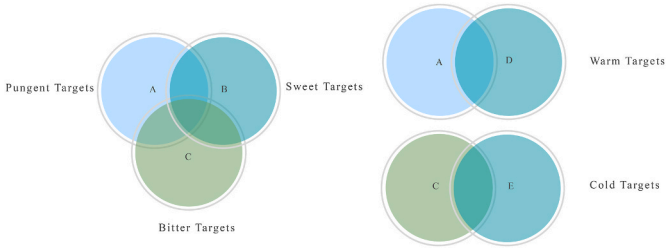

Fig. 1. The screening of Natures and Flavors targets
(**A**)"Pungent-Neutral" common targets; (**B**)"Sweet-Neutral" common targets; (**C**)"Bitter-Neutral" common targets; (**D**)"Pungent-Warm" common targets; (**E**) The "Bitter-Cold" common targets.

"Pungent-Neutral","Sweet-Neutral and "Bitter-Neutral" TCMs for characterizing the "Flavors" (Pungent, Sweet and Bitter), and "Pungent--Warm" and "Bitter-Cold" TCMs for characterizing the "Natures" (Warm and Cold). due to ADME screening and target prediction tools only support small molecule compound analysis, so we can only select plant TCMs, and exclude animal, mineral and fungal TCMs. we preferentially selected the plant TCM with as many chemical ingredients as possible as the candidate TCMs in this work.

4.2. Collection and screening of ingredients, PPI network, and tool TCMs

The results of collecting and screening TCM information are shown in Table 1. In "Sweet-Neutral" Group, there are fewer PPI targets for SN7 and SN8, as well as fewer active ingredients in SN9. In "Bitter-Neutral" group, the PPI targets of BN6 are fewer, and the chemical components of BN7, BN8 and BN9 are fewer. Among "Pungent-Warm" group, there are fewer PPI targets for PW7, PW8 and PW9. Among "Bitter-Cold" group, there are fewer active ingredients in BC7 and BC8. There are fewer PPI targets for BC9. Finally, five "Pungent-Neutral", Six "Sweet-Neutral", five "Bitter-Neutral", six "Pungent-Warm" and six "Bitter-Cold" candidate TCMs were selected as tool TCMs. The selected tool TCMs are marked with an asterisk (*) in the following tables.

4.3. Natures and flavors targets network and characteristic targets

Firstly, the "Pungent-Neutral" common target network was established by the same targets of five "Pungent-Neutral" tool TCMs PPI network targets using Merge function of Cyctospae 3.9.1. The Merge function can merge and extract the same targets of multiple TCM target networks and preserve the relationship between the targets. Than, the "Pungent-Neutral" network contains 5732 target proteins and 131533 interaction relationships. Using the same method, we obtained "Sweet-Neutral" common target network with 4749 target proteins and 126280 interaction relationships. The "Bitter-Neutral" common target network has 3404 target proteins and 90427 interaction relationships. The "Pungent-Warm" common target network has 5521 target proteins and 130840 interaction relationships. The "Bitter-Cold" common target network has 6116 target proteins and 141319 interaction relationships.

After merging the "Pungent-Neutral", "Sweet-Neutral" and "Bitter-Neutral" common target networks, the "Pungent" target network contains 1316 target proteins and 891 interaction relationships. The "Sweet" target network contains 487 target proteins and 596 interaction relationships. The "Bitter" target network contains 157 target proteins and 67 action relationships. The "Warm" target network contains 481 target proteins and 594 interaction relationships by merging "Pungent-Warm" and "Pungent-Neutral" common target networks. The "Cold" target network contains 2896 target proteins and 8039 interaction relationships by merging "Bitter-Cold" and "Bitter-Neutral" common target networks. Through network topology analysis, the core targets were screened as characteristic targets. As shown in Table S2 and **Tabl S3**, there are 175 characteristic targets for "Pungent', 195 characteristic targets for "Sweet", 95 characteristic targets for "Bitter', 194 characteristic targets for "Warm", and 199 characteristic targets for "'Cold". The results are shown as follows.

4.4. Analysis of natures and flavors ingredients sets

The chemical ingredients corresponding to the characteristic targets were obtained. As shown in Table S4, the "Pungent" group includes 30 chemical ingredients and 6 related targets. The analysis results of the "Pungent" group are shown in Fig. 2. Among them, The amount of flavonoids are the most, followed by alkaloids, monoterpenes and sesquiterpenes. It has been reported statistically that the main components of "Pungent' TCMs are volatile oil (such as, monoterpenes and sesquiterpenes) and alkaloids.[5,6] In this study, it was found that the major components were flavonoids. Flavonoids, such as quercetin, apigenin, naringenin, fisetin and luteolin, mostly have anti-inflammatory, antibacterial, antiviral effects.[27–30] These effects are correspond to the "Pungent" can relieve exterior syndrome. Ricinine and atropine have central nervous system excitatory effect,[31,32]which emboding the inducing resuscitation effect of "Pungent". Carvacrol has vasodilating effects and ellagic acid can treat cardiovascular diseases,[33,34] Acacetin has the effect to protect the heart.[35] Shikimic acid and its derivatives have antibacterial, antitumor, antithrombotic and anti-cerebral ischemia effects,[36] which these components effects on cardiovascular diseases coincide with the promoting blood circulation effect of

Table 1
Collection and Screening of candidate TCMs ingredients and targets.

TCM	Chemical components	SwissADME Screening	Active ingredients	Predicting targets	PPI targets
PN1*	165	116	82	305	7826
PN2*	136	38	31	233	7162
PN3*	180	87	64	306	8334
PN4*	66	20	20	182	6510
PN5*	251	163	97	310	8038
SN1*	289	158	94	331	8390
SN2*	357	246	202	447	9249
SN3*	264	114	77	370	7558
SN4*	96	65	36	221	7146
SN5*	198	123	85	358	8270
SN6*	146	93	64	331	8395
SN7	174	88	61	247	6877
SN8	131	93	52	246	6424
SN9	133	57	20	–	–
BN1*	131	79	53	259	7032
BN2*	119	55	42	198	6495
BN3*	107	49	38	197	4958
BN4*	164	104	68	319	7824
BN5*	113	79	49	225	7087
BN6	87	63	31	97	2853
BN7	46	–	–	–	–
BN8	52	–	–	–	–
BN9	42	–	–	–	–
PW1*	306	187	124	294	8088
PW2*	293	178	96	314	7640
PW3*	234	131	68	285	7631
PW4*	191	104	69	327	7870
PW5*	183	131	79	338	7963
PW6*	178	99	72	214	7566
PW7	237	95	60	277	7333
PW8	222	143	80	257	6613
PW9	235	147	89	286	5517
BC1*	229	147	102	419	8592
BC2*	166	107	83	234	7638
BC3*	116	66	61	260	7856
BC4*	181	100	69	288	7908
BC5*	231	129	106	368	7742
BC6*	203	150	120	227	8452
BC7	129	50	35	302	5424
BC8	147	60	43	212	6531
BC9	105	74	57	294	5102

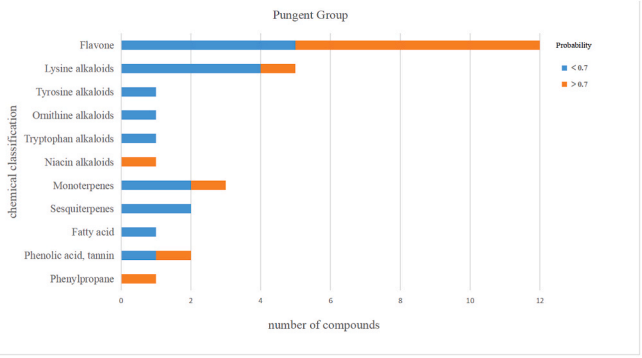

Fig. 2. Classification of chemical compositions of the "Pungent".

"Pungent'.

As shown in Table S5, the "Sweet" group includes 106 chemical ingredients and 19 related targets. The analysis results of the "Sweet" group are shown in Fig. 3. Among them, The amount of isoflavones are the most, followed by glucosides, amino acids, phenols, steroids, terpenes, coumarins and others. The "Sweet" substance group has many nutrients, which may be related to the fact that 'Sweet" TCMs have a tonifying effect and can supplement the substances needed by the body. Among the isoflavones, Formononetin and Ononin have antibacterial, anti-apoptosis and anti-inflammatory activities, and can improve myocardial damage in diabetic mice through the AKT/FoxO1 signaling pathway.[37,38] Tectorigenin has antioxidant and scavenging free radicals, regulating immunity, anti-inflammatory and protecting liver effects. Paeonol has neuroprotective and hepatoprotective effects,[39] L-glutamic acid, L-tryptophan and L-citrulline and other amino acid nutrients have immune regulation, protection of the cardiovascular system, and other extensive health benefits. The effects of these components are related to enhancing the body's physical fitness, reflecting the gain compensation characteristic effect of "Sweet".

As shown in Table S6, the "Bitter" group includes 12 chemical ingredients and 4 related targets. The analysis results of the "Bitter" group are shown in Fig. 4. It cannot be found which chemical compositions are most strongly associated with the "Bitter" by the component classification. It may be related to the high molecular weight of many "Bitter" chemical components, which cannot predict the targets by the selected screening tool. On the other hand, pharmacological studies have shown that "Bitter" TCMs mainly excite or inhibit the pathological decline or hyperactivity.[40] These may cause fewer same targets of "Bitter". Furthermore, studies have indicated that the chemical ingredients of "Bitter-Neutral" TCMs are complex and the chemical compositions cannot fully reflect their main characteristics.[41] As a result, there were fewer chemical components of the "Bitter" group in this study.

As shown in Table S7, the "Warm" group includes 82 chemical ingredients and 15 related targets. The analysis results for the "Warm" group are shown in Fig. 5. The amount of fatty acids in the "Warm" group are the most, followed by alkaloids, terpenes and phenolic acids. Fatty acid metabolism can provide the energy required by the body, and is closely related to glycolipid metabolism and energy metabolism.[42,43] This reflects that the material basis of "Warm" is to directly provide the energy substances mainly fatty alcohols, such as, octanol, heptanol, nonanol, decanol, undecanol, dodecanol, thirteenol, tetradecyl alcohol, pentadecanol, cetyl alcohol and heptadecanol. In addition to fatty acids, "Warm" is also closely related to volatile oil (such as, monoterpenes and sesquiterpenes), as well as tryptophan-derived and lysine-derived alkaloids.

As shown in Table S8, the "Cold" group includes 43 chemical ingredients and 9 related targets. The analysis results for the "Cold" group are shown in Fig. 6. The amount of flavonoids in the "Cold' group are significantly more than the other components. Flavonoids, such as, quercetin, apigenin, Chrysoeriol, kaempferol, (S)-naringenin, luteolin have good antibacterial and antiviral effects.[29,30,38] Emodin and chrysophanic acid have hypoglycemic and lipid-reducing effects.[44,45] Baicalin has antibacterial and antiviral effects.[46] Eriodictyol can improve glycolipid metabolism.[47] The analysis results showed that the "Cold" can regulate the body's glycolipid and energy metabolism and also has the effect of eliminating pathogens through antibacterial and antiviral effects to eliminate pathogens and relieve fever symptoms, which exerting its "Cold" properties.

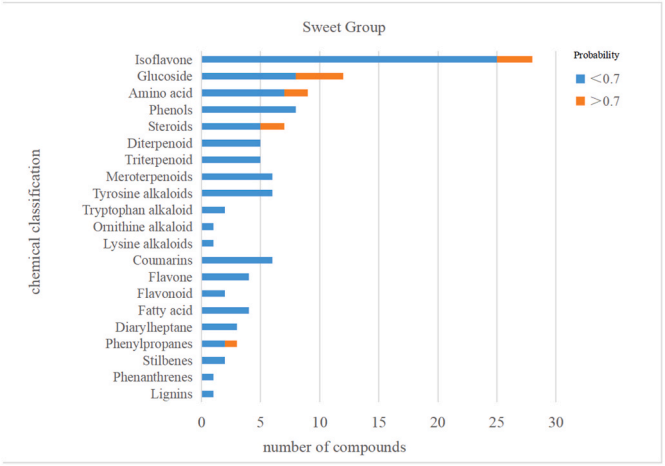

Fig. 3. Classification of chemical compositions of the "Sweet".

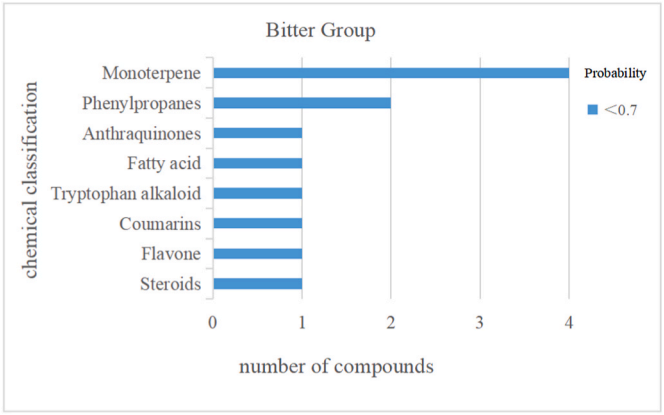

Fig. 4. Classification of chemical compositions of the "Bitter".

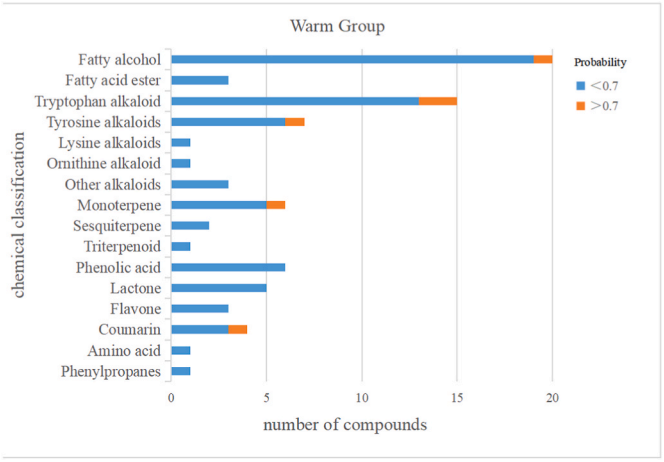

Fig. 5. Classification of chemical compositions of the "Warm".

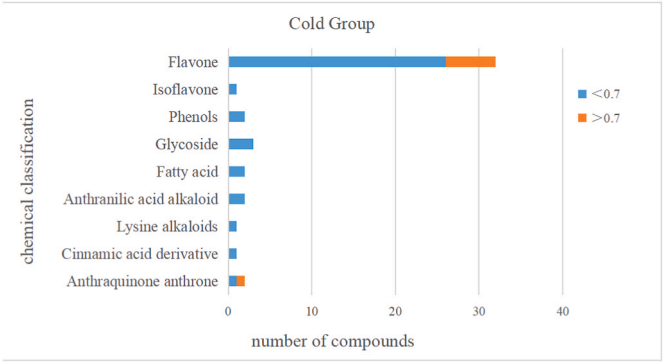

Fig. 6. Classification of chemical compositions of the "Cold".

4.5. GO enrichment and KEGG pathway analysis of "natures and flavors" related targets

The results of "Pungent" targets analysis are shown in Fig. S1. The spontaneous aggregation of β-amyloid proteins results in formation numerous toxic oligomers, which can cause neuronal death in the brain and lead to cognitive impairment.[48] The regulation of β-amyloid proteins by "Pungent" reflects its potential enlightening effect. The active regulation of myocardial cell contraction reflects the blood-activating effect of "Pungent". The type I interferon (IFN–I) signaling pathway is a key element of the innate immune signaling pathway,[49] and its positive-regulation suggests that "Pungent" may have an immune regulatory effect. The KEGG analysis results showed that "Pungent" may affect the folate antagonist resistance metabolism. Folic acid inhibitors can inhibit biological processes such as cell division, DNA and RNA synthesis, as well as protein synthesis. Theey are frequently used in tumor chemotherapy.[50] "Pungent" show the potential anticancer activity.

The results of "Sweet" related targets analysis are shown in Fig. S2. The GO enrichment analysis indicate that the regulation of biological processes such as cell stress response, nutrient level response and cell proliferation, which reflect the "Pungent" plays an important role in supplementing the body's deficiencies. At the molecular function level, threonine is related to protein serine/Threonine kinase activity. Threonine is an essential amino acid that acts as a nutritional enhancer. It has been shown to alleviate fatigue and promote growth and development in humans. Its regulation reflects "Sweet" nourishing effect.

The results of "Bitter" related targets analysis are shown in Fig. S3. Similar to the results of the "Bitter" composition analysis, no significant "Bitter" characteristics were found. Some study have reported that "Bitter-Neutral" TCM have many pharmacological effects, including dispelling wind and dampness, reducing blood pressure, improving blood circulation and hemostasis.[40] Therefore, when using the "Bitter-Neutral" TCM to summarize the characteristics of "Bitter", The "Bitter" characteristics are not clear.

The results of "Warm" related targets analysis are shown in Fig. S4. The GO enrichment analysis showed that the "Warm" intervention may be reflected in the regulation of cardiac contraction, the process of cellular protein catabolism, the process of Messenger RNA metabolism, the process of mRNA catabolism, the positive regulation of protein hydrolysis and other biological processes. The impacts of "Warm" on these catabolic biological processes are related to the promotion of material and energy metabolism in the body. The regulation of cardiac contraction suggests that "Warm" may play a role in regulating the circulatory system. At the cellular component level, β-Endorphins have a strong analgesic effect and can promote the release of Glucagon[51] and α1-adrenergic receptors are mainly distributed in vascular smooth muscle and can cause vasoconstriction when stimulated. The KEGG metabolic analysis results showed that "Warm" is closely related to the pancreatic hyperglycemia signaling pathway, as well as the linoleic acid metabolism and fatty acid degradation. Glucagon is secreted by islet A cell. Its primary function is to stimulate the conversion of the glycogenolysis and non-glucose substances into glucose, thereby increasing blood glucose levels. Fatty acids can undergo oxidation in liver cells to produce linoleic acid. Linoleic acid is bound to the glycoester metabolism-related kinases in the cytoplasm, which activate the process of glucose and lipid metabolism, and release energy to improve blood glucose levels.[52] "Warm" may promote the metabolism of substances and thermogenesis in the body through these pathways.

The results of "Cold" related targets analysis are shown in Fig. S5. The GO enrichment analysis indicated that both "Cold" and "Warm" have impact on the breakdown and metabolism of various proteins and fatty acid in the body. It is speculated that "Warm" promotes metabolism, while "Cold" inhibits metabolism. At the same time, "Cold" is also related to the Hippo signaling pathway. The Hippo signaling pathway is a signaling pathway that inhibits cell growth. The Hippo signaling pathway also plays an crucial role in cancer development, tissue regeneration and the regulation of stem cell function.[53] In molecular function analysis, "Cold" is associated with functions such as ATP dependent activity and ATPase-coupled ion transmembrane transport protein activity. It is speculated that the possible mechanism of "Cold" is that it can directly inhibit the functional regions related to energy metabolism.[54,55] The KEGG enrichment analysis revealed that "Cold" may directly inhibit the metabolism of fatty acids and other substances, leading to reduction in energy release and heat production.

In general, upon analyzing the selected related "Natures and Flavors" targets, there are still numerous unexplainable results. However, some certain characteristics of "Natures and Flavors" have been identified, which providing the theoretical basis for the contemporary comprehension of TCM's characterization of "Natures and Flavors".

5. Conclusion

The theory of TCM properties involves multiple-dimensions. It is difficult to control variables and obtain expected multi-dimensional correlation results when conducting synchronous research at high dimensions. Although network pharmacology can not reflect the dose-effect relationship between compounds and pharmacological actions, it provides a new research model from the whole perspective in TCM modernization initial stage. At present, "Natures and Flavors" is not clear, but we has revealed some scientific and regularity of them. In this study, the "Pungent-Neutral", "Sweet-Neutral", "Bitter-Neutral", "Pungent-Warm", "Bitter-Cold" TCMs were used to screen the "Natures" and "Flavors" characterized compositions and targets using network pharmacology integrated strategy. In the "Pungent" group, flavonoids are the most. "Pungent" may have immune-regulatory effects and potential anticancer activity. In the "Sweet" group, isoflavones are the most. "Sweet" are related to effectively invigorate health. Fatty acids in the "Warm" group are the most. Flavonoids in the "Cold' group are far more than other components. "Warm" and "Cold" are both related to fatty acid and energy metabolism.The "Pungent", "Sweet", "Bitter", "Warm" and "Cold" characterized compositions and targets were identified, which providing the foundation for further understanding of the rules and identification of the "Natures" and "Flavors".

Credit author statement

Yu Zhiguo: Conceptualization, Methodology **Zhao Yunli:** Conceptualization **Wang Hong:** Methodology, Formal analysis, Investigation, Writing - Original Draft **Wei Wenfeng:** Investigation **Liu Jing:** Investigation **Zhang Shuang:** Investigation.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtcme.2023.12.004.

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