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Version updates of strategies for drug discovery based on effective constituents of traditional Chinese medicine

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Abstract

The discovery of effective constituents of traditional Chinese medicine (TCM) is an important approach in new drug development. Several well-known drugs, such as artemisinin, berberine, and ephedrine have been developed using this approach. However, the efficacy and safety of TCM, two key issues for drug development based on TCM clinical experience, remain unclear worldwide. The discovery strategy of relevant constituents is the most important step for determining efficacy and safety, which still a key scientific problem that restricts the development of new drugs. Furthermore, TCM formulas used as clinical drugs address a specific TCM syndrome (*Zheng*), and the complexity of the formula and vagueness of the syndrome make the identification of the effective constituents related to clinical effectiveness challenging. Over decades, researchers have developed transdisciplinary technologies and research methodologies to identify effective constituents *in vivo*. In this paper, the history of strategy development for identifying the effective constituents related to the clinical efficacy of TCM is reviewed and summarized. The main approaches include the phytochemical method, which involves the classical systematic separation and screening (extraction, separation, purification, structure identification, and activity test); bioactivity-guided separation; serum pharmacochemistry of TCM *in vivo*; and Chinmedomics, which connects *in vivo* constituents with the biomarkers of the relevant TCM syndrome. Chinmedomics is a promising strategy that conforms to the theory and characteristics of TCM. By clarifying the effective constituents, targets and pathways of medicines, it can promote the discovery of lead compounds and the research of innovative drugs, and continuously promote the modernization of TCM.

Keywords: Chinmedomics, Effective constituents of TCM, New drug discovery strategy, Serum pharmacochemistry of TCM, Traditional Chinese medicine

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Introduction

Traditional Chinese medicine (TCM) has played an important role in the medical and health system in China for thousands of years, and the discovery of effective TCM constituents is a critical step in drug innovation[1]. For example, artemisinin, an antimalarial drug originating from *Artemisia Annua* herba, has saved 200 million people worldwide in recent years[2]; berberine, an isoquinoline alkaloid isolated from the Chinese herb *Coptidis rhizoma* and other *Berberis* plants, has been demonstrated to be a promising multifunctional drug that can be used to treat digestive, metabolic, cardiovascular, and neurological diseases[3–4]; paclitaxel (Taxol) is a natural antineoplastic drug that is widely used in treating breast, ovarian, and lung cancers[5]. Moreover, in the prevention and control of serious infectious diseases, such as COVID-19, TCM has exhibited an extensive advantage[6]. New formulas such as Qingfei Paidu granules, Huashi Baidu granules, and Xuanfei Baidu granules have emerged[7], and a novel effective compound wogonoside in Qingfei Paidu granule has been demonstrated to exert anti-inflammatory effects through the downregulation of USP14 to promote the degradation of activating transcription factor 2[8]. In addition, TCM has unique advantages in treating cardiovascular, cerebrovascular[9], and complex metabolic diseases[10]. Tanshinone IIA, the main fat-soluble component of *Salviae miltiorrhizae* Radix et Rhizoma, has shown remarkable cardioprotective effects through enhancing angiogenesis, which may serve as an effective treatment against cardiovascular diseases; it affects multiple signaling pathways including antiproliferative, antiapoptotic, anti-inflammatory, and antioxidative stress pathways[11]. The efficacy and treatment concept of TCM has attracted

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increasing attention worldwide. The growing international demand for TCM has also provided a broad scope for the development of TCM and drug discovery.

However, the theoretical system, diagnosis, and treatment methods of TCM are notably different from those of Western medicine. From the perspective of TCM, the disease is mostly described as a syndrome (*Zheng*), and treatment is based on a combination of herbal medicines called formula; the correspondence between *Zheng* and formula is the premise of efficacy[12]. Different syndromes require different formulas to reverse imbalance and restore homeostasis[13]. Moreover, the complexity of the formula and the vagueness of the syndrome make the identification of the effective constituents and mechanisms related to clinical efficacy challenging. Discovering the active constituents of TCMs, evaluating their efficacy, and elucidating the targets and pathway are crucial steps for interpreting the principles of TCM, revealing the internal law of formula compatibility, and designing new formulas for treating current chronic diseases.

Over decades, researchers have developed strategies for identifying effective constituents. With the progress of modern analytical technology and systems biology, the methods for identifying the effective constituents of TCM are based on systematicity and integrity. From ephedrine, an active drug for relaxing tracheal smooth muscles[14], to artemisinin, a new antimalarial drug that is safe and effective and can resolve drug resistance[15], the method had advanced from the phytochemical approach to bioactivity-guided separation. More recently, the emergence of TCM serum pharmacochemistry established a foundation for rapid screening of *in vivo* effective constituents of TCMs[16]. The development of metabolomics, the most phenotypic omics research, has enabled characterization of metabolic profiles and biomarkers of TCM syndromes and created the research model of correlation analysis between active formula constituents *in vivo* and endogenous biomarkers of TCM syndromes. Therefore, the research strategy of Chinmedomics was born[17]. The present review based on CNKI and PubMed, summarized different versions of effective constituent discovery methods of TCM by time and technological advancement, which helps clarify the effective substances of TCM, promotes the discovery of lead compounds and innovative drugs, and assists the modernization of TCM.

Version updates of strategies for the effective constituent discovery of TCM

Phytochemical method based on extraction, separation, purification, structure identification, and activity testing: version 1.0

Initially, the discovery of effective constituents was mainly based on the phytochemical method. Extraction is the first step to identify effective constituents of herbal medicines by decoction, refluxion, and organic solvent ultrasound. The extracts had to be separated and purified using columns according to the properties (solubility, pH, adsorption, molecular weight) to obtain pure compounds, followed by identification of the chemical structure using infrared spectrum, ultraviolet-visible absorption spectrum, nuclear magnetic resonance, and mass spectrometry. Subsequently, biological methods were used to test the activity of the single compounds to ultimately determine the active constituents[18]. Common separation technologies to purify compounds include low-medium-pressure column[19], thin-layer[20], and preparative chromatography[21]. The specific workflow is shown in Figure 1. This method is characterized by strong maneuverability, which can separate and prepare a large number of compounds and has been the common widely used method in the early stage of searching for effective constituents of TCM, achieving remarkable results.

The most representative and earliest example of the application of this strategy is the discovery of ephedrine in TCM *ephedra herba* (*Ephedra sinica* Stapf, *Ephedra intermedia* Schrenk et C.A. Mey., *Ephedra equisetina* Bge.). Nagai isolated an alkaloid from this *Ephedra sinica* in 1887, named it ephedrine, and discovered that it has a mydriatic effect; Amatsu and Kubota described a rise in blood pressure and relaxation of the intestines after ephedrine in 1918[22]. The Chinese pharmacologist Chen K.K. conducted a systematic study on the pharmacological effects of ephedrine in 1927, and found that ephedrine is clinically stable, so the age, exposure to light, or boiling does not alter its action; ephedrine has a persistent and uniform action, in contrast with that of adrenaline; ephedrine has low toxicity and an exceptionally wide margin of safety, which can be conveniently administered, either orally or through intramuscular injection without local irritation[23].

Camptothecin, an alkaloid, was isolated from the tree *Camptotheca acuminata* Nyssaceae in 1966. The stem wood was extracted using the standard method of continuous, hot hexane–heptane extraction from dried plant material followed by similar extraction with 95% ethanol. The ethanol extract concentrate is partitioned between water and chloroform. Silica gel chromatography of the methanol-insoluble material from the chloroform extract followed by crystallization from methanol–acetonitrile showed camptothecin as pale yellow needles. Camptothecin (0.25–1.0 mg/kg/day) tested in L1210 leukemic mice prolonged survival by up to 100%; in Walker 256 (intramuscular) rat tumor, concentrations as low as 1.25 mg/kg significantly inhibited growth; moreover, it showed moderate cytotoxicity against KB cell culture (ED_{50} = 0.07 μg/mL)[24].

Schizandrin provides another example; its plane structure was elucidated by Kochetkov et al. in 1961; subsequently, gomisin A, B, C, F, and G in Schisandrae chinensis Fructus (*Schisandra chinensis* (Turcz.) Baill.) were gradually isolated[25]. Assessment of sleep time, activity, and muscle state of mice revealed that both schizandrin and gomisin A had sedating or tranquilizing effects. The efficacy of gomisin A was durable and intense and that of schizandrin was transient. However, the analgesic effect of gomisin A was inferior to that of schizandrin[26]. Other representative studies are shown in Table 1[27–46].

In addition to the above-mentioned achievements, this method also has some disadvantages: cumbersome operation, long experimental cycle, lower clarity, and greater consumption. Moreover, the separated compounds usually have no or weak activity. In addition, some active ingredients with low content may be lost in the process of separation and purification. Owing to the yield limitation of the separated compounds and activity testing methods, several ingredients barely stay at the level of structural identification. In contrast, most screening methods using the systematic separation focus on novel chemical structures and the discovery of new compounds. Furthermore, the activity evaluation model has a low correlation with traditional efficacy; therefore, the research results provide limited assistance with elucidating effective constituents of TCMs.

Bioactivity-guided separation: version 2.0

Bioactivity-guided separation is a pharmacological activity-oriented screening method for the effective constituent of TCMs. The main method started with total extraction under the guidance of sensitive and reliable activity evaluation and tested the activity of the fractions/constituents separated at each step, tracked and separated the active constituents, and finally, obtained the effective constituents[47]. The process can be simply summarized into two steps. The first step involves rough separation; TCMs are successively further extracted using different polar solvents to obtain corresponding extraction sites or macroporous adsorption resin, ion exchange resin, and glucan gel to separate the components. All obtained components are detected using an activity test, and the crudely separated components, which show biological activity, are called effective components. The second step involves fine separation; the effective components are loaded into a separation column and extracted with an eluent, and the monomer compound is finally obtained using repeated column chromatography separation. Subsequently, the constituents obtained from each separation are tested for activity. The structures of the single compounds obtained through tracking and separation are determined based on their physical and chemical properties and spectral data. The workflow is summarized in Figure 2. Bioactivity-guided separation with simultaneous chemical separation and activity evaluation greatly reduced the blindness of the analysis and saved unnecessary separation and preparation of the inactive fraction. Based on this method, numerous active constituents have been discovered, with clear structures, significant pharmacological effects, and relatively clear mechanisms. Some of them have been developed into clinical medicine and a new drug development model using natural products was gradually created.

Artemisia annua L. (Qinghao) was first indicated to reduce fevers relevant to the symptoms of malaria in Handbook of Prescriptions for Emergency Treatments (Zhǒu Hòu Bèi Jí Fāng ,肘后备急方) written by Ge Hong in 340 AD. The antimalarial activity of the extract obtained *via* the conventional heating extraction method is not optimal. The investigators proposed that active constituents of *Artemisia annua* L. may be destroyed during the extraction process under heating. A low-temperature extraction method for the plant was then proposed, inspired by Ge Hong's handbook, instructing to "immerse a handful of Qinghao in 2 L water, squeeze out the juice, and drink it all." Ether extracts produced encouraging results in mice infected with *Plasmodium berghei* and finally, a nontoxic neutral extract was obtained on October 4, 1971, by immersing or distilling Qinghao using ethyl ether[2], with 100% efficacy in mice infected with *P. berghei* and in monkeys infected with *Plasmodium cynomolgi*. The extraction was further separated by antimalarial

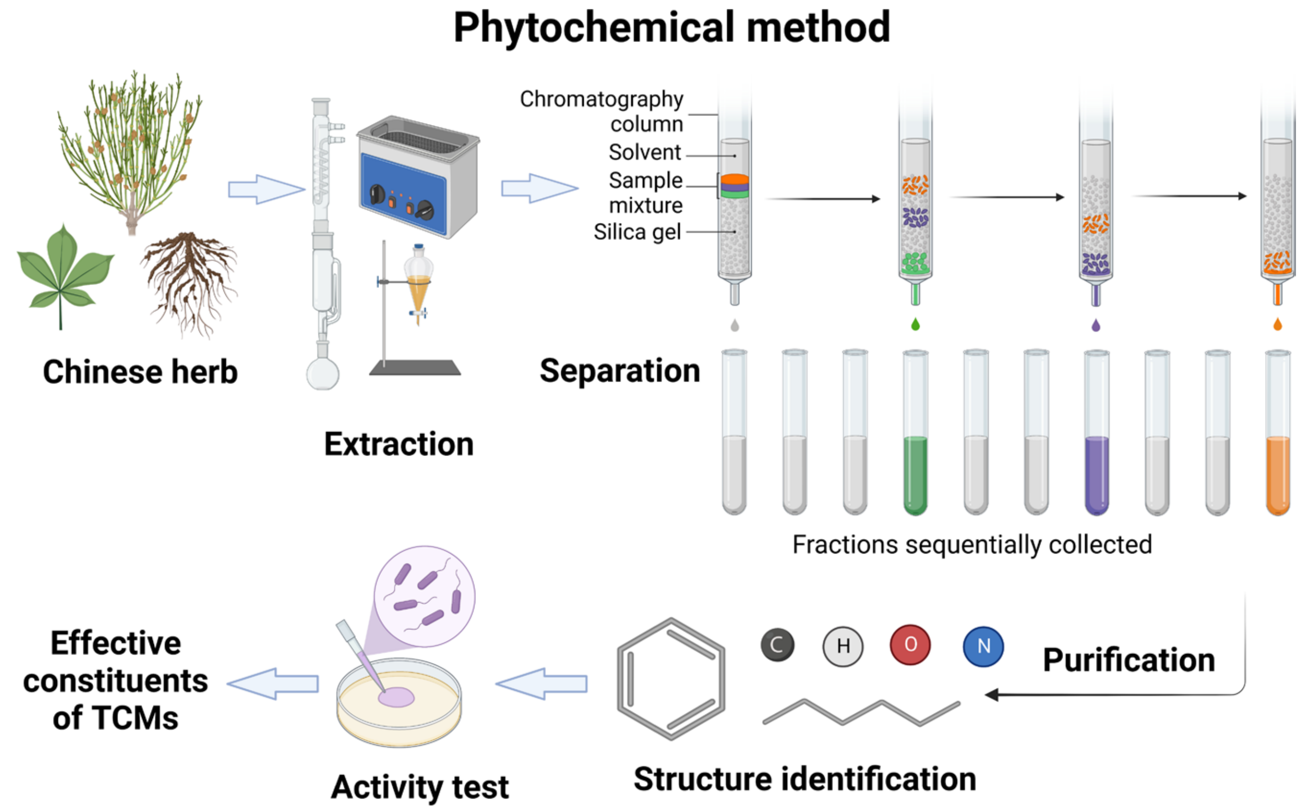

Figure 1. Workflow of the classical phytochemical method for screening effective constituents of TCMs. TCM: Traditional Chinese medicine. Created by Biorender.com.

Table 1.

Representative studies on the effective constituents of TCM using classical systematic separation and screening methods

Herb name	Monomer compound	Effective constituent	Activity evaluation	References
Ephedrae Herba (Mahuang)	Ephedrine	Ephedrine	Animal *in vivo* experiments (dogs, rabbits, rats, etc)	[23]
Camptotheca acuminata (Xishu)	Camptothecin	Camptothecin	Leukemia L1210 in mice, Walker 256 (intramuscular) tumor (rats)	[24]
Schisandra chinensis (Wuweizi)	Schizandrin, gomisin A, gomisin B, gomisin C, gomisin F, and gomisin G	Schizandrin, gomisin A	Animal *in vivo* experiments (mice, pigs, and rats)	[25–26]
Berberis vulgaris (Sankezhen)	Berberine	Berberine	Berberine hydrochloride (0.5%) inhibited the growth of *Bacterium coli*, inhibited the formation of indol and volatile fatty acids in protein decomposition, and acted as a strong poison to rabbits, frogs, hens, and mice, causing leucocytosis and nephritis and affecting the nervous system	[27]
Rehmannia glutinosa (Dihuang)	Catalpol, ajugol, aucubin, melittoside, rehmannioside A, rehmannioside B, rehmannioside C, and rehmannioside D	Rehmannioside D	Rehmannioside D showed a weak hypoglycemic action in spontaneously diabetic mice	[28]
Chelidonium majus (Baiqucai)	Chelidocystatin	Chelidocystatin	Chelidocystatin exhibited a strong inhibition of cathepsin L, papain, and cathepsin H	[29]
Poria cocos (Fuling)	3 β-p-hydroxybenzoyldehydrotumulosic acid	3 β-p-hydroxybenzoyldehydrotumulosic acid	TPA-and AA-induced ear inflammation in mice	[30]
Gentiana tibetica (Xizangqinjiao)	8-Hydroxy-10-hydrosweroside, isomacrophylloside and ethyl N-docosanoylanthranilate, β-sitosterol, daucosterol, oleanolic acid, loganic acid, gentiopicroside, sweroside, 2′-(2,3-dihydroxybenzoyl) sweroside, trifloroside, rindoside, and macrophylloside A	Ethyl N-docosanoylanthranilate	Ethyl N-docosanoylanthranilate inhibited the growth of the human pathogenic fungi *Candida albicans* and *Aspergillus flavus*	[31]
Ganoderma lucidum (Lingzhi)	Ganoderic acid alpha, ganoderic acids A, ganoderic acids B, ganoderic acids C1, ganoderic acids H, ganoderiols A, ganoderiols B, ganoderiols F, ganodermanontriol, ergosterol, ergosterol peroxide, cerevisterol, 3 β-5 alpha-dihydroxy-6 β-methoxyergosta-7,22-diene	Ganoderiol F, ganodermanontriol, ganoderic acid B, ganoderiol B, ganoderic acid C1, 3 β-5 alpha-dihydroxy-6 β-methoxyergosta-7,22-diene, ganoderic acid alpha, ganoderic acid H, and ganoderiol A	Active as anti-HIV-1 agents, moderately active inhibitors against HIV-1 PR	[32]
Plumbago zeylanica (Baihuadan)	3′-O-β-glucopyranosyl plumbagic acid, 3′-O-β-glucopyranosyl plumbagic acid methylester, plumbagin, chitranone, maritinone, elliptinone and isoshinanolone, seselin, 5-methoxyseselin, suberosin, xanthyletin, and xanthoxyletin	Plumbagin	Cytotoxicity of all compounds against various tumor cells lines was evaluated, and plumbagin was found to significantly suppress growth of Raji, Calu-1, HeLa, and Wish tumor cell lines	[33]
Scutellaria barbata (Banzhilian)	Barbatin A, barbatin B, barbatin C, scutebarbatine B	Barbatins A, Barbatins B, Barbatins C, scutebarbatine B	Human cancer lines, namely, HONE-1 nasopharyngeal, KB oral epidermoid carcinoma, and HT29 colorectal carcinoma cells	[34]
Salvia miltiorrhiza (Danshen)	Salvianonol, salviamone, 2a-acetoxysugiol, palmitoyl arucadiol, (E)-4-[5-(hydroxymethyl) furan-2-yl] but-3-en-2-one, along with 13 known compounds	Palmitoyl arucadiol	Cancer cell lines (HeLa and OVCAR-3 cells)	[35]
Gentiana rigescens (Dianlongdancao)	Gentirigenic acid, five glycosides, gentirigeosides A–E	Gentirigeosides A, Gentirigeosides C, Gentirigeosides E	Antifungal activity against the plant pathogen *Glomerella cingulata*	[36]
Datura metel (Yangjinhua)	Withametelins I-P, 1,10-seco-withametelin B, and 12β-hydroxy-1,10-seco-withametelin B, together with seven known withanolides	Withametelin I, withametelin K, withametelin L, withametelin N	Exhibited cytotoxic activities against A549 (lung), BGC-823 (gastric), and K562 (leukemia) cancer cell lines	[37]

(Continued)

Table 1.
(Continued)

Herb name	Monomer compound	Effective constituent	Activity evaluation	References
Sinomenium acutum (Qingfengteng)	2,2′-disinomenine, 7′,8′-dihydro-1,1′-disinomenine, 1, 1′-disinomenine	2,2′-disinomenine, 1,1′-disinomenine	Weak inhibition against A549 and Hela cells	[38]
Uncaria rhynchophylla (Gouteng)	18,19-dehydrocorynoxinic acid B, 18,19-dehydrocorynoxinic acid, corynoxeine, isocorynoxeine, rhynchophylline, isorhynchophylline, vincoside lactam	Corynoxeine, isocorynoxeine, rhynchophylline, isorhynchophylline, vincoside lactam	Exhibited inhibitory activity on LPS-induced NO release in primary cultured rat cortical microglia (IC 50: 13.7–19.0 μM)	[39]
Dioscorea septemloba (Mianbixie)	Cholestane glycosides, dioseptemlosides A and B, together with six spirostane glycosides, dioseptemlosides C–H	None	These compounds did not show considerable inhibitory antitumor activities at a concentration of 10 mM	[40]
Euphorbia helioscopia (Zeqi)	Four new jatrophane-type diterpenoids, together with 16 known related compounds: euphornin, euphornin A, euphornin B, euphornin G, euphoheliosnoid A, euphoheliosnoid B, euphoheliosnoid C, euphoscopin A, euphoscopin B, euphoscopin C, euphoscopin E, euphoscopin J, helioscopinolide A, dehydrovomifoliol, ficusic acid, loliolide	Helioscopinolide A and euphornin	Cytotoxicity against HeLa and MDA-MB-231 cells was evaluated, with only helioscopinolide A and euphornin showing activity	[41]
Schisandra propinqua (Wuweizi)	14 new lignans, tiegusanins A–N, together with 13 known compounds	Tiegusanin G	Anti-HIV-1 activity with an EC_{50} value of 7.9 mM and a TI of more than 25	[42]
Gentiana loureirii (Huananlongdan)	Iridoid glycosides, 2′,3′,6′-tri-O-acetyl-4′-O-trans-p-(O-β-d-glucopyranosyl) coumaroyl-7-ketologanin, 2′-O-caffeoylloganic acid, 2′-O-p-hydroxybenzoylloganic acid 2′-O-trans-p-coumaroylloganic acid, and 2′-O-cis-p-coumaroylloganic acid, along with six known iridoids, 7-ketologanin, loganin, loganic acid, sweroside, boonein, isoboonein, and three other known compounds	None	The isolated iridoids were evaluated for antibacterial and antioxidant activities but were either inactive or very weakly active	[43]
Capparis spinosa (Cishangan)	Capparisine A, capparisine B, capparisine C, 2-(5-hydroxymethyl-2-formylpyrrol-1-yl) propionic acid lactone, and N-(3′-maleimidy1)-5-hydroxymethyl-2-pyrrole formaldehyde	None	The five compounds had no inhibitory effect on the human hepatocyte cell line HL-7702 apoptosis induced by Act D and TNF-α	[44]
Valeriana jatamansi (Zhizhuxiang)	Jatamanins A-M, (+)-9′-isovaleroxylariciresinol, together with seven known iridoids and 13 lignans	(+)-9′-isovaleroxylariciresinol	Showed significant *in vitro* cytotoxicity against PC-3M and HCT-8 cell lines	[45]
Asparagus filicinus (Yangchitianmendong)	Filiasparoside E, filiasparoside F, filiasparoside G, stachysterone A-20, 22-acetonide, asparagusin, filiasparoside A, filiasparoside B, aspafilioside A, aspafilioside B, and filiasparoside C	Aspafilioside A, aspafilioside B, filiasparoside C	Human breast adenocarcinoma MDA-MB-231 cell line	[46]

AA: Arachidonic acid; LPS: Lipopolysaccharide; PR: Protease receptor; TCM: Traditional Chinese medicine; TI: Therapeutic index; TNF: Tumor necrosis factor; TPA: 12-O-tetradecanoylphorbol-13-acetate.

activity; a sesquiterpene active constituent was identified as artemisinin and reported in 1979[48].

Taxol, isolated from the stem bark of the western yew, *Taxus breuifolia*, has potent antileukemic and antitumor properties; it is the first compound possessing a taxane ring that has been demonstrated to have such activities. The alcohol extract of the stem bark was concentrated and partitioned between water and chloroform. Guided by assay in 9 KB and various leukemia systems, three successive chromatographies of the residue from the chloroform extract on Sephadex LH-20, and silica gel followed by crystallization from aqueous methanol showed taxol as needles[15]. Further representative studies are summarized in Table 2[49–84].

In general, the majority of single compounds isolated using bioactivity-guided separation have adequate activities, and the hit rate of the effective constituents found is considerably higher than that of version 1.0 (phytochemical method). However, this method has certain limitations; extensive separation and preparation is still required, which is time-consuming and labor-intense. In addition, TCM have multi-component and multi-target

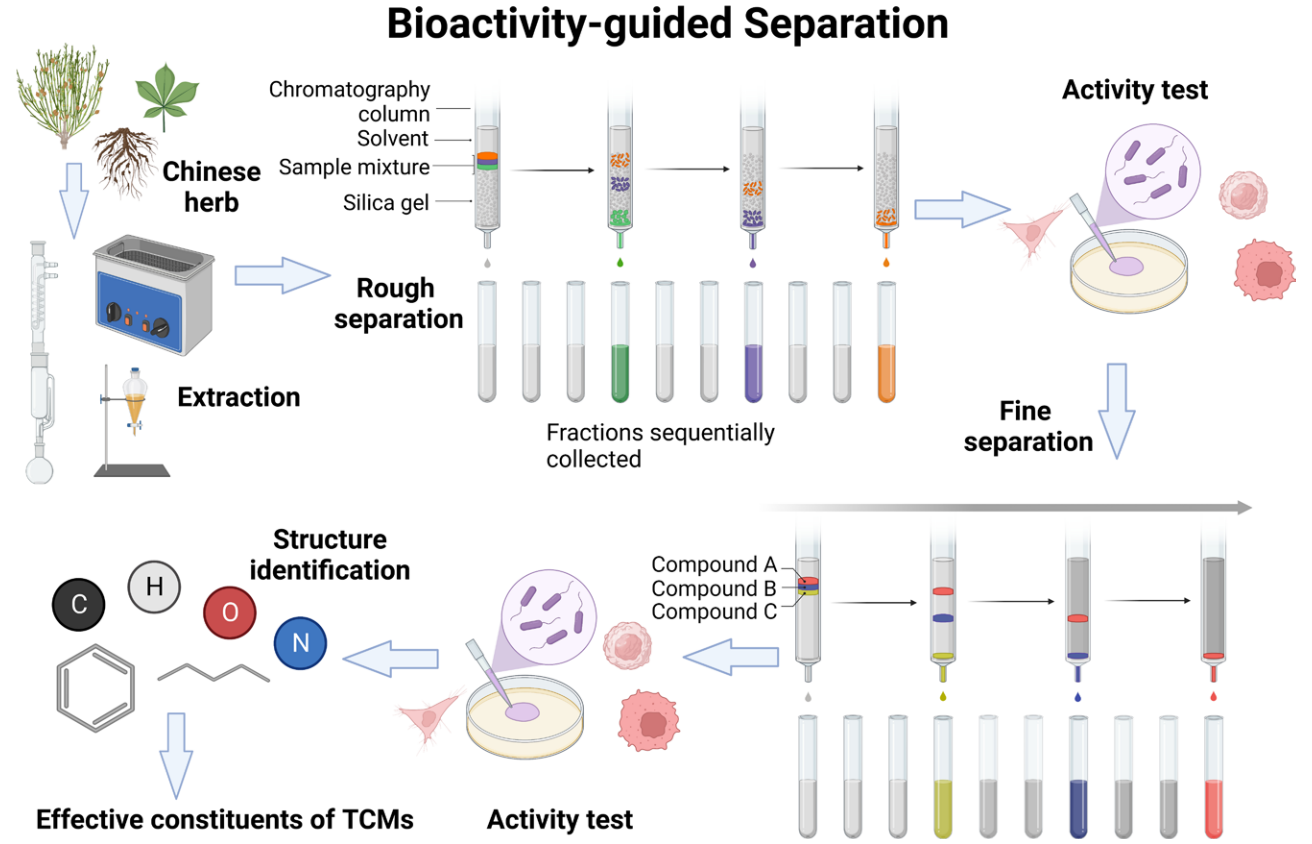

Figure 2. Workflow of the bioactivity-guided separation method for screening effective constituent of TCMs. TCM: Traditional Chinese medicine. Created by Biorender.com.

characteristics; therefore, the active ingredients screened using a single or a few indicators cannot fully reflect all effective constituents of TCM. Importantly, this method is still in the *in vitro* stage, which cannot reflect the biotransformation of various constituents in the body, nor can it demonstrate the interaction among drugs during the process of absorption, distribution, metabolism, and elimination *in vivo*. Therefore, bioactivity-guided separation is a basic method for initial screening of active TCM constituents.

Serum pharmacochemistry of TCM: version 3.0

In the 1980s, Japanese scholar Masakazu Tashiro first proposed the concepts of "serum pharmacochemistry" and "serum pharmacology." Some constituents are selectively absorbed after oral administration of crude drugs, and those compounds reach their target by circulation and play an effective role[85]. Based on serum pharmacochemistry, Professor Xijun Wang performed a large number of studies on TCM formulas, such as Yin Chen Hao Tang (YCHD)[86], Yinchen Wuling powder[87], Liuwei Dihuang pill[88–89], among others. By integrating serum pharmacochemistry with TCM theory and clinical practice, he proposed and established the theory and method of TCM serum pharmacochemistry[16]. This method involves TCM sample preparation and quality control, sample administration, blood collection and serum preparation, analysis, and detection. In general, the method uses modern separation technology to analyze and identify the constituents in human/animal blood after oral administration of TCM formulas to identify the compounds in blood that originate from the formula. This approach reflects the synergistic effect of the combination of the formula drug and its ingredients and the interaction between the herbal drugs, intestinal flora, and human body, ultimately identifying the directly effective constituents *in vivo*[90]. The specific steps are shown in Figure 3. Serum pharmacochemistry of TCMs can effectively avoid the false positives or false negatives caused by the blind separation of chemical constituents *in vitro*. Based on this method, numerous herbal medicines and TCM formulas have been analyzed. Studies on YCHD and Ciwujia (Acanthopanax senticosus（Rupr. etMaxim.）Harms, AS) are used as typical examples to provide details of the method.

YCHD, recorded in "Treatise on Cold Damage Diseases (Shāng Hán Lùn, 伤寒论)" written by Zhang Zhongjing during the Eastern Han Dynasty (25–220 AD), is the representative classical formula for treating the jaundice syndrome (Yang Huang syndrome, YHS). The formula comprises three Chinese herbs: Artemisiae scopariae Herba (*Artemisia scoparia* Waldst.etKit./*Artemisia capillaris* Thunb.), Gardeniae Fructus (*Gardenia jasminoides* Ellis), and Rhei Radix et Rhizoma (*Rheum palmatum* L./*Rheum tanguticum* Maxim. ex Bal£./*Rheum officinale* Bail1.). Under strict quality control, a total of 45 compounds were detected and 21 compounds were identified in blood, including picrocrocinic acid, chlorogenic acid, 6-methaxy coumarin-7-hydroxyl sulfate, 7-methoxy coumarin-6-hydroxyl sulfate, genipingentiobioside, geniposide, saffloryellow A, capillaridin A, isofraxidin, quercetin-3-O-glycoside, 6,7-dimethoxy coumarin, 2,5-dimethyl-7-hydroxy chromone, chimaphylin,

Table 2.

Representative studies on the effective constituents of TCM using bioactivity-guided separation

Herb name	Monomer compound	Effective constituent	Activity evaluation model	References
Artemisia annua (Qinghao)	Artemisinin	Artemisinin	Antimalarial activity in a mouse model of infection	[48]
Taxus breoifolia (Duanyehongdoushan)	Taxol	Taxol	Various leukemia systems	[15]
Gentiana olivieri (Xiewanqueqinjiao)	Isoorientin	Isoorientin	Glucose-hyperglycemic and streptozotocin-induced diabetic rats	[49]
Kaempferia galanga (Shannai)	Ethyl cinnamate, ethyl p-methoxycinnamic acid	Ethyl cinnamate	Anesthetized rats (determination of mean arterial pressure)	[50]
Spatholobus suberectus (Jixueteng)	3′,4′,7-trihydroxyflavone, eriodictyol, plathymenin, dihydroquercetin, butin, neoisoliquiritigenin, dihydrokaempferol, liquiritigenin, 6-methoxyeriodictyol	Butin	HEMn	[51]
Senecio scandens (Qianliguang)	Jacaranone glycoside 1, jacaranone glycoside 2, jacaranone glycoside 3, jacaranone glycoside 4	Jacaranone glycoside 1, jacaranone glycoside 2, jacaranone glycoside 3, jacaranone glycoside 4	Moderate cytotoxicities against five tumor cell lines	[52]
Dipsacus asper (Chuanxuduan)	Caffeic acid, 2,6-dihydroxycinnamic acid, vanillic acid, 2′-O-caffeoyl-D-glucopyranoside ester, caffeoylquinic acid, loganin, cantleyoside, triplostoside A, lisianthioside, 6′-O-β-D-apiofuranosyl sweroside, as well as triterpenoids oleanic acid and akebiasaponin D, dipsanosides C-G, 3′-O-β-D-glucopyranosyl sweroside	Caffeic acid, 2,6-dihydroxycinnamic acid, vanillic acid, 2′-O-caffeoyl-D-glucopyranoside ester, and caffeoylquinic acid	Five human tumor cell lines including lung carcinoma A549, hepatoma Bel7402, gastric carcinoma BGC-823, colon cancer HCT-8, and ovary cancer A278052	[53]
Tripterygium wilfordii (Leigongteng)	Tripterygiol, (–)-syringaresinol, triptolide, tripdiolide, triptriolide, euonine, wilforine, cangorinine E-I, and 3,4,5-trimethoxyphenol	Tripterygiol, (–)-syringaresinol, triptolide, tripdiolide, triptriolide, euonine, wilforine, cangorinine E-I, and 3,4,5-trimethoxyphenol	Mouse monocyte/macrophage cell line RAW 264.7	[54]
Valeriana sorbifolia (Xiecao)	Sorbifolivaltrates A–D, isovaltrate, valtrate, seneciovaltrate, valtrate hydrine B3, and valtrate hydrine B7	Sorbifolivaltrates A–D, isovaltrate, valtrate, seneciovaltrate, valtrate hydrine B3, and valtrate hydrine B7	All compounds exhibited weak to moderate cytotoxicity against the human metastatic prostate cancer cell line, PC-3M	[55]
Rabdosia coetsa (Xiangchacai)	Yielded ethyl caffeate, rosmarinic acid, and methyl rosmarinate	Yielded ethyl caffeate, rosmarinic acid, and methyl rosmarinate	Inhibited ACE activity	[56]
Scrophularia ningpoensis (Xuanshen)	6″-O-caffeoylharpagide, 6″-O-feruloylharpagide, 6″-O-β-glucopyranosylharpagoside, 2-(3-hydroxy-4-methoxyphenyl) ethyl O-alpha-arabinopyranosyl-(1→6)-O-alpha-rhamnopyranosyl-(1→3)-O-β-glucopyranoside, phenyl O-β-xylopyranosyl-(1→6)-O-β-glucopyranoside, 3-methylphenyl O-β-xylopyranosyl-(1→6)-O-β-glucopyranoside, 6-O-cinnamoyl β-fructofuranosyl-(2→1)-O-alpha-glucopyranosyl-(6→1)-O-alpha-glucopyranoside, 6-O-feruloyl β-fructofuranosyl-(2→1)-O-alpha-glucopyranosyl-(6→1)-O-alpha-glucopyranoside, 6″-O-alpha-D-galactopyranosyl harpagoside, 6″-O-(p-coumaroyl) harpagide, harpagoside, angoroside C	6″-O-caffeoylharpagide, phenyl O-β-xylopyranosyl-(1→6)-O-β-glucopyranoside, 3-methylphenyl O-β-xylopyranosyl-(1→6)-O-β-glucopyranoside, 6″-O-(p-coumaroyl) harpagide, and harpagoside	Rat cardiac myocytes	[57]

(Continued)

Table 2.
(Continued)

Herb name	Monomer compound	Effective constituent	Activity evaluation model	References
Saussurea lappa (Yunmuxiang)	Dehydrocostus lactone, 11β,13-dihydrodehydrocostus lactone, costunolide, 11bsta,13-dihydrocostunolide,11β,13-dihydrosantamarin, 11β, 13-dihydro-reynosin, a-cyclocostunolide, 11β,13-dihydro-acyclocostunolide, 1β-hydroxycolartin, ilicol, 11β,13-dihydro-b-cyclocostunolide, alantolactone, isoalantolactone	Dehydrocostus lactone, costunolide, α-cyclocostunolide	Mouse macrophage RAW 264.7 cells	[58]
Angelica sinensis (Danggui)	Z-ligustilide, 11-angeloylsenkyunolide F, coniferyl ferulate, and ferulic acid	Z-ligustilide, 11-angeloylsenkyunolide F, coniferyl ferulate, and ferulic acid	Aggregated amyloid β-peptide (agg Aβ1-40) induced damage of differentiated PC-12 cells	[59]
Mori Cortex Radicis (Sangbaipi) / Morus Bombycis (Sangye)	Moracin O, moracin P, moracin Q, moracin M, mulberrofuran H, mulberrofuran G, sanggenon O, sanggenon C, albafuran A, mulberrofuran D, mulberrofuran W, kuwanon J, kuwanon Q, kuwanon R, kuwanon V	Moracin O, moracin P, moracin Q, mulberrofuran H, sanggenons O, albafuran A, mulberrofurans D, kuwanons J	Human hepatocelluar carcinoma cell-line Hep3B cells	[60]
Polygonum cuspidatum (Huzhang)	Citreorosein, together with emodin and its glucoside	Citreorosein	Recombinant yeast was used to assess the estrogenic activity	[61]
Garcinia oblongifolia (Lingnanshanzhuzi)	Oblongixanthones A-C, oblongifolins E-G, and 12 known compounds	Oblongifolin C	All isolates were assayed for their apoptosis-inducing effects against HeLa-C3 cells	[62]
Paeonia lactiflora (Chishao)	1,2,3,4,6-penta-O-galloyl-D-glucopyranose	1,2,3,4,6-penta-O-galloyl-D-glucopyranose	*In vitro* enzyme assay with human recombinant PTP1B	[63]
Actaea racemosa (Shengma)	Actein, 26-deoxyactein, 23-epi-26-deoxyactein, 23-O-acetylshengmanol 3-O-β-D-xylopyranoside, cimiracemoside F, cimigenol 3-O-β-D-xylopyranoside, cimiracemoside D, 25-O-acetylcimigenol 3-O-α-L-arabinopyranoside, 25-O-anhydrocimigenol 3-O-α-L-arabinopyranoside, 25-O-anhydrocimigenol 3-O-β-D-xylopyranoside, cimiracemoside K	23-O-acetylshengmanol 3-O-β-D-xylopyranoside, actein, cimigenol 3-O-β-d-xylopyranoside, and 25-O-acetylcimigenol 3-O-α-l-arabinopyranoside	GABA(A) receptor-modulating constituents in Xenopus laevis oocytes	[64]
Euphorbia kansui (Gansui)	Kansuinine B, kansuinine A, kansuiphorin C, 3-O-benzoyl-20-deoxyingenol, and 3-O-(2'E,4'Z-decadienoyl)-20-O-acetylingenol	Kansuinine B, kansuinine A, 3-O-(2'E,4'Z-decadienoyl)-20-O-acetylingenol	Models of inflammation using exoteric mice SPL and rat peritoneal macrophages (PMphi)	[65]
Glycyrrhiza uralensis (Gancao)	Glycyrrhisoflavone	Glycyrrhisoflavone	Alpha-glucosidase inhibitory activity	[66]
Tinospora sagittata (Qingniudan)	Palmatine, columbamine, and columbinyl glucoside	Palmatine, columbamine, and columbinyl glucoside	RAW264.7 macrophage cells	[67]
Salvia miltiorrhiza (Danshen)	Royleanone, horminone, 7-O-methyl-horminone and 7-acetyl-horminone, erythrodiol-3-acetate, β-sitosterol	Horminone, 7-acetyl-horminone and erythrodiol-3-acetate, 7-O-methyl-horminone	Human cervix adenocarcinoma (HeLa), skin carcinoma (A431), and breast adenocarcinoma (MCF7) cells using the MTT assay	[68]
Acanthopanax senticosus (Ciwujia)	Eleutheroside E, eleutheroside E (2)	Eleutheroside E, eleutheroside E (2)	Sleep-deprived mice (mental fatigue model)	[69]

(Continued)

Table 2.
(Continued)

Herb name	Monomer compound	Effective constituent	Activity evaluation model	References
Gardenia jasminoides (Zhizi)	Shanzhiside, gardenoside, geniposide, geniposidic acid, chlorogenic acid, 10-O-acetyl geniposide, shanzhiside methyl ester, scandoside methyl ester or deacetyl asperulosidic methyl ester, 6″-O-E-caffeoyl deacetyl asperulosidic methyl ester, 6″-O-sinapoyl gardoside, 3-O-caffeoyl-5-O-sinapoyl quinic acid or 3-O-caffeoyl-4-O-sinapoyl quinic acid, 3,4-di-O-caffeoyl quinic acid or 3,5-diO-caffeoyl quinic acid, 6″-O-sinapoyl geniposide	Shanzhiside, gardenoside, geniposide, geniposidic acid, chlorogenic acid, 10-O-acetyl geniposide, shanzhiside methyl ester, scandoside methyl ester or deacetyl asperulosidic methyl ester, 6″-O-E-caffeoyl deacetyl asperulosidic methyl ester, 6″-O-sinapoyl gardoside, 3-O-caffeoyl-5-O-sinapoyl quinic acid or 3-O-caffeoyl-4-O-sinapoyl quinic acid, 3,4-di-O-caffeoyl quinic acid or 3,5-diO-caffeoyl quinic acid, 6″-O-sinapoyl geniposide	Antiviral activity: influenza virus strain A/FM/1/47-MA in rats	[70]
Salvia miltiorrhiza (Danshen)	15,16-dihydrotanshinone I, cryptotanshinone, tanshinone I, tanshinone IIA, and dansenspiroketallactone	15,16-dihydrotanshinone I, cryptotanshinone, tanshinone I, tanshinone IIA	*In vitro* FAS inhibitory activity	[71]
Ligusticum chuanxiong (Chuanxiong)	3,8-Dihydro-diligustilide, 4,5-dehydro-diligustilide, levistolide A, tokinolide B, riligustilide	3,8-Dihydro-diligustilide, riligustilide	Estrogenic activity was measured *via* ERa and ERb expression in the presence of the estrogen-responsive MMTV-ERE-Luc reporter gene (HeLa cells)	[72]
Uncaria rhynchophylla (Gouteng)	Corynoxine, corynoxine B, corynoxeine, isorhynchophylline, isocorynoxeine, rhynchophylline	Rhynchophylline, isorhynchophylline	Cellular model of Alzheimer disease, β-amyloid-(Aβ-) induced neurotoxicity in PC12 cells	[73]
Garcinia nujiangensis (Nujiangtenghuang)	Nujiangexanthones A and B, nujiangefolins A–C, and 10 known related analogs	Isojacareubin	11 cancer cell lines and immortalized MIHA normal liver cells	[74]
Polygonum cuspidatum (Huzhang)	2-methoxystypandrone, as well as three anthraquinones	2-Methoxystypandrone	A potent inhibitory effect on STAT3 activation and significant inhibition of cell proliferation in human breast cancer cells, especially those with constitutively activated STAT3	[75]
Astragalus membranaceus (Huangqi)	Formononetin, calycosin, and astragaloside IV	Formononetin	Mouse macrophage RAW 264.7 cells (anti-inflammatory effect)	[76]
Picriafel terrae (Kuxuanshen)	Picfeltarraenin IA, picfeltarraenin IB, picfeltarraenin IV, picfeltarraenin X, picfeltarraenin XI, and one unknown compound	Picfeltarraenin IA, picfeltarraenin IB, picfeltarraenin IV, picfeltarraenin X, picfeltarraenin XI, and one unknown compound	One 96-well plate was used for a bioassay (AChE-inhibitory assay)	[77]
Scutellaria barbata (Banzhilian)	Scutebatas P–R, scutebata E, and scutebarbatine B	All compounds except scutebata R showed weak cytotoxicity	K562 cell lines, HL60 cell lines	[78]
Ligustrum lucidum (Nvzhenzi)	Tyrosol, tyrosyl acetate, hydroxytyrosol, salidroside, oleoside dimethyl ester, oleoside-7-ethyl-11-methyl ester, nuzhenide, and G13	Tyrosol, tyrosyl acetate, hydroxytyrosol, salidroside, oleoside dimethyl ester, oleoside-7-ethyl-11-methyl ester, nuzhenide, and G13	Osteoblast-like UMR-106 cells	[79]
Cinnamomum cassia (Rougui)	Cinnamoids A–D, cinnamoid E, and 18 known compounds	Cinnamoid E, (–)-15-hydroxy-T-muurolol, 10α-dihydroxy aromadendrane, and cinnacasside A	High-glucose-stimulated mesangial cells (diabetic nephropathy)	[80]
Limonium bicolor (Ersebuxuecao)	Luteolin, acacetin, quercetin, isorhamnetin, kaempferol, eriodictyol, kaempferol-3-O-α-L-rhamnoside, kaempferol-3-O-β-D-glucoside, quercetin-3-O-α-L-rhamnoside, quercetin-3-O-β-D-glucoside, quercetin-3-O-β-D-galactoside, myricetin-3-O-α-L-rhamnoside, kaempferol-3-O-(6″-O-galloyl)-β-D-glucoside, hesperidin, rutin	Luteolin, quercetin, kaempferol	Human colon cancer cells, human breast cancer cells, and osteosarcoma cell lines	[81]

(Continued)

Table 2.
(Continued)

Herb name	Monomer compound	Effective constituent	Activity evaluation model	References
Cimicifuga yunnanensis (Yunnanshengma)	Actein, cimigenol-3-O-β-d-xylopyranoside (23R,24S), 26-deoxyacetylacteol-3-O-α-l-arabinopyranoside, 23-epi-26-deoxyactein, cimifugin, cimifugin glucoside, norcimifugin glucoside, norkhellol, 25-O-acetylcimigenol, 26-deoxyacetylacteol, acetylacteol, 25-O-methylcimigenol, cimigenol, 25-O-acetylcimigenol-3-O-β-d-xylopyranoside, 24-O-acetylhydroshengmanol-3-O-β-d-xylopyranoside, 26-deoxyactein	23-epi-26-deoxyactein and cimigenol	Triple-negative breast cancer cells	[82]
Uncaria gambier (Erchagouteng)	Uncariitannin	Uncariitannin	Mouse models of intraperitoneal infection (*Staphylococcus aureus*)	[83]
Morinda officinalis (Bajitian)	Two inulin-type fructans with a backbone consisted of α-D-Glcp-(1→, 44 →1)-β-D-Fruf-(2→ and β-D-Fruf-(2→ residues (DP = 7 and 13, respectively)	Two inulin-type fructans	Classic ovariectomized rat model and MC3T3-E1 cells	[84]

ACE: Angiotensin-converting enzyme; FAS: Fatty acid synthase; HEMn: Human epidermal melanocytes; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; MIHA: cell code, without extension; PYP1B: Protein tyrosine phosphatase 1B; SPL: Splenic lymphocytes; TCM: Traditional Chinese medicine.

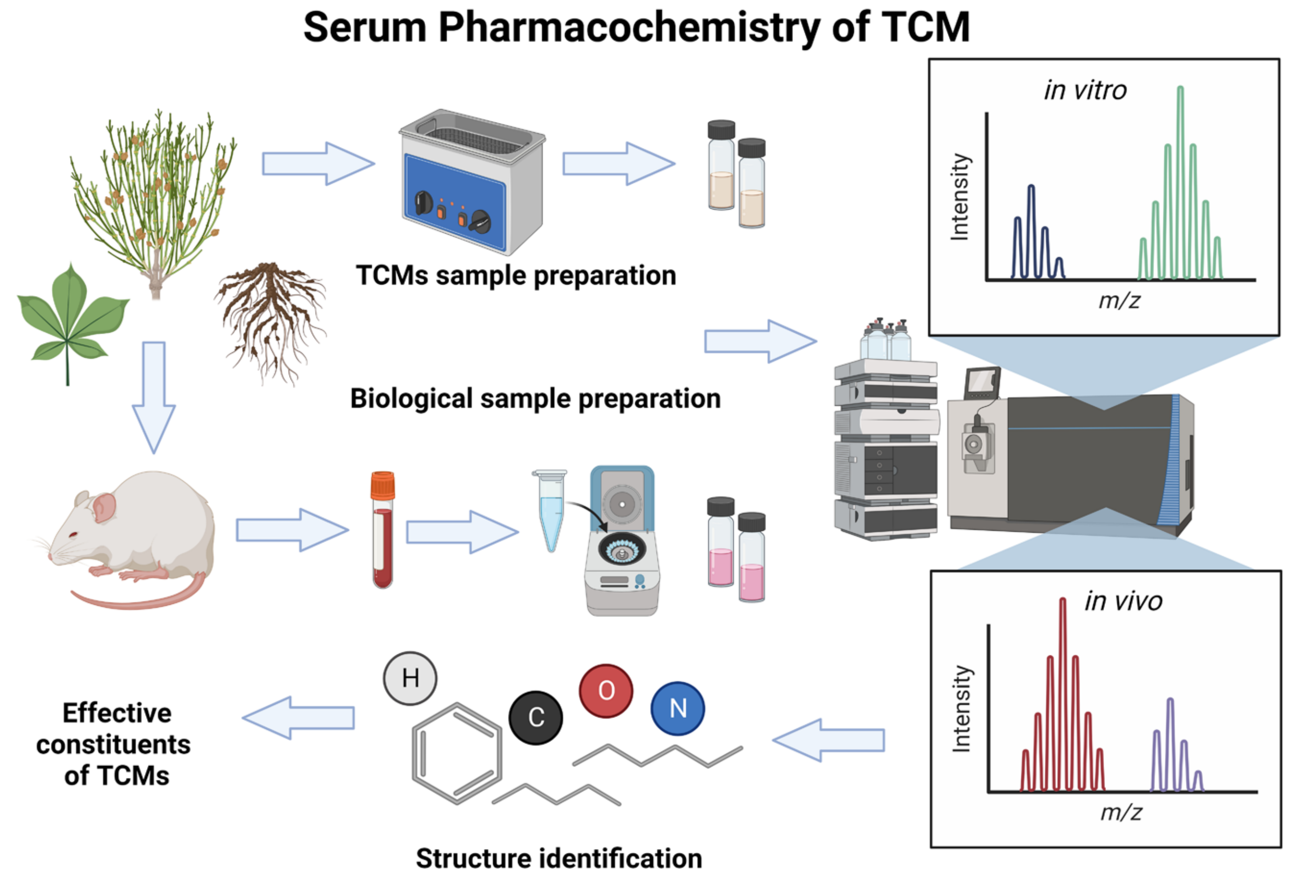

Figure 3. Workflow of serum pharmacochemistry of TCM method for screening effective constituent of TCMs. TCM: Traditional Chinese medicine. Created by Biorender.com.

6-dementhoxycapillarisin, capillarisin, rhein, and emodin[91]. Serum sample analysis after the administration of YCHD with different compositions (complete formula, removal of one combination herbal drug or single herbal drug) showed that eight of the 21 compounds were absorbed from the complete formula alone; interestingly, these eight compounds have stronger hepatoprotective and choleretic activities than those of the others. These findings illustrated the scientific significance of TCM formula compatibility[92]. Pharmacokinetic analysis of the main compounds in blood, namely 6,7-dimethoxyesculetin, geniposide, and rhein, demonstrated considerably better pharmacokinetic properties of these compounds from the complete formula; the three herbal drugs work synergistically *in vivo*[93]. Bioanalysis Zone described this phenomenon as "Three's a crowd: but could three be better than one"[94].

AS has anti-stress, antiulcer, anti-irradiation, anticancer, anti-inflammatory, and hepatoprotective activities[95]. After oral administration of AS extracts, 19 compounds were detected and identified in the blood; of which, 11 were prototype constituents, for example, protocatechuic acid, eleutheroside B, chlorogenic acid, caffeic acid, eleutheroside E, isofraxidin; eight were metabolites, mostly metabolites of eleutheroside B. In addition, the results showed that the content of isofraxidin was small *in vitro* but was highly increased *in vivo*. The time course of isofraxidin in serum after oral administration of AS extract exhibited a bimodal absorption, which is completely different from single compound administration of isofraxidin; this finding may be the combined behavior of free-type isofraxidin and the biotransformation of related precursor compounds (eleutheroside B1). The results demonstrated that the AS extract is a natural slow-release system of isofraxidin, which shows a higher bioavailability and longer-lasting effect compared to-with that of the single compound. These results explain the difference between administration of the AS extract and single isofraxidin and reveals the inherent lasting effect of TCM[96] and shows the advantages of serum pharmacochemistry of TCM. Furthermore, with the continuous progress of detection technology, serum pharmacochemistry of TCM has been widely used; the relevant studies are shown in Table 3[97–120].

The emergence of serum pharmacochemistry of TCM was a revolutionary breakthrough in the research on active constituents of TCM and a leap from separation *in vitro* to analysis *in vivo*. The results reflect not only the interaction between formula and herbal drugs but also the effect of the human body on the drugs. The method has been widely used in the field of pharmacodynamics and pharmacokinetics of TCM. Nevertheless, some limitations persist. The most crucial weakness is that the identified components found in the blood are still not associated with disease targets and pathways; in particular, these components are not associated with the biomarkers of TCM syndromes or reflect the clinical efficacy of the TCM formula. This weakness will be overcome by integrating the serum pharmacochemistry of TCM with syndrome metabolomics, establishing associations between *in vivo* (not exclusively in blood) chemical constituents from herbal medicines and syndrome markers to find the constituents highly associated with clinical efficacy. Moreover, other problems remain to be solved. For example, some constituents of herbal medicines with strong activity may not be absorbed; instead, they affect the intestinal flora, and those constituents are ignored using this approach. The enrichment and detection of low-content constituents in serum is also a challenge for active constituent screening, and the time for each TCM constituent to reach peak concentration in serum is not uniform; therefore, more comprehensive and accurate methods for detecting constituents in blood need to be established to improve the theory and method of serum pharmacochemistry of TCM.

Chinmedomics method: version 3.0 plus

The effective constituents of TCM are compounds that show clinical efficacy *in vivo* and are related to the targets of disease. According to TCM theory and clinical practice, the disease is described as a syndrome (*Zheng*), and the combination of herbal drugs called formula used as a clinical drug must correspond to the syndrome[121]. One herbal drug may contain thousands of chemical constituents with individual bioactivities; nevertheless, owing to the different combinations in formulas for different syndromes, the absorbed constituents for the same herbal drug are different, resulting from formula compatibility and the corresponding syndrome[122–123]. Therefore, analyzing the *in vivo* constituents when the formula shows efficacy for the syndrome is a better choice for identifying the compounds related to the treatment of the disease. However, for a long time, TCM syndromes did not have objective diagnostic criteria, and the diagnosis depended on the subjective cognitive analysis and judgment by the practitioner[124]. The absence of diagnostic parameters for TCM syndromes resulted in the inability to accurately evaluate the effectiveness of the formula; therefore, identification of the effective constituents of the formula *in vivo* related to clinical efficacy was not possible. Hence, interpretation of the biological characteristics of a syndrome and syndrome biomarkers are the premise for evaluating the efficacy and discovering effective constituents of TCMs[125].

Traditional Chinese medicine syndromes are functional disorder states, which are the body's response to various environmental changes and internal and external pathogenic factors. The essence of the syndrome is based on the metabolic network changes caused by body imbalance[121]. Metabolites represent both the downstream output of the genome and the upstream input from the environment, and they can provide unique insights into the fundamental causes of disease[126]. Therefore, metabolomics technology can facilitate the discovery of biomarkers of syndromes/diseases and the exploration of targets and metabolic pathways of a syndrome through non-discriminatory analysis of endogenous small-molecule metabolites. Professor Xijun Wang integrated serum pharmacochemistry of TCM and metabolomics, thereby proposing and establishing the research strategy of "Chinmedomics"[127]. On one hand, metabolomics technology is used to clarify the biomarkers of TCM syndromes and evaluate the efficacy of the formula based on metabolomic profiles and biomarkers of the syndromes. On the other hand, serum pharmacochemistry of TCM is used to identify the active constituents *in vivo* when the formula shows efficacy, further mining the correlation between formula constituents *in vivo* and endogenous biomarkers of syndromes using big data analytics and bioinformatics to identify constituents highly related to the efficacy of the formula as the effective constituents of TCM[128]. The flow chart of the strategy is shown in Figure 4. This method consists of four key techniques: identification

Table 3.

Representative studies on the effective constituents of TCM using serum pharmacochemistry

Single herb/ formula	Chemical component (*in vitro*)	Constituent (*in vivo*)	References
Yin Chen Hao decoction	45 constituents in Yin Chen Hao Tang	Picrocrocininc acid, chlorogenic acid, 6-methoxy coumarin-7-hydroxyl sulfate, 7-methoxy coumarin-6-hydroxyl sulfate, genipingentiobioside, geniposide, safflor yellow A, capillaridin A, 5,6-dimethoxy-7-hydroxy coumarin, quercetin-3-O-glycoside, 6,8-dimethoxy-7-hydroxycoumarin, 6,7-dimethylesculetin, 2,5-dimethyl-7-hydroxychromone, chimaphylin, 6-demethoxycapillarisin, capillarisin, rhein, emodin	[91]
Liuwei Dihuang pill	-	11 constituents in rat plasma, seven constituents in the original form, namely, loganin, paeonol, and four metabolites	[89]
Shexiang Baoxin pill	-	Gamabufotalin, ginsenoside Re, 17-hydroxyprogesterone, 11-hydroxyprogesterone, ginsenoside Ra_1/Ra_2, 1β-hydroxylbufalin, ginsenoside Rb_1, resibufaginol, ginsenoside Rc, ginsenoside Rb_2, ginsenoside Rb_3, ginsenoside Rd, bufalin, 3-epi-bufalin, cholic acid, hyodeoxycholic/ursodeoxycholic acid, cinobufagin, chenodeoxycholic acid, deoxycholic acid	[97]
Shaofu Zhuyu decoction	16 peaks were detected; 12 peaks, including flavones, organic acids, and terpene glycosides, were identified by comparing with references	Nine metabolites, including quercetin glucuronide sulfates, quercetin diglucuronides, isorhamnetin sulfates, methyl-ferulic acid, hydroxyl-paeonimetabolin, isorhamnetin glucosides, quercetin, isorhamnetin glucuronides, isorhamnetin	[98]
Yinchen Sini decoction	86 compounds were identified or tentatively characterized	6-Methoxycoumarin-7-O-glucuronide, isotalatizidine, karakoline, 7-methoxycoumarin-6-O-glucuronide, 6-methylesculetin sulfate, 7-methylesculetin sulfate, liquiritin-7-O-glucuronide, neoline, 5, 6-dimethoxycoumarin-7-O-glucuronide/6,8-dime-thoxy coumarin-7-O-glu-curonide, 6-methoxy-7-hydroxyl coumarin, 7-methoxy-6-hydroxyl coumarin, talatizamine, 5,6-dimethoxy-7-hydroxycoumarin, Liquiritigenin-4′-O-glucuronide, liquriritigenin-7-O-glucuronide, quercetin glucuronide, 6,8-dimethoxy-7-hydroxy coumarin, 6,7-dimethylesculetin, naringenin glucuronide, kaempferol glucuronide, isorhamnetin glucuronide, liquiritigenin, formononetin glucuronide, isoliquiritigenin glucuronide, chimaphylin, rhamnocitrin glucuronide, 6-demethoxycapillarisincapillarisin, 6-gingerol-4′-O-glucuronide, hypaconitine, cirsimaritin, and one remained unknown	[99]
Fufang Danshen tablet	43 constituents; 37 constituents were identified by comparing with reference standards and reference literature; the other six constituents were tentatively assigned solely based on the MS data	14 constituents: notoginsenoside R_2, ginsenoside Rg_2, ginsenoside Rd, danshexinkun B, etc and eight metabolites were observed in plasma, glycine conjugate of danshensu, glucuronic acid conjugate of protocatechuic acid, protocatechuic acid, 18-methyl hydroxycryptotanshinone, protopanaxatriol, protopanaxadiol, methylated conjugate of danshexinkun B, and one remained unknown	[100]
Huanglian Jiedu decoction	-	Gardenoside B, chlorogenic acid A1, phellodendrine, magnoflorine, jasminoidin, lotusine, berberubine A2, columbamine, epiberberine, jatrorrhizine, palmatine, berberine, baicalin, wogonoside, obakulactone, wogonin, genipingentiobioside, chrysin-6-c-arabinosy)-8-c-gucoside, chrysin-6-C-gfucosy-8-arabonoside, oroxyli4-7-0-glucuronide, 5,6,7-trihydroxy-8-methoxy flavone-7-O-glucuronide, 5,7-dihydroxy-6,8-dimethoxy flavone-7-O-glucuronide, glucuronide conjugate of demethyl tetrahydropalmatine, glucuronide conjugate of demethyleneberberine, jatrorrhizine 3-O-glucuronide, berberubine 9-O-glucuronide, baicalin-6-O-3-glucopyranuronoside-7-O-3-glucopyranuronoside, baicalin-5-O-B-glucopyranuronoside-7-O-3-glucopyranuronoside, 6-O-methyl-baicalin-7-O-3-glucopyranuronoside, baicalin-6-O-3-glucopyranuronoside	[101]
Gegen Qinlian pill	62 constituents were identified or tentatively characterized, including 42 flavonoids, 8 alkaloids, 6 triterpenoids, 3 phenylethanoid glycosides, and three other types	Puerarin-4′-O-glucoside, puerarin-O-glucuronidle, 3′-hydroxypuerarin, daidzein-O-sulfate-O-glucuronide, 3′-hydroxypuerarin xyloside, 6″-O-xylosylpuerarin, puerarin, mirificin, magnoflorine, 3′-methoxypuerarin, 3′-methoxy puerarin, 6-O-β-apionoside, daidzein-O-glucuronide, apigenin, 6-C-glucoside-8-C-arabinoside, demethyleneberberine-O-glucuronidle, jatrorrhizine-O-glucuronice or columbamine-O-glucuronide, genistein-8-C-apiosyl(1–6)-glucoside, p-hydroxyphenylpropionic acid, pueroside A, demethylberberine-O-glucuronide, puerarin isomer, liquiritin apioside, didemethylberberine-O-glucuronide, chrysin, 6-C-arabinoside-8-C-glucoside, liquiritigenin-O-glucuronide, isomer of s2, formononetin-8-C-apiosyl(1-6)-glucoside, 4-methoxypuerarin, baicalin, daidzein, methylbaicalein-O-glucuronide, liquiritingenin, formononetin monohydroxylate, oroxylin A 7-O-glucuronide, chrysin-7-O-glucuronide, baicalein-O-glucuronide, wogonoside, genistein, baicalein, glycyrrhizic acid, wogonin, oroxylin A	[102]

(Continued)

Table 3.
(Continued)

Single herb/ formula	Chemical component (*in vitro*)	Constituent (*in vivo*)	References
Shuang Huang Lian formula	46 peaks were obtained and tentatively characterized	Qunic acid, 3,4-dihydroxyphenylglycolsulfate, dihydrosecologanic acid, N-acetyl cysteine S conjugate, isomer of secoxyloganin2-(3,4-dihydroxyphenyl) ethanol sulfate, 3,4-dihydroxyphenylethanol, isomer of secologanic acid, dihydrosecologanic acid, secologanoside-7-methyl ester, demethyldihydrophillygeninglucuronide, secologanic acid, isomer of sweroside, caffeic acid, isomer of secoxyloganin, demethyldihydrophillygenin glucuronide, sweroside, secoxyloganin, demethyldihydrophillygenin glucuronide, p-hydroxyphenyl-propionic acid, chrysin-6-C-arabinosyl-8-C-glucoside, 5,7,3,2′,6′-Ppntahydroxyflavone, (+)-pinoresinol glucuronidehydroxylphillygenin glucuronide, (+)-pinoresinol glucuronide, (+)-dehydropinoresinol, (+)-hydroxylpinoresinol, dihydroxy-trimethoxy-flavanone, baicalin, 3,4′-dihydroxy-5,6-dimethoxy-7-O-glucuronide, phillygenin glucuronide, isomer of baicalin, phillygenin glucuronide, 5,7,8-trihydroxy-8-methoxyflavone-7-O-glucuronide, norwogonin-7-O-glucuronide, chrysin-7-O-glucuronide, oroxylin A-7-O-glucuronide, 5,6,7-trihydroxy-8-methoxy-flavone-7-O-glucuronide, dedydrophillygenin, isomer of baicalin, wogonoside, dihydrowqogonin, 5,7-dihydroxy-2′-methoxyflavanone-7-O-glucuronide, dihydroxy-dimethoxy flavoneglucuronide, dihydroxy-dimethoxy flavoneglucuronide, matairesinol, hesperetin, skullcapflavon II glucuronide, 5,7,3-trihydroxy-4’-methoxyflavone, norwogonin, 5,7,4′-trihydroxy-8-methoxyflavone, trihydroxy-dimethoxy flavone, baicalein, trihydroxy-dimethoxy flavone, trimethoxy flavone, (+)-epipinoresinol5,2′-dihydroxy-7,8,6′-trimethoxy flavone, wogonin, chrysin, 5,7-dihydroxy-6,8-dimethoxyflavone, scullcapflavone II, oroxylin A	[103]
Zhibai Dihuang pill	93 constituents were identified or tentatively characterized, 23 of which were from Phellodendri amurensis Cortex	Hydroxymethyl armepavine-O-glucuronide, hydroxymethyl armepavine-O-glucuronide, phellodendrine-O-glucuronide, phellodendrine, armepavine metabolites, 3-O-feruloylquinic acid-O-glucuronide, magnoflorine, tetrahydrojatrorrhizine-O-glucuronide, 3-O-feruloylquinic acid, demethyleneberberine-O-glucuronide, jatrorrhizine-O-glucuronide, menisperine, N-methyltetrahydrocolumbamine, berberine-O-sulfate-O-glucuronide, hydroxyberberine-O-glucuronide, hydroxyberberine, hydroxyjatrorrhizine-O-glucuronide, palmatine, berberine, γ-hydroxybutenolide deniatives II, sanleng acid-O-glucuronide, sanleng acid, rutaevin, obaculactone, obacunone, 3-hydroxyl feruloylquinic acid, tri-hydroxyjatrorrhizine, rutaevin-O-sulsate 3-O-feruloylquinic acid-O-glucuronide, hydroxyjatrorrhizine, sanleng acid-O-sulsate, rutaevin-O-glucuronide	[104]
Phellodendri Amurensis Cortex	A total of 46 peaks were obtained, 41 of which were tentatively characterized from GHB	24 ions of interest were extracted, 12 prototype constituents of GHB, phellodendrine, magnoflorine, 3-O-feruloylquinic acid, menisperine, tetrahydropalmatine, palmatine, berberine prototype, sanleng acid, rutaevin, obaculactone, obacunone, and 12 metabolites	[105]
Shaoyao Gancao decoction	-	Gallic acid, albiflorin, liquiritigenin-4-O-glucuronide, liquiritin, formononetin glucuronide, pallidiflorin, liquiritigenin, isoliquiritigenin, formononetin, isolicoflavonol, licoricone, one unknown (12 constituents including nine prototype constituents and two metabolites)	[106]
Xianling Gubao capsule	-	Diphylloside A, epimedoside E, diphylloside B, epimedoside A, epimedin A, epimedin B, epimedin C, icariin, wanepimedoside A, ikarisoside B, ikarisoside F, 2″-O-rhamnosylkarisoside A, ikarisoside A, sagittatoside A, icariside I, sagittatoside B, 2″-O-rhamnosylicariside I, icariside II, icaritin, psoralenolIsobavachin, neobavaisoflavone, bavachin, corylin, bavachalcone, isobavachalcone, bavachinin, corylifol A, bavachromene, 4-methylbavachalcone, neocorylin, psoralidin, psoralenoside, isopsoralenoside, psoralen, isopsoralen, asperosaponin VI, hederagenin B, sophorosyl ester, akebia saponin PA, timosaponin B-II, timosaponin B-III, timosaponinA- III, timosaponin A-I, protocatec huicaldehyde, caffeic acid, danshensu, ferulic acid, isoerulic acid, 15,16-dihydrotanshinone I, tanshinol B, 1,2-dihydrotanshinone I, tanshinone v, cyptotanshinone, dehydrotanshinone II, A, tanshinone IIA, magnoflorine, cycloolivil, 3-hydroxybakuchiol, bakuchiol, loganin, sweroside, cis-hinoliresinol, etc (57 prototype components and 77 metabolites)	[107]
Sheng Mai powder	-	7,8-dihydroxy-3-demethyl schizandrin, 7,8-dihydroxy-2-demethyl schizandrin, 7,8-dihydroxy-2-demethyl schizandrinisomer, 7,8-dihydroxy-schizandrin, schisandrin, gominsin D, schisandrol B, angeloygomisin H, schisantherin A, schisantherin B, deoxyschisandrin, γ-schisandrin, schisandrin B, 20(S)-ginsenoside Rh1, 20(R)-ginsenoside Rh1, ginsenoside Rk3, ginsenoside Rh4, gomisin P isomer, gomisin H isomer, isoschisandrin, schisandrin isomer, tigloylgomisin H, epi-gomisin O, benzoylgomisin Q, angeloylgomisin Q, gomisin F, schisandrin C	[108]

(Continued)

Table 3.
(Continued)

Single herb/ formula	Chemical component (*in vitro*)	Constituent (*in vivo*)	References
Kai Xin powder	-	Bergapten, mannose, valine, dimethyl(R)-(+)-malate, sucrose, nicotinic acid, benzoic acid, adenine, adenosine, sibiricose AI, ethyl benzoylacetate, salicylic acid, polygalaxanthone III, 1-(3,4-dimethoxyphenyl) ethan-1-one, 2-O-methyl-a-D-glucopyranose, 2-hydroxybenzonic acid[(2S)-5-oxotetrahydro-2-furanyl] methyl, benzoate, dibutyl oxalate, 3,4,5-trimethoxy cinnamic acid, acoronene, poricoic acid H, poricoic acid G, gomisin A, 3β,16a-dihydroxylanosta-7,9(11),24-trien-21-oic acid, poricoic acid B, 6,7-dehydroporicoic acid H, 16-deoxyporicoic acid B, tumulosic acid, cis/trans-methylisoeugenol, 3β-hydroxy-16a-acetoxy-lanosta-7,9(11),24-trien-21-oic acid, daedaleanic acid A, (5E,20S)-24-methylene-3-oxolanosta-7,9(11)-dien-21-oic acid, dehydroeburiconic acid, dehydrotumulosic acid, 2,6-di-sec-buty1-4-methylphenol, pachymic acid, 3-(2-furyl)-3-oxopropanenitrile, N-(2,5-dimethoxyphenyl)-2-methoxyacetamide	[109]
Zi Shen pill	92 peaks were characterized	7-O-glucopyranosyl mangiferin, amurenlactone A/B, mangiferin/isomangiferin, phellodendrine, mangiferin/isomangiferin, magnoflorine, lotusine, menisperine, tetrahydropalmatine, jatrorrhizine, palmatine, berberine,γ-hydroxybutenolide deniatives II, anemarsapon in I/anemarsapon in II, sanleng acid, rutaevin, obaculactone, obacunone	[110]
Acanthopanacis senticosi Radix et Rhizoma Seu Caulis (Leaf)	131 constituents were identified or tentatively characterized including triterpenoid saponins, phenols, flavonoids, lignans, coumarins, polysaccharides, and other compounds	Protocatechuic acid, 1-O-butyryl-xylopyranose, quinic acid, caffeic acid, feruloyl quinic acid, rutin, hyperoside, quercitrin, 2,3,5,4-tetrahydroxystilbene 2-O-glucopyranoside, 4,15-dioxo-5,8,11,14-tetraoxaoctadecane-1,18-dioic acid, malyngic acid, 3-{[2-O-(6-deoxy-mannopyranosyl)- arabinopyranosyl] oxy} olean-12-ene-28,29-dioic acid	[111]
Da Bu Yin pill	70 constituents including alkaloids, flavonoids, polysaccharide, limonoids, flavonoids	*Z*-6,7-epoxyligustilide, ginsenoside Re, butylidenephthalide, 3-butylphthalide, ginsenoside Rc, glycyrrhizin, ginsenoside Rd, gypenoside XVI, zingibroside R1, paconol + O + SO_3, liquiritigenin + SO_3 + GluA, liquiritigenin + GluA, liquiritigenin + GluA, davidigenin + GluA + SO_3, liquiritigenin + SO_3, liquiritigenin + SO_3 + GluA, davidigenin + GluA + SO_3, davidigenin + O + GluA, davidigenin + GluA, davidigenin + GluA, isoliquiritigenin + GluA, isoliquiritigenin + SO_3, hydroxyglycyrrhetinic acid, hydroxyglycyrrhetinic acid, glyasperinD + GluA, hydroxyglycyrrhetinic acid, hydroxyglycyrrhetinic acid	[112]
Xianling Gubao capsule	-	Sweroside, magnoflorine, psoralenoside, isopsoralenoside, psoralen, timosaponin B II, isopsoralen, epimedin C, icariin, akebia saponin D, icariside I, 2″-O-rha-icariside II, neobavaisoflavone, icariside II, corylin, akebia saponin PA, psoralidin, bavachinin	[113]
Moutan Cortex	46 constituents	b-glucogallin, gallic acid, mudanoside B, (+)-catechin, hyemaloside B, oxypaeoniflorin, dihydroxy acetophenone, ethyl gallate, dihydroxy methoxy acetophenone, paeonol, paeoniflorin, ethyl gallate, suffruticoside B or D, galloyl paeoniflorin, paeonol, benzoyloxypaeoniflorin, mudanpioside C, dihydroxy methoxy acetophenone	[114]
Wen Xin formula	97 constituents	Syringic, acid-4-B-D-glucopyranoside, protocatechuic, acid-3-glucoside, oxypaeoniflorin, magnoflorine, albiflorin, paeoniflorin, linalool 3,6-oxide 6-O-β-D-xylopyranosyl-β-D-glucopyranoside, berberrubine, galloylpaeoniflorin isomer, coptisine, epiberberine, jatrorrhizine, berberine, palmatine, 3-methyl ellagic acid, 3,3-di-O-methyl ellagic acid, ginsenoside Rg1, 9(S),12(S),13(S)-trihydroxy-10(E),15(Z)-octadecadienoic acid, ginsenoside Rb1, 5-O-feruloylquinic acid/3-O-feru-loylquinic acid, berberrubine-9-O-3-D-glucuronide, jatrorrhizine-3-O-B-D-glucuronide, demethyleneberberine-2-O-3-D-glucuronide, demethylberberrubine-2-O-3-D-glucuronide, demethyleneberberine, 9(S),10(S),1 1(R)-trihydroxy-12(Z),15(Z)-octadecadienoic acid, ginsenoside Rf, 9(S),10(S),13(S)-trihydroxy-11(E),15(Z)-octadecadienoic acid	[115]
Acanthopanacis senticosi Radix et Rhizoma Seu Caulis (Stem)	115 constituents	Vanillic acid glucoside, protocatechuic acid, vanillic acid, neochlorogenic acid, coniferol, sinapyl alcohol, ferulic acid, sinapyl alcohol, caffeic acid, isofraxidin, vanillic acid, isofraxidin, caffeic acid, isofraxidin, guaiacylglycerolconiferol glucoside, guaiacylglycerol hydroconiferolglucoside, 2-{2-hydroxy-5-(1E)-3-hydroxy-1-propen-1-yl]-3-methoxyphenyl}-3-(4-hydroxy-3-methoxyphenyl) propylhexopyranoside, isofraxidin, guaiacylglycerol coniferol glucoside, salvadoraside, guaiacylglycerolhydroconiferolglucoside, 2-{2-hydroxy-5-[(1E)-3-hydroxy-1-propen-1-yl-3-methoxyphenyl}-3-(4-hydroxy-3-methoxyphenyl) propylhexopyranoside, guaiacylglycerolconiferol glucoside, guaicylglycerol hydro-coniferol glucoside, feruloylquinic acid, (2R,3R)-2,3-bis (4-hydroxy-3,5-dimethoxybenzyl)-1,4-butanediol, ciwujiatone, secoisolariciresinol, dimethoxyl lariciresinol, ciwujiatone, lariciresinol 3-α-O-β-D-glucopyranoside, dimethoxyl lariciresinol, methoxyl lariciresinol, isofraxidin, pinoresinol glucoside, syringaresinol, 4-O-β-D-glucopyranoside, matairesinol	[116]

(Continued)

Table 3.
(Continued)

Single herb/ formula	Chemical component (*in vitro*)	Constituent (*in vivo*)	References
Schisandrae chinensis Fructus	-	Schisandrin, gomisin D, schisandrol B, benzoylgomisin H, angeloylgomisin Q, gomisin G, gomisin K, gomisin E, deoxyschizandrin, schisandrin B, and 39 metabolites	[117]
Ding Kun pellet	A total of 234 constituents were identified, including 47 triterpenoid saponins, 55 flavonoids, and 38 alkaloids	1,2,3-tri-O-methyl-D-arabino-L-erythro-4-octulose, safflomin A, isovanillic acid, D-phenylalanine, (+)-laurotetanine, albiflorin, picrocrocin, mudanpioside E, liquiritin, ferulic acid, rotundinum, coryphenanthrine, homochelidonine, tetrahydropalmatine, tetrahydrocoptisine, notoginsenoside R$_1$, baicalin, ginsenoside Rg$_1$, corydaline, liquiritigenin, oroxylin-A-7-O-glucuronide, hispidulin-7-O-β-D-glucuronopyranoside, wogonoside, ginsenoside Rb1, glyasperins M, isoliquiritigenin, ginsenoside Rd, 3-(2,4-dihydroxyphenyl)-8-(1,1 dimethylprop-2-enyl)-7-hydroxy- 5-methoxy-coumarin, glycycoumarin or isomer, palmitic acid, senkyunolide C, phytosphingosine, yunnankadsurin A, genkwanin, acetoxyl oxokadsurane, heteroclitin G, ligustilide, eudesm-4 (15) en-6-one, glycyrin, angeloylgomisin R, interiotherin A, senkyunolide O, levistolide A	[118]
Zuo Jin pill	90 constituents were identified in ZJP	3,4-dihydroxybenzoic acid, magnoflorine, berberrubine, 5-O-feruloylquinic acid, coptisine, dehydroevodiamine, epiberberine, columbamine, jatrorrihizine, berberine, palmatine, dehydrocorydaline, diosmin, skimmianine, formyldihydrorutaecarpine, 8-oxo-epiberberine, 8-oxocoptisine, evodiamine, rutaecarpine, 1-Methyl-2- undecyl – 4(1H)-quinolone, evocarpine, dihydroevocarpine	[119]
Da Cheng Qi decoction	-	5,7-dihydroxychromone, rhein, diglucoside, scolymoside, cinnamic acid, 2-cinnamoyl-glucose, benzylideneacetone, safrole, (–)-epicatechin gallate, rhein-8-O-3-D-glucopyranoside, physcion 8-glucoside, toralactone, narirutin, naringin, aloe-emodin-glucoside, hesperidin, emodinanthrone, 5,7,4′-trimethylapigenin, tetramethoxyluteolin, 2-methoxy-4-vinylphenol, hesperetin, quercetin, chrysophanol, glucoside, neohesperidin, 4′,5,7,8-tetramethoxyflavone, anthraglycoside B, eupatin, apigenin, naringenin, poncirin, magnolignan C, bergapten, rheochrysin, nobiletin, randaiol, luteolin, ammidin, magnolignan E, isosinensetin, tangeretin, aloeemodin, rhein, 5-O-demethylnobiletin, emodin, obovatol, chrysophanol, physcion	[120]

GHB: Guanhuangbo (Phellodendri amurensis Cortex); MS: Mass spectrum; TCM: Traditional Chinese medicine.

of syndrome biomarkers, precise efficacy evaluation of formula, characterization of constituents of formula *in vivo*, and mining the correlation between constituents and syndrome biomarkers[17]. This method has been widely accepted and cited; *Nature* recommended it as a powerful approach for the efficacy evaluation of TCM, a biological language between TCM and Western medicine[129]. Professor Rahman described it as an emerging technology that could be used for disease fingerprinting or predicting drug effects[130]. In 2015, Professor Xijun Wang published a monograph on Chinmedomics by Elsevier[131], and he published six series of monographs in yearly volumes of Research Progress in Chinmedomics by Science Press (2016–2021)[132–137].

Chinmedomics has been used to evaluate the efficacy of approximately 20 classical TCM formulas and elucidate their mechanisms, a summary of these is provided in Table 4[138–154]. We will use YHS and YCHD as examples to introduce the detail of Chinmedomics. YHS is a damp-heat phenotype of jaundice syndrome, and its clinical symptoms are fever; polydipsia; bright yellowish discoloration of the whites of the eyes, mucous membranes, and the skin; strong-tea-colored dark urine; accompanied by loss of appetite or nausea and vomiting[155]. YCHD is a classical prescription commonly used in the treatment of YHS; thousands of years of experiments on human subjects indicate that the clinical effect is remarkable[156]. However, it remains unclear which specific active ingredients act on which targets to produce therapeutic effects, and Chinmedomics provides a new research paradigm for solving this problem. For the first time, researchers introduced the metabonomics technology for small-molecule metabolite-level characterization of the TCM syndrome. Comparing patients accompanied YHS with healthy subjects, 40 urinary metabolic differential markers were successfully identified; these mainly included kinetin, porphobilinogen, and indoleacrylic acid[157]. The approach achieved accurate quantitative characterization of the TCM syndrome, which laid a foundation for an accurate evaluation of the TCM curative effect. In a recent study of blood metabolomics in jaundice patients, 14 different metabolites were successfully identified, including taurocholic acid, bilirubin glucuronide, bilirubin, and biliverdin, which suggests that they can be used as an effective supplement to clinically diagnose jaundice. In addition, after treatment with YCHD, the clinical symptoms of patients were markedly improved, and the contents of 13 blood metabolic biomarkers were notably altered, returning to normal levels. Moreover, 26 prototype components and three metabolites were successfully identified *in vivo* using serum pharmacochemistry of YCHD in clinical subjects with significant efficacy, and the correlation analysis with biomarkers revealed that geniposide, scoparone, isorhamnetin, quercetin, naringenin, rhein, chlorogenic acid, and kaempferol were related to clinical efficacy[158].

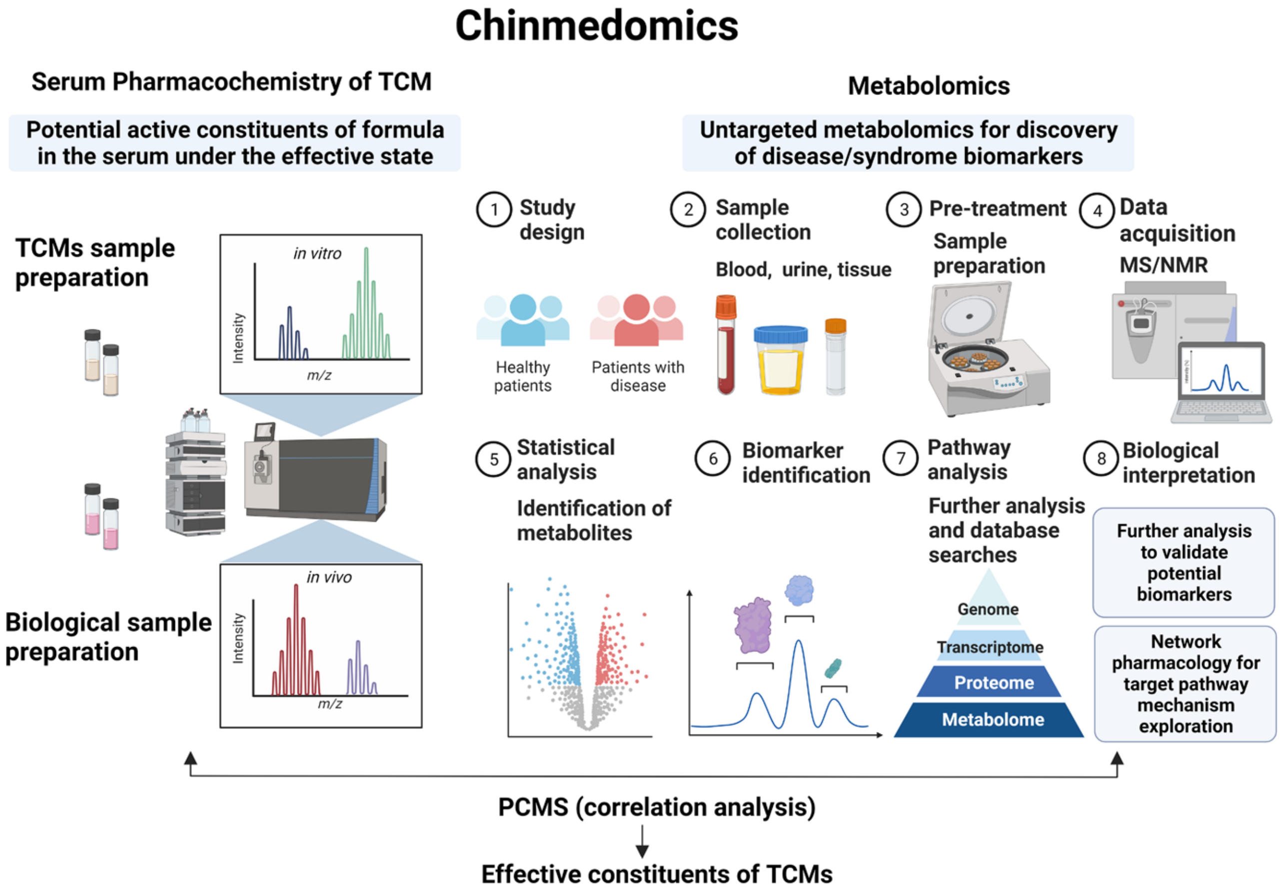

Figure 4. Workflow of Chinmedomics for screening effective constituents of TCMs. PCMS: Plotting of Correlation between Marker metabolites and Serum constituents; TCM: Traditional Chinese medicine. Created by Biorender.com.

In relevant studies using YHS animal models, 33 biomarkers of mouse urine metabolism were successfully identified. Among these, 10 biomarkers were consistent with the results of clinical studies, including epinephrine, 5-methoxytryptophan, kynurenic acid, 5,6-dihydrouridine, gluconic acid, phenylpyruvic acid[159]. In this way, the animal model successfully replicated the clinical TCM syndrome. In blood metabolomics studies, 12 biomarkers were characterized; among these metabolites, three common markers, namely bilirubin, biliverdin, and taurocholic acid, were found in both the human patient and mouse serum samples. After intragastric administration of YCHD, the contents of 10 serum biomarkers were significantly reduced, and the liver histopathology and metabolic profile of mice were similar to those of the control group. In addition, a total of 33 prototype components and three metabolites were characterized in mouse serum; among which, 21 chemical components were analogous with the results of clinical samples. In conclusion, through multidimensional integrated analysis, network database mining, and experimental verification of protein expression levels, the effective constituents of YCHD were identified, including geniposide, scoparone, isorhamnetin, quercetin, naringenin, rhein, chlorogenic acid, and kaempferol. These regulate CYP7A, ABCC2, ABCC3, UGT1A1, FXR protein targets, which synergistically regulate primary bile acid biosynthesis, porphyrin and chlorophyll metabolism, and biliary secretion, exerting their therapeutic role as cholagogic and anti-jaundice agents[158]. Furthermore, numerous other research findings support the above research results[146,160–163].

In summary, Chinmedomics combines the most advanced ideas of integration and technological means, creating new ideas and strategies for the in-depth analysis of the efficacy and mechanisms of TCM prescriptions[128]. Chinmedomics focuses on TCM syndrome and formula and considers clinical efficacy, avoiding the blindness of simple separation of chemical components, which was the hallmark of the early stages. Particularly, this method solved the scientific problem of the mismatch between TCM components *in vivo* and the targets and pathways of disease. It explores the underlying mechanism of the formula on syndrome biomarkers and related metabolic pathways, finding the target and pathway of effective constituents, and further elucidating the characteristics of TCMs with multiple components and multiple targets. Some components of TCMs do not need to be absorbed into the blood to play a therapeutic role; some of them can improve the disease by directly regulating the intestinal flora[164–166]. Therefore, Chinmedomics also integrates gut microbiota metabolism to identify the constituents at multiple sites *in vivo*, such as feces and intestinal content. In addition, systems biology methods, such as genomics[167], transcriptomics[168], proteomics[169], and other technologies, can be comprehensively used to enrich Chinmedomics and to supplement the experimental results with multiple indicators and perspectives. Moreover, big data analytics and artificial intelligence should be introduced into Chinmedomics to improve its scientificity and reliability. The expanded research on Chinmedomics is shown in Figure 5.

Table 4.

Representative studies on the effective constituents of TCMs using Chinmedomics

Prescription	Syndrome/ diseases	Effective constituent	References
Wen Xin formula	Myocardial ischemia syndrome	Ginsenosides, paeoniflorin, galloyl glucose, berberis alkaloids, phenolic, phenolic glycosides and unsaturated fatty acids, glucuronide products of original berberis alkaloids	[138]
Shen Qi pill	Kidney-*yang* deficiency syndrome	Azelaic acid-O-glucuronide, jionoside D, azelaic acid, poricoic acid B-O-sulfate, tumulosic acid-O-glucuronide, poricoic acid A, eugenol methyl ether, and dehydroeburicoic acid	[139]
Nan Shi Oral liquid	Kidney-*yang* deficiency syndrome	Scandoside, monotropeine, gallic acid, stevioside C, steviolbioside, and morroniside glucuronide conjugation	[140]
AS1350	Kidney-*yang* deficiency syndrome	Schizandrin, gomisin S, βine, scoparone, clovene, stepharine, longipedunin C, gomisins, auxin A, and 1,11-undecanedicarboxylicacid	[141]
Nan Shi capsule	Kidney-*yang* deficiency syndrome	Caffeic acid, 2-hydroxy-1-methoxy-anthraquinone, 1-hydroxy-2-methoxyanthraquinone, ferulic acid glucuronide conjugation, deacetylasperulosidic acid, cynaroside, βine, and umbelliferone	[142]
Phellodendri amurensis Cortex (GHB)	Prostate cancer	Magnoflorine-O-glucuronide, (p-hydroxybenzyl)-6,7-dihydroxy-N-methyltetrahydro isoquinoline-7-O-p-D-glucopyranosid, magnoflorine, menisperine-O-glucuronide, menisperine, berberine, jatrorrhizine, obaculactone, and obacunone	[143]
Sheng Mai powder	Alzheimer disease	Schisandrin, isoschisandrin, angeloylgomisin Q, gomisin D, angeloylgomisin H, gomisin M2, ginsenoside F1, and 20(R)-ginsenoside Rg3	[144]
Kai Xin powder	Alzheimer disease	Ginsenoside Rf, ginsenoside F1, 20-O-glucopyranosyl ginsenoside Rf, dehydropachymic acid, and E-3, 4,5-trimethoxycinnamic acid	[145]
Yin Chen Hao decoction	Dampness-heat jaundice syndrome	2-ethyl-2-hexenal, isofraxidin, 2,5-dimethyl-7-hydroxy chromone, 6,7-dimethoxy coumarin, geniposide, capillarisin, neochlorogenicacid, chimaphylin, and isorhamnetin-3-glucoside	[146]
Yun Nan Bai Yao	Blood stasis syndrome	Karacolidine, senbusine B, isotalatizidine, karakoline, denudatine, talatisamine, and chasmanine	[147]
Gelan Xinning capsule	Coronary heart disease	11 compounds as quality markers responsible for the efficacy of Ge Lan Xin Ning capsule	[148]
Fangji Huangqi decoction	Nephrotic syndrome	(+)-Tetrandrine demethylation, fenfangjine G hydrogenation, tetrandrine, N-methylfangchinoline, tetrandrine demethylation, fangchinoline, glycyrrhetic acid, astragaloside II alcohol dehydration, atractylenolide III demethylation + hydrogenation, atractylenolide III demethylation + hydrogenation, and licoricone-N-acetylcysteine conjugation	[149]
Baizhu Shaoyao powder	Ulcerative colitis	Atractylenolide I-M5, hesperidin-M2, cimifugin, albiflorin-M2, cimifugin-M3, 5-O-methylvisamminol-M2, sec-O-glucosyl-hamaudol, atractylenolide II, atractylenolide III-M5, and atractylenolide III-M4	[150]
Compound Zao fan pill (consist of Panacis quinquefolii Radix)	*Qi*-blood deficiency syndromes	24-methyl-7-cholesten-3β-ol, zizybeoside II, betulin, ginsenoside Rd, cinnamyl alcohol, and pseudoginsenoside F11 considered a potential Q-marker for the compound Zao fan pill; pseudoginsenoside F11 and ginsenoside Rd, considered a potential Q-marker for Panax quinquefolium L	[151]
Si Jun Zi decoction	Spleen *qi* deficiency syndrome	Malonyl-ginsenoside Rb2 and ginsenoside Ro as the q-markers of ginseng; dehydrotumulosic acid and dihydroxy lanostene-triene-21-acid as the q-markers of poria; glycyrrhizic acid, isoglabrolide, and glycyrrhetnic acid as the q-markers of licorice; and 2-atractylenolide as the q-marker of macrocephala	[152]
GHB in Zhi Bai Di Huang pill	Kidney-*yin* deficiency	Magnoflorine and jatrorrhizine	[122]
Zhizi Baipi decoction	Damp-heat jaundice syndrome	Isoformononetin, 3-O-feruloylquinic acid, glycyrrhizic acid, oxyberberine, obaculactone, and five metabolites	[123]
Ke Luo Xin capsule	Diabetic retinopathy	Emodin, rhein, astragaloside IV, chrysophanol, chrysophanol-8-O-β-d-glucopyranoside, aloe-emodin, aloin, biochanin A, 10-hydroxyoleuropein, 3,4′,7-trihydroxyflavone, and 3′-O-methyl-(−)-epicatechin 7-O-glucuronide	[153]
Danggui Jianzhong decoction	Primary dysmenorrhea	Ferulic acid, zizyphusin, cinnamic acid, protocatechuic acid-3-glucoside, and azelaic acid	[154]

GHB: Guanhuangbo (Phellodendri amurensis Cortex); TCM: Traditional Chinese medicine.

Conclusion

Analysis and identification of effective constituents from the complex mega system of TCMs are crucial for interpreting the principles of TCMs. They are also key steps to the discovery of new drugs based on clinical experience. With the continuous innovation of technology, researchers have innovatively explored and established a series of strategies for TCM. The discovery methods

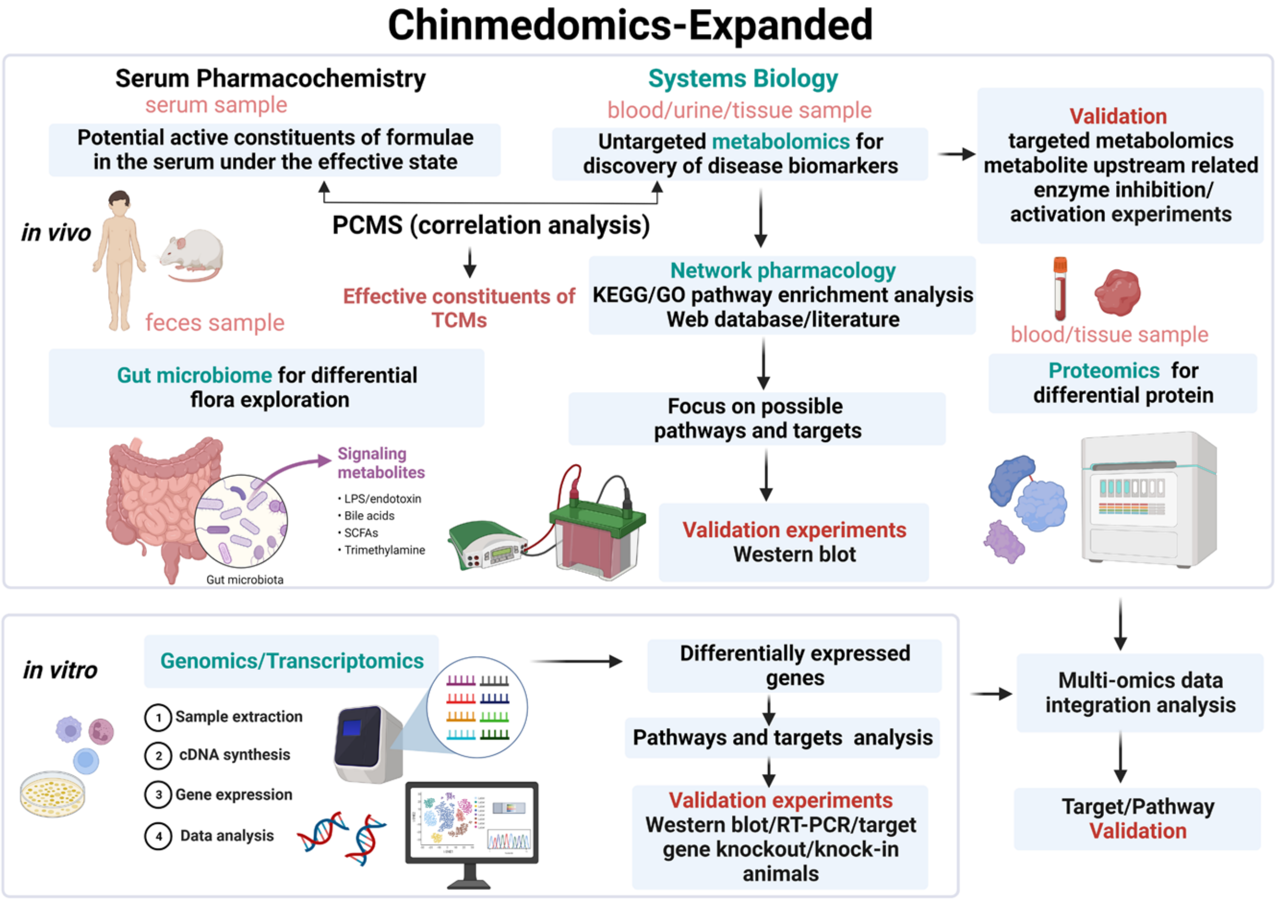

Figure 5. Workflow for Chinmedomics-expanded, an integrated validation analytical method of serum pharmacochemistry and systems biology. GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; LPS: Lipopolysaccharide; PCMS: Plotting of Correlation between Marker metabolites and Serum constituents; RT-PCR: Reverse Transcription-Polymerase Chain Reaction; SCFA: Short Chain Fatty Acids. Created by Biorender.com.

summarized in this study were formed during different historical development stages and played different roles. They have all made historical contributions to the research on the material basis of the efficacy of TCMs and will continue to play an important role in the future. Chinmedomics has created a new and comprehensive strategy for effective constituents; however, further improvements along with the progress of analytical technology and systems biology are required. The classical phytochemical methods and bioactivity-guided separation will still be used in different periods and stages and will remain indispensable to the research on active TCM constituent identification.

In the future, with the continuous development of comprehensive multidisciplinary analytic techniques and the continuous emergence of new biological sample analysis techniques, the research methods for mining effective constituents of TCMs will continue to be developed and improved based on Chinmedomics. With the consideration of clinical efficacy, the combination of virtual screening, experimental verification, non-discriminatory multi-target screening, and targeted screening is expected to take the technological leap from inefficient to efficient discovery. Particularly, the application of big data and artificial intelligence technology will help build optimal and suitable syndrome diagnostic criteria, pharmacodynamic analysis models, biological information models, and data integration methods. Future research will bring the discovered effective constituents much closer to clinical efficacy, and their targets and pathways will be clearer. Based on TCM theory and clinical practice, new effective natural medicines will be discovered and created. Furthermore, the discovery of effective constituents of TCM and clinical translational application will continue to promote the high-quality development of TCM and its role in addressing major diseases and acute infections, and finally, realize the modernization and international development of TCM.

Conflict of interest statement

Xijun Wang are editorial board members of this journal. None of the other authors declare any conflicts of interest.

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Author contributions

Xijun Wang and Hui Sun proposed the idea and designed the structure of the paper. Nan Ge and Guangli Yan participated in the writing of the paper. Le Yang, Ling Kong, Ye Sun, Ying Han, Qiqi Zhao, and Shuyu Kang participated in the performance of some research and reference material collection.

Ethical approval of studies and informed consent

Not applicable.

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None.

Data Availability

All data generated or analyzed during this study are included in this published article.

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