



Review

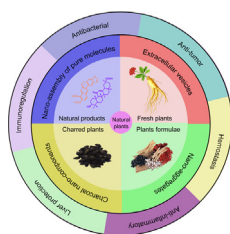
Carrier-free nanoplatfroms from natural plants for enhanced bioactivity

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HIGHLIGHTS

- Carrier-free nanoplatfroms with self-assembly from natural plants.
- Nano self-assembly of pure small molecules for enhanced bioactivity.
- Extracellular vesicles containing active substances from fresh plants.
- Charcoal nanocomponents with carbon skeleton structure from charred plants.
- Nanoaggregate particles with wide curative effects from decoction of plants formulae.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:

Received 15 June 2022

Revised 15 September 2022

Accepted 28 September 2022

Available online 5 October 2022

Keywords:

Natural plants
Carrier-free nanoplatfrom
Extracellular vesicle
Charcoal nanocomponent
Nanoaggregates

ABSTRACT

Background: Natural plants as well as traditional Chinese medicine have made outstanding contributions to the health and reproduction of human beings and remain the basis and major resource for drug innovation. Carrier-free nanoplatfroms completely self-assembled by pure molecules or therapeutic components have attracted increasing attention due to their advantages of improved pharmacodynamics/pharmacokinetics, reduced toxicity, and high drug loading. In recent years, carrier-free nanoplatfroms produced by self-assembly from natural plants have contributed to progress in a variety of therapeutic modalities. Notably, these nanoplatfroms based on the interactions of components from different natural plants improve efficiency and depress toxicity.

Aim of Review: In this review, different types of self-assembled nanoplatfroms are first summarized, mainly including nanoassemblies of pure small molecules isolated from different plants, extracellular vesicles separated from fresh plants, charcoal nanocomponents obtained from charred plants, and nanoaggregates from plants formulae decoctions.

Key Scientific Concepts of Review: We mainly focus on composition, self-assembly mechanisms, biological activity and modes of action. Finally, a future perspective of existing challenges with respect to the clinical application of plant-based carrier-free nanoplatfroms is discussed, which may be instructive to further develop effective carrier-free nanoplatfroms from natural plants in the future.

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Peer review under responsibility of Cairo University.

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Introduction

In the course of fighting against disease, mankind first apply natural plants to relieve pain [1]. Therefore, in the early history of various ancient civilizations, there is no lack of records of herbal medicines. In particular, working Chinese people have accumulated rich experience in the processes of long-term production practices and fighting against diseases and have gradually formed unique traditional Chinese medicine (TCM) [2]. Natural plants have made outstanding contributions to the health and reproduction of all mankind. Furthermore, natural ingredients from medicinal plants are characterized by abundant scaffold diversity and structural complexity, which have played important roles in drug discovery [3]. Among the 1,881 drugs approved by the U.S. Food and Drug Administration from 1981 to 2019, approximately 65 % were natural products or natural product-based drugs [4]. Some well-known drugs, including artemisinin, paclitaxel, camptothecin, Guanfu base A, and huperzine A, have all been discovered in natural plants. Recently, natural active molecules have been used for the treatment of coronavirus disease 2019 [5,6]. Therefore, natural plants are receiving increasing attention in treating diseases. Discovery of natural lead compounds from plants has been, and will continue to be, the frontier of fundamental research of biological medicine and a promising path to drug research and development.

Nanodrug delivery and targeting systems have provided advanced opportunities in recent years and can significantly improve therapeutic efficacy for the treatment of human diseases [7,8]. The drug molecules are loaded into nanocarriers and ultimately released to the diseased region after active or passive targeting. Thus, nanotechnology is widely used to deliver active ingredients from natural plants to improve their solubility and chemical stability and prolong their blood circulation time [9,10]. However, the preparation of nanocarriers is too laborious for clinical translation, and most nanocarriers pose the risks of undesirable immune responses and carrier-related toxicity. Moreover, their low drug-loading capacity and premature drug leakage restrict their therapeutic efficacy. Fortunately, carrier-free nanoplateforms completely self-assembled from pure drugs or therapeutic components in the absence of exotic nontherapeutic carriers have been developed [11–13]. No additional nanocarriers are involved in the preparation, which can enhance drug-loading efficiency (even up to 100 %), avoid toxicity of nanocarriers, and facilitate large scalable production and clinical translation. Therefore, carrier-free nanoparticles have been developed as an emerging promising platform for treating diseases [14–16].

Natural medicinal plants are inclined to cure some certain diseases and can be a source for potential drugs, which are increasingly recognized by researchers and clinicians [1,3,4]. Thus, the emerging carrier-free nanoplateforms with self-assembly from natural plants over the past decade urgently need to be summarized and reviewed. In this review, an emphasis will be placed on four types of nanoplateforms (Fig. 1): (i) nanoassembly of pure small molecules isolated from different plants; (ii) extracellular vesicles separated from fresh plants; (iii) charcoal nanocomponents obtained from charred plants; and (iv) nanoaggregates from the decoction of plant formulae. We will focus on the sources of small molecules, the self-assembly mechanisms, the compositions, and the biological activities. Finally, we discuss the prospects and future challenges of carrier-free nanoplateforms from natural plants.

Carrier-free nanoplateforms of pure natural molecules

Plant alkaloid-based nano self-assembly

Natural alkaloids, which are widely distributed in plants, are a group of important bioactive ingredients containing basic nitrogen atoms that exhibit multiple biological activities [17,18]. Berberine (BBR), a famous natural isoquinoline alkaloid, is isolated from *Coptis chinensis* and other *Coptis* species plants. BBR possesses remarkable antibacterial action and other pharmacological activities [19,20]. Lei's group [21] discovered that BBR could interact with baicalin (BA) to form nanoparticles (BA-BBR) by self-assembly, while wogonoside (WOG) interacted with BBR to form nanofibers (WOG-BBR) by self-assembly (Fig. 2A). BA and WOG are flavonoid glycosides which are the main active components from *Scutellaria baicalensis*. BA-BBR displayed significantly enhanced bacteriostatic activity, whereas WOG-BBR showed a weaker effect than BBR. This was because BA-BBR formation was governed by electrostatic and hydrophobic interactions to orient hydrophilic glucuronic acid toward the outside. Thus, BA-BBR were attached much easier onto bacteria and sustained release BBR in large quantities, thereby inducing the collapse of the bacterial population and the decrease in biofilm. As for WOG-BBR, they were difficult to attach onto bacteria owing to their hydrophobic properties. Furthermore, it was found [22] that BA-BBR nanoparticles could promote a synergistic effect on diarrhea-predominant irritable bowel syndrome mice compared with simple mixing. This synergistic effect might be related to brain-gut peptides, immune inflammation, and intestinal flora, which are important interrelated components of the

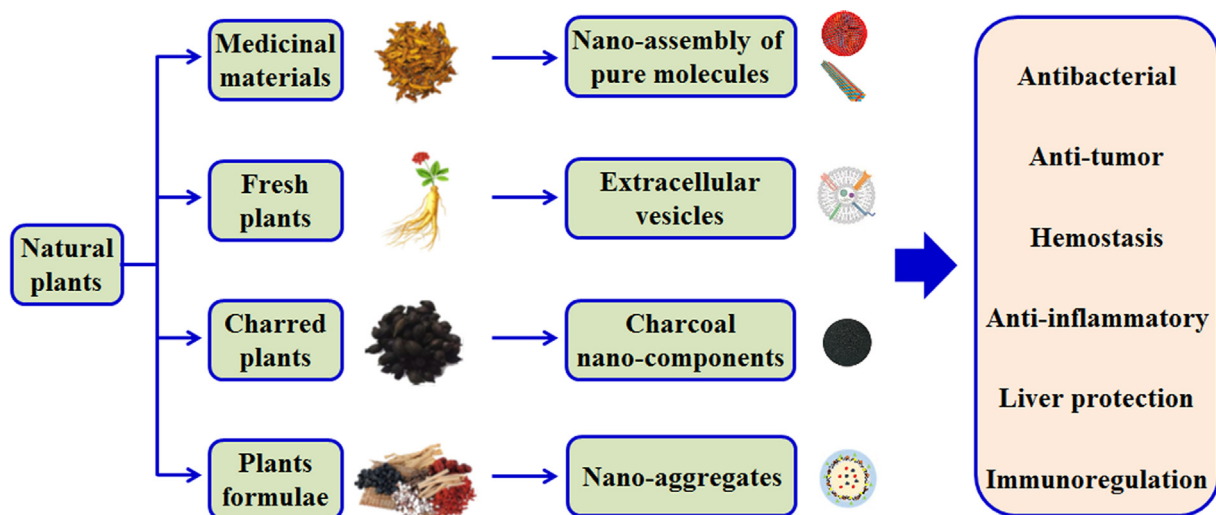


Fig. 1. Carrier-free nanoplateforms from natural plants with enhanced bioactivity.

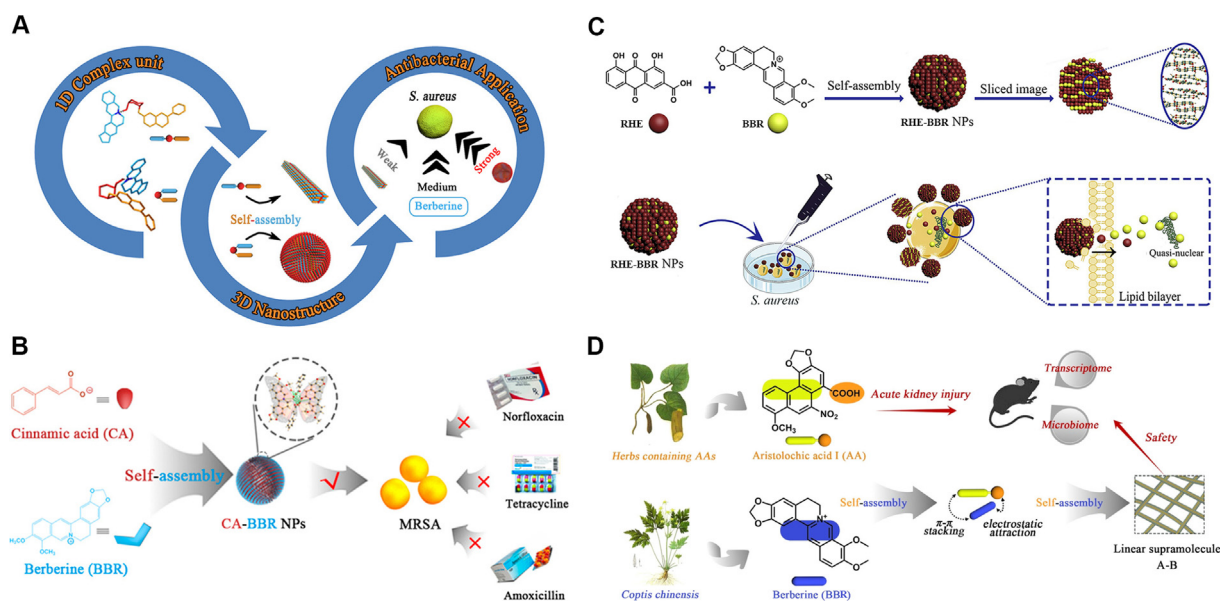


Fig. 2. (A) Self-assembly between berberine (BBR) and flavonoid glycosides (baicalin/wogonoside) for antibacterial application. Reproduced with permission from Ref. [21]. Copyright 2019, American Chemical Society; (B) Self-assembly between cinnamic acid (CA) and BBR for inhibiting multidrug-resistant *Staphylococcus aureus*. Reproduced with permission from Ref. [24]. Copyright 2020, American Chemical Society; (C) Self-assembly between rhein (RHE) and BBR for antibacterial activity. Reproduced with permission from Ref. [29]. Copyright 2020, Elsevier Publishing Group; (D) Self-assembly between aristolochic acid (AA) and BBR for neutralizing acute nephrotoxicity. Reproduced with permission from Ref. [31]. Copyright 2021, American Chemical Society.

microbiota–gut–brain axis. Cinnamic acid (CA) is a representative compound from *Cinnamomum cassia* [23]. CA could also directly self-assemble into nanoparticles with BBR by hydrogen bonds and $\pi - \pi$ stacking interactions [24]. Compared with first-line antibiotics (norfloxacin, amoxicillin, and tetracycline), CA-BBR nanoparticles showed a better inhibitory effect on multidrug-resistant *Staphylococcus aureus*, owing to their preferential adherence to the surface of *S. aureus* leading to synergetic converging bacterial attack (Fig. 2B). Next, 3,4,5-methoxycinnamic acid (3,4,5-TCA), a derivative of CA, was isolated from *Polygala tenuifolia* as an effective antibacterial agent with a phenylpropanoid skeleton [25]. 3,4,5-TCA-BBR nanoparticles [26] were almost uniform spherical particles, and their size (diameter: 92.6 nm) was larger than that of CA-BBR (diameter: 66.0 nm).

Rheum palmatum is a medicinal plant with purgation effects. Its main active ingredient is rhein (RHE), which has an anthraquinone skeleton [27,28]. Tian et al. [29] found that RHE could also interact with BBR to accomplish nano self-assembly (Fig. 2C). In spherical RHE-BBR particles, RHE served as a layered framework by hydrogen bonding interactions, and BBR was inserted into the backbone by electrostatic interactions. RHE-BBR nanoparticles had stronger inhibitory effects on *S. aureus* than single components. Aristolochic acid (AA), from *Aristolochia debilis*, has been shown to cause a variety of severe side effects, such as acute kidney injury, AA nephropathy, and liver cancer [30]. AA and BBR can assemble into linear heterogeneous supramolecules in aqueous solution [31]. The hydrophilic groups were inside and the hydrophobic groups were outside in the AA-BBR nanostructure, which might play an impor-

tant role in blocking the toxic site of AA (Fig. 2D). *In vivo* assays showed that AA-BBR supramolecules significantly reduced AA-induced acute kidney injury compared with AA. This method offered a novel strategy to overcome the toxicity problems of medicinal plants containing AA. Hu's group [32] further found that BBR alkylation (BBRA) could form nanodrugs with rhamnolipids (RHL) via self-assembly. Four types of BBRA-RHL nanoparticles with RHL shielding of positive charge and increasing hydrophilicity were successfully obtained. BBRA-RHL nanoparticles could penetrate the mucus barrier and effectively eradicate *Helicobacter pylori* biofilms to achieve antibacterial activity. Moreover, the authors discovered that BBRA nanoparticles exerted antitumor effects by activating mitochondrial apoptosis pathways [33]. Next, BBR was linked together with paclitaxel (PTX) by a disulfide bond to obtain a drug-drug conjugate (PTX-ss-BBR) [34]. This conjugate could be self-assembled to form nanoparticles through π - π stacking and hydrophobic interactions. PTX-ss-BBR nanoparticles exerted a synergistic antitumor effect due to BBR's mitochondria-targeting delivery and GSH-responsible drug release, which dissipated mitochondria membrane potential and upregulated ROS levels to induce apoptosis of cancer cells. In conclusion, BBR provided a new template for small molecules to self-assemble into nanostructures to enhance bioactivity. The main driving force of the formation of self-assembly between BBR and small molecules in aqueous solution is the electrostatic interaction and π - π stacking. After the formation of one-dimensional structure, they continue to form three-dimensional nanostructures under the action of hydrogen bonding and π - π stacking. Thus, these small molecules should include carboxyl group which can interact with the quaternary ammonium ion of BBR. Besides, the aromatic structure of small molecules is also essential, which can interact with isoquinoline ring of BBR. However, whether small molecules can self-assemble with BBR needs further experimental verification.

PTX, first separated from *Taxus chinensis*, is a widely applied chemotherapy agent with an extensive spectrum of antitumor activity [35,36]. Pei et al. [37] synthesized a PTX dimer (PTX-S-PTX) via a mono thioether linker. PTX-S-PTX nanovesicles, formed by self-assembly, exhibited effective cytotoxicity and served as

carriers to load fluorescent molecules for simultaneous bioimaging. Indomethacin (IDM) is a nonsteroidal anti-inflammatory drug that restrains the synthesis of prostaglandin E2 and increases the proinflammatory macrophage ratio and immune response to cancer cells [38,39]. Zhang et al. [40] built carrier-free IDM-PTX nanocrystal self-assemblies (Fig. 3A). In the IDM-PTX nanostructure, PTX nanocrystals were cast with IDM, similar to a "brick cement" architecture. IDM-PTX assemblies showed enhanced synergistic antitumor effects based on chemotherapy and immunotherapy. Cabazitaxel (CTX) [41], a derivative of PTX, is a hydrophobic drug, while dasatinib (DAS) [42], a polytyrosine kinase inhibitor, is an amphiphile. Therefore, Chen et al. [43] assembled nanoparticles constructed from CTX and DAS (Fig. 3B). DAS-CTX nanoparticles showed enhanced synergistic antitumor efficiency and alleviated systemic toxicity. Meanwhile, DAS exhibited aggregation-induced emission that could be surveyed for bioimaging.

Camptothecin (CPT), isolated from *Camptotheca acuminata*, is a typical quinoline-based alkaloid. It has been widely proven to inhibit the DNA topoisomerase I enzyme and exhibit prominent antitumor activity against various cancers [44]. CPT could self-assemble into helical nanoribbons, whereas CPT derivatives (10-hydroxy CPT and carboxylic CPT) self-aggregated into flat nanoribbons and cylindrical nanorods, respectively [45]. CPT-based self-assemblies with J-type patterns were stable in aqueous solution, which could avoid hydrolysis of CPT-based drugs to increase their antitumor activity. Zhou et al. [46] synthesized an aminated CPT prodrug (CPT-NH₂), which could self-assemble into nanofibers with length of several micrometers and width of 100 nm. These CPT-NH₂ nanofibers could rapidly enter cancer cells and efficiently release the active CPT to exhibit cytotoxicity. Next, amphipathic CPT derivatives were designed and synthesized, and these CPT derivatives could self-assemble into nanocapsules for drug self-delivery. A CPT-floxuridine conjugate amphiphile with a hydrolysable ester linkage was developed to prepare liposome-like nanocapsules (CFNs) [47]. CFNs showed high drug loading and no premature drug release owing to the highly stable codelivery without the need for any carrier. *In vivo* delivery of CFNs resulted in longer

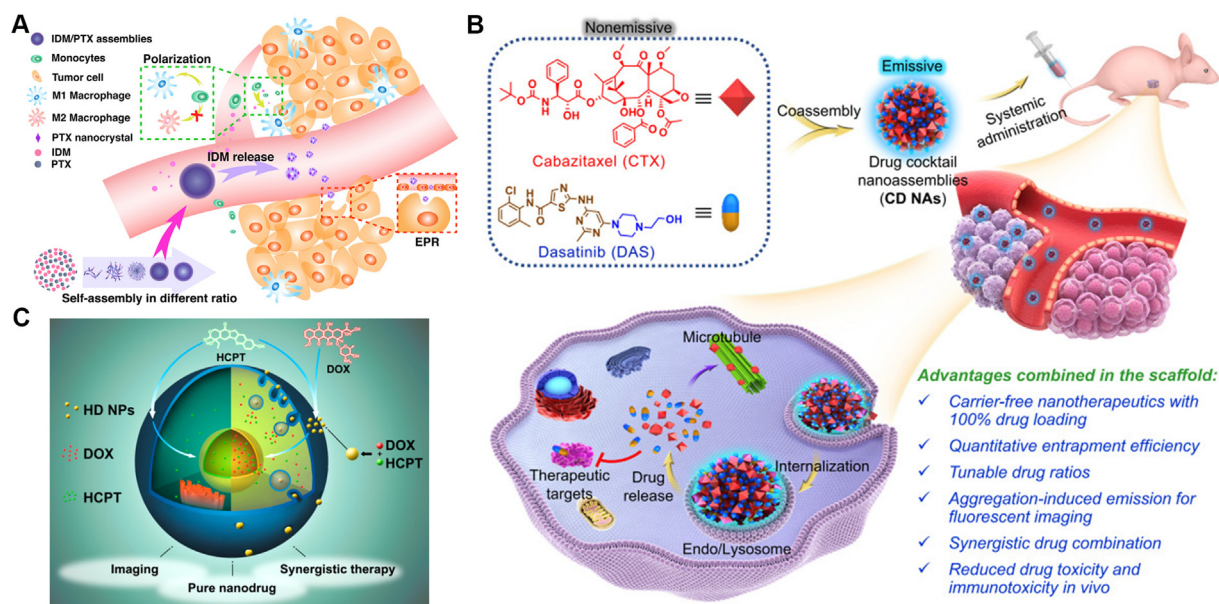


Fig. 3. (A) Self-assembly between indomethacin (IDM) and paclitaxel (PTX) for synergistic antitumor effect. Reproduced with permission from Ref. [40]. Copyright 2019, American Chemical Society; (B) Schematic illustration of self-assembly between cabazitaxel (CTX) and dasatinib (DAS) for combination cancer therapy. Reproduced with permission from Ref. [43]. Copyright 2021, Ivyspring International Publisher; (C) Illustration of different intracellular drug accumulation of nano-assembly between 10-hydroxycamptothecin (HCPT) and doxorubicin (DOX). Reproduced with permission from Ref. [52]. Copyright 2015, American Chemical Society.

blood circulation, higher tumorous accumulation of drugs, and enhanced efficacy in murine tumor models. Pei's group [48,49] established a lactose-modified CPT amphiphile that self-assembles into a carrier-free supramolecular structure for targeted drug delivery. These CPT amphiphiles were synthesized with ROS- or GSH-responsive bonds to target the release of CPT in cancer cells. More importantly, the assembled supramolecular structure could load other chemotherapeutics or fluorescent molecules to exert synergistic therapy or enable theranostics. Curcuminoids are extracted from *Curcuma longa* with the traditional effect of activating blood circulation [50]. Xiao et al. [51] constructed carrier-free self-delivery systems based on curcuminoids and CPT derivatives (irinotecan and topotecan) for targeted cancer therapy. The surface charges of nanoparticles changed by approximately -10 mV under neutral conditions to $+40$ mV under acidic environments. Next, Liang's group [52] used a simple "green" reprecipitation procedure to fabricate dual-drug nano self-assemblies. 10-Hydroxycamptothecin (HCPT) could interact with doxorubicin (DOX), a common clinical chemotherapy drug, by self-nanocrystallization (Fig. 3C). HCPT-DOX nanoparticles showed enhanced synergistic antitumor effects due to the increased accumulation of HCPT in the nucleus, while HCPT and DOX directly mixed and exhibited antagonistic effects. Further study [53] showed that HCPT-DOX nanoparticles improved the intracellular drug retention of DOX to as much as 2-fold in drug-resistant MCF-7R breast cancer cells to resist P-glycoprotein (P-gp) efflux and enhanced synergistic antiproliferation efficiency against drug-resistant cancer cells.

Natural triterpene-based nano self-assembly

Natural triterpenoids consisting of six isoprene units are widely distributed in plants and have a range of useable biological effects [54]. Recent studies [55,56] showed that triterpenoids could self-assemble into supramolecular structures with a variety of shapes, such as nanofibers, nanotubes, vesicles, and spheres. Cyclic triterpenes have rigid skeletons and many chiral carbon atoms, and the position and number of hydroxyl groups are also different. They can be assembled into organogels by π - π stacking, intermolecular van der Waals force, hydrogen bond, hydrophobicity, dipole and other non-covalent bond interactions. In the one-dimensional direction, long fibrous, ribbon-like, tubular or helical

nanostuctures are formed. These longer structures are then wound to form a three-dimensional network structure in space, and the organic solvent is captured in this spatial structure, which usually shows the assembly morphology of fiber, sheet, vesicle and so on. These phenomena could be applied to the areas of drug delivery to enhance drug stability, solubility, and bioactivity. Oleanolic acid (OA), which exists in *Ligustrum lucidum*, has remarkable antioxidant and antitumor activity [57,58]. OA could self-assemble into nanospheres with sizes of < 200 nm, and the diameter increased slightly when loaded with other molecules [59–61]. When the OA carrier interacting with molecules became saturated, the excessive molecules coexisted in a physically mixed form with the nanospheres. Thus, OA nanosphere had a complete multilayer spherical shell nanostructure, which could be used as ideal drug delivery vehicle. Lupeol (Lup), betulinic acid (BTA), and betulinol (Bet) are lupine-type pentacyclic triterpenoids and are separated from *Lupinus luteus* [62]. Lup nanoparticles showed a nanospherical structure with interspersed nanofibers [59,63]. BTA- and Bet-based nanoparticles showed nanofibers or slab-like shapes with sizes of greater than 500 nm, of which all surface charges were slightly negative [59,60]. Furthermore, Wang et al. [64] constructed a supramolecular structure formed by BTA and PTX via hydrogen bonding and hydrophobic interactions (Fig. 4A). BTA-PTX nanoparticles exhibited biosafety and low toxicity and exhibited synergistic enhancement of antitumor efficacy. BTA induced Bcl-2 down-regulation or p53 up-regulation to block the cell cycle in the S phase, while PTX prompted microtubule polymerization to mediate the induction of apoptosis. Phytosterols such as stigmasterol (Sti), ergosterol (Erg), and β -sitosterol (BS) are important secondary metabolites in many medicinal plants [65]. Sti and Erg could form nanorods in aqueous solution, while BS nanoparticles showed irregular morphology [59,63]. Compared with Sti- and BS-based nanopatforms, Erg was the ideal carrier molecule to load the photosensitizer chlorin e6 (Ce6) [66]. The Erg-Ce6 nanodrugs (Fig. 4B) could increase reactive oxygen species (ROS) generation by promoting type I photoreactions for cancer therapy. Poricoic acid A is a tricyclic triterpenoid, and dehydrotumulosic acid are tetracyclic triterpenoids that are separated from *Poria cocos*. These triterpenoids could self-assemble into gel scaffolds, which showed excellent controlled gelation, sustained release, and good safety [63]. Yang et al. [63] assembled oral PTX-loaded DTA nanoparticles through hydrogen

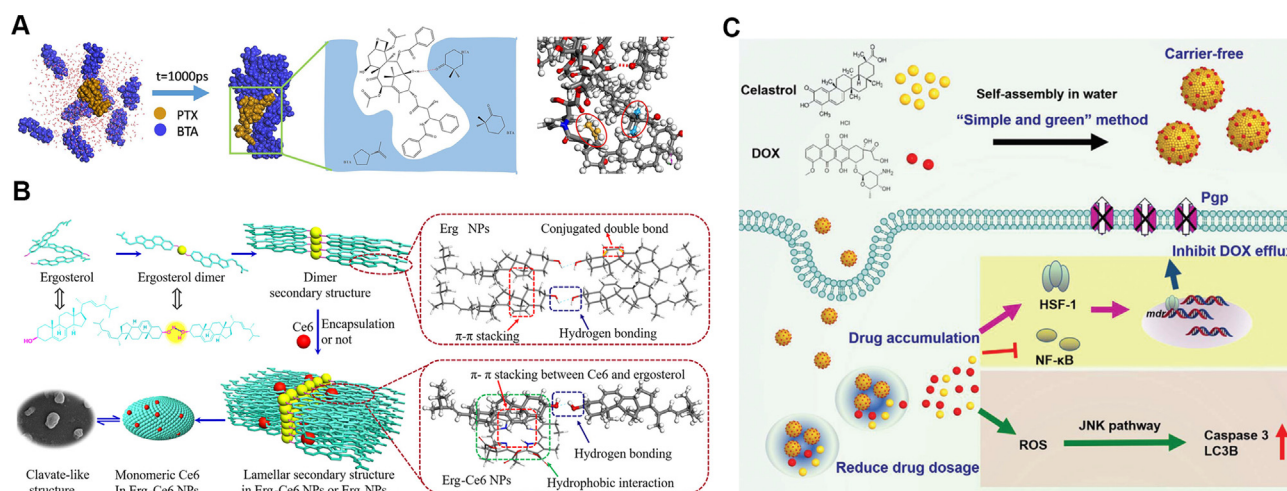


Fig. 4. (A) Combination mode diagram and molecular simulation of interaction between betulinic acid (BTA) and paclitaxel (PTX). Reproduced with permission from Ref. [64]. Copyright 2020, Elsevier Publishing Group; (B) Schematic representation of self-assembly behavior of ergosterol (Erg) and molecular stacking models of co-assembled Erg-Ce6 nanoparticles. Reproduced with permission from Ref. [66]. Copyright 2019, American Chemical Society; (C) Schematic diagram of celestrol-doxorubicin nanoparticles reversing drug resistance in tumor cells. Reproduced with permission from Ref. [69]. Copyright 2018, The Royal Society of Chemistry.

bonding. These nanoparticles could penetrate the gastrointestinal tract by hijacking the apical sodium-dependent bile transporter-based intestinal transport system, thereby treating disease through oral drug delivery. Liquidambaric acid (LA), separated from *Liquidambar formosana*, exhibits excellent anticancer activity [67]. Celastrol (CST), a friedelane-type pentacyclic triterpenoid [68], is isolated from *Tripterygium wilfordii*. Xiao et al. [69] assembled carrier-free nanoparticles with CST and DOX to overcome DOX resistance for synergistic combination chemotherapy (Fig. 4C). The spherical CST-DOX nanoparticles could significantly increase cellular DOX accumulation by restraining nuclear factor kappa-B to depress P-gp expression and then led to autophagy and apoptosis of multidrug-resistant cells via the ROS/JNK signaling pathway.

Panax ginseng is used for reinforcement of vital energy in its traditional functions [70,71]. Ginsenoside Ro (Ro), an active ingredient of ginseng, could increase the solubility of saikosaponin a (SSa), which is derived from *Bupleurum chinense* [72]. Ro preferentially formed nanovesicles with SSa. Moreover, some hydrophobic molecules without hydrophilic groups (such as quercetin and coumarin) were mainly inserted into the hydrophobic layer of the Ro vesicle, and molecules including both hydrophilic and hydrophobic fragments (such as BA and SSa) were located in the palisade layer of the Ro vesicle [73]. Next, interactions between other ginsenosides (Rb1 and Rg1) from ginseng with SSa were further explored [74]. Rg1 could form spherical micelles with SSa in aqueous medium. Compared with Rg1, a greater number of sugars in Rb1 established more binding sites with SSa. Thus, worm-like micelles were formed by Rb1 and SSa molecules. Platycodin, isolated from *Platycodon grandiflorum*, was forecasted to be an ideal carrier in pharmaceutical fields to increase the solubility of hydrophobic molecules [75]. The concentration-dependent structural variation of platycodin was observed, and the platycodin-based aggregate morphologies included spherical, elliptical, tubular, oblate, and necklace-like micelles and multilamellar and multicompart ment vesicles.

Glycyrrhizic acid (GL) and glycyrrhetic acid (GA) are found in *Glycyrrhiza uralensis* [76]. In terms of chemical structure, GL is an amphiphilic molecule with a hydrophilic glucuronic acid residue and hydrophobic triterpenoid aglycone (GA residue). Zhao et al.

[77,78] found that GL could form an injectable low-molecular-weight hydrogel with anisotropic nanoclusters in aqueous solution. The GL hydrogel could restrain the growth of Gram-positive *S. aureus*, while it showed no effect on Gram-negative *Escherichia coli*. Similar to other triterpene glycosides, GL could also form host-guest complexes by self-assembly, thereby being used as a carrier to improve the water-solubility of hydrophobic drugs. Molecular dynamics simulation [79] showed that GL aggregation occurred with PTX to form GL-PTX clusters at a ratio of 3:1 with a size of approximately 10 nm. Next, baicalein-loaded GL nanomicelles [80] were also prepared, which improved the solubility of baicalein in water by more than 4,500 times and generated a sustained-release effect of baicalein. GA is the aglycone of GL. Bag et al. [81] reported that GA could form nanosized spherical and flower-like nanostructures by self-assembly with fibrillar networks, yielding thermoreversible gels. Wu et al. [82] considered dipole-dipole interactions between sodium and carboxylates to be the main driving force for the formation of GA hydrogels. Furthermore, the GA hydrogel exhibited the properties of adsorption and sustainable release of some fluorescent dyes. Yang's group [59] reported a nanomedicine-cum-carrier drug delivery system with GA and OA (Fig. 5A). GA-OA nanoparticles, established through noncovalent interactions, not only showed high drug loading and excellent stability but also exhibited a synergistic antitumor effect. In addition, PTX was loaded into GA-OA nanoparticles to further improve the antitumor effect. Compared with free drugs, GA-OA-PTX nanoparticles could reduce liver damage caused by chemotherapy drugs via upregulating key antioxidant pathways, enhance pharmacological activity, and improve the antitumor efficacy.

Ursolic acid (UA) is discovered in many natural plants, including *Rosmarinus officinalis*, *Cornus officinalis*, and *Prunella vulgaris*, which show a wide range of biological properties, such as antitumor, anti-inflammatory, and antidiabetic properties [83,84]. Fan et al. [85] established a carrier-free nanodrug by the self-assembly of UA. UA nanoparticles could cause apoptosis in A549 cells by decreasing the expression of COX-2/VEGFR2/VEGFA and offer the potential for liver disease prevention. Additionally, the nanoparticles could also increase immunostimulatory activity to improve the activation of

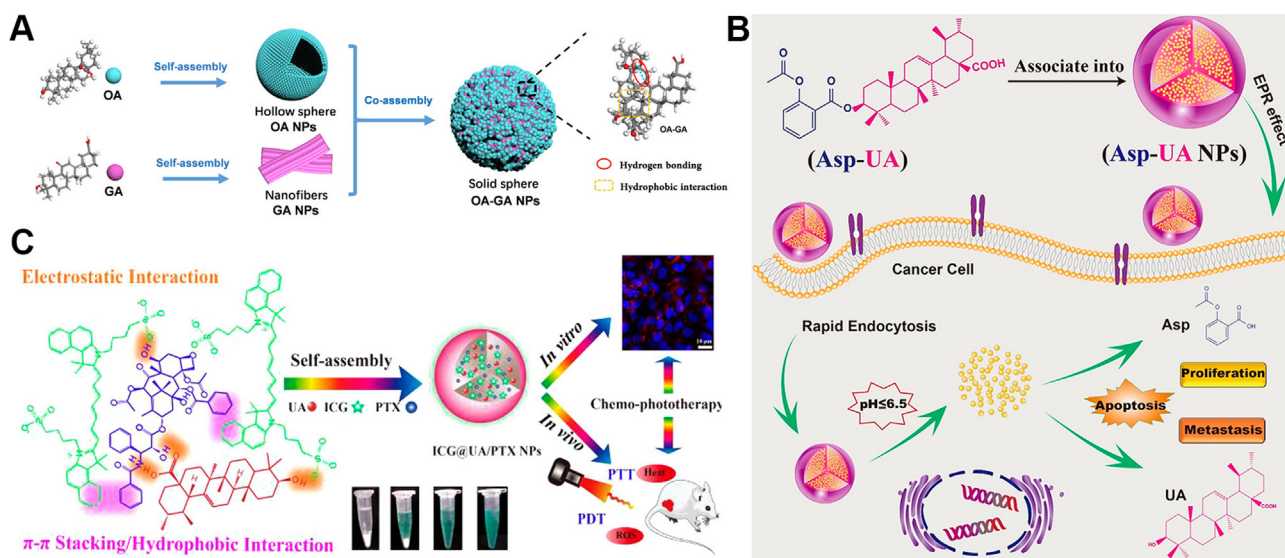


Fig. 5. (A) Self-assembly between oleanolic acid (OA) and glycyrrhetic acid (GA). Reproduced with permission from Ref. [59]. Copyright 2020, American Chemical Society; (B) Schematic illustration of formation of aspirin-ursolic acid (Asp-UA) nanoparticles and their inhibitory effect on tumor metastasis. Reproduced with permission from Ref. [86]. Copyright 2018, American Chemical Society; (C) Illustration of self-assembly between UA, paclitaxel (PTX), and indocyanine green (ICG) for synergistic antitumor effect. Reproduced with permission from Ref. [90]. Copyright 2017, American Chemical Society. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

CD4 + T cells, which proved the potential of UA nanoparticles for immunotherapy. Next, UA interacted with aspirin (Asp), an anti-inflammatory drug, to assemble nanodrugs, which displayed outstanding physicochemical characteristics, such as a controlled release rate, suitable mean diameter, and good surface zeta potential [86]. Asp-UA nanoparticles inhibited tumor metastasis with low toxicity (Fig. 5B). Zhao et al. [87] developed multimodal nanoparticles with UA, indocyanine green (ICG), and lactobionic acid (LBA). UA-LBA-ICG nanoparticles not only exhibited antiproliferative activities on HepG2 cells by increasing ROS, but also self-monitored nanodrugs targeting tumors by a near-infrared (NIR) laser. Jiang et al. [88] developed a carrier-free dual-drug nanodelivery system with UA and DOX, which was further modified with a HER2 aptamer. These coassembled nanodrugs increased the tumor targeting of drugs and significantly inhibited tumor growth in HER2-overexpressing cancers. In addition to DOX, a fluorophore dye was also assembled into UA-DOX nanoparticles for cancer treatment and diagnosis [89]. PTX could also interact with UA to establish a “self-contained bioactive nanocarrier” system [60]. The hydrophobic interactions and hydrogen bonding played leading roles in the formation of UA-PTX nanoparticles. Reduction of hydrogen bonding sites and increased steric hindrance could influence drug loading capacity. Similarly, the fluorescent molecule ICG was also loaded in UA-PTX nanoparticles to establish a therapeutic nanoplatform [90]. The developed nanodrugs showed efficient accumulation in the tumor sites of subcutaneous H22 cell xenograft mice through enhanced permeability and retention effects; this accumulation was then imaged by NIR fluorescence of ICG (Fig. 5C). Finally, UA-PTX-ICG nanoparticles synergistically suppressed tumor growth by photodynamic therapy, photothermal therapy and chemotherapy. Zhang et al. [91] constructed a “core-shell” carrier-free nanodrug with UA and epigallocatechin gallate, which was modified by EpCAM-aptamer for hepatocellular carcinoma treatment. The nanocomplex with low cytotoxicity and good biosafety could activate innate immunity and acquired immunity, leading to a synergistic therapeutic effect. Ou et al. [92] synthesized poly(ursolic acid) (PUA) by polycondensation of UA. PUA could self-assemble into nanoparticles with PTX. PUA-PTX nanoparticles enhanced tumor accumulation, prolonged blood circulation time, improved antitumor efficacy, and showed no obvious toxicity.

Other natural molecule-based nano self-assembly

Wang et al. [93] prepared lollipop-like dual-drug-loaded nanoparticles (GPDs), which were developed based on the self-assembly of gossypol, polydopamine, and DOX via π - π stacking. Polydopamine filled the gaps between DOX and gossypol to form the super-compact long-circulating nanoparticles. Therefore, GPD might broaden the therapeutic window of drugs for various tumors, indicating great potential for the treatment of cancer. With the aid of electronic interactions, DOX could also self-assemble into a carrier-free nanotransformer (DTIG) [94] with tannic acid [95] and ICG. DTIG first transitioned to oversized hydrophobic particles in acidic lysosomes to escape from lysosomes and then rapidly returned to smaller hydrophilic particles to release drugs in the cytoplasm (Fig. 6A). This reversible hydrophilic-hydrophobic conversion and reassembly process showed enhanced antitumor efficacy and an obvious advantage for prognosis. Epigallocatechin gallate (EGCG), a condensed tannin isolated from green tea, has shown therapeutic effects against tumors [96]. The nanoplatform [97] combining EGCG and melittin, derived from the venom of *Apis mellifera* [98], induced ROS generation to offer a new strategy for cancer therapy. Emodin, a natural anthraquinone similar to RHE, could fabricate a kidney-specific nanocomplex with chitosan and metal ions by coordination-driven assembly, which manifested obvious attenuation of fibrotic progression in mice [99]. On the other hand, RHE could self-assemble into nanofiber network-based hydrogels without structural modifications or the use of any carriers [100]. The RHE hydrogels, with little cytotoxicity, accomplished dephosphorylation of $\text{I}\kappa\text{B}\alpha$, restrained nuclear translocation of p65 in the NF- κB cellular signaling pathway, and alleviated neuroinflammation with a long-lasting effect (Fig. 6B). Luteolin (Lut), a flavonoid separated from *Lonicera japonica* [101], possesses multiple pharmacological activities. Liu et al. [102] reported a coordination-driven assembly strategy to construct Lut-based nanoparticles in combination with ferric ions (Fe^{3+}) (Fig. 6C). These Lut/ Fe^{3+} nanoparticles not only notably enhanced Lut's solubility but also broadened the absorption spectrum to the NIR region, which achieved efficient antitumor effects with chemotherapy and photothermal therapy. Quercetin, which exists in *Sophora japonica*, has a powerful antioxidant capacity [103]. Shang et al. [104] developed a quercetin-mediated self-

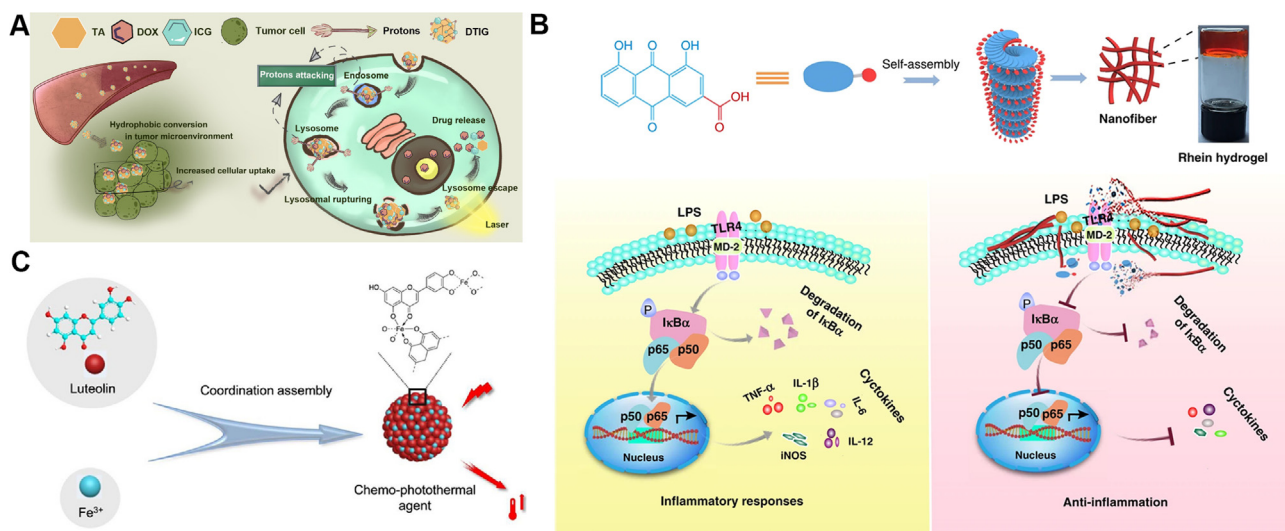


Fig. 6. (A) Illustration of carrier-free nanoplatform DTIG for improving tumor drug delivery programmatically. Reproduced with permission from Ref. [94]. Copyright 2020, American Chemical Society; (B) Schematic depiction of neuroinflammatory prevention induced by rhein nano-assembly. Reproduced with permission from Ref. [100]. Copyright 2019, Springer Nature Limited; (C) Schematic illustration of self-assembly of luteolin and Fe^{3+} with proposed supramolecular structure. Reproduced with permission from Ref. [102]. Copyright 2020, Elsevier Publishing Group.

assembly nanomedicine consisting of DOX and ferrous ions (Fe^{2+}). This nanomedicine increased accumulation of DOX at tumor sites and exhibited effective synergistic antitumor effects on triple-negative breast cancer cells via the mitochondrial damage pathway. Quercetin with powerful antioxidant capacity could relieve the cardiotoxicity of DOX. All-*trans* retinoic acid (ATRA), an acyclic diterpenoid, has been clinically used for the treatment of acute promyelocytic leukemia [105]. Aggregation or deposition occurred after stirring ATRA in aqueous solution. Therefore, retinoic hydroxamic acid (RHA), a hydroxamic derivative of ATRA, was obtained, which contained a lipophilic hydrocarbon backbone and hydrophilic hydroxamic group. These functional groups could prompt RHA to form nanoparticles by self-assembly [106]. Thereafter, RHA nanoparticles showed potent antitumor effects against A-375 melanoma cells.

Extracellular vesicles from fresh plants

Similar to the exosomes secreted into the extracellular space by mammalian cells [107], fresh natural plant-derived extracellular vesicles (EVs) are membranous vesicles that contain a lipid bilayer as a skeleton and can load various proteins, miRNAs, polysaccharides, and other active substances [108–110]. EVs can serve as extracellular messengers to regulate cell–cell and interspecies communication. In recent years, EVs from fresh plants have been regarded as natural therapeutics against a variety of human diseases and nanoplatforms to efficiently deliver specific drugs [111–113].

Zingiber plant-derived EVs

Ginger (*Zingiber officinale*) has been used to treat a diversity of health issues, such as colds, migraines, nausea, and gastrointestinal disorders [114]. Zhang et al. [115] isolated ginger-derived EVs (GEVs) and demonstrated their efficient colon targeting through oral administration. GEVs with a negative zeta potential and a size of 230 nm involved lipids, proteins, microRNAs, and some active compounds (such as 6-gingerol and 6-shogaol). GEVs could enhance anti-inflammatory cytokines (IL-10 and IL-22) and reduce proinflammatory cytokines (TNF- α , IL-6 and IL-1b), thus reducing acute colitis and preventing colitis-associated cancer. Next, GEVs contained microRNAs that targeted various genes in *Lactobacillus*

rhamnosus [116] or showed anti-inflammatory effects in intestinal Caco-2 cells [117]. GEV-RNAs or indole-3-carboxaldehyde (I3A) were sufficient to induce the production of IL-22; thus, GEVs could ameliorate mouse colitis through IL-22-dependent mechanisms by reshaping the gut microbiota (Fig. 7A). Chen et al. [118] studied the inhibitory effects of GEVs against the activation of the nucleotide-binding domain and leucine-rich repeat-containing family pyrin domain-containing 3 (NLRP3) inflammasome in macrophages (Fig. 7B). The lipids in GEVs block the assembly of the NLRP3 inflammasome and restrain pathways downstream of inflammasome activation including caspase cleavage, secretion of IL-1 β and IL-18, and pyroptotic cell death. Together, GEVs could be considered new potent agents to block NLRP3 inflammasome assembly and activation in disease settings. Zhuang's group [119] found that oral GEVs could protect mice against alcohol-induced liver damage. GEVs participated in the activation of nuclear factor erythroid 2-related factor 2 (Nrf2), which resulted in the expression of some liver detoxifying/antioxidant genes and inhibited ROS production. The active ingredient in GEVs, shogaol was shown to play a role in the induction of Nrf2 in a TLR4/TRIF-based manner, which further contributed to liver protection. Moreover, the authors [120] proved that GEVs could be selectively recognized by the periodontal pathogen *Porphyromonas gingivalis* through interactions between phosphatidic acid (PA) in GEVs and hemin-binding protein 35 (HBP35) on the surface of *P. gingivalis*. In addition, the degree of unsaturation of PA played an important role in the GEV-mediated interaction with HBP35. The cargo molecules in GEVs interacted with multiple pathogenic factors in *P. gingivalis* simultaneously, and GEVs were deemed to prevent/treat chronic periodontitis.

Next, GEVs were used as a delivery platform for DOX to treat colon cancer [121]. In addition to loading with DOX, GEVs were decorated with folic acid. The nanoparticles could be efficiently taken up by colon cancer cells, had an excellent pH-based DOX-release profile compared with DOX liposomes, and increased the inhibition of tumor growth compared with free DOX. Next, GEVs were used to deliver siRNA against CD98 (siRNA-CD98) to colon tissues [122]. siRNA-CD98 reduced the expression of CD98, and this reduction played a crucial role in colitis and colitis-associated cancer. The siRNA-loaded GEVs were effectively targeted to the colon and reduced the expression of CD98, which offers great promise as an efficient siRNA-delivery vehicle. Therefore, GEVs are not just an attractive treatment strategy for human

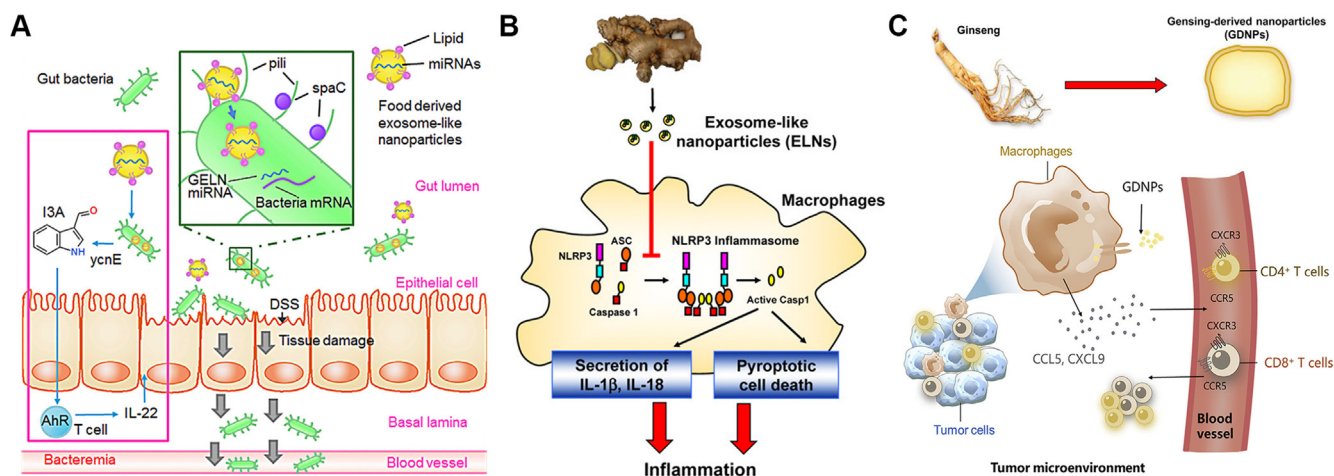


Fig. 7. (A) Ginger-derived extracellular vesicles regulating gut microbiota composition and host physiology, thereby enhancing gut barrier function to alleviate colitis. Reproduced with permission from Ref. [116]. Copyright 2018, Elsevier Inc.; (B) Ginger-derived extracellular vesicles inhibiting NLRP3 inflammasome activation. Reproduced with permission from Ref. [118]. Copyright 2019, American Chemical Society; (C) Ginseng-derived extracellular vesicles enhancing immune checkpoint antibody efficacy by reprogramming cold tumor microenvironment. Reproduced with permission from Ref. [140]. Copyright 2022, The American Society of Gene and Cell Therapy.

diseases but also potential lipids to deliver active molecules, siRNA, and even proteins to different types of cells [123]. Moreover, GEVs showed multiple benefits, such as low toxicity, tissue-specific targeting, and minimal hazardous effects on the environment.

Citrus plant-based EVs

Citrus plants, such as *Citrus reticulata*, *Citrus aurantium*, and *Citrus grandis*, are widely used as medicinal plants to regulate the flow of vital energy. Pocsfalvi et al. [124] isolated EVs from the fruit juice of *C. aurantium*, in which patellin-3-like, clathrin heavy chain, HSP70, 14-3-3 protein, G3PD and FBA6 were highly expressed. EVs with a size of 50–70 nm from *Citrus limon* [125–127] containing citrate, vitamin C, and miRNAs had a significant protective effect against oxidative stress on mesenchymal stromal cells. *C. limon* EVs could also inhibit cell proliferation through the TRAIL-mediated pathway in different tumor cell lines. Lei et al. [128] discovered an elegant three-way interaction between gut microbes, bile, and *C. limon*-derived EVs. Daily consumption of these EVs increased the percentage of probiotic *Lactobacillus rhamnosus* GG in the small intestine by enhancing bile resistance.

Xiao et al. [129] obtained EVs from grapefruit (*Citrus paradisi*), and they found that highly expressed miRNAs in EVs could potentially regulate the expression of inflammatory cytokines and cancer-related genes *in vitro*. Grapefruit EVs could be selectively taken up by intestinal macrophages [130] and then inhibit the production of IL-1 β and TNF- α and increase the expression of heme oxygenase-1. Therefore, grapefruit EVs ameliorated dextran sulfate sodium-induced colitis in mice. Stanly et al. [131] demonstrated that grapefruit EVs specifically inhibited the proliferation of lung, skin and breast cancer cells. Grapefruit EVs reduced the expression of cyclins B1 and B2, intercellular cell adhesion molecule-1, cathepsins and cleaved PARP-1 to inhibit Akt and ERK signaling. Grapefruit EVs also served as nanovehicles to deliver chemotherapeutic agents, proteins, and short interfering RNAs to different types of cells [132–135]. MiR-18a encapsulated in grapefruit EVs possessed an antimetastatic effect. The miR-18a-mediated macrophage IFN γ was necessary for the subsequent induction of IL-12, and then IL-12 activated natural killer T cells to inhibit colon tumour liver metastasis. Next, miR17 could also be carried by grapefruit EVs for rapid delivery to the mouse brain via intranasal administration. These EVs were selectively taken up by GL-26 tumor cells, and showed therapeutic efficacy in mouse brain tumors.

Vitis plant-derived EVs

Grape (*Vitis vinifera*) was used to relieve muscle pain and promote eruption in ancient China. EVs separated from grape contain proteins, lipids, and microRNAs [136]. Grape EVs can be taken up by intestinal macrophages and then induce the expression of the antioxidation gene heme oxygenase-1 and the anti-inflammatory cytokine IL-10 [137]. Thereafter, Wnt signaling is activated, which is critical for maintaining intestinal homeostasis. Grape EVs can also be taken up by intestinal stem cells by penetrating the intestinal mucus barrier [138]. These EVs caused significant induction of Lgr5^{hi} intestinal stem cells via the Wnt/ β -catenin pathway. Oral administration of grape EVs resulted in protection of mice from dextran sulfate sodium-mediated colitis through induction of intestinal stem cells.

Other plant-based EVs

Cao's group [139,140] isolated EVs from fresh roots of ginseng that contained lipids, nucleotides, proteins, and active molecules (such as ginsenoside Rg3). Ginseng EVs could promote polarization

of M2 to M1-like macrophages through the Toll-like receptor-4/myeloid differentiation antigen 88 signaling pathway and then increase ROS production to lead to apoptosis of mouse melanoma cells. The authors also explored a combinatorial strategy with both ginseng EVs and PD-1 monoclonal antibody, which showed the ability to alter the cold tumor microenvironment to enhance immune checkpoint inhibitor antitumor treatment (Fig. 7C). Separated from *Asparagus cochinchinensis*, mushrooms, and broccoli, EVs inhibited the proliferation of hepatocellular carcinoma cells, alleviated liver damage in mice, and inhibited mouse colitis by activating dendritic cell AMP-activated protein kinase, respectively [141–143]. Perut et al. [144] isolated EVs from strawberry juice, which had a high content of anthocyanins, folic acid, flavonols, and vitamin C. *Fragaria*-derived EVs prevented oxidative stress in a dose-dependent manner in human mesenchymal stromal cells. Thus, *Fragaria*-derived EVs might be used in food with potential health-promoting activity. Tea flower-derived EVs contain large amounts of functional proteins, polyphenols, flavonoids, and lipids [145]. *In vitro* and *in vivo* experiments revealed that these EVs induced ROS amplification to trigger mitochondrial damage and arrest the cell cycle, resulting in anti-proliferation and anti-invasion activities. Next, cabbage- and cucumber-derived EVs could be used as alternative drug delivery vehicles to load lipophilic molecules to manifest biological activities [146,147]. Furthermore, EVs can also be isolated from *Dendropanax morbifera* [148], coconut water [149], and sunflower [150]. *D. morbifera*-derived EVs were deemed to act as an anti-melanogenic agent to promote the development of natural cosmetics.

Charcoal nanocomponents from charred plants

Charred plants have a history of more than 2,000 years and are still widely used in clinical practice because of their special therapeutic effects. Charred plants are referred to as charcoal drugs in TCMs and possess extensive pharmacological effects, especially for hemostasis, and are formed by carbonization at similar high temperatures. In recent years, many scholars have separated charcoal nanocomponents (CNs) from charred plants [151,152]. These CNs contain a carbon skeleton structure with a size of < 10 nm, which is similar to the structure and properties of carbon quantum dots [153] in nanomaterials.

CNs with hemostatic effects

The *Cirsium* plants *Cirsium setosum* and *Cirsium japonicum* are clinically used for cooling blood and hemostasis in traditional functions. Luo et al. [154] separated CNs from charred *C. setosum*, which had a nearly spherical shape with a diameter of 2.6 nm and luminescence and fluorescence emission properties. These CNs could activate the fibrinogen system and stimulate the extrinsic blood coagulation system. Next, CNs from charred *C. japonicum* with diameters of 2–11 nm showed remarkable inhibition of hemorrhage induced by mouse tail amputation or liver scratching [155]. Charred pollen of *Typha angustifolia* (CPTA) is a type of calcined drug that has been used to promote hemostasis for thousands of years. CPTA-CN could increase fibrinogen to activate partial thromboplastin time [156]. Furthermore, transformation rules of flavonoids during the heating process of CPTA could be used to control the quality, and seven minutes was determined to be the optimal processing time with a heating temperature of 272 °C [157,158]. Charred *Juncus effusus* (CJE) is the charcoal-processed product obtained by high-temperature heating of dried stem pith of *J. effusus* and has been used to treat hemorrhagic conditions. Cheng et al. [159] separated hydrophobic CJE-CN from CJE. CJE-CN not only showed outstanding hemostatic effects but

also protected against hemorrhage-induced liver injury. Meanwhile, CJE-CNs reduced the serum levels of biochemical indicators of liver damage, such as total bilirubin, direct bilirubin, alanine amino transferase, aspartate aminotransferase, and alkaline phosphatase. Qu and Zhao's group [160–164] studied a series of CNs for hemostasis from charred *Phellodendron chinense*, charred *Schizonepeta tenuisfolia*, charred spica of *S. tenuisfolia*, and charred *Selaginella tamariscina*. CNs derived from these charcoal plants presented similarities and differences in structural features, physicochemical properties and hemostatic bioactivity. All derived CNs showed remarkable hemostatic effects in tail amputation and liver scratch models, which increased fibrinogen and platelet contents and decreased prothrombin time by activating the fibrinogen system and stimulating the extrinsic blood coagulation system. Further studies [165,166] showed that flavonoids were the main active ingredients in charcoal plant-derived CNs, and the key proteins related to hemostatic diseases might include SERPINC1, FVIII, FX, FII and FXII [167]. The hemostatic mechanism might reverse the imbalanced metabolites through alanine, aspartate and glutamate metabolism and the citrate cycle pathway [168]. These results provide new insights into the material basis of charred plants for hemostatic bioactivity.

CNs with anti-inflammatory and antioxidant activities

CNs exist in charred flowers of *Lonicera japonica* (CFLJ), ranging from 1.0 to 10.0 nm in diameter [169]. CFLJ-CNs alleviated lipopolysaccharide-induced inflammation by reducing the expression of tumor necrosis factor- α , IL-1 β , and IL-6 and relieved the fever and hypothermia caused by inflammation. Similarly, charred mulberry silkworm cocoon-derived CNs exhibited remarkable anti-inflammatory activity, which was likely mediated by the inhibition of TNF- α and IL-6 [170]. Hu et al. [171] separated CNs from the charred root of *Sophora flavescens* (CRSF). CRSF-CNs showed anti-inflammatory and antioxidative effects, which could reduce the levels of NF- κ B, TNF- α , IL-6, glutathione, superoxide dismutase, catalase, malondialdehyde, glutathione peroxidase, and inducible nitric oxide synthase to inhibit ethanol-induced acute gastric ulcers. Zhao et al. [172] found that CNs from charred roots of *Paeonia lactiflora* could increase superoxide dismutase levels and reduce malondialdehyde in a carbon tetrachloride-induced acute liver injury model. Therefore, the CNs could inhibit free radical-induced liver cell lipid peroxidation and scavenge oxygen free radicals to exhibit hepatoprotective effects.

CNs with anti-gout effect

Pueraria lobata is commonly used for dispelling wind and removing heat in TCM. CNs from the charred root of *P. lobata* (CRPL) were nearly spherical with diameters of approximately 5 nm [173]. CRPL-CNs reduced blood uric acid levels in rat models by inhibiting the activity of xanthine oxidase and reduced the degree of swelling and pathological damage in gouty arthritis. Most interestingly, CRPL-CNs were able to increase the water solubility of BA [174]. Finally, charred fruit of *Citrus aurantium*-derived CNs could also relieve gouty arthritis induced by monosodium urate crystals, and lowered serum uric acid by restraining xanthine oxidase activity [175].

CNs with other bioactivities

Jiaosanxian (JSX) is a “blind” medicine in TCM and is obtained by the carbonization of three plants, including the fruit of *Crataegus pinnatifida*, the fruit of *Hordeum vulgare*, and *Massa medicata fermentata*. JSX-CNs were proven to regulate blood sugar levels [176]. Moreover, CNs from charred fruit of *C. pinnatifida* also

exerted hypoglycemic effects and thus might act as disaccharidase inhibitors to restrain the bioactivities of sucrase and maltase [177]. *Glycyrrhiza uralensis*-based CNs with a spherical structure and size of 2–10 nm were synthesized by an environmentally-friendly one-step pyrolysis process and possessed an explicit anti-GU effect [178]. Zhao et al. [179] synthesized environmentally-friendly fluorescent *Forsythia*-derived CNs (F-CNs) in a one-pot method. F-CNs showed natural anti-wood rot fungus activity against two fungi (*G. trabeum* and *C. versicolor*), which indicated that F-CNs might be used as environmentally protective wood preservatives. Charred leaves of *Artemisia argyi*-derived CNs (CLAA-CNs) obtained by a smoking simulation method exhibited the antibacterial ability of Gram-negative bacteria by inhibiting cell wall activity and changing the enzymatic secondary structure [180]. However, CLAA-CNs showed no significant antibacterial function against Gram-positive bacteria. On the other hand, CLAA-CNs could strengthen anti-frostbite ability by reducing the concentrations of IL-1 β and TNF- α and decreasing the levels of blood glucose caused by frostbite [181]. Qu's group [182] found that CNs from charred root of *Zingiber officinale* had remarkable analgesic effects in thermal and chemical stimulus tests, which were possibly mediated by 5-hydroxytryptamine levels and opioid-like mechanisms. They [183] also found that CNs from cigarette mainstream smoke exhibited anti-anxiety and sedative effects, which might be associated with the reduction in glutamate and dopamine and the increase in norepinephrine in the mouse brain and serum. Finally, GL-based and curcumin-based CNs were also prepared by high-temperature heating of GL and curcumin, respectively [184,185]. These small molecule-based CNs could inhibit the propagation of some viruses, such as porcine reproductive and respiratory syndrome virus, enterovirus 71, pseudorabies virus, and epidemic diarrhea virus.

Nanoaggregates from plants formulae

Plant formulae are one of the most widely used dosage forms of TCMs in the clinic. A variety of complex chemical and physical changes often occur in the process of decoction. Recently, nanoaggregate particles (NAs) with nanometer sizes and different dispersed states with wide curative effects have been discovered in plant formula decoctions, which usually contain proteins, polysaccharides, and micromolecules [186–188].

Bai-Hu decoction (BHD), a traditional Chinese decoction used for the treatment of fever, contains three medical plants and one mineral, including the root of *Anemarrhena asphodeloides*, the root of *Glycyrrhiza uralensis*, Japonica rice, and gypsum. NAs from BHD with a size of 97 nm were formed through cooperation of the chemical constituents of these four herbs [189,190]. Liquiritin from licorice and polysaccharides from Japonica rice could enhance the solubility of mangiferin and neomangiferin from *A. asphodeloides*, while inorganic ions, including Ca²⁺, Mg²⁺, and Zn²⁺ in gypsum, acted as zeta potential modifiers. BHD-NAs exhibited remarkable antipyretic effects by inhibiting the expression of IL-1 β , TRPV4, NF- κ B, and TNF- α . Ke and Rao's group [191] isolated NAs from Ma-Xing-Shi-Gan decoction (MXSGD) and detected their inhibition of cell proliferation. MXSGD, which contains four herbs (*Ephedra sinica*, the seed of *Prunus armeniaca*, the root of *Glycyrrhiza uralensis*, and gypsum), has been used for clearing lung and relieving asthma for thousands of years in TCM. The formation of MXSGD-NAs was dependent on the interaction of amphiphilic molecules (ephedrine and pseudoephedrine), which could further interact with other molecules through ionic or hydrophobic interactions. Next, Ge-Gen-Qin-Lian decoction (GGQLD)-derived NAs [192] exhibited stronger hypoglycemic and antioxidant activities, which could promote the permeation of active monomers (e.g., BA) into

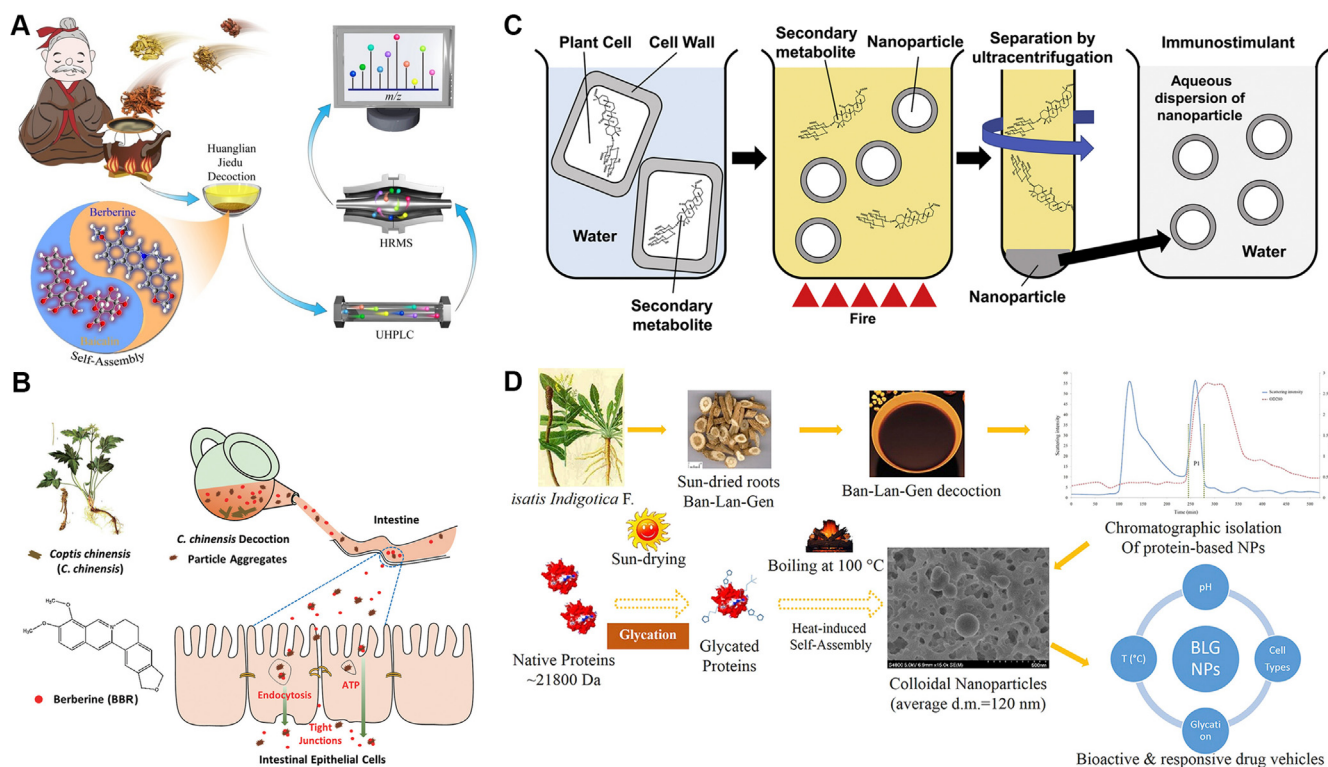


Fig. 8. (A) Self-assembled phytochemical complex with naturally-occurring modality in Huang-Lian-Jie-Du decoction. Reproduced with permission from Ref. [194]. Copyright 2021, Elsevier Publishing Group; (B) Illustration of promotion mechanisms of particle aggregates in *Coptis chinensis* decoction on absorption of berberine in small intestine. Reproduced with permission from Ref. [198]. Copyright 2020, The Royal Society of Chemistry; (C) Scheme of immune efficacy of herbal decoction based on nano-aggregate particles. Reproduced with permission from Ref. [201]. Copyright 2018, Elsevier Publishing Group; (D) Formation of nano-aggregate particles in boiling *Isatis indigotica* decoction. Reproduced with permission from Ref. [202]. Copyright 2017, Elsevier Publishing Group.

cells. Naoluo Xintong Decoction (NXD), consisting of six herbs and one animal medicine, is a clinically proven prescription used to treat ischemic stroke. NXD-derived NAs with typical characteristics of 200 nm size were mainly composed of polysaccharides, proteins, and saponins [193]. They could improve nerve function, have brain-protective effects, reduce oxidative stress, and inhibit cell apoptosis.

Huang-Lian-Jie-Du decoction (HLJDD) contains the root of *C. chinensis*, the bark of *P. chinense*, the root of *S. baicalensis*, and the fruit of *Gardenia jasminoides* in a proportion of 3:2:2:3 and is widely used for purging heat by removing toxins. HLJDD-NAs had 14 marker compounds, and BA and BBR were significantly higher in NAs due to self-assembly complexation (Fig. 8A) [194–196]. HLJDD-NAs showed neuroprotective effects in cobalt chloride-treated differentiated PC12 cells. Next, the NAs of licorice protein-BBR were obtained in the decocting process of *C. chinensis* and licorice [197], which could improve the antimicrobial activity of BBR. Moreover, NAs were also achieved by decocting the root of *C. chinensis* itself [198]. These NAs with sizes of 200–400 nm and negative charges were mainly composed of polysaccharides and small molecules, which promoted the absorption of BBR in the intestine (Fig. 8B). Meanwhile, the NAs could also dynamically regulate intestinal tissue permeability and the expression of tight junction proteins in intestinal epithelial cells by activating Peyer's patch-associated immunity [199]. Similar to *C. chinensis*, NAs could also be obtained during the process of licorice decoction [200]. These NAs mainly contained licorice protein in contrast to *C. chinensis*. Licorice-derived NAs solubilized astragaloside IV by encapsulation and promoted the proliferation of normal hepatocytes. Iitsuka et al. [201] found that sugar-based NAs were also separated in boiling licorice water extracts (Fig. 8C). Phagocytosis of sugar-

based NAs could lead to immunostimulant effects by increasing the expression of proteins and genes of inflammatory cytokines in macrophage cells.

Zhou et al. [202] isolated protein-based NAs from a decoction of the root of *Isatis indigotica*. Two glycosylated proteins (BLGP1 and BLGP2) were identified in these NAs, and they could facilitate the growth of normal cells and inhibit that of carcinogenic cells (Fig. 8D). At the same time, six groups of NAs with sizes ranging from 57 to 300 nm were successfully isolated from the *I. indigotica* decoction [203]. All of the obtained NAs had a high contents of polysaccharides and high similarities in other compositions. However, four of these six NAs showed significant antiviral activity against influenza virus H1N1 and distinct cytotoxicity toward MDCK cells *in vitro*. Furthermore, protein-based NAs could also be obtained from decoction of the root of *Pseudostellaria heterophylla* [204], *Phaseolus angularis* [205], and the seed of *P. armeniaca* [206], which might be used to encapsulate hydrophobic drugs to improve their efficacy. Li et al. [207] obtained heat-stable decoctosomes from a decoction of the root of *Rhodiola crenulata*. These decoctosomes comprised lipids, chemical compounds, proteins, and sRNAs (e.g., HJT-sRNA-m7 and PGY-sRNA-6), which showed potent antifibrotic and anti-inflammatory effects. The herbal decoctosome provided an effective oral delivery route for nucleic acid therapy.

Conclusions and perspectives

Natural plants as well as TCMs are attracting increasing attention from researchers and clinical scientists due to their effective protection against illnesses with lower toxicity and higher synergistic potential. Despite the appearance of chemically synthesized

Table 1
Nano-assembly of pure small molecules isolated from natural plants.

Molecule 1	Molecule 2	Morphology	Interaction forces of self-assembly	Bioactivity	Research level	Refs.
Berberine	Baicalin	Nanosphere	Electrostatic and hydrophobic interactions	Antibacterial activity/ Treatment of diarrhea	<i>In vitro</i> / Zebrafish model/ Mice model	[21,22]
Berberine	Wogonoside	Nanofiber	Electrostatic and hydrophobic interactions	Antibacterial activity	<i>In vitro</i> / Zebrafish model	[21]
Berberine	Cinnamic acid	Nanosphere	Hydrogen bond/ π - π stacking interaction	Antibacterial activity	<i>In vitro</i> / Zebrafish model	[24]
Berberine	3,4,5-Methoxycinnamic acid	Nanosphere	Hydrogen bond/ π - π stacking interaction	Antibacterial activity	<i>In vitro</i>	[26]
Berberine	Rhein	Nanosphere	π - π interaction/ Electrostatic interaction	Antibacterial activity	<i>In vitro</i> / Zebrafish model	[29]
Berberine	Aristolochic acid	Network nanofiber	Electrostatic interaction/ π - π interaction	Neutralizing acute nephrotoxicity	<i>In vitro</i> / Zebrafish model/ Mice model	[31]
Berberine alkylation	Rhamnolipid	Nanosphere	Electrostatic and hydrophobic interactions	Antibacterial activity	<i>In vitro</i> / Mice model	[32]
Paclitaxel dimer	None	Nanovesicle	Hydrophobic interaction	Antitumor	<i>In vitro</i> / Mice model	[37]
Paclitaxel	Indomethacin	Nanosphere	π - π stacking/ H-bonding/ Hydrophobic interaction	Antitumor	<i>In vitro</i> / Mice model	[40]
Cabazitaxel	Dasatinib	Nanosphere	π - π stacking/ hydrogen bonding/ Van der Waals interaction	Antitumor	<i>In vitro</i> / Mice model	[43]
Camptothecin/ Camptothecin derivative	None	Helical nanoribbon/ Flat nanoribbon/ Cylindric nano-rod	π - π interaction/ Hydrogen bond	Antitumor	<i>In vitro</i> / Mice model	[45–49]
Irinotecan/ Topotecan	Curcuminoids	Nanosphere	Intermolecular non-covalent interactions	Antitumor	<i>In vitro</i> / Mice model	[51]
10-Hydroxycamptothecin	Doxorubicin	Nanosphere	π - π stacking/ Hydrophobic interactions	Antitumor drug resistance	<i>In vitro</i>	[52,53]
Oleanolic acid	Paclitaxel	Nanosphere	Hydrogen bonding/ Hydrophobic interaction	Antitumor	<i>In vitro</i> / Mice model	[59,60]
Lupeol	None	Nanosphere with nanofiber interspersed	Hydrogen bonding/ Van der Waals force/ π - π stacking	Drug delivery vehicle	<i>In vitro</i> / Mice model	[59,63]
Betulinol	Ursolic acid	Nanosphere	Hydrogen bonding/ Van der Waals force/ π - π stacking	Drug delivery vehicle	<i>In vitro</i>	[59,60]
Betulinic acid	Paclitaxel	Nanofiber	Hydrogen bond/ Hydrophobic interaction	Antitumor	<i>In vitro</i> / Mice model	[64]
Ergosterol	Chlorin e6	Clavate-shaped nanoparticle	π - π stacking/ Hydrophobic interaction	Antitumor	<i>In vitro</i> / Mice model	[66]
Dehydrotrametenolic acid	Paclitaxel	Nanosphere	Hydrogen bonding/ Van der Waals force/ π - π stacking	Antitumor	<i>In vitro</i> / Mice model	[63]
Celastrol	Doxorubicin	Nanosphere	π - π stacking/ Electrostatic interaction	Antitumor drug resistance	<i>In vitro</i>	[69]
Ginsenoside Ro	Saikosaponin a	Nanosphere	–	–	Virtual prediction	[72,73]
Glycyrrhizic acid	Paclitaxel/ Baicalein	–	–	–	Virtual prediction/ Preparation and characterization	[79,80]
Glycyrrhetic acid	None/ Oleanolic acid and/or Paclitaxel	Nanosphere with fibrillar network	Hydrogen bonding/ Van der Waals force/ π - π stacking	Antitumor	<i>In vitro</i> / Mice model	[59,81,82]
Ursolic acid	Aspirin	Nanosphere	Hydrogen bond/ Hydrophobic interaction	Tumor metastasis therapy	<i>In vitro</i> / Mice model	[86]
Ursolic acid	Indocyanine green and lactobionic acid	Nanosphere	π - π stacking/ Hydrophobic interaction/ Electrostatic interaction	Tumor theranostics	<i>In vitro</i> / Mice model	[87]
Ursolic acid	Doxorubicin/ Paclitaxel	Nanosphere	Electrostatic interaction/ π - π interaction/ Hydrophobic interaction	Antitumor	<i>In vitro</i> / Mice model	[88–90]
Doxorubicin	Indocyanine green and tannic acid	Nanosphere	π - π interaction/ Electronic interaction	Antitumor	<i>In vitro</i> / Mice model	[94]
Rhein	None	Network nanofiber	π - π interaction/ Hydrogen bond/ Electrostatic interaction	Treating neural inflammation	<i>In vitro</i>	[100]

–Not applicable.

Table 2
Nanoplatforms existed in fresh plants, charred plants, and plants formulae.

Nanoplatform	Source	Bioactivity	Research level	Refs.	
Extracellular vesicles	<i>Zingiber officinale</i>	Anti-inflammatory/ Treating colitis-based cancer/ Liver protection/ Antibacterial activity	<i>In vitro</i> / Mice model	[115–120]	
	<i>Citrus aurantium</i>	–	Isolation and characterization	[124]	
	<i>Citrus limon</i>	Antioxidative effect/ Antitumor	<i>In vitro</i> / Mice model	[125–128]	
	<i>Citrus paradisi</i>	Anti-inflammatory/ Antitumor	<i>In vitro</i> / Mice model	[129–135]	
	<i>Vitis vinifera</i>	Treating colitis	<i>In vitro</i> / Mice model	[136,138]	
	<i>Panax ginseng</i>	Antitumor	<i>In vitro</i> / Mice model	[139,140]	
	<i>Asparagus cochinchinensis</i>	Antitumor	<i>In vitro</i> / Mice model	[141]	
	Shiitake Mushroom	Liver protection	<i>In vitro</i> / Mice model	[142]	
	Broccoli	Treating colitis	<i>In vitro</i> / Mice model	[143]	
	<i>Dendropanax moribifera</i>	Anti-melanogenic activity	<i>In vitro</i> / Mice model	[148]	
	Charcoal nano-components	charred <i>Cirsium setosum</i> / charred <i>Cirsium japonicum</i> / charred <i>Typha angustifolia</i> / charred <i>Juncus effusus</i>	Hemostatic effect	<i>In vitro</i>	[154–156,159]
		charred <i>Phellodendron chinense</i> / charred <i>Schizonepeta tenuifolia</i> / charred <i>Selaginella tamariscina</i>	Hemostatic effect	<i>In vitro</i>	[160–164]
charred <i>Lonicera japonica</i> / charred <i>Sophora flavescens</i> / charred <i>Paeonia lactiflora</i>		Antiinflammatory/ Antioxidant activity	<i>In vitro</i> / Mice model	[169–172]	
charred <i>Pueraria lobata</i> / charred <i>Citrus aurantium</i>		Anti-gout effect	<i>In vitro</i> / Mice model	[173,175]	
Jiaosanxian/ charred <i>Crataegus pinnatifida</i>		Hypoglycemic activity	<i>In vitro</i> / Mice model	[176,177]	
charred <i>Artemisia argyi</i> / charred <i>Zingiber officinale</i>		Antibacterial activity Analgesic effect	<i>In vitro</i> / Mice model	[180] [182]	
Bai-Hu decoction		Antipyretic effect	<i>In vitro</i> / Mice model	[189,190]	
Ma-Xing-Shi-Gan decoction		Inhibiting cell proliferation	<i>In vitro</i>	[191]	
Ge-Gen-Qin-Lian Decoction		Antioxidant activity	<i>In vitro</i>	[192]	
Huang-Lian-Jie-Du decoction		Neuroprotective Effect	<i>In vitro</i>	[194–196]	

-Not applicable.

drugs, natural molecules remain the basis and major resource for drug innovation. Currently, more than half of the drugs on the market are based on natural products and related molecules. Artemisinin, derived from *Artemisia annua* for the treatment of malaria, is a notable example. Furthermore, the China National Medical Products Administration approved the inclusion of Jinhua Qinggan granules, Lianhua Qingwen granules and capsules, and Xuebijing injection among new treatments for coronavirus disease 2019. Undoubtedly, natural plants or herbal medicines from TCMs are receiving unprecedented attention and are now reaching a new golden age.

Nanoassembly of pure small molecules isolated from natural plants into nanomedicine without any carriers may open an alternative avenue and offer a new perspective for medicinal plant research (Table 1). Natural pentacyclic triterpenoids are widely studied for self-assembly through the intermolecular forces of hydrogen bonds of carboxyl and hydroxyl groups, as well as the interactions of the triterpenoid skeleton. In addition, some natural alkaloids can be interacted with specific small molecules to assemble nanoplatform. BBR, the most studied, can self-assemble into nanoparticles with carboxyl-based natural molecules through electrostatic interactions and $\pi - \pi$ interactions. EVs separated from fresh plants have also been used for therapeutic purposes for various diseases (Table 2). EVs can be obtained on a large scale from

beneficial renewable plants and they may reduce cytotoxicity or any other negative side effect due to being evolved in plant cells. Furthermore, Plants-derived EVs have the potential as drug delivery nanocarriers which can be easily modified to target specific ligands. Charred plants are one kind of distinctive TCM that have been widely used to treat various bleeding syndromes in the clinic. CNs isolated from charred plants are widely studied and found to exhibit hemostatic, anti-inflammatory, antioxidant, and anti-gout effects, as well as other activities. In addition, decoction of plant formulae is another traditional clinical method of TCM. In particular, the combination spirit of TCM has been widely accepted in the clinic. Complex interactions always occur at the time of the water decoction process of plant formulae after dissolving active ingredients, and most significantly, they also generate NAs. The phenomenon of NAs in water decoction may be caused by direct noncovalent combination between the active ingredients or the primary metabolites, such as proteins, polysaccharides, and nucleic acids.

Despite the therapeutic benefits of natural plants-derived carrier-free nanoplatforms, there are still some downsides. Carrier-free self-assembly of pure natural molecules have shown the potentiality for therapeutic activities. However, there are still lots of problems to be solved for their successful clinical application. The nanoassembly of other natural compounds from plants

is still rare. The determination of whether a natural product can be self-assembled or coassembled still relies on test screening. Moreover, modulating the proportions of multiple compounds within one nanoplatform to improve the synergetic efficiency remains challenging. Finally, how to increase the active targeting capability of nanodrugs through surface modification is still an issue to be considered. Although EVs separated from fresh plants were discovered earlier, research on plant EVs is not in-depth enough, especially in the biomedical field, and most of them are in the laboratory research stage. Studies on fresh plant-based EVs need to be expanded further and are mainly focused on ginger at present. There are regional and seasonal differences in plant herbs, and whether EVs should be obtained in different regions and seasons needs systematic evaluation. Especially when plant-based EVs are used as therapeutic agents or drug carriers in the biomedical field, the safety, effectiveness, stability and quality standards of EVs need to be controlled. Moreover, many plant-based EVs are derived from fresh juice, and how to preserve extracted EVs is the limiting factor to determine suitability for large-scale processing. There is still a lack of research in this area, and some reports even include contrary aspects. The internal reasons for this necessitate further research. As for charred plants-derived CNs, there has not been a unified standard conclusion regarding the detailed mechanism of the pharmacological action in clinic application. It is only speculated that it may be related to the different structures and properties of CNs formed by different medicinal plants in different preparation processes, and further principles remain to be studied and discussed. Related studies on CNs must integrate multidisciplinary research ideas and explore more suitable research technologies and methods for CNs to decode charred plants. As for NAs from decoction of plants formulae, knowledge of the formation mechanism of NAs is not sufficiently mature enough. In particular, the energy changes and structural characteristics of self-precipitation in the process of decoction still need to be further explored. In addition, determination of whether NAs generated by water decoction have special pharmacological effects and clinical application value are also necessary directions of future research. The synergistic effect of NAs are the future research highlights.

In summary, carrier-free nanoplatforms with self-assembly from natural plants may be a promising alternative treatment strategy for human diseases. With more research on plant-based carrier-free nano self-assembly, we believe that there will be clinical trials and even medical products in the near future, which can be safely and reliably produced on a large scale.

CRedit authorship contribution statement

Zhongrui Li: Investigation, Conceptualization, Writing – original draft. **Xiao Xu:** Visualization, Writing – review & editing. **Yun Wang:** Writing – review & editing. **Lingyi Kong:** Conceptualization, Validation, Writing – original draft. **Chao Han:** Investigation, Conceptualization, Visualization, Writing – review & editing, Validation, Supervision.

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.

Acknowledgements

This work was co-supported by National Natural Science Foundation of China (82104359), the Fundamental Research Funds for the Central Universities (2632021ZD24), the China Postdoctoral

Science Foundation (2021M691647), and the Open Project of State Key Laboratory of Natural Medicines (SKLNMKF202207).

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