

The Difference between White and Red Ginseng: Variations in Ginsenosides and Immunomodulation*

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ABSTRACT

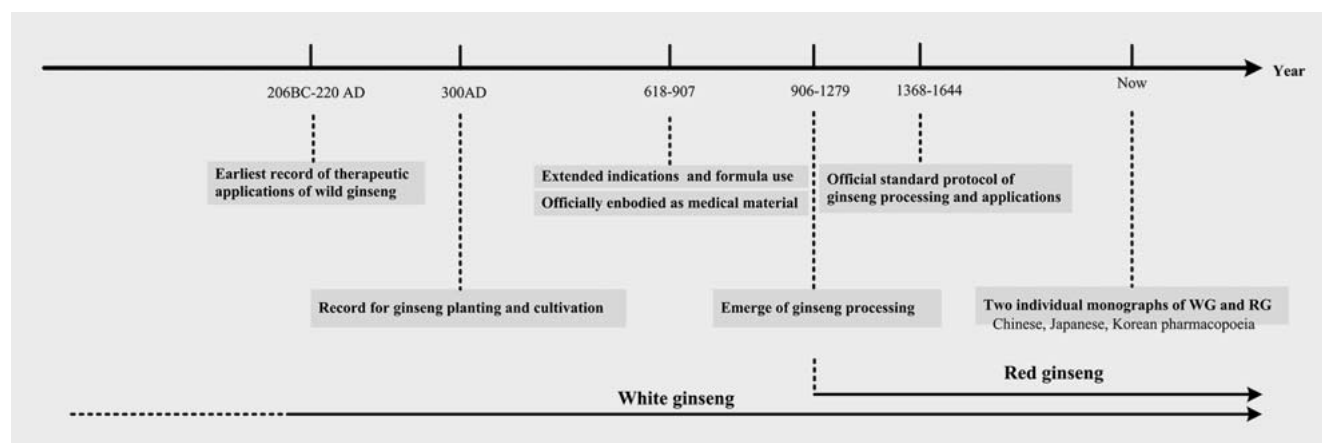
Ginseng Radix (*Panax ginseng*) is one of the most commonly used herbs worldwide for the treatment of inflammation-related diseases among others, supported by ancient historical records. Throughout this long history, the large-scale cultivation of ginseng created an increasing demand for long-term storage of the harvested plant material, accelerating the development of post-harvesting procedures. Dried white ginseng and processed (steamed) red ginseng are the products of the two most common traditional post-harvest processes. Although there are a significant number of reports on practice-based therapeutic applications of ginseng, science-based evidence is needed to support these uses. Using a reverse pharmacology approach in conjunction with high-throughput techniques and animal models may offer clear, simple paths for the elucidation of the mechanisms of activity of herbal medicines. Moreover, it could provide a new and more efficient method for the discovery of potential drug candidates. From this perspective, the different chemical compositions of white ginseng and red ginseng could very likely result in different interactions with signaling pathways of diverse biological responses. This paper provides an overview of white ginseng and red ginseng, mainly focusing on their chemical profile and immunomodulation activities. Synergistic effects of ginseng herbal drugs with combinations of other traditional herbal drugs or with synthetic drugs were reviewed. The use of the zebrafish model for bioactivity testing greatly improves the prospects for future ginseng research.

Introduction

Ginseng Radix (*Panax ginseng* C. A. Meyer, Araliaceae) is one of the most widely used medicinal herbs in the world, particularly in the Far East, China, Japan, and Korea. The most frequently used part of this plant for traditional therapeutic applications is the root (rhizome and/or radix). Ginseng has a long history of traditional applications, with records of its medicinal use as an adaptogen, for the restoration, repair, or healing of the human body, or prevention of disease. Both market demand and academic research have increased significantly in recent years, while its efficacy has been disputed in many cases. One of the potentially most important reasons for these controversies might lie in the absence of a

correct differentiation of two types of processed ginseng material. Besides that, other critical factors such as the geographical origin, development stage or age, storage, type of extract, among others, may also contribute to variations in the results. In this manuscript we focus on the two traditional ginseng preparations that are mainly used nowadays, i.e., white ginseng (WG) and red ginseng (RG). After searching the databases Pubmed/MEDLINE, Embase, and Biosis (for English papers) as well as CNKI (for Chinese papers), with the key words “ginseng” or “white/unpro-

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► **Fig. 1** Historical development and uses of white/red ginseng in China. Stage 1: The earliest records of therapeutic applications of wild ginseng can be found in a Chinese ancient book, Shen-Nong's Classic of Materia Medica (also known as "Shen-Nung Pen-Ts'ao Ching" or "Shin-Nong-Bon-Cho-Kyung", in Chinese: "Shen-Nong bencao jing"). The texts were compiled during the Han Dynasty (206 BC–220 AD) and were considered to be a documentation of Shen-Nong who lived around 2800 BC [2]. Stage 2: With a period of enlarged demands, the emergence of ginseng planting/cultivation began at the end of the Jin Dynasty (~ 300 AD), as mentioned in an ancient Chinese history book "Book of Jin–Shi Le Part" (texts compiled and re-edited during the Tang dynasty around 648 AD). Stage 3: During the Tang Dynasty (618–907), ginseng was characterized by a flourishing of indications for different therapeutic purposes and with multi-formulations in Beiji Qianjin yaofang (Invaluable prescriptions, finished in 652 AD); this was later transmitted to Japan, Korea, and other Asian countries. In addition, ginseng was recorded as a plant in the "Materia Medica", the first Chinese pharmacopoeia (newly revised canon of Materia Medica; in Chinese: Xinxu Bencao) published in 659 AD by the Chinese officials. Stage 4: The earliest record of processed ginseng corresponds to the Song Dynasty (960–1279 AD) [3]. Stage 5: The cultivation, processing, and applications of ginseng were detailed in Compendium of Materia Medica (in Chinese: Bencao gangmu), which was written by Shizhen Li in the Ming Dynasty (1368 ~ 1644 AD) and is considered to be the most important source of Chinese herbal medicine for the modern Chinese pharmacopoeia. Stage 6: This is the current developing stage of official individual monographs of WG and RG in the CP 2015. The differences between WG and RG in functional characters and therapeutic applications as well as their similar applications such as their benefits for blood nourishing and weakness (lack of energy) are recorded in detail [4].

cessed ginseng" or "red/processed/steamed ginseng" or "ginsenosides", along with "immune" or "immunomodulation", there were approximately 3400 papers (349 in English and 3209 in Chinese) that have been published since the 1980s. Within these papers, 267 were about *P. ginseng* C.A. Meyer extracts (35 in English and 242 in Chinese) and ginsenosides from ginseng (105 in English and 278 in Chinese) under the topic of immunomodulation against cancer or inflammation. Based on the information from the literature, both types of ginseng, WG and RG, possess different chemical components that could result in different therapeutic activities, especially in cases such as the modulation of the immune system. This paper presents a review of the comparative studies of WG and RG, focusing mainly on their composition and efficacy.

Historical Development of White and Red Ginseng

P. ginseng C.A. Meyer has many synonyms, such as Ren shen, Mountain Ginseng, Korean Ginseng, or Asian ginseng. The name "ginseng" is also used for many other plants, such as American ginseng (*Panax quinquefolius* L., in Chinese: Xiyangshen), Noto (pseudo) ginseng [*Panax notoginseng* (Burk) F.H. Chen, in Chinese: Sanchi/Sanqi], and Japanese ginseng [*Panax japonicus*, (T. Nees.) C.A. Meyer], but *P. ginseng* C.A. Meyer is the still most commonly used species [1]. The historical development of ginseng as a me-

dicinal plant can be summarized in six major stages (► **Fig. 1**): Stage 1, 206 BC–220 AD: The earliest records of therapeutic applications of wild ginseng date back to this period; Stage 2, ~ 300 AD was characterized by an increase in the demand and consequent emergence of planting and cultivation; Stage 3, 618–907, during this time the indications for its use were extended, and multi-formulas were created having been included in the official Materia Medica; Stage 4, 960–1279 AD witnessed the emergency of ginseng processing, accelerated by the increased demand of indications and the need for long-term storage of harvested material; Stage 5, 1368 ~ 1644 AD marked the appearance of the official standard protocol of ginseng processing and applications; and Stage 6, now is the current developing stage in which separate monographs of WG and RG have been included in the Chinese Pharmacopoeia (CP 2015) [2], the Korean Pharmacopoeia [3], and the Japanese Pharmacopoeia [4].

As summarized above, throughout thousands of years, ginseng evolved from a wild herb to being the product of large scale plantations, requiring the development of post-harvesting and long-term storage methods to prevent its degradation due to insects, moulds, and fungi. Most of the herbs used in traditional Chinese medicine must be submitted to some type of post-harvesting processing. This processing is not only directed at ensuring a better stability during storage, but may have other purposes according to the herb, such as achieving consistent quality and better efficacy, reducing toxic or undesired side effects, modifying the chemical composition to increase solubility, the removal of

the unpleasant smell of certain drugs, or even the preparation of drug material with completely different therapeutic properties to the original raw material [5]. The simplest post-harvesting procedure is the dehydration of the material by drying in the sun (naturally under sunshine). In the case of ginseng, the result of this treatment is WG (dried ginseng/unprocessed ginseng, Chinese: Bai shen). This traditional procedure may not always guarantee the long-term storage of plant material due to climatic variations and transportation times. Thus, over time, a number of alternative processing procedures appeared, such as steaming with or without additional excipients (e.g., alcohol or honey), boiling, and stir-frying. Among these, the most common process is steaming the ginseng as following: after harvest ginseng, the side roots of ginseng will be removed and washed in clean water, then the cleaned ginseng will be steamed (100 °C) for 2–3 h until the roots color becomes yellow. Then heat-dry or sun-dry the steamed ginseng [5]. This procedure can be repeated for several cycles. When the steaming is done at a high temperature for sterilization, followed by drying, a Maillard reaction [6] occurs and the ginseng acquires a reddish or brown tinge that gives the material the name red ginseng (RG, processed ginseng/steamed ginseng; in Chinese: Hong shen/Hongsam). The longer the steaming, the darker the resulting color of the ginseng. Black ginseng is in fact the product of a 9-cycle steaming process. However, WG and RG are the two traditional preparations that are most commonly used.

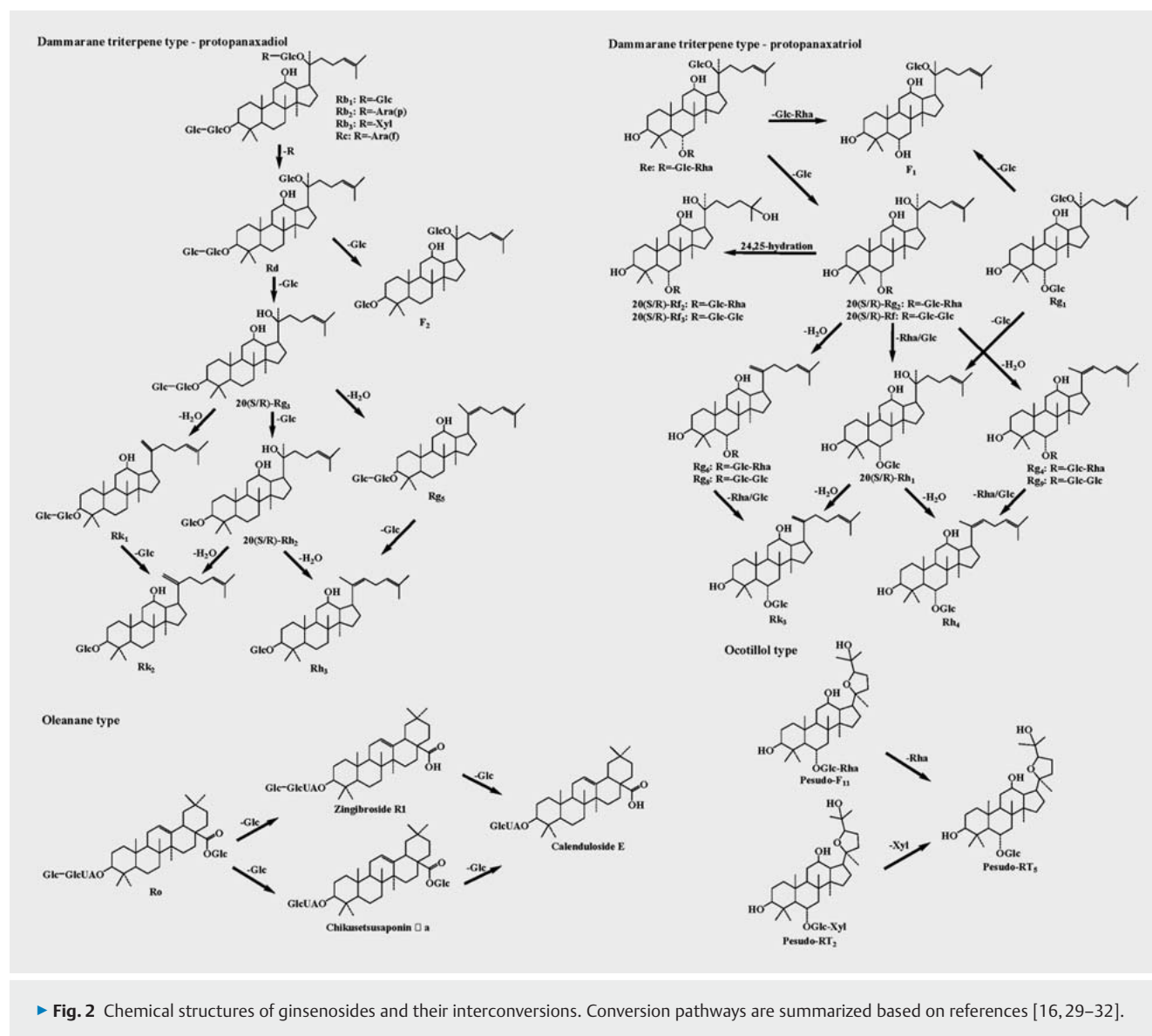
Red ginseng was originally developed with the only purpose of improving the preservation of ginseng for long-term storage. However, as has been observed in general for most plant material, the difference in post-harvest treatments can result in changes in the chemical composition and biological activities of the material [7]. This proved to be the case for ginseng also. Chinese herbal medicines are traditionally classified according to the sensations they evoke in the patient and the patient's response, resulting in descriptive characterizations such as taste, warm/cold, and toxic/nontoxic. Such classifications have now been scientifically validated [8, 9]. Based on this theory, both WG and RG are considered to have "warm" functional characters, but this property may be enhanced by the processing of RG [10, 11]. While WG is traditionally considered relatively mild and has the specific function of quenching thirst (one of the main symptoms of diabetes in ancient descriptions), it is also beneficial for the treatment of insomnia and pulmonary disease. RG is more suitable for weakness in aging patients, for syndromes of excessive bleeding during puerperium, or postsurgical weakness. These very different functional characters of WG and RG and their resulting therapeutic applications are also recognized in the CP 2015 to the extent that there are now two separate monographs for WG and RG, reflecting their differences in therapeutic indications. However, according to the CP 2015 [2], WG and RG share some similar applications such as their benefits for blood nourishing and fatigue (lack of energy) [2]. There is, however, a need for much more scientific evidence to support the different therapeutic applications that are proposed, as well as an explanation of the mechanisms of their purported pharmacological activities. Nonetheless, when searching for justifications for the inconsistency in results of pharmacological or clinical studies with ginseng, it is important to consider that many scientific

researchers may have been unaware of the existence of these two processed forms of ginseng, and published results actually correspond to different materials. This could account for the variation of the scientific results reported so far. We thus considered that it was very important to provide information that could contribute to distinguish these two forms of ginseng, creating awareness of the importance of their differences.

Differences in Chemical Composition of White Ginseng and Red Ginseng

Ginseng saponins, or ginsenosides, are the major active principles of ginseng. They are triterpenoidal glycosides with a high chemical variation, depending on the linkage position and number of sugars on the aglycone skeleton. In general, based on the structure of aglycone, ginsenosides can be classified into three different types: 1) dammarane type, that includes protopanaxadiols (PPDs) with sugar substituted on C-3, C-20, or both positions, such as Rb₁, Rb₂, Rb₃, Rc, Rd, Rg₃, Rh₂, and compound K; and protopanaxatriols (PPTs) with sugar substitutes on C-6, C-20, or both positions, such as Re, Rf, Rg₁, Rg₂, and Rh₁; 2) oleanane (oleanolic acid) type, such as Ro and polyacetyleneginsenoside Ro; and c) ocotillol type, such as majonosides R₁, majonosides R₂, vina-R₁, vina-R₂, vina-R₆, vina-R₁₄, 24-pseudoginsenoside RT₄, and pseudoginsenoside F₁₁ [12–15]. These ginsenosides can be interconverted (► Fig. 2). In *P. ginseng* C.A. Meyer, only dammarane-type (both PPD and PPT type) and oleanane-type ginsenosides are detectable, while the ocotillol type exist mainly in the *P. quinquefolius* L. To date, more than 50 different ginsenosides have been identified in ginseng [16–19]. The content of ginsenosides in ginseng varies according to the growth/harvest location, harvest season, age, and part of the plant [18–28].

Many chemical reactions can occur during the processing (especially when submitted to heat), and these structural conversions of ginsenosides lead to the main differences between RG and WG. The structural conversions depend both on the temperature used and the duration of the process. The relative differences in the ginsenoside content of WG and RG are displayed in ► Table 1. In general, PPD-type ginsenosides (Rb₂, Rb₃, Rc, Rd, Re₁, F₂, Ro) as well as PPT-type ginsenosides (Re, Rf, Rg₁, Noto-R₁) are relatively more abundant in WG [26]. Conversely, ginsenosides (20S- and 20R-) Rg₃, Rh₁, and Rh₂ are considered to be characteristic components of RG [29, 30]. Higher levels of PPD-type ginsenosides (Ra₁, Ra₂ and Ra₃, Rb₁) and PPT-type ginsenosides (Rg₄) are also found in RG [26]. In WG, malonyl ginsenosides mRa₁/Ra₂, mRa₃, mRb₁, mRb₂, mRb₃, mRc, mRd, mRe, and mRg₁ are considered to be characteristic components that are decreased by processing [26, 31, 32]. The ginsenoside 20S-Rg₂ is relatively higher in WG, while 20R-Rg₂ is higher in RG [26]. Many acetylated ginsenosides (quinenoside R₁ and ginsenosides Rs₁ and Rs₂) are relatively abundant in RG because of the inactivation of the deacetylating enzyme with steaming [30, 33]. In addition, contents of the less polar ginsenosides (F₄, F₅, Rk₁, Rg₂, Rg₅, Rg₆, 20R-Rs₃, 20S-Rs₃, and Rs₄) are observed in RG because of their bioconversions during processing at high temperatures [34–37]. It was also reported that the bioaccessibility of WG and



RG may be different [38]. Therefore, not only the differences in qualitative composition, but also differences in quantitative bioavailability and qualitative bioconversion should be considered when further studying the pharmacological differences between WG and RG.

Apart from ginsenosides, polysaccharides are another class of important active components in ginseng. The concentration of reducing sugars (acidic polysaccharides) detected in RG are higher than in WG due to the degradation of sugar components during processing [39, 40]. Maltol and its glucosides have been considered artificially synthesized products because of the Maillard reaction in the processing of RG [41]. On the other hand, the total level of phenolic acids and insoluble-bound phenolic acids are relatively higher in WG, while trans-ferulic acid and esterified phenolic acids are higher in RG [42]. The volatile flavor compounds 2-furanmethanol and 3-hydroxy-2-methyl-4H-pyran-4-one are considered to be the major characteristic components of WG, while

RG contains 1,2-benzenedicarboxylic acid dibutyl-ester and 2-furanmethanol instead [43]. Alkaloids have also been detected in both types of ginseng.

Pharmacology: Immunomodulation

Among the many bioactivities of ginseng reported in the literature, in this review we have focused on immunomodulation. There are a number of reasons for our choice. In the first place, the traditional indications of ginseng as an adaptogenic comprise activities directed mainly at nourishing, strengthening, or restoration of the homeostasis of the body after a long illness or due to aging. From a modern perspective, these activities are related to regulations of the immune system [44]. Secondly, based on scientific research, ginseng has multiple biological bioactivities, such as vasorelaxation (angio-modulation), but it also has antioxidant, anti-inflammatory, anticancer, and antidiabetic-related (for obe-

► **Table 1** Relative content of ginsenosides in WG and RG based on literature studies (references [26, 33–41]).

PPD-type	WG	RG	PPT-type	WG	RG	Malonyl ginsenosides	WG	RG	Other transformed ginsenosides	WG	RG
Ra1	+	++	Rg4	+	++	mRb1	++	+	Quinquenoside R1	N/A	+
Ra2	+	++	Rg4	+	++	mRb2	++	+	Rs1	N/A	+
Ra3	+	++	Rh1	+	++	mRc	++	+	Rs2	N/A	+
Rb1	+	++	Re	++	+	mRd	++	+	Rk1	–	++
Rg3	+	++	Rf	++	+	mRe	++	+	20R-Rg2	–	++
Rh2	+	++	Rg1	++	+	mRg1	++	+	Rg5	–	++
Rb2	++	+	Noto-R1	++	+	mRa1/mRa2	++	+	Rg6	–	++
Rb3	++	+	20S-Rg2	++	+	mRa3	++	+	Rs3	–	++
Rc	++	+							Rs4	–	++
Rd	++	+							F4	+	++
Re1	++	+							F5	+	++
F2	++	+									
Ro	++	+									

–: Not detected; N/A: not reported; +: detected with relatively lower concentration; ++: detected with relatively higher concentration

sity) properties. All these biological effects are closely connected to inflammation and the immune system. Lastly, the structures of the terpenoid saponins currently reported have some similarities to steroids, which play a role in the anti-inflammatory effects or the modulation of the immune system [45–49]. The partial overlapping of the chemical composition of WG and RG suggests that while they may have some similar biological activities, they also have many differences. These differences in immunomodulatory activities should be more exhaustively evaluated since they could be exploited for better personalized applications and treatments, and also eventually lead to the discovery of new active molecules or treatment strategies.

The vast majority of published reports do not specify whether the investigations were done with WG or RG. We thus selected literature data related to immunomodulation (► **Table 2**). In this summary, it is clear that some studies reported effects consistent with potential immunoactivation, while others pointed towards immunosuppression. Could these differences be explained by the use of different ginseng materials (RG/WG) and/or a variation of the content/ratio of ginsenosides? It has been reported, for example, that variations in the ratio of Rg1/Rb1 can lead to an opposing effect on angiogenesis [50]. As mentioned, the post-harvest processing can alter the ratio of various ginsenosides. Other possible explanations could be totally unrelated to the processing method, lying rather in differences in the biological responses when using different cell-based assay systems. Therefore, *in vivo* assays should be favored since they are more reliable for the validation of pharmacological effects.

The significant differences in the relative content of certain ginsenosides (► **Table 2**) in RG and WG could provide an opportunity to analyze the biological effects of different ginsenosides contained in them. In ► **Fig. 3**, we summarized the cross-talking of the immunomodulatory effects of ginsenosides classified by their relative concentration in WG and RG. This data is based on litera-

ture information, including enzymes, genes, proteins, and inflammatory-based factors. This summary focuses mainly on antioxidant capacity, anti-inflammatory, anticancer activity, and angiogenesis, as well as their detailed potential pathways (► **Fig. 3**) [14]. As there are significant differences in the ratio and content of various ginsenosides (► **Table 1**), the integrated systems view may reveal some differences in targets and effects of WG and RG (► **Fig. 3**).

In particular, in the field of inflammation, most studies have focused mainly on the anti-inflammatory activities of the individual ginsenosides Rb1, Rh1, Rg1, Rg5, and Re [51]. The mechanisms of action that have been revealed include the inhibition of enzyme expression (such as cyclooxygenase) and the production of pro-inflammatory chemokines, cytokines, prostaglandins, and interleukins [44]. Among these ginsenosides, Rg1 and Re are more abundant in WG, while the rest are all relatively more abundant in RG. This may explain the fact that both WG and RG have potential anti-inflammatory activities that relate to immunomodulation. The significantly greater anti-inflammatory effects exhibited by RG might be due to their higher inhibitory effect on the synthesis of NO and IL-6, but also of more inflammatory-related pathways/mediators such as NF- κ B, TLR, MyD88, IgE level, and their radical scavenging capacity [34, 52–57]. The relationship between angio-modulation, inflammation, and the immune system has been well established. Pharmacological studies of ginseng on angio-modulation refer mainly to ginsenosides Rb1, Rg1, Rg3, Re, Rd, and Rh1. The mechanisms that have been described involve the activation of nitric oxide (NO) production to reduce reactive oxygen species (ROS) production, as well as the activation of PI3K, AKT/PKB, and the β -catenin/T cell factor-dependent pathway via the glucocorticoid receptor (GR) [49, 58, 59]. In the field of cancer research, a great number of studies have shown the anticancer effects of ginseng or different ginsenosides, though to date no comparison between the anticancer effects of WG

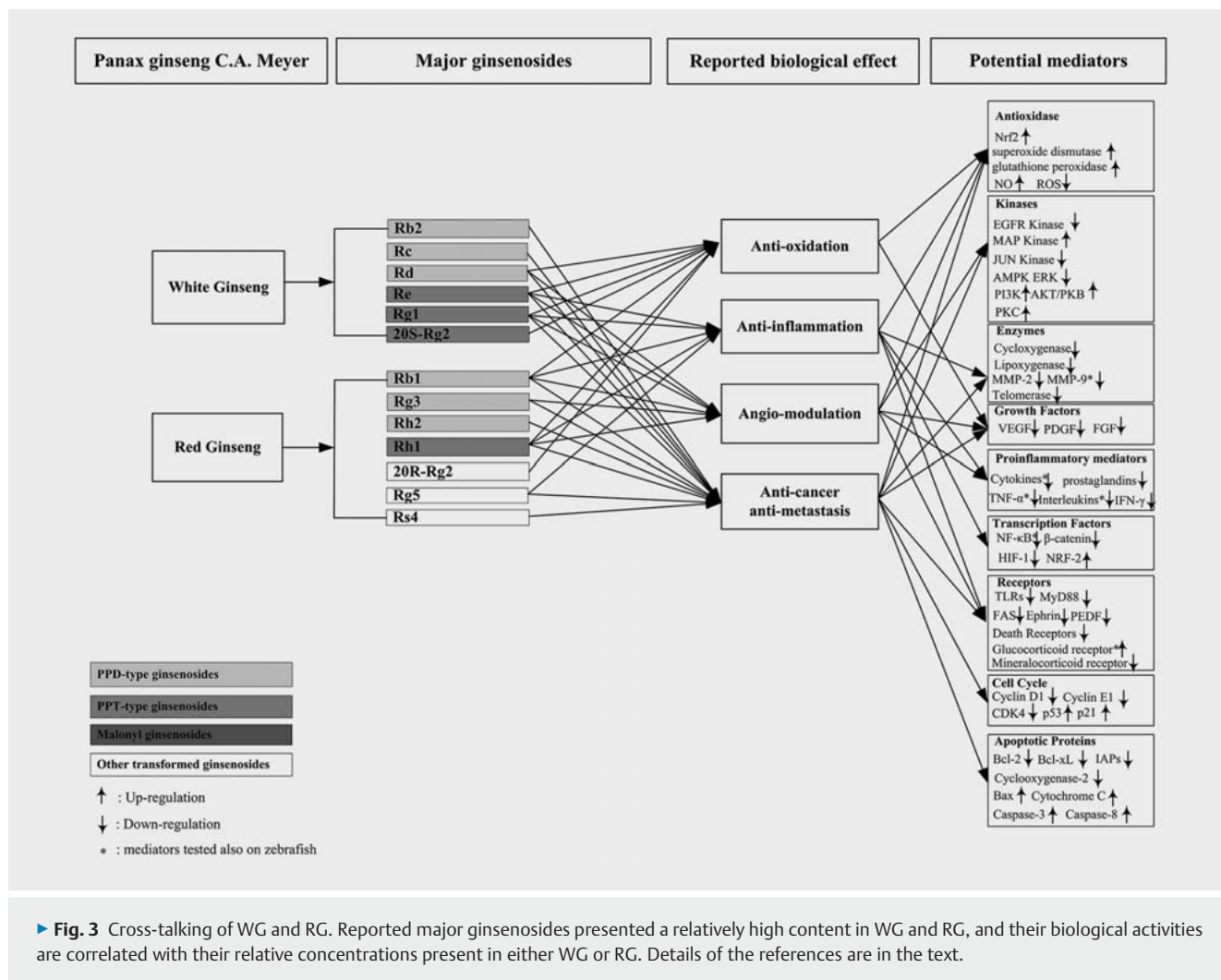
► **Table 2** Multiple immunomodulation pathways of ginseng extract/pure ginsenosides (no distinction of WR/RG is made).

Ginseng extract/ ginsenoside	Reported activation of factors related with immunomodulation	Ref.	Reported suppression of factors related with immunomodulation	Ref.
Ginseng extract (including ginsenosides)	Stimulate proliferation of macrophages; enhance production of IL-12 (both in mRNA and protein levels) via the Th1 response; enhance immunoglobulin IgM, IgG, and IgA, and IL-2, IFN- γ , IL-4, IL-10 levels; activation of TLR-4	[53–58]		
	increases endocytosis of dendritic cells		Inhibits maturation of dendritic cells; inhibits expression of CD-40, CD86, HLA-DR, and CD1a; inhibition on production of IL-12 in dendritic cells	[59]
	Increase innatural killer cell activity	[57, 60, 61]		
			Inhibits expression of CD-40, CD86, HLA-DR, and CD1a; decrease of T-lymphocytes CD3+, CD4+8-, CD4-8+	[57, 59]
Polysaccharides	Enhances proliferation of T and B cells; activation of macrophages to produce NO, H ₂ O ₂ , IL-1b, RNF-a, IL-6, TNF- α , IL-12, IL-17 and IFN- γ ; activation of NF- κ B pathways (NF- κ B-p65 expression); increases phagocytic activity of macrophages	[62–69]	Inhibits expression of TNF- α , IL-1b, IL-6, IL-12, IFN-gamma, IL-18, interferon-gamma, and TLR2, TLR4, TLR9, MyD88, phosphor-JNK-1/2, phospho-p38 MAPK, and NF- κ B in macrophages	[68, 69]
	Increases production of IL-12 and TNF- α in dendritic cells	[67]		
			Promotes generation of immunosuppressive regulatory T cells (Tregs)	[70]
Rb1	Enhances serum levels of IL-4 and IL-10 as well as IFN- γ , IL-2, IL4, TNF- α , IgG1, IgG2a, and IgG2b	[71]	Inhibition of TNF- α	[72]
Rd	Enhances expression of IgG, IgG1, and IgG2b, IL-2, IFN- γ , IL4, and IL-10	[73]		
Rh2			Inhibition of NO production	[74]
Compound K	Enhances nuclear protein binding to CRE	[75]	Inhibits production/expression of iNOS, ROS, IL-1, IL-6, MCP-1, mmp-3 and mmp-9 via MAPK, NF- κ B pathways	[75]
Rh1			Inhibits production of NO, ROS, TNF- α ; inhibits expression of NF- κ B, IRF-1, STAT1, JAK1, STAT1, STAT3, ERK	[74, 76]
Rg1	Increases production of IL-1 by macrophages; enhances mRNA expression of IL-2 and IL-4; stimulates VEGF and PI3K/Akt and β -catenin/ T cell factor-dependent pathway via GR	[51, 77]		
	Increases natural killer cell activity	[77]		
	Enhances proliferation of lymphocytes; enhances IL-2 and IL-4 to induce Th2 in CD4+ T cells	[78, 79]	Decrease IFN-r in CD4+ T cells	[79]
Rg5			Inhibition of IL-1b and TNF- α , iNOS, and COX-2 in macrophages	[80]
Re	Activation of the insulin signaling pathway via inhibition of JNK and NF- κ B activation and inhibitor of NF- κ B degradation	[81]		

and RG has been made [14, 60–64]. The antitumor effect of individual ginsenosides refers mainly to ginsenosides that are abundant in RG (Rb1, Rg3, Rh1, Rh2, Rg5, and Rs4), and also to some ginsenosides that are abundant in WG (Rb2, Rc, Rd, Re, Rg1) [14, 26, 60, 61, 65–69]. These results suggest that both WG and RG may have an anticancer effect, though their mechanisms or pathways may differ [70]. Additionally, polysaccharides showed an immunostimulatory effect to activate macrophages via the pathways of NF- κ B and AP-1, as well as ERK, JNK, and TLR2 [71]. Water-soluble ginseng oligosaccharides help with lymphocyte

proliferation [72]. The immunomodulatory and anti-inflammatory effects of ginseng polysaccharides as well as potential pathways have been reviewed by Liao et al. [73]. Only one paper compared the effect of polysaccharides from white and red ginseng, which stated that there was a better effect of red ginseng polysaccharides against cell death [74].

It is clear that both WG and RG may share biological activities at a cellular level (► **Fig. 3**). At a human clinical level, however, the traditional therapeutic indications of these two herbal drugs differ (CP 2015). Based on the data we collected, we cannot fully justify



why and how these two herbal forms have so many multiple biological effects that overlap in some cases and differ in others. Possibly, from a systems biology perspective, we can analyze our data using a reverse pharmacological approach that may provide an opportunity to reveal and explore “how” and “why” from the known traditional experience by integrating systems information. It is also important to consider that all current pharmacological studies of ginseng focus mainly on a limited number of ginsenosides that are available commercially or readily acquired, such as Rg1, Rb1, Rh1, Rh2, and Rg3, while the vast majority of ginsenosides have not been evaluated. Additionally, Cui et al. [75] mentioned that the oral bioavailability of ginsenosides is low for protopanaxatriol and protopanaxadiol in human urine. The other important aspect is the role of microflora in the human digestive system for the bioconversion/metabolism of ginsenosides, as Kong et al. reported [17]. Therefore, bioavailability is also very important and may contribute to the bioactivities of WG and RG [38]. A further comparative study between RG and WG should also be taken into consideration from this perspective. In short, investigating WG and RG individually may shed light on the difference in a potential mechanism(s) that correlates to their therapeutic applications and characteristic component(s). Furthermore, investigations on

WG and RG may contribute to the development of strategies for new personalized interventions and the discovery of novel drugs.

Interactions of Ginseng with other Drugs

Ginseng is a popular herbal drug that is used worldwide. Thus, from the consumer safety perspective, it is important that full information and awareness of the interactions of ginseng with other herbs and medicines is readily accessible. According to the ancient book *Shennong bencao jing*, ginseng is considered to be a superior herbal drug with low toxicity. However, based on the records of Chinese medical applications, the use of ginseng together with some specific herbs such as *Veratrum nigrum* L. should be avoided altogether as it can reduce or even reverse the therapeutic function of ginseng. Such interactions have recently been supported by scientific evidence [76]. There are also some other descriptions of interactions between ginseng and food in Chinese practice, for instance, the consumption of radish (*Raphanus sativus* L.) may reduce the nourishing effect of ginseng. Further scientific research focused on these topics is needed to reveal other contraindications or interactions of ginseng. In addition, some clinical case reports have mentioned the interaction between ginseng with

some chemical drugs such as warfarin with RG [77] and phenelzine [78].

Effect of the Combination of Ginseng Extracts with other (herbal) Medicines

Few studies related to combinations with ginseng include any reference to which type, WG or RG, was used for the research. According to Chinese medicinal practice, ginseng can be used alone or in combination with other herbs. For example, the formula “Zhu-Xiang”, an herb mixture that contains ginseng and other herbs such as safflower (*Carthamus tinctorius* L., Compositae), has an antitumor effect to inhibit the proliferation of breast cancer cells [79]. Not only as an anticancer drug, but also as a drug against other diseases, ginseng combined with other herbal medicines has been reported to have benefits. Gincosan is a famous mixture of *P. ginseng* C. A. Meyer and *Ginkgo biloba* L. extracts that is frequently used to improve cognitive functions [80]. For instance, it can increase serotonin and decrease noradrenaline levels in the brain, and increase serum ACTH levels, thus being beneficial for the brain and improving learning behavior and secondary memory [81–83]. A combination of a ginseng extract with guarana [*Paullinia cupana* (Mart.) Ducke] has been reported to improve attention and memory [84]. Dry ginseng extracts with aluminium hydroxide or mineral oil have been found to synergistically act as vaccine adjuvants that can enhance immune responses, while Rb1 and Rg1 were observed to act antagonistically and partially inhibit each other [85,86]. In another case, combination therapy with ginseng and sodium ozagrel was reported to be more effective as a neuroprotective for the treatment of transient cerebral ischemia since it enhanced neuronal cell survival and inhibited astrocytes expansion [87].

Red ginseng acidic polysaccharides, in combination with chemotherapeutic agents such as cyclophosphamide, pidotimod, and paclitaxel (taxol), have multiple immunomodulatory effects as well as antitumor and chemoprotection activities [39,88,89]. The RG acidic polysaccharide in combination with IFN- γ can stimulate macrophage function by increasing the production of IL-1, IL-6, NO, and TNF- α and activating NF- κ B [90,91]. The activation of SCF and GM-CSF are also involved in these mechanisms [88]. In combination with rIL-2, the acidic polysaccharide can also induce Th1 cell and macrophage cytokines and generate LAK cells [92].

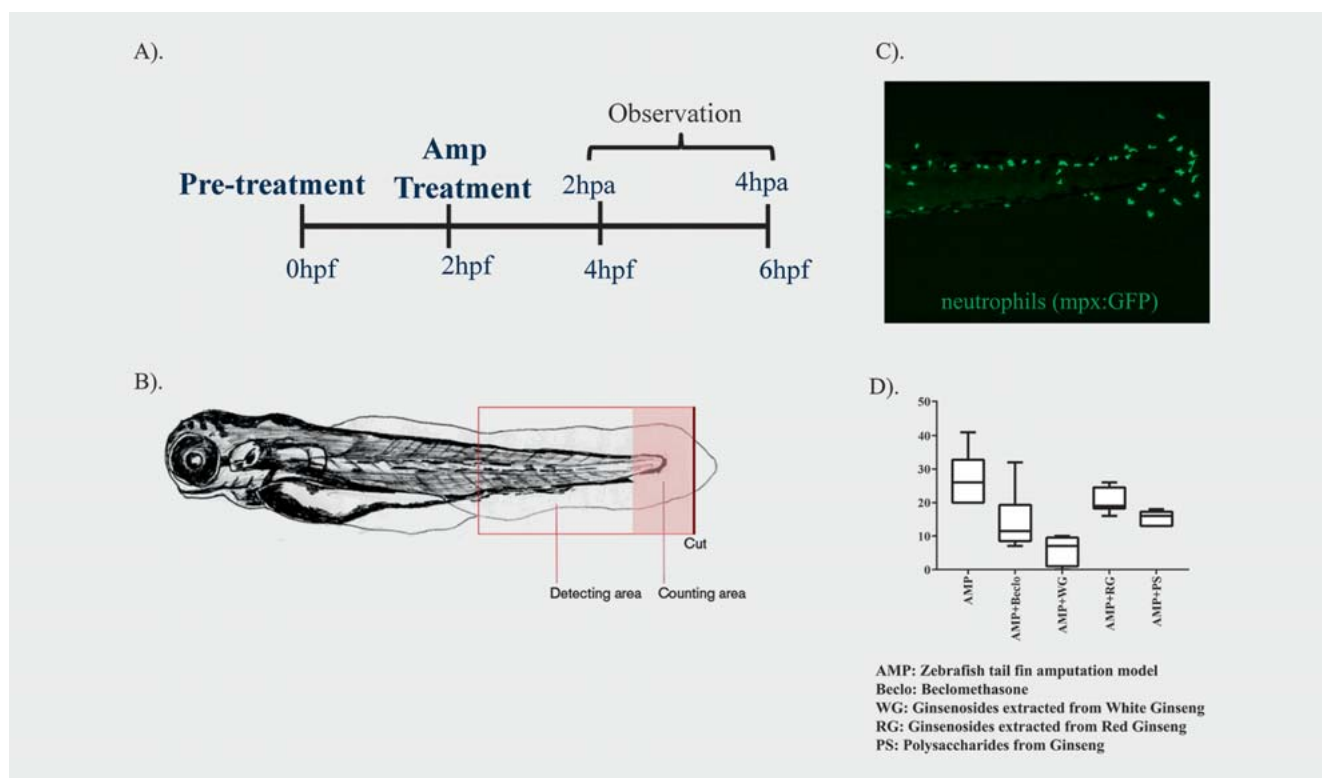
Synergistic Effects of Ginsenoside(s) with synthetic Pharmaceuticals

The simultaneous administration of Rg3 with docetaxel, paclitaxel, cisplatin, and doxorubicin showed a synergistic effect against colon cancer cell growth and apoptosis via inhibition of NF- κ B [93], while its combination with cyclophosphamide exhibited a synergistic effect against ovarian cancer, prolonging the life of mice and reducing microvessel density and VEGF levels [94]. Combining Rg3 with gemcitabine or cyclophosphamide was reported to inhibit angiogenesis and growth of lung cancer and improve the survival and quality of life of mice [95,96]. Compound K in combination with gamma ray radiation was observed to induce apoptosis, nuclear fragmentation, loss of mitochondrial membrane potential, and activate caspase 3, and enhance regression of tumor xenografts for cancer therapy [97]. The combination of

Rg1 with Bt2cAMP was reported to synergistically downregulate the GR by induction of the luciferase reporter gene [46]. Mixtures of 20-protopanaxatriol with Rg1 or Rb1 acted synergistically, showing antioxidant activity inducing the Nrf2-antioxidant response element [98]. The abundance of Rg1 in WG and Rg3 and Rb2 in RG may contribute to their antitumor activity, and RG plus 5-fluorouracil has been reported to have a better antiproliferative effect on human colorectal cancer than the combination of WG combined with 5-fluorouracil [70]. This difference in antitumor effect of WG and RG may reflect different bioresponses. This goes to prove that it is critical to distinguish which ginseng, WG or RG, is used in bioactivity studies since the results will provide better guidance for the clinical applications of ginseng, as well as for the design of synergistic combinations for cancer treatment and chemotoxic prevention.

Zebrafish as a Model for Research on the Effect of Red Ginseng and White Ginseng on Immunomodulation

As can be observed in our summary in ► **Table 2**, different and even contradictory biological responses have been reported for ginseng. As mentioned before, the use of different ginseng materials (RG or WG) or cell-based assay systems could account for this. Alternatives such as *in vivo* investigations combined with the reverse pharmacology approach could provide novel insight into the biological effects of herbal medicine to obtain more accurate and consistent results. This requires the use of more advanced techniques and high-throughput models that provide sufficient reliable data. Among these, animal models such as zebrafish fulfill many of these requirements. Zebrafish is a widely used model that has emerged in recent years for the study of multiple disorders and is considered to be a rapid and high-throughput drug screening system [99,100]. According to the strict European and American animal welfare regulations on the protection of animals used for scientific studies, the zebrafish (particularly the earlier life stage embryos/larvae) model is considered a replacement or refinement method, and has become a popular model for biomedical and toxicology/pharmacology research. The immune system of zebrafish is remarkably similar to that of humans, and thus this animal model is increasingly used to study inflammatory and infectious diseases or other disorders in which the immune system plays a major role, such as diabetes and cancer [101–103]. Zebrafish embryos develop into the larval stage within only 3 days. The first cells of the immune system appear on the first day after fertilization of the eggs, and within the next 2 days, both macrophages and neutrophils are present. The millimetric size of zebrafish embryos and larvae makes the experimental conditions easier, since only low quantities of drugs are needed and administration through the medium is simple. Macrophages and neutrophils, which are the main immune cell types orchestrating the innate immune response, can be fluorophore-marked using different transgenic lines, such as Tg (mpeg:mcherry) and Tg (mpx:GFP) [104,105]. Such transgenic models are convenient for the dynamic visualization of the behavior and interactions of



► **Fig. 4** Recent results of a pilot study on the comparative activity of WG and RG extracts on neutrophil migration using the zebrafish tail-fin amputation model. **A** General scheme of the tail-fin amputation (Amp) zebrafish model with drug treatment. The zebrafish larvae used for this assay are at 3 days post-fertilization. After 2 h pretreatment with either WG, RG, or beclomethasone (positive control), the larvae tail fin is cut and the same drug treatment is repeated immediately. At least two time points (2 or 4 h post-amputation) are selected for further microscopic observation or bioassays. **B** Schematic figure of the zebrafish larva indicating the position of the tail cut and the areas used for detecting and counting the neutrophils. **C** Representative images showing the migration of neutrophils towards the cut edge after tail-fin amputation (4 h post-amputation). **D** Preliminary results of the anti-inflammatory activity of WG vs. RG, compared to beclomethasone; the y-axis indicates the number of neutrophils that migrated to the tail cut edge at 4 h post-amputation.

innate immune cells in a living organism, which is especially useful for inflammation and cancer studies [106, 107].

The zebrafish tail-fin amputation model is a validated inflammatory model with visible innate immune system activation (migration of macrophages and neutrophils) [106]. Such a model is well suited for studies of ginseng/ginsenoside(s) to visually screen for anti-inflammatory activity. ► **Fig. 4** shows an example of preliminary results using this model, suggesting that WG has an anti-inflammatory effect that is stronger than that of beclomethasone, while RG shows no significant effect. Potentially different mechanisms might be involved, and other indicators of inflammation, such as the expression of proinflammatory and anti-inflammatory genes, level of protein and lipid mediators of inflammation, and post-translation modification of key enzymes, have yet to be studied. The potential phagocytic function of macrophages of ginseng extracts has been evaluated recently using a different zebrafish model [108].

Summary and Conclusions

Current scientific methods for drug discovery and development include the use of advanced techniques such as genomics, pro-

teomics, metabolomics, and combinations of biochemistry, biomedicine, computing statistics, and high-throughput assays. Despite the success of these in many fields, a shift in paradigm in the approach to novel drug discovery is urgently needed, and the reverse pharmacology approach provides a very interesting option. Natural herbal medicines that have been validated by ancient wisdom derived from traditional use offer great potential for novel drug discovery or treatment strategies thanks to their long history of clinical applications on humans with renown effectiveness but lack a clear knowledge of the molecular basis for their mechanisms of action. Reverse pharmacology can provide alternative routes to identify significant candidates and to understand potential bioactivities, as well as to enhance the efficacy and reduce the toxicity for advanced treatment strategies [109].

Ginseng is a widely used herb with diverse indications in Asian countries and its chemical composition and pharmacological activities have attracted the attention of researchers in the past decades. It is rich in many different ginsenosides and polysaccharides, the content of which may vary both qualitatively and quantitatively according to the steaming processing used for post-harvest treatments. These differences may be beneficial since they can presumably also have different bioactivities, participating in vari-

ous signaling pathways related to immunomodulation. The emphasis of ginseng studies on efficacy in the future should distinguish the material that is being tested, WG or RG, since results would be more comparable and could provide a more reliable guide in the search of new active compounds as well as contribute to the discovery of synergistic effects and their multiple targeting mechanisms on immunomodulation.

Extending the knowledge of the cellular level to organs and the human body requires a multilevel approach. This can be achieved using reverse pharmacology, an emerging approach that has revolutionized the experimental design of studies aimed at discovering potential drugs or treatments and has been applied successfully to traditional medicine. Additionally, the need of high-throughput techniques and animal models is imperative to understand efficacy and the mechanisms of action of component(s)/combinations. For this, the development of a vertebrate animal model such as zebrafish has provided a visible, simple way to evaluate drug efficacy on immunomodulation, requiring relatively small amounts of samples that are easily administered. The combination of this pharmacological approach and the availability of an animal model such as the zebrafish should greatly increase our understanding of the biological activities of both WG and RG at an integrated system level.

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Conflict of Interest

The authors declare no conflict of interest.

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