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Network pharmacology integrated molecular docking reveals the bioactive components and potential targets of ginseng anti-skin ageing mechanism

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ABSTRACT

In this study, we adopted a comprehensive approach to study ginseng's preliminary anti-skin ageing molecular mechanism via network pharmacology integrated molecular docking strategy. The results showed that 15 active components and 70 potential targets of ginseng were obtained through screening. 238 biological process (BP) items, 36 cell composition (CC) items and 57 molecular function (MF) items were shown in 97 signalling pathways. They were obtained by Gene Ontology (GO) functional enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment. Moreover, molecular docking was done to validate the strong binding ability between the core target (AKT1 and PTGS2) and active ingredients (panaxadiol, β-sitosterol, frutinone A and girinimbin). These results proved that ginseng plays a vital role in anti-skin ageing through multiple targets and pathways. Besides, provided a deeper insight into the anti-skin ageing action mechanisms of ginseng.

Keywords: Molecular docking, molecular mechanisms, network pharmacology, *Panax ginseng*, skin ageing.

INTRODUCTION

Skin is the largest tissue covering the body surface. It effectively blocks the invasion of pathogenic microorganisms and protects the body from cold and heat and maintains the body temperature through sweating the largest immune organ of the body (34,51). For a long time, skin ageing is considered as the inevitable physiological degeneration. Due to internal physiological factors (genetic, endocrine and others) combined with external environmental factors (ultraviolet (UV) radiation and others) may cause skin diseases (25). The more serious diseases decrease the immunity of the body and cause skin cancer (24). Notably, long-term exposure to UV radiation is the primary factor of epidermis ageing (30). UV radiation destroys the integrity of skin connective tissue and disturb the dynamic balance of oxidative stress caused by the increase in reactive oxygen species (ROS) leading to skin ageing (48). Studies have shown that skin ageing can be delayed by regulating genes, neuroendocrine, biological metabolism and immune system (2,6,28,52). Presently there are many researches on the anti-skin ageing. Wang *et al*. (46) studied the 'dieckol', which suppressed the airborne particulate matter-induced skin ageing by inhibiting the pro-inflammatory cytokines and matrix metalloproteinases (MMPs)

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through regulating the NF-kappa B (NF-κB), activator protein 1 (AP-1) and MAPKs signalling pathways, Gendrisch *et al*. (14) studied the luteolin, it can suppress the proinflammatory mediators (IL-1β, IL-6, IL-8, IL-17, IL-22, TNF-α and COX-2) and regulate various signalling pathways (NF-κB, JAK-STAT as well as TLR signaling pathway). In this way, luteolin can modulate many inflammatory processes of skin, against skin ageing.

Ginseng (*Panax ginseng* C. A. Mey. Araliaceae family) is well known medicinal herb. Since ancient times, *Panax ginseng* has been used to slow down the skin ageing process in China and other Asian countries, but its mechanisms is not known. Ginseng medicine has little toxic and side effects on human body as per Shen-nong's Herbal Classic of Han Dynasty (31). It is one of the most traded medicinal herbs for health and disease treatment and is currently sold in 35 countries (22,27). But in recent years, continuous cropping problems of ginseng has drastically hindered the sustained development of ginseng industry (5), hence, it is an important research area. Ginseng contains variety of active ingredients, such as ginsenosides, polypeptides, volatile oils, and polysaccharides (15), which improves the immunity, promotes material metabolism, scavenge free radicals, anti-tumor, anti-ageing and immune stimulation in human tissues (7,21,42). Recently due to its skin whitening, anti-wrinkle and anti-ageing effects (32,38,55), ginseng is widely used as natural cosmetics for beauty and skin care in Asian and other countries (20,23,50). Presently there are few researches on these aspects mainly on anti-ageing effects.

Now, network pharmacology has emerged in the field of traditional Chinese medicine to find the mechanism of action, how multi-components and targets occurs during the treatment course with systematic biology integrated network topology, multi-level pharmacology and computational biology. In this study, 15-active components of ginseng were screened by oral bioavailability (OB), drug-like (DL) parameters and the network pharmacological method, and then the results were used to construct the active components-targets-diseases network relationship to explore the anti-skin ageing mechanism of its active components. Based on this, molecular docking technology was used to preliminarily verify the mechanism of ginseng anti-skin ageing, which laid the foundation for the development of ginseng drugs for anti-skin ageing.

MATERIALS AND METHODS

Chemical constituents and targets of ginseng

The main chemical components of ginseng were extracted by traditional Chinese medicine system pharmacology platform database (TCMSP, http://tcmspw.com) and the main active components were screened under the conditions of oral bioavailability (OB) $\geqslant$ 30% and drug-like (DL) $\geqslant$ 0.18 (47). At the same time, targets of the main active components were screened by TCMSP database. The active component target was transformed into the corresponding GeneSymbol by Uniprot (https://www.uniprot.org) database and the species was limited to "Human".

According to the screening results of TCMSP data platform, 15-effective components (Table 1) in ginseng were screened excluding the compounds lacking targets as per the screening criteria of OB ⩾ 30 % and DL ⩾ 0.18 for analysis.

Table 1. Information about the main active components of Ginseng

TCMSP number	Compound name	OB (%)	DL	Alog*P*
MOL000449	Stigmasterol	43.83	0.76	7.64
MOL000359	β-sitosterol	36.91	0.75	8.08
MOL003648	Inermin	65.83	0.54	2.44
MOL000422	Kaempferol	41.88	0.24	1.77
MOL005308	Aposiopolamine	66.65	0.22	1.39
MOL005317	Deoxyharringtonine	39.27	0.81	3.13
MOL005318	Dianthramine	40.45	0.20	2.05
MOL005320	Arachidonate	45.57	0.20	6.41
MOL005321	Frutinone A	65.9	0.34	2.70
MOL012632	Ginsenoside Rh2	36.32	0.56	4.04
MOL005348	Ginsenoside Rh4	31.11	0.78	5.59
MOL005356	Girinimbin	61.22	0.31	4.60
MOL005376	Panaxadiol	33.09	0.79	5.46
MOL005384	Suchilactone	57.52	0.56	3.73
MOL000787	Fumarine	59.26	0.83	2.95

OB : Oral Bioavailability, DL : Drug-like

Acquisition of skin ageing-related targets

GeneCards disease (https://www.genecards.org) database has wide range of data sources, integrating Drugbank (https://go.drugbank.com) and other database resources, literature, experimental data and is one of the few comprehensive disease databases at present (5,13,39). Genecards disease database and Online Mendelian Inheritance in Man (OMIM, https://david.ncifcrf.gov/home.jsp) database were used to search the related genes with "skin ageing" as the keyword and relevance score ⩾ 10, then the potential targets of anti-skin ageing of ginseng were obtained by intersecting the corresponding targets of ginseng active components and skin ageing-related targets.

Similarity analysis of potential targets of bioactive components was done based on TCMSP. A total of 188 potential targets of ginseng were predicted from 15 obtained bioactive components, 92 potential targets were obtained after removing duplicates. A total of 3220 skin ageing-related disease genes were collected from Genecards disease database and OMIM database. The number of common targets obtained from the intersection of active component targets and disease targets was 70 (Table 2), which were predicted to be the target genes of ginseng anti-ageing skin.

Active components-targets-diseases network construction and target screening

Cytoscape 3.7.1 software was used to construct ginseng active components-targets-ageing disease network map and the degree value of target genes in the network was calculated by the function of "Network Analyzer". The degree value of target gene represents the active component interacting with the target. The higher the degree, the

Table 2. Anti-skin ageing targets of the active components of Ginseng

No.	Uniprot	Target	No.	Uniprot	Target	No.	Uniprot	Target
1	P35228	NOS2	**25**	P06401	PGR	**49**	P35968	KDR
2	Q08209	PPP3CA	**26**	P55211	CASP9	**50**	P27338	MAOB
3	P21397	MAOA	**27**	P01584	IL1B	**51**	P05177	CYP1A2
4	P10415	BCL2	**28**	P20309	CHRM3	**52**	P01579	IFNG
5	P08709	F7	**29**	P25963	NFKBIA	**53**	P05362	ICAM1
6	P04798	CYP1A1	**30**	Q16678	CYP1B1	**54**	Q13698	CACNA1S
7	P46098	HTR3A	**31**	Q01959	SLC6A3	**55**	Q04206	RELA
8	P09211	GSTP1	**32**	P09960	LTA4H	**56**	P14672	SLC2A4
9	P25100	ADRA1A	**33**	P23975	SLC6A2	**57**	P11137	MAP2
10	P35869	AHR	**34**	P00749	PLAU	**58**	P35372	OPRM1
11	P29466	CASP1	**35**	P31645	SLC6A4	**59**	P19320	VCAM1
12	P35354	PTGS2	**36**	P09917	ALOX5	**60**	P06213	INSR
13	P00326	ADH1C	**37**	P19793	RXRA	**61**	P31749	AKT1
14	P45985	MAP2K4	**38**	P27169	PON1	**62**	P23219	PTGS1
15	P03956	MMP1	**39**	O95150	TNFSF15	**63**	O75469	NR1I2
16	P42224	STAT1	**40**	Q12809	KCNH2	**64**	P05412	JUN
17	P14867	GABRA1	**41**	Q14790	CASP8	**65**	P10275	AR
18	P08235	NR3C2	**42**	P37231	PPARG	**66**	Q07812	BAX
19	P09601	HMOX1	**43**	P16581	SELE	**67**	P17252	PRKCA
20	P18509	ADCYAP1	**44**	P04150	NR3C1	**68**	P08684	CYP3A4
21	P09488	GSTM1	**45**	P03973	SLPI	**69**	Q14524	SCN5A
22	O14920	IKBKB	**46**	P07550	ADRB2	**70**	P07477	PRSS1
23	P45983	MAPK8	**47**	P22303	ACHE			
24	P15121	AKR1B1	**48**	P42574	CASP3			

higher the probability that the target will be the main target. To elucidate the protein interactions of the common targets, the STRING (http://www.string-db.org) database was used and the species was limited to "Homo sapiens". The protein-protein interaction (PPI) network map of common targets was obtained and then exported from the website, the Cytoscape software was used to visualize the PPI network, then, according to the median of degree values $\geq$ 2 times, the key targets were selected and the PPI network map was drawn.

GO and KEGG pathway enrichment analyses of components-targets-diseases PPI network

Gene Ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of key anti-skin ageing targets of ginseng by DAVID database (https://david.ncifcrf.gov/home.jsp), the top 20 biological processes and pathways involving numerous targets were screened with their threshold $P < 0.05$, the visual processing was done using the software of Prism 7.0 and the website of OmicShare Tools (www.omicshare.com/tools/index.php).

Molecular docking

The PDB format files of 3D structure of the targets, including protein kinase 1 (AKT1) and prostaglandin synthase 2 (PTGS2) were downloaded from the Protein Data Bank (PDB, https://www.rcsb.org) of Research Collaboration for Structural Bioinformatics (RCSB). Subsequently, Pymol software was used to remove water molecules and original ligands in the protein crystal, then the hydrogenation and electron addition operations were done by AutoDock software, then converted to PDBQT format using AutoDock Tools as docking proteins. The structural formula of ginseng active components was downloaded from the PubChem database (https://pubchem.ncbi.nlm.nih.gov) and the ChemDraw 19.0 (PerkinElmer, USA) was used for energy minimization. Then, AutoDock software was used for hydrogenation, electron addition and selection of rotatable chemical bonds, which was saved as the PDBQT format file as the docking ligand. Finally, the docking site was selected and AutoDock was used for molecular docking to obtain the binding energy of protein and ligand. The docking results of the two groups of proteins with the lowest binding energy were analyzed by Pymol software.

RESULTS AND DISCUSSION

Active components-diseases-targets network construction and target screening

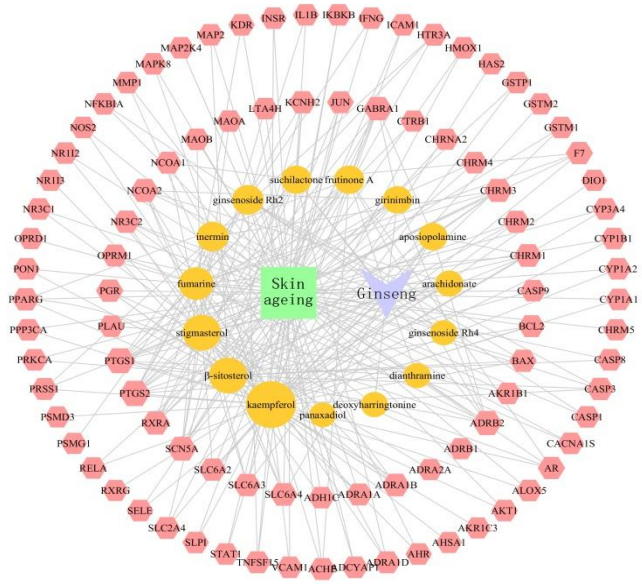

Figure 1. The active components-disease-targets network of ginseng anti-skin ageing (There were 87 nodes and 232 edges, in which "regular hexagon" represents 70 common targets, "circle" represents 15 kinds of active components, "rectangle" represents disease, "inverted triangle" represents traditional Chinese medicine and the edges represent the interaction between active components of ginseng and anti-skin ageing)

As shown in Figure 1, the results showed that 15 active components corresponded to 18, 20, 9, 40, 6, 2, 2, 2, 9, 11, 1, 6, 1, 7 and 13 targets. Five active components including kaempferol, β-sitosterol, stigmasterol, fumarine and Ginsenoside Rh_2 with more targets, kaempferol was 40, β-sitosterol was 20, stigmasterol was 18, fumarine was 13 and Ginsenoside Rh_2 was 11 targets and the 3-targets PTGS2 (11 active components), PTGS1 (10 active components), ADRB2 (8 active components) which corresponded to more active components, may be the key active components and the key targets of the ginseng anti-skin ageing. Among them, 4-active components (Ginsenoside Rh_2, kaempferol, β-sitosterol and stigmasterol) were related to anti-skin ageing. Ginsenoside Rh_2 can inhibit ROS production in HaCaT cells induced by UVB to achieve the anti-photoageing effect (33). Kaempferol, β-sitosterol and stigmasterol play a crucial role in anti-oxidant, anti-inflammatory, inhibiting the cell damage of skin fibroblasts and has the anti-skin ageing activities (1,3,17,18,41).

The PPI network of potential targets of ginseng anti-skin ageing is shown in Figure 2A. 10 important targets including AKT1, PTGS2, JUN, MAPK8, CASP3, IL1B, ICAM1, HMOX1, PPARG and RELA were screened (Figure 2B) by the median of degree $\geqslant$ 2. They can interact with 43, 34, 34, 34, 34, 32, 25, 25, 25, 25 compounds, respectively.

AKT1 is one of the three protein kinases, which participates in multiple signalling pathways related to inflammation, immunity, metabolism and cell proliferation (11,16,45). PTGS2 plays a special role in inflammation. Overexpression of PTGS2 can lead to skin inflammation and skin cancer (37). Interleukin-1β (IL-1β), intercellular adhesion molecule 1 (ICAM-1) and peroxisome proliferator-activated receptor gamma (PPAR) are useful as anti-inflammatory of skin (8,9, 13,19). Proto-oncogene (c-Jun) is an important member of the activator protein 1 (AP-1) transcription factor family, which plays a major regulatory role in the occurrence of skin melanoma (40). Mitogen-activated protein kinase 8 (MAPK8) and caspase-3 (CASP3) are vital for regulating cell apoptosis (29,49). Heme oxygenase 1 (HMOX1) is a key enzyme in the response of cells to tissue damage and oxidative stress of the skin. Previous research concluded that HMOX1 can also suppress skin inflammation (10). Transcription factor p65 (RELA) is a dimeric complex that forms NF-κB. NF-κB is a major regulator of inflammation and cell death. Previous studies have shown that NF-κB blocks the occurrence of skin inflammation in mice (36). Taken together, PPI results indicated that ginseng could achieve the anti-ageing effects of the skin by regulating oxidative stress, inflammatory response and apoptosis. In a word, it can be concluded that Chinese herbal ginseng has the mechanism of multi-component and multi-target interactions, one target can act on different compounds, as well as one compound can act on multiple targets.

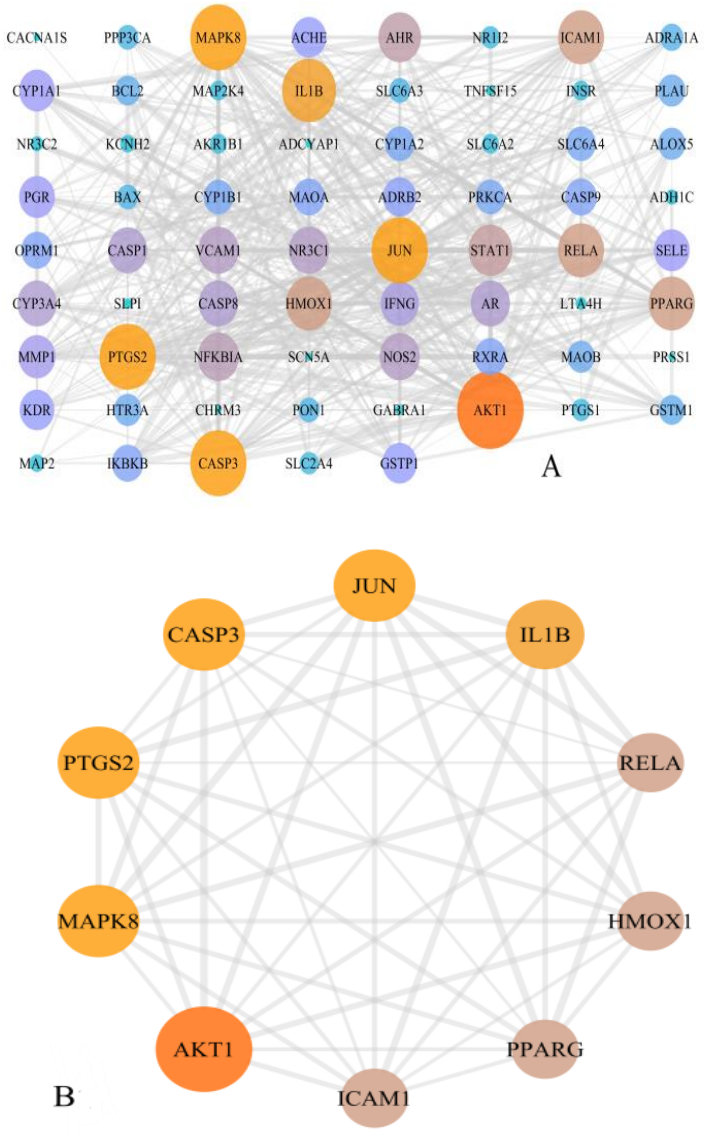

Figure 2. Protein-protein interaction (PPI) network of potential targets of ginseng anti-skin ageing (A:70 targets, B:10 key targets, "circle" represents the protein, and the "connection lines" indicate the association between proteins, involving 70 proteins and 475 combinations. The size of "circle" stands for the degree value. The larger the "circle" is, the larger the degree value of the corresponding protein is. The "combined score" is considered as the thickness of the connection. The larger the "combined score", the stronger is the connection)

Gene function and pathway analysis

GO annotation analysis and KEGG pathway analysis were performed on 70 potential targets of ginseng anti-skin ageing by using DAVID database, the threshold was set as $P < 0.05$ (43), it was found that the anti-skin ageing effects of ginseng involves biological processes such as apoptosis, metabolism and stress response. As shown in Figure 3 and Figure 4, the top 20 analysis results were visualized through GraphPad Prism 7.0 and Omicshare Tools websites. As shown in Figure 3, GO function enrichment analysis yielded a total of 331 entries: the 238 biological processes (BP) mainly involved apoptosis process, oxidation-reduction process, response to the drug, toxic substance, etc. The 36 cell components (CC) mainly involved membrane raft, cytosol, cell surface, nucleus, etc. The 57 molecular functions (MF) mainly involved enzyme binding, oxidoreductase activity and peptidase activity. Thus ginseng may play role by regulating the apoptosis and proliferation of epidermal cells, inhibiting oxidative stress and regulating the synthesis and metabolism of collagen in the skin. Studies have found that the reduction of epidermal cell apoptosis and proliferation, oxidative stress that ROS is involved and collagen synthesis and metabolism are the impact factors affecting the skin ageing (1,26,44).

To further explore the shared key signaling pathways, the enrichment analysis of KEGG signalling pathway was well performed on the common targets. The results of KEGG pathway analysis (Fig. 4) showed that ginseng anti-ageing targets mainly involved cancer-related pathways (20 targets/29%), TNF signalling pathway (14 targets/20%), Toll-like receptor signalling pathway (10 targets/14%), NF-κB signalling pathway (9 targets/13%), apoptosis signalling pathway (9 targets/13%) and others. Accordingly, these pathways may be involved in the process of skin ageing. Toll-like receptor, TNF and NF-κB signalling pathways are mainly involved in the inflammatory response and immune regulation. It showed that the main active components of ginseng are distributed in different metabolic pathways. The anti-ageing effects of skin results from the multi-components and multi-target interactions.

Next, we choose NF-κB signaling pathway as the candidate signal for further study. The nuclear factor-κB (NF-κB) transcription factor family is the principal mediator of the inflammatory process and a key participant in innate immunity responses, adaptive immune responses, inflammatory response and tumor progression (12), the concept prevails that NF-κB should be a valuable pharmaceutical target. UV can contribute to the accumulation of ROS and disturb the dynamic balance of oxidative stress. At the same time, excessive free radicals activate the NF-κB signalling pathway and MAPK signalling pathway, contributing to the activation of AP-1 and NF-κB. Then it increased the level of TNF-α and the expression of MMPs. MMPs, as a member of the superfamily of zinc metallo-proteinases, due to its catalytic activity, can induce the degradation of extracellular matrix (ECM) in the skin, such as degrade collagen and elastic fibers, which may lead to wrinkle formation and accelerated skin ageing (25). Excess of MMP-1 and decrease in collagen synthesis are regarded as the crucial features of the skin ageing diseases and various inflammations (35). Therefore, inhibition of NF-κB signal transduction is an essential way to inhibit the skin ageing.

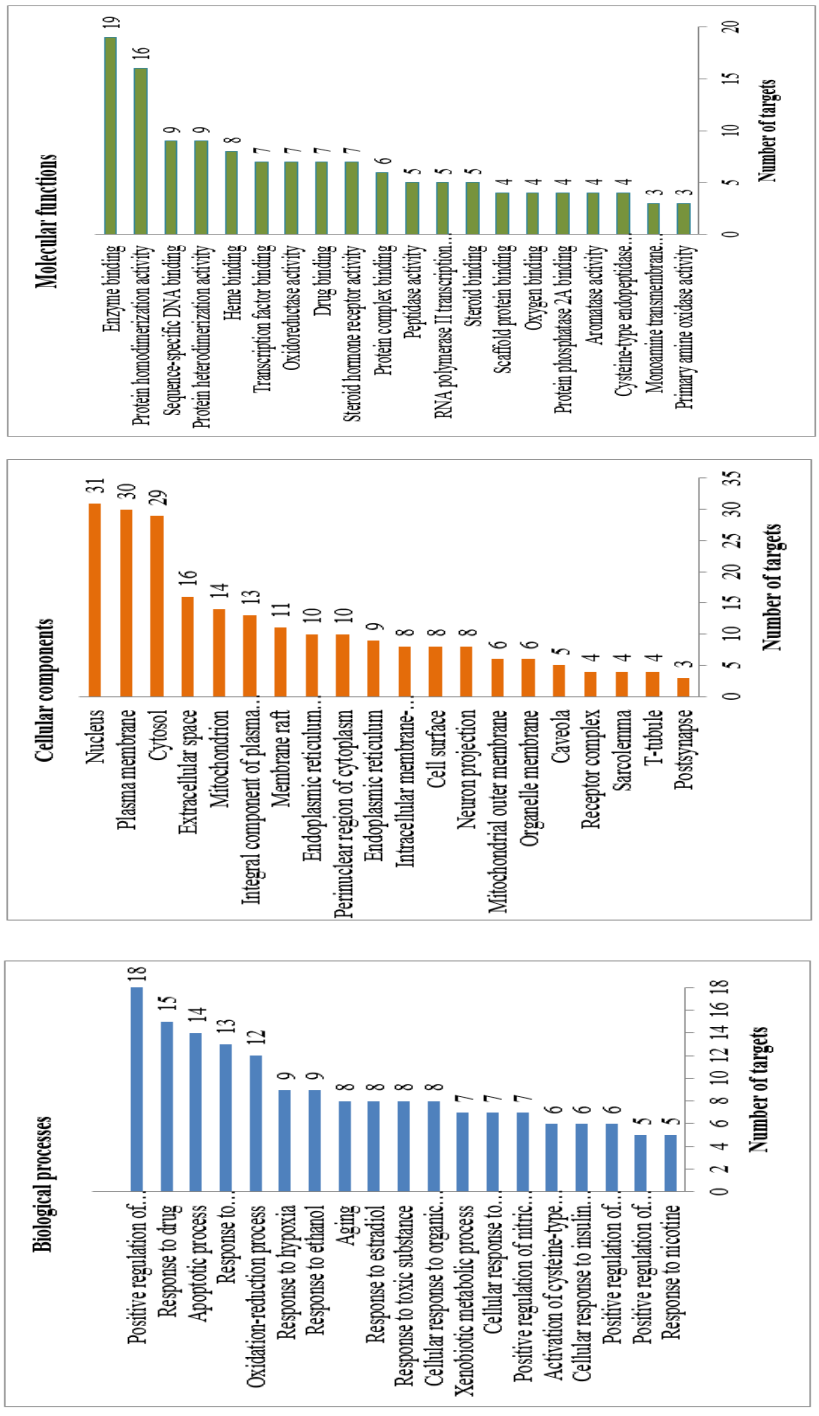

Figure 3. GO functional analysis for Biological processes (BP), Cellular components (CC), Molecular functions (MF) of anti-skin ageing targets of ginseng active components

Verification of the interactions between active ingredients and target genes

It is generally accepted that when the conformation of the ligand and receptor is stable, the lower the energy, the greater the possibility of action (4). The molecular docking results (Figure 5) showed that the two compounds with the lowest binding energy to protein kinase 1 (AKT1) were panaxadiol (-11.3 kcal/mol) and β-sitosterol (-9.6 kcal/mol), respectively. Two compounds with the lowest binding energy to prostaglandin synthase 2 (PTGS2) were frutinone A with the binding energy of -10.9 kcal/mol and girinimbin with the binding energy of -10.5 kcal/mol, respectively, the docking mode is shown in Figure 6. The compounds with the lowest binding energy with PTGS2 were frutinone A and girinimbin, and the binding energies were -10.9 kcal/mol and -10.5 kcal/mol, respectively. With binding energy ≤ -5.0 kcal/mol as the screening standard, the binding energy of anti-skin ageing active components of ginseng with AKT1 and PTSG2 were less than -5.0 kcal/mol. The results showed that the anti-skin ageing active components of ginseng have low conformational energy, stable structure and high binding activity with protein kinase 1 and prostaglandin synthase 2.

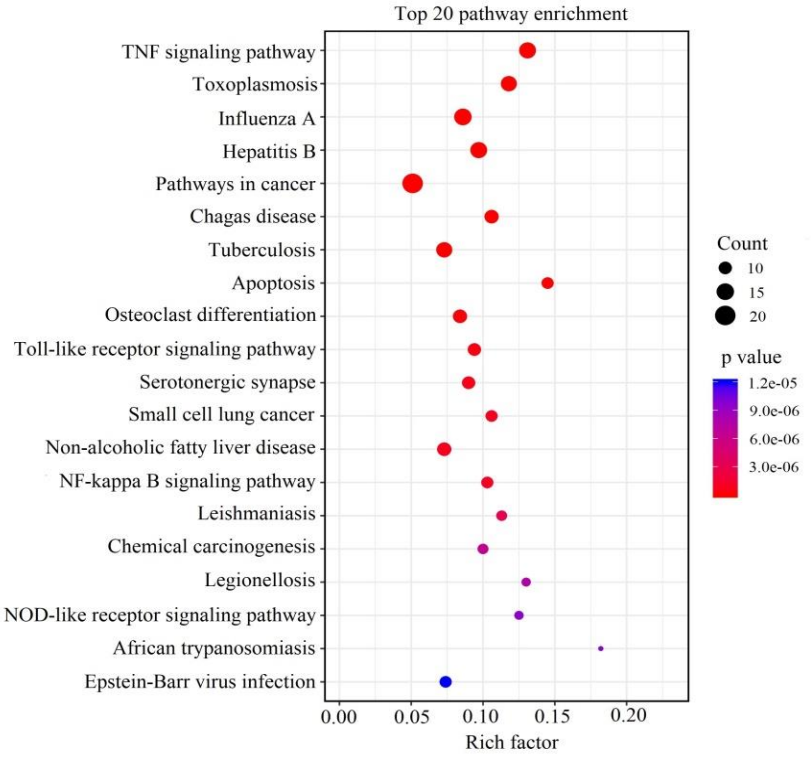

Figure 4. KEGG enrichment analysis of anti-skin ageing targets of ginseng active components

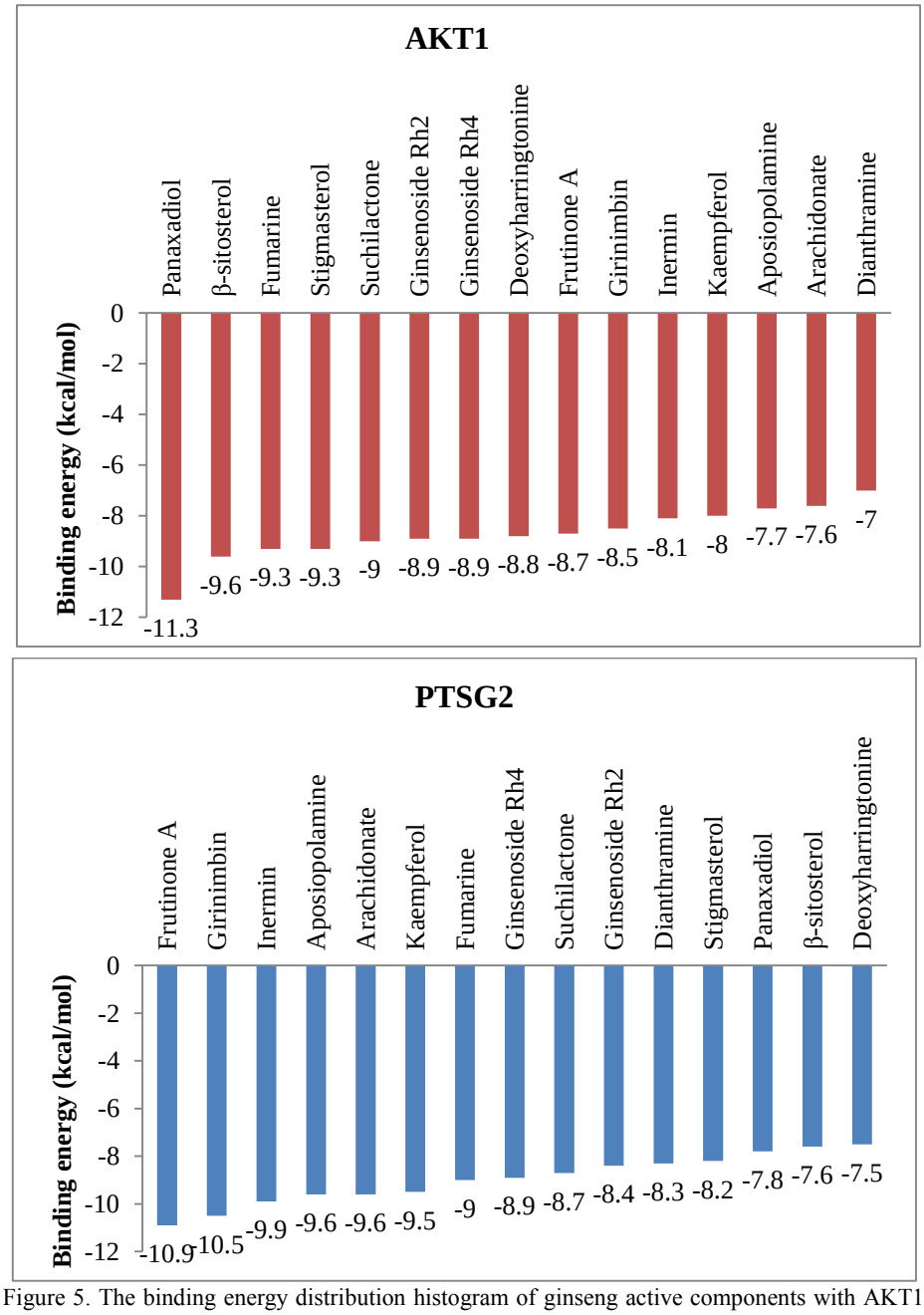

Figure 5. The binding energy distribution histogram of ginseng active components with AKT1 protein and PTGS2 protein

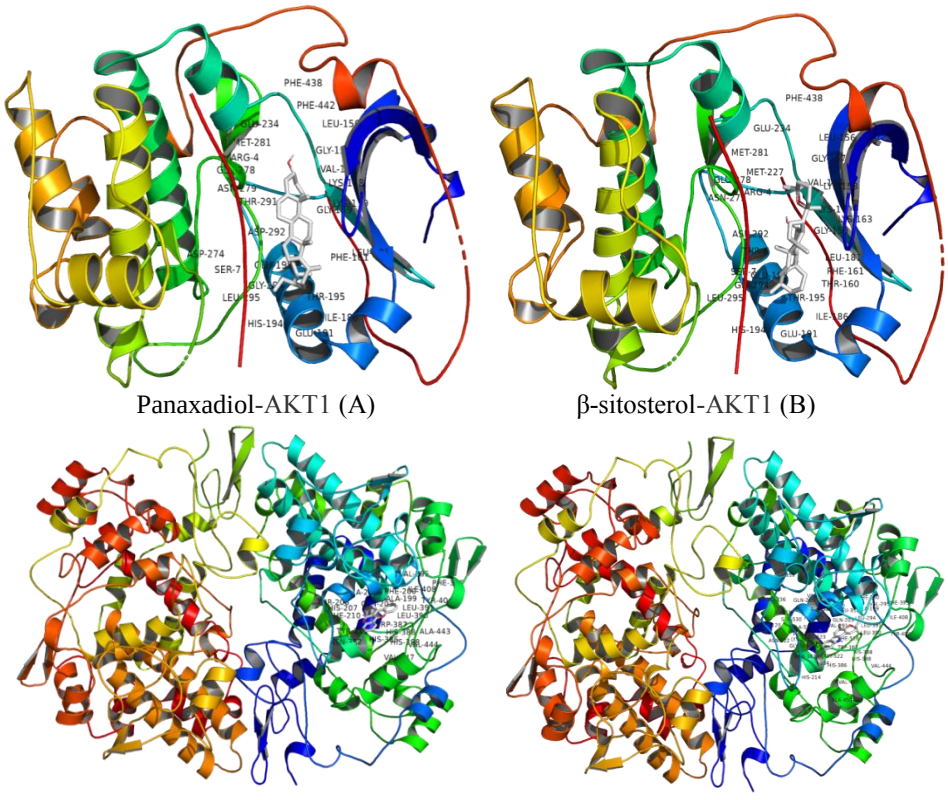

Figure 6. Molecular docking modes of panaxadiol with AKT1 (A), β-sitosterol with AKT1 (B), frutinone A with PTGS2 (C) and girinimbin with PTGS2 (D)

CONCLUSIONS

We found that the signalling pathways of ginseng anti-skin ageing may be closely related to cell apoptosis, collagen synthesis and metabolism, immune inflammatory response and oxidative stress through network pharmacology prediction, which is consistent with the mechanism reported in current studies, indicating the reliability and accuracy of the prediction results of network pharmacology to some extent. The anti-skin ageing effects of ginseng were systematically analyzed by network pharmacology and its potential targets were found. The relationship between active components, targets and pathways of ginseng was clarified, which provided a theoretical basis for further verification experiment, on the effects of ginseng for the prediction of key targets and signalling pathways. In short, this study provided a new idea and vision for the effective development of the ginseng industry in the current situation of decreasing ginseng yield due to continuous cropping problems.

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CONFLICT OF INTEREST

The authors announce that they have no conflict of interest.

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