Identification of the Active Compounds and Significant Pathways of Artemisia Annua in the Treatment of Non-Small Cell Lung Carcinoma based on Network Pharmacology

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
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Background: Artemisia annua exerts powerful effects in non-small cell lung carcinoma (NSCLC). Some studies have shown that Artemisia annua possesses the characteristics of new therapeutic drugs for NSCLC patients. However, the underlying molecular mechanism of Artemisia annua anti-NSCLC is not yet fully elucidated because Artemisia annua contains hundreds of ingredients. This study aimed to conduct network pharmacological analysis on the mechanism of action of Artemisia annua against NSCLC.

Material/Methods: The active ingredients and corresponding potential targets of Artemisia annua were searched and screened in the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP). Then through The Cancer Genome Atlas (TCGA) and the National Center for Biotechnology Information (NCBI) databases to establish NSCLC related targets. Based on the matching results of Artemisia annua potential targets and NSCLC targets, a protein–protein interaction (PPI) network was constructed to analyze the interactions between these targets and topologically screen the central targets. Furthermore, Gene Ontology (GO) biological functions analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) signal pathways enrichment were carried out.

Results: There were 19 main active ingredients of Artemisia annua screened for target prediction; 40 NSCLC-related common targets were identified via multiple NSCLC databases. The node area and corresponding degree value of AKT1, MYC, CCND1, VEGFA, JUN, MAPK1, EGFR, and ESR1 were large and could be easily found in the PPI network. The aforementioned results were further verified by the analysis of GO biological function and KEGG enrichment analysis.

Conclusions: The network pharmacology analysis reveals the molecular biological mechanism of Artemisia annua anti-NSCLC via multiple active components, multi-channels, and multi-targets. This suggests that Artemisia annua might be developed as a promising anti-NSCLC drug.

MeSH Keywords: **Antineoplastic Agents • Carcinoma, Non-Small-Cell Lung • Medicine, Chinese Traditional • Protein Interaction Maps**

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Background

Lung cancer is one of the most lethal and most common malignant tumors in the world. The incidence of non-small cell lung cancer (NSCLC) in lung cancer overall incidence is about 80%, and it is the main cause of cancer-related deaths in China [1]. Despite advances in treatment options, including radiation, surgery, targeted therapies, and chemotherapy, prognosis remains poor due to the presence of locally advanced or extensively metastatic tumors at diagnosis in most patients [2]. Therefore, scientists are persistently looking for novel drugs to improve the effectiveness of NSCLC treatment.

Traditional Chinese medicines (TCMs), unique biomedical and pharmaceutical resources have been widely used in the prevention and treatment of NSCLC [3–5]. Artemisia annua is an ancient antipyretic traditional Chinese medicine, which is used as tea or press juice to treat malaria. Since 2015 when the Nobel Prize in Physiology or Medicine was conferred to a Chinese scientist Youyou Tu, Artemisia annua has drawn worldwide attention [6]. Artemisia annua and its derivatives have been shown to have the anti-cancer activity *in vitro* and *in vivo* [4–6]. Mechanisms for anti-cancer activity of Artemisia annua rely mainly on the generation of reactive oxygen species (ROS) resulting from the interaction of the endoperoxide bridge with either free ferrous iron or heme iron [7,8]. Increased requirements for iron in cancer cells and higher intake of iron through transferrin receptors, typically upregulates in cancer cells, making them more susceptible to damage by ROS from an iron-catalyzed reaction with Artemisia annua [9–11]. Some fundamental research studies have shown that Artemisia annua possesses qualities of a novel therapeutic for patients with NSCLC [12,13]. In addition, Artemisia annua and its derivatives also exert synergistic anti-tumor effects on NSCLC with a wide array of clinically established drugs [14–16]. However, Artemisia annua contains hundreds of ingredients; its active ingredients, targets, and mechanism of anti-NSCLC effects have not been apparent.

Due to the multi-chemical components, multi-pharmacological effects, and multi-action targets of TCM in disease treatment, it is difficult for traditional research methods to explain its mechanism of action adequately [3,6]. However, in recent years, network pharmacology generated by the combination of bioinformatics and pharmacology can clarify the principle of action of these herbs [17,18]. Therefore, we used the network pharmacology method to study the mechanism and material basis of Artemisia annua treatment of NSCLC to provide a new theoretical basis and direction for the systematic development of Artemisia annua.

Material and Methods

Database and software

The databases involved in this study were the following: Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP, *http://tcmspw.com/tcmsp.php*), PubChem (*http://pubchem.ncbi.nlm.nih.gov*), Unipot (*https://www.uniprot.org*), STRING (*https://string-db.org/*), National Center for Biotechnology Information (NBCI) (*https://www.ncbi.nlm.nih.gov/*), and The Cancer Genome Atlas (TCGA) (*https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga*). The data analysis software includes Cytoscape 3.7.2 (plug-ins ClueGo 2.5.2 and CluePedia 1.5.5), discovery studio, and Venn diagrams (OmicShare tools).

Screening the active ingredients of Artemisia annua and predicting the component-targets

All the active ingredients of Artemisia annua were collected from the TCMSP platform, and the screening conditions were based on drug-likeness (DL) ≥0.18 and oral bioavailability (OB) ≥30%. Enter the active ingredients into PubChem to search for the corresponding molecular structure and record the corresponding PubChem CID. Then, based on TCMSP database, the potential targets of the active components were matched one by one, and then the target protein and gene information were corrected through UniProt database to select the object of "Homo sapiens," which is the prediction target of the main active ingredients of Artemisia annua.

Seeking out NSCLC-related targets

With "Non-small-cell lung carcinoma" as the keywords, NBCI (*https://www.ncbi.nlm.nih.gov/*) and TCGA (*https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga*) were used to search and screen the known disease-targets for the subsequent study, and the repeated targets in the search results were discarded. UniProtKB (*http://www.uniprot.org/*) was used to get the standard targets' names with the organism selected as "homo sapiens." Then, the predicted targets of Artemisia annua primary active ingredients and NSCLC related targets were intersected, and the Wayne map was drawn to extract common target genes.

Construction of a protein–protein interaction (PPI) network

The common target genes were uploaded to the STRING platform to obtain protein interaction data. Then use Cytoscape software to establish and analyze the protein-protein interaction (PPI) network, and classify according to the MCODE algorithm, which is a method to automatically find molecular complexes in large protein interaction networks [19]. The size

Table 1. Main active components of artemisia annua.

TCMSP ID	PubChem CID	compound	Target number
MOL000006	5280445	Luteolin	57
MOL000098	5280343	Quercetin	154
MOL000354	5281654	Isorhamnetin	37
MOL000359	12303645	Sitosterol	3
MOL000422	5280863	Kaempferol	63
MOL000449	5280794	Stigmasterol	31
MOL002235	5317287	EUPATIN	16
MOL004083	5281699	Tamarixetin	15
MOL004112	5281678	Patuletin	11
MOL004609	158311	Areapillin	17
MOL005229	5320351	Artemetin	23
MOL007274	188323	Skrofulein	11
MOL007401	160237	Cirsiliol	10
MOL007404	N/A	Vitexin_qt	15
MOL007412	5281603	DMQT	10
MOL007415	10026486	[(2S)-2-[[(2S)-2-(benzoylamino)-3-phenylpropanoyl]amino]-3-phenylpropyl] acetate	5
MOL007423	N/A	6,8-di-c-glucosylapigenin_qt	16
MOL007424	N/A	Artemisinin	15
MOL007426	N/A	Deoxyartemisinin	1

and color depth of the nodes were set according to the degree, that is, the larger the node was, the higher the degree of the target gene corresponding to the darker the color was. The thickness of the edge expressed the score of the relationship value between the 2 target genes, that is, the higher the combination score, the thicker the line of the edge.

Gene Ontology (GO) biological function and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis

Using the ClueGo and CluePedia plug-ins on the Cytoscape software, and using $P<0.05$ as the screening condition for statistical differences, Gene Ontology (GO) biological function and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of the potential targets of Artemisia annua against NSCLC were performed. Furthermore, the main signal pathway and the biological process involved in the pharmacological action of Artemisia annua against NSCLC were obtained, and the topological network diagram of the active component target genes of Artemisia annua was constructed.

Results

Active ingredients and target selection of Artemisia annua

After screening, a total of 19 main active ingredients were included in the study, target prediction was carried out for these 19 ingredients, and 510 targets were extracted after de-duplication, as shown in Table 1.

Screening of NSCLC targets

The database of NCBI and TCGA were used to retrieve 1520 and 1042 NSCLC related targets respectively, and then the human gene name transformation was carried out through the UniProt database. The NCBI as aforementioned related target genes and 185 predicted target genes, were input into Venn Diagrams (OmicShare tools) software for intersection and construction of Venn diagrams as shown in Figure 1. There were 40 common target genes, namely Artemisia annua potential anti-NSCLC targets obtained as shown in Table 2.

Construction of PPI network

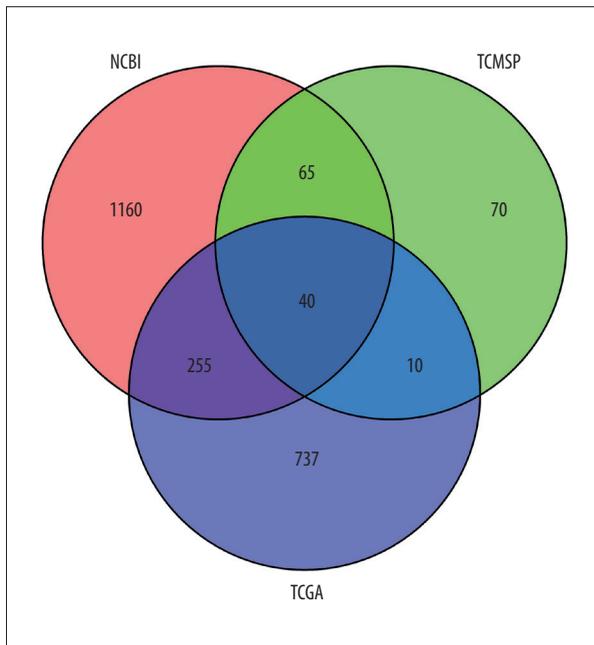As shown in Figure 2, the aforementioned 40 target genes were uploaded to the STRING platform, and the species was

Figure 1. Screening of potential anti-NSCLC targets of Artemisia annua. NSCLC – non-small cell lung cancer.

set as "Homo sapiens" and the rest parameters were defaulted to obtain the protein interaction data information; then, they were imported into the software of Cytoscape to draw the PPI network. In the PPI network, 25 nodes (target genes) interact through 412 edges. The area of a node was positively related to the degree of the node that indicates the number of relationships between the node and other nodes in the network. AKT1, MYC, CCND1, VEGFA, JUN, MAPK1, EGFR, and ESR1 have a large node area and can be easily found in the PPI network (Figure 1), and their corresponding degrees were 36, 35, 34, 33, 32, 32, and 31 respectively (Table 2).

Construction of anti-NSCLC target network of Artemisia annua active ingredients

We took the 40 common targets as the anti-NSCLC targets of Artemisia annua (Table 2), and we import them into Cytoscape 3.7.2 to construct the network diagram of active components and targets of Artemisia annua for NSCLC treatment (Figure 3). The rectangle represents the active ingredient of Artemisia annua for NSCLC treatment, and the ellipse represents the target. The same related biological processes and molecular functions were obtained.

GO biological process analysis

Based on the plug-ins "ClueGo" of Cytoscape software platform, GO biological process enrichment analysis was carried out on the anti-NSCLC target points of Artemisia annua obtained in "2.4", 10 biological processes with $P<0.05$ were screened, as shown

in Table 3, and the visualization results are shown in Figure 4. The biological process of Artemisia annua against NSCLC mainly involved the regulation of oxidative stress-induced intrinsic apoptotic signaling pathway, regulation of anoikis, negative regulation of hydrogen peroxide-induced cell death, and DNA damage response, signal transduction by p53 class mediator resulting in transcription of p21 class mediator. This suggested that the active ingredients of Artemisia annua may act on NSCLC by intervening in the above biological processes.

Enrichment analysis of KEGG signaling pathway

Based on the plug-ins "ClueGo" of Cytoscape software platform, the enrichment analysis of KEGG signal path of Artemisia annua anti-NSCLC target obtained in "2.4" was carried out, and signal paths with $P<0.05$ were screened, as shown in Table 4, and the visualization results are shown in Figure 5. The signal pathways involved in Artemisia annua anti-NSCLC included PI3K/Akt signal pathway, apoptosis, JAK/STAT signal pathway, VEGF signal pathway, etc.

Discussion

The effectiveness of traditional Chinese medicine (TCM) and its derivatives in treating cancer has been more and more affirmed, and it has been widely used in the clinic [3,20]. A large number of clinical studies have reported the beneficial effects of TCM on the survival, immune modulation, and quality of life of cancer patients, which indicates that TCM has a significant therapeutic value and represents an excellent resource for drug discovery [21,22]. As we all know, TCM contains complex ingredients to enhance their therapeutic effects or reduce their toxicity. Therefore, it is crucial to find out and make rational use of the characteristics of TCM with multi-components, multi-targets, and multi-channels.

Network pharmacology looks at biological networks, analyzing the links among drugs, targets, and diseases in these networks, which provides a comprehensive and systematic study with the characteristics of a holistic view; the holistic view is the main feature of traditional Chinese medicine. Therefore, network pharmacology can be used to explore the synergistic effects of various targets and pathways of traditional Chinese medicine [17,18]. Artemisia annua and its derivatives have been found to have anti-cancer activity *in vitro* and *in vivo* [4,22–24]. Artemisia annua and its derivatives are not only widely used for the treatment of various tumors, but also the prevention of tumors [6,23–25]. Artemisia annua and its derivatives also exert synergistic anti-tumor effects with a wide array of clinically established drugs [26–28]. However, the target and mechanism of Artemisia annua against NSCLC have not been apparent.

Table 2. The potential anti-non-small cell lung cancer target of artemisia annua.

| UniprotID	Target protein	Name	Degree
P31749	RAC-alpha serine/threonine-protein kinase	AKT1	36
P01106	Myc proto-oncogene protein	MYC	35
P15692	Vascular endothelial growth factor A	VEGFA	34
P24385	G1/S-specific cyclin-D1	CCND1	34
P05412	Transcription factor AP-1	JUN	33
P28482	Mitogen-activated protein kinase 1	MAPK1	32
P00533	Epidermal growth factor receptor	EGFR	32
P03372	Estrogen receptor	ESR1	31
Q07817	Bcl-2-like protein 1	BCL2L1	28
P04626	Receptor tyrosine-protein kinase erbB-2	ERBB2	28
Q16665	Hypoxia-inducible factor 1-alpha	HIF1A	27
P38936	Cyclin-dependent kinase inhibitor 1	CDKN1A	26
Q07820	Induced myeloid leukemia cell differentiation protein Mcl-1	MCL1	26
Q00987	E3 ubiquitin-protein ligase Mdm2	MDM2	26
O15519	Caspase-8	CASP8	25
P37231	Peroxisome proliferator activated receptor gamma	PPARG	22
P42224	Signal transducer and activator of transcription 1-alpha/beta	STAT1	22
P09874	Poly [ADP-ribose] polymerase 1	PARP1	21
P22301	Interleukin-10	IL10	20
P06400	Retinoblastoma-associated protein	RB1	19
P35968	Vascular endothelial growth factor receptor 2	KDR	18
P49841	Glycogen synthase kinase-3 beta	GSK3B	17
P04049	RAF proto-oncogene serine/threonine-protein kinase	RAF1	17
O14757	Serine/threonine-protein kinase Chk1	CHEK1	17
P08581	Hepatocyte growth factor receptor	MET	17
P25963	NF-kappa-B inhibitor alpha	NFKBIA	15
O96017	Serine/threonine-protein kinase Chk2	CHEK2	15
P01344	Insulin-like growth factor II	IGF2	15
P10415	Apoptosis regulator Bcl-2	BCL2	14
Q13950	Runt-related transcription factor 2	RUNX2	14
O14920	Inhibitor of nuclear factor kappa-B kinase subunit beta	IKBKB	13
Q07812	Apoptosis regulator	BAX	13
P21860	Receptor tyrosine-protein kinase erbB-3	ERBB3	13
P11387	DNA topoisomerase 1	TOP1	13
Q16236	Nuclear factor erythroid 2-related factor 2	NFE2L2	12
Q9H3D4	Cellular tumor antigen p53	TP63	11
P02452	Collagen alpha-1 (I) chain	COL1A1	11
P19793	Retinoic acid receptor RXR-alpha	RXRA	9
Q15596	Nuclear receptor coactivator 2	NCOA2	7
P06213	Insulin receptor	INSR	6
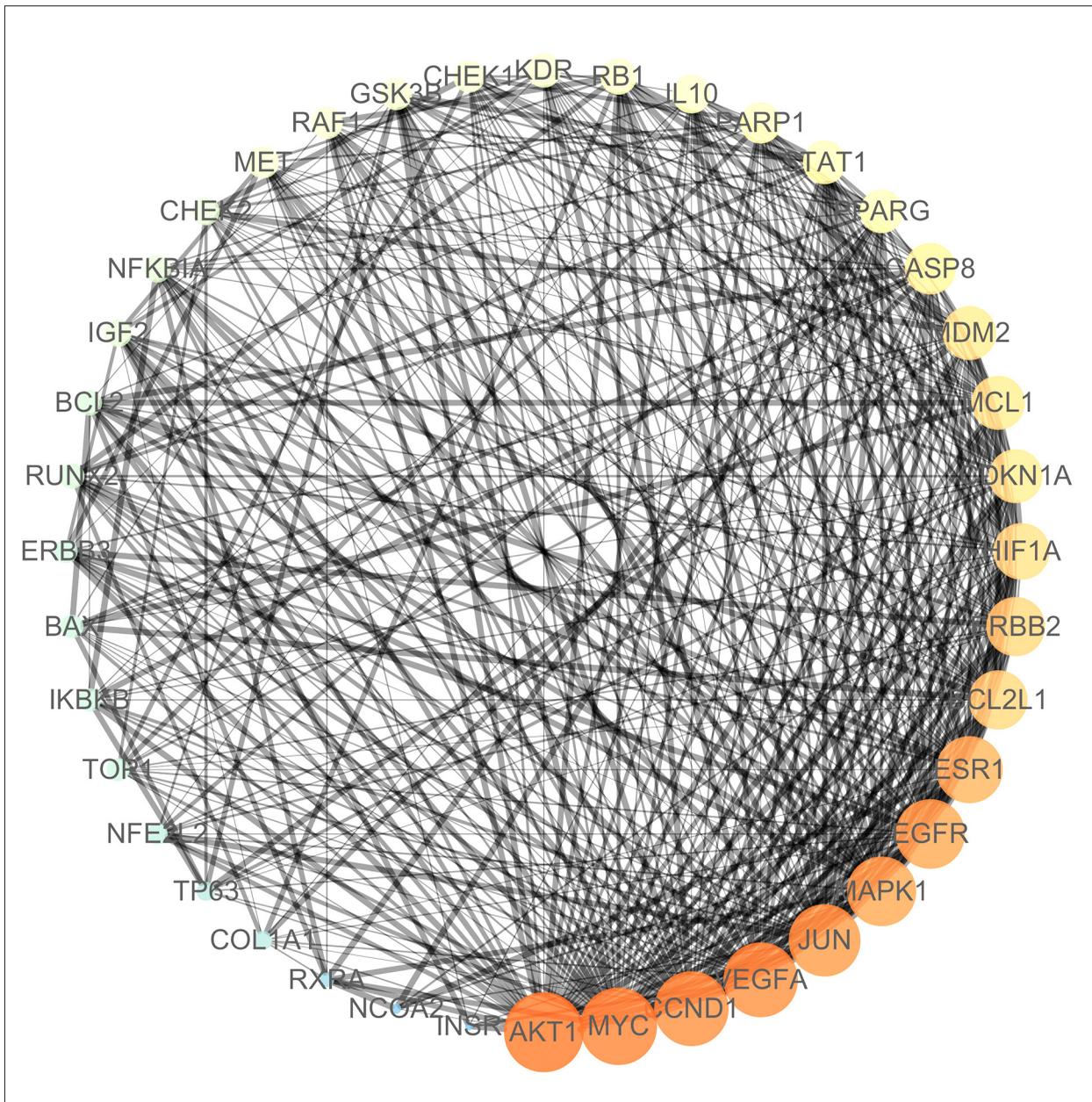
Figure 2. PPI network of Artemisia annua potential target for NSCLC treatment. PPI – protein–protein interactions; NSCLC – non-small cell lung cancer.

In this study, 19 active ingredients of Artemisia annua were screened out via TCMSP database as potential targets. These potential ingredients were as follows: Luteolin, Quercetin, Isorhamnetin, Sitosterol, Kaempferol, Stigmasterol, EUPATIN, Tamarixetin, Patuletin, Areapillin, Artemetin, Skrofulein, Cirsiliol, vitexin_qt, DMQT, [(2S)-2-[[(2S)-2-(benzoyl amino)-3-phenylpro-panoyl]amino]-3-phenylpropyl] acetate, 6,8-di-c-glucosylapigen-in_qt, Artemisinin, and deoxyartemisinin. Then, we screened out 40 common target genes that might have potential anti-NSCLC function by the integration of a multi-source database.

In networks, the basic unit is a node, which can be a disease, target, or a drug; the connection between any 2 nodes is termed an edge. A particular group of related nodes is called a module and the connection between any 2 modules is termed a bridge. The degree and hubs are promising targets or parameters for drug discovery, and the complex relationships among diseases, targets, and drugs in network pharmacology can thus be visu-alized. In this study, we established a visual PPI network to an-alyze the interaction between these targets, and we based on the matching results between the potential targets of Artemisia annua and NSCLC tumor targets, screen the central targets
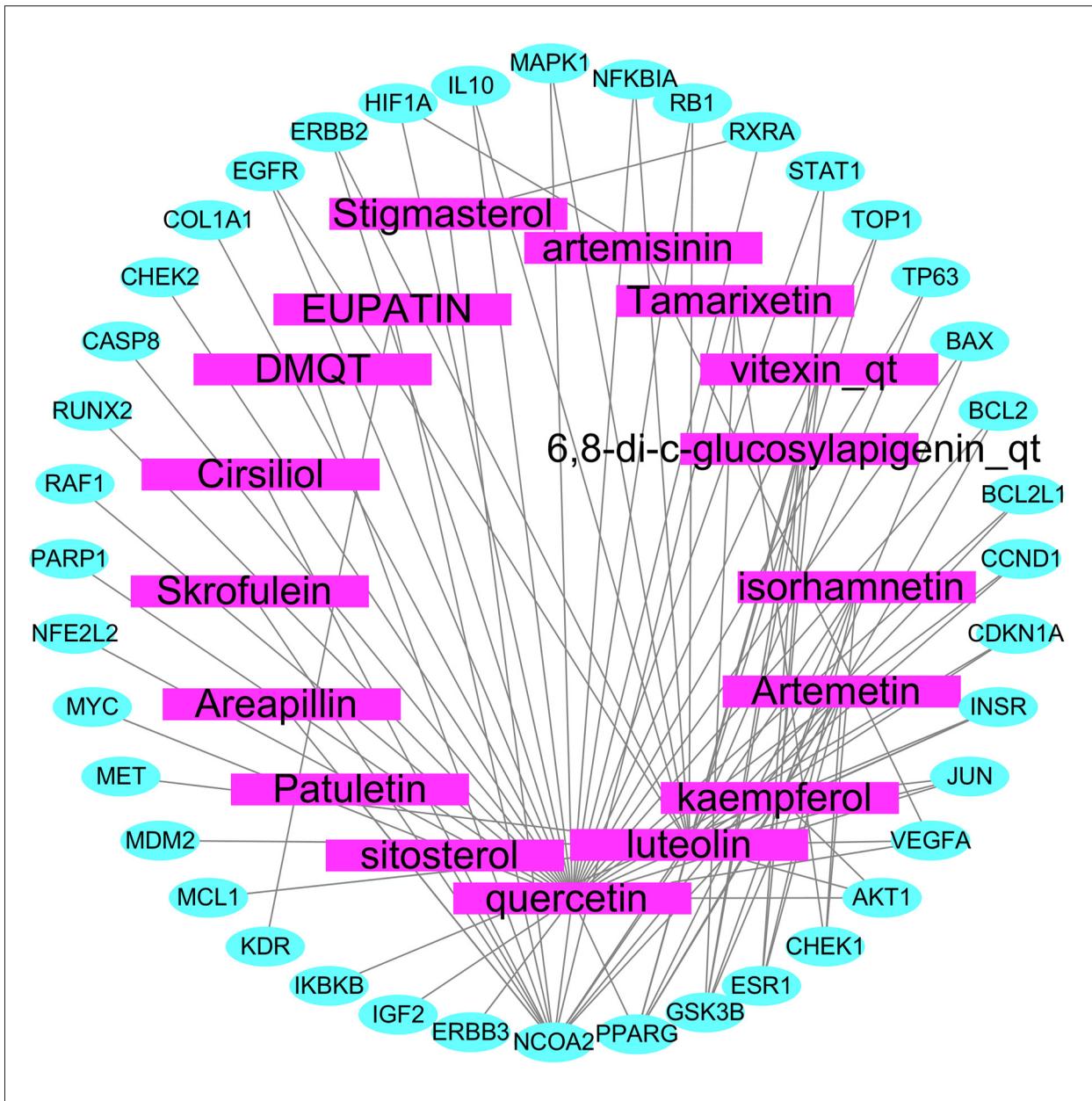
Figure 3. Network map of active ingredients and targets of Artemisia annua in the treatment of NSCLC. NSCLC – non-small cell lung cancer.

by topology. We found that AKT1, MYC, CCND1, VEGFA, JUN, MAPK1, EGFR, ESR1 nodule area, and corresponding degree values were large, and could be easily found in the PPI network. We also found that AKT1, MYC, CCND1, VEGFA, JUN, MAPK1, EGFR, ESR1, and other genes are involved in 2 or more pathways as crosstalk genes, suggesting that these genes or pathways may be the main targets of Artemisia annua against NSCLC. As we all know, these pathways and genes are mainly involved in tumor cell proliferation, apoptosis, cell cycle regulation, epithelial-stromal transformation, angiogenesis, tumor invasion and metastasis, tumor signal transduction, immune regulation,

drug resistance and other critical biological processes [27–30]. More importantly, these pathways and genes might cooperate in promoting metastasis of NSCLC through crosstalk between cancer and immune microenvironment [29–31].

Furthermore, GO enrichment analysis was used to analyze the biological process of Artemisia annua's potential anti-NSCLC targets. We found that Artemisia annua's anti-NSCLC target involved 10 biological processes, including the regulation of oxidative stress-induced intrinsic apoptotic signaling pathway, regulation of mitochondrial depolarization, negative regulation

Table 3. The biological process of artemisia annua on non-small cell lung cancer target.

| *P*-value	Description	Group genes
6.51E-06	Regulation of oxidative stress-induced intrinsic apoptotic signaling pathway	AKT1, MCL1, NFE2L2, PARP1
8.93E-05	Regulation of mitochondrial depolarization	BCL2, KDR, PARP1
1.85E-04	Cellular response to vitamin	COL1A1, MDM2, PPARG
1.85E-04	Negative regulation of hydrogen peroxide-induced cell death	IL10, MET, NFE2L2
1.85E-04	Regulation of anoikis	BCL2, CHEK2, MCL1
3.91E-04	Cytoplasmic sequestering of NF-kappaB	IL10, NFKBIA
4.02E-04	Regulation of oxidative stress-induced neuron intrinsic apoptotic signaling pathway	MCL1, PARP1
8.23E-04	Monocyte differentiation	JUN, PPARG
1.27E-03	Ositive regulation of glycogen biosynthetic process	AKT1, INSR
1.57E-03	DNA damage response, signal transduction by p53 class mediator resulting in transcription of p21 class mediator	CDKN1A, CHEK2
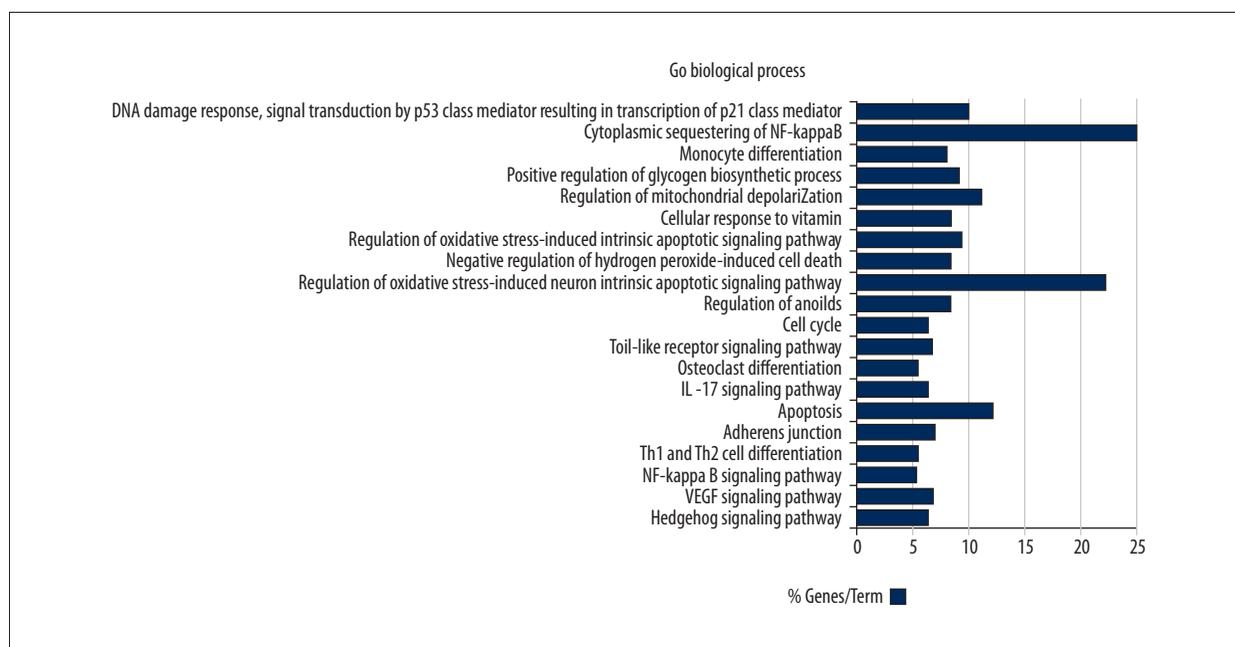
Figure 4. GO biological process analysis. Go – Gene Ontology.

of hydrogen peroxide-induced cell death, regulation of anoikis, cytoplasmic sequestering of NF-kappaB, regulation of oxidative stress-induced neuron intrinsic apoptotic signaling pathway, and monocyte differentiation. These biological processes are related to the occurrence of NSCLC, reflecting the characteristics of Artemisia annua as a multi-component, multi-target, and multi-channel treatment of NSCLC.

According to the analysis of KEGG signal pathway enrichment, there were 68 signal pathways related to the effect of Artemisia annua on NSCLC, including PI3K, Akt, JAK stat, erbB, FOXO, p53, HIF-1, toll-like receptor, TNF, and VEGF, which are strictly related to the pathogenesis of cancer. According to reports, inhibition of NSCLC mediated by IFN-γ is regulated by PI3K-Akt signal transduction, and the inhibition of PI3K can downregulate the expression of PD-L1 and enhance the anti-tumor effect of IFN-γ, which indicates that the inhibition of PI3K can maximize the anti-tumor impact mediated by IFN-γ [32]. Xu et al. found that the expression of JAK2 gene was upregulated in cancer tissues, and was related to lymph node metastasis, and

Table 4. Enrichment analysis of KEGG signal pathway of artemisia annua anti-small cell lung cancer targets (top 20).

KEGG ID	Pathway	P-value	Group genes
KEGG: 04151	PI3K-Akt signaling pathway	3.28E-17	AKT1, BCL2, BCL2L1, CCND1, CDKN1A, COL1A1, EGFR, ERBB2, ERBB3, GSK3B, IGF2, IKBKB, INSR, KDR, MAPK1, MCL1, MDM2, MET, MYC, RAF1, RXRA
KEGG: 04210	Apoptosis	4.69E-11	AKT1, BAX, BCL2, BCL2L1, CASP8, IKBKB, JUN, MAPK1, MCL1, NFKBIA, PARP1, RAF1
KEGG: 04012	ErbB signaling pathway	2.45E-10	AKT1, CDKN1A, EGFR, ERBB2, ERBB3, GSK3B, JUN, MAPK1, MYC, RAF1
KEGG: 04115	p53 signaling pathway	1.76E-09	BAX, BCL2, BCL2L1, CASP8, CCND1, CDKN1A, CHEK1, CHEK2, MDM2
KEGG: 04625	C-type lectin receptor signaling pathway	1.79E-09	AKT1, CASP8, IKBKB, IL10, JUN, MAPK1, MDM2, NFKBIA, RAF1, STAT1
KEGG: 04510	Focal adhesion	3.56E-09	AKT1, BCL2, CCND1, COL1A1, EGFR, ERBB2, GSK3B, JUN, KDR, MAPK1, MET, RAF1
KEGG: 04630	JAK-STAT signaling pathway	6.86E-09	AKT1, BCL2, BCL2L1, CCND1, CDKN1A, EGFR, IL10, MCL1, MYC, RAF1, STAT1
KEGG: 04068	FoxO signaling pathway	1.72E-08	AKT1, CCND1, CDKN1A, EGFR, IKBKB, IL10, INSR, MAPK1, MDM2, RAF1
KEGG: 04218	Cellular senescence	1.05E-07	AKT1, CCND1, CDKN1A, CHEK1, CHEK2, MAPK1, MDM2, MYC, RAF1, RB1
KEGG: 04066	HIF-1 signaling pathway	6.47E-07	AKT1, BCL2, CDKN1A, EGFR, ERBB2, HIF1A, INSR, MAPK1
KEGG: 04110	Cell cycle	2.95E-06	CCND1, CDKN1A, CHEK1, CHEK2, GSK3B, MDM2, MYC, RB1
KEGG: 04620	Toll-like receptor signaling pathway	1.28E-05	AKT1, CASP8, IKBKB, JUN, MAPK1, NFKBIA, STAT1
KEGG: 04380	Osteoclast differentiation	4.29E-05	AKT1, IKBKB, JUN, MAPK1, NFKBIA, PPARG, STAT1
KEGG: 04657	IL-17 signaling pathway	8.36E-05	CASP8, GSK3B, IKBKB, JUN, MAPK1, NFKBIA
KEGG: 04215	Apoptosis	2.21E-04	BAX, BCL2, BCL2L1, CASP8
KEGG: 04520	Adherens junction	2.65E-04	EGFR, ERBB2, INSR, MAPK1, MET
KEGG: 04658	Th1 and Th2 cell differentiation	7.11E-04	IKBKB, JUN, MAPK1, NFKBIA, STAT1
KEGG: 04064	NF-kappa B signaling pathway	7.37E-04	BCL2, BCL2L1, IKBKB, NFKBIA, PARP1
KEGG: 04370	VEGF signaling pathway	1.31E-03	AKT1, KDR, MAPK1, RAF1
KEGG: 04340	Hedgehog signaling pathway	6.15E-03	BCL2, CCND1, GSK3B

upregulated expression of JAK2 could enhance the ability of tumor cell proliferation, metastasis, and invasion. In contrast, downregulated expression of JAK2 had the opposite effect [33]. Kachroo et al. found that interleukin-27 (IL-27) could induce epithelial cell transformation and inhibit the generation of angiogenic factors through STAT1 in NSCLC cell lines [34]. Another study found that STAT3 could induce MDSCs and macrophages to kill CD4+ and CD8+T cells, induce the growth and inhibition of dendritic cells (DCS), and make macrophages differentiate from M1 to M2 in lung tumorigenesis, thus promoting the development of lung cancer [35]. Quercetin is the active ingredient in Artemisia annua. It exerts anticancer effects on cancer cells and tumors by regulating PI3K/Akt/mTOR, Wnt/β-catenin, and MAPK/ERK1/2 pathways. Quercetin promotes the loss of cell viability, apoptosis, and autophagy in cancer by reducing the stability of β-catenin and HIF-1α, inducing caspase-3 activation and inhibiting Akt, mTOR, and ERK phosphorylation [36]. Kaempferol is the active ingredient that also inhibits apoptosis, autophagy, and proliferation in NSCLC cells, which might function through the upregulation of PTEN and inactivation of the PI3K/AKT pathway [37]. Therefore, it is speculated that Artemisia annua might exert an anti-NSCLC effect by acting on the related signaling pathways or targets.
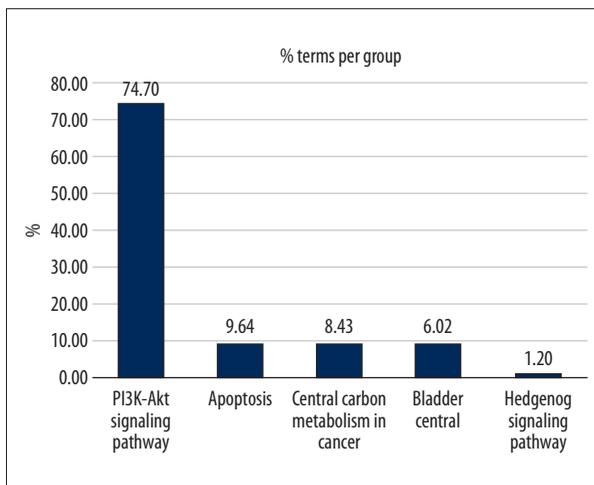
Figure 5. Enrichment analysis of KEGG signaling pathway.
KEGG – Kyoto Encyclopedia of Genes and Genomes.

Although the present research revealed the mechanism of Artemisia annua in the treatment of NSCLC from the perspective of network pharmacology, it still has several limitations. The main limitation of this study is the lack of experimental verification, which will be further expanded in future research.

Conclusions

In summary, bioinformatics and network pharmacology analysis reveals the molecular biological mechanism of Artemisia annua anti-NSCLC via multiple active components, multi-channels, and multi-targets. The targets identified included AKT1, MYC, CCND1, VEGFA, JUN, MAPK1, EGFR, ESR1, et al. in the network of AN and NSCLC, indicating potential as therapeutic biomolecules for treating NSCLS. It is suggested that Artemisia annua might be developed as a promising anti-NSCLC drug.

Conflicts of interest

None.

References:

1. Zhang XC, Wang J, Shao GG et al: Comprehensive genomic and immunological characterization of Chinese non-small cell lung cancer patients. Nat Commun, 2019; 10: 1772

2. Altorki NK, Markowitz GJ, Gao DC et al: The lung microenvironment: An important regulator of tumor growth and metastasis. Nat Rev Cancer, 2019; 19(1): 9–31

3. Kasymjanova G, Tran AT, Cohen V et al: The use of a standardized Chinese herbal formula in patients with advanced lung cancer: A feasibility study. J Integr Med, 2018; 16(6): 390–95

4. Efferth T: From ancient herb to modern drug: Artemisia annua and artemisinin for cancer therapy. Semin Cancer Biol, 2017; 46: 65–83

5. Abba ML, Patil N, Leupold JH et al: Prevention of carcinogenesis and metastasis by Artemisinin-type drugs. Cancer Lett, 2018; 429: 11–18

6. Zhang Y, Liang Y, He C: Anticancer activities and mechanisms of heat-clearing and detoxicating traditional Chinese herbal medicine. Chin Med, 2017; 12; 20

7. O'Neill PM, Posner GH: A medicinal chemistry perspective on artemisinin and related endoperoxides. J Med Chem, 2004; 47(12): 2945–64

8. Wang JG, Zhang CJ, Chia WN et al: Haem-activated promiscuous targeting of artemisinin in Plasmodium falciparum. Nat Commun, 2015; 6: 10111

9. Shterman N, Kupfer B, Moroz C: Comparison of transferrin receptors, iron content and isoferritin profile in normal and malignant human breast cell lines. Pathobiology, 1991; 59(1): 19–25

10. Kwok J, Richardson DR: The iron metabolism of neoplastic cells: Alterations that facilitate proliferation? Crit Rev Oncol Hematol, 2002; 42(1): 65–78

11. Lai H, Singh NP: Selective cancer cell cytotoxicity from exposure to dihydroartemisinin and holotransferrin. Cancer Lett, 1995; 91(1): 41–46

12. Zhai DD, Supaibulwatana K, Zhong JJ: Inhibition of tumor cell proliferation and induction of apoptosis in human lung carcinoma 95-D cells by a new sesquiterpene from hairy root cultures of Artemisia annua. Phytomedicine, 2010; 17(11): 856–61

13. Rassias DJ, Weathers PJ: Dried leaf Artemisia annua efficacy against non-small cell lung cancer. Phytomedicine, 2019; 52: 856–61

14. Abba ML, Patil N, Leupold JH et al: Prevention of carcinogenesis and metastasis by Artemisinin-type drugs. Cancer Lett, 2018; 429: 11–18

15. Zhang ZY, Yu SQ: [Artesunate combined with vinorelbine plus cisplatin in treatment of advanced non-small cell lung cancer: A randomized controlled trial.] Zhong Xi Yi Jie He Xue Bao, 2008; 6(2): 134–38

16. Li SG, Chen HY, Ou-Yang CS et al: The efficacy of Chinese herbal medicine as an adjunctive therapy for advanced non-small cell lung cancer: A systematic review and meta-analysis. PLoS One, 2013; 8(2): e57604

17. Zhang Y, Li XY, Xu XL, Yang NX: Mechanisms of *Paeonia lactiflora* in treatment of ulcerative colitis: A network pharmacological study. Med Sci Monit, 2019; 25: 7574–80

18. Luo B, Que ZJ, Zhou ZY et al: Feiji recipe inhibits the growth of lung cancer by modulating T-cell immunity through indoleamine-2,3-dioxygenase pathway in an orthotopic implantation model. J Inegr Med, 2018; 16(4): 283–89

19. Min S, Chao G, Liu MZ et al: Therapeutic targets of vitamin C on liver injury and associated biological mechanisms: A study of network pharmacology. Int Immunopharmacol, 2019; 66: 383–87

20. Luo H, Vong CT, Chen HB et al: Naturally occurring anti-cancer compounds: shining from Chinese herbal medicine. Chin Med, 2019; 14: 48

21. Shen SJ, Zhang YH, Gu XX et al: Yangfei Kongliu formula, a compound Chinese herbal medicine, combined with cisplatin, inhibits growth of lung cancer cells through transforming growth factor-β1 signaling pathway. J Inegr Med, 2017; 15(3): 242–51

22. Rasheed SA, Efferth T, Asangani IA, Allgayer H: First evidence that the antimalarial drug artesunate inhibits invasion and *in vivo* metastasis in lung cancer by targeting essential extracellular proteases. Int J Cancer, 2010; 127: 1475–85

23. Odaka Y, Xu BS, Luo Y et al: Dihydroartemisinin inhibits the mammalian target of rapamycin-mediated signaling pathways in tumor cells. Carcinogenesis, 2014; 35(1): 192–200

24. Tong Y, Liu Y, Zheng H et al: Artemisinin and its derivatives can significantly inhibit lung tumorigenesis and tumor metastasis through Wnt/beta-catenin signaling. Oncotarget, 2016; 7: 31413–28

25. Mi YJ, Geng GJ, Zou ZZ et al: Dihydroartemisinin inhibits glucose uptake and cooperates with glycolysis inhibitor to induce apoptosis in non-small cell lung carcinoma cells. PLoS One, 2015; 10: e0120426

26. Saeed MEM, Breuer E, Hegazy MEF, Efferth T: Retrospective study of small pet tumors treated with Artemisia annua and iron. Int J Oncol, 2020; 56(1): 123–13

27. Efferth T: Cancer combination therapies with artemisinin-type drugs. Biochem Pharmacol, 2017; 139: 56–70

28. Petrek H, Yu AM: MicroRNAs in non-small cell lung cancer: Gene regulation, impact on cancer cellular processes, and therapeutic potential. Pharmacol Res Perspect, 2019; 7(6): e00528

29. Dragoj M, Bankovic J, Podolski-Renic A et al: Association of overexpressed MYC gene with altered PHACTR3 and E2F4 genes contributes to non-small cell lung carcinoma pathogenesis. J Med Biochem, 2019; 38(2): 188–95

30. Zhao MY, Xu P, Liu Z et al: Dual roles of miR-374a by modulated c-Jun respectively targets CCND1-inducing PI3K/AKT signal and PTEN-suppressing Wnt/β-catenin signaling in non-small-cell lung cancer. Cell Death Dis, 2018; 9(2): 78

31. Zhang XC, Wang J, Shao GG et al: Comprehensive genomic and immunological characterization of Chinese non-small cell lung cancer patients. Nat Commun, 2019; 10: 1772

32. Yi G, Yang JJ, Cai YX et al: IFN-γ-mediated inhibition of lung cancer correlates with PD-L1 expression and is regulated by PI3K-AKT signaling. Int J Cancer, 2018; 143(4): 931–43

33. Xu XJ, Jin J, Xu JW et al: JAK2 variations and functions in lung adenocarcinoma. Tumour Biol, 2017; 39(6): 1010428317711140

34. Kachroo P, Lee MH, Zhang L et al: IL-27 inhibits epithelial-mesenchymal transition and angiogenic factor production in a STAT1-dominant pathway in human non-small cell lung cancer. J Exp Clin Cancer Res, 2013; 32: 97

35. Zhou JJ, Qu ZX, Sun F et al: Myeloid STAT3 promotes lung tumorigenesis by transforming tumor immunosurveillance into tumor-promoting inflammation. Cancer Immunol Res, 2017; 5(3): 257–68

36. Marjorie RF, Catalina CP: The anti-cancer effect of quercetin: molecular implications in cancer metabolism. Int J Mol Sci, 2019; 20(13): 3177

37. Han X, Liu CF, Gao N et al: Kaempferol suppresses proliferation but increases apoptosis and autophagy by up-regulating microRNA-340 in human lung cancer cells. Biomed Pharmacother, 2018; 108: 809–16