

ORIGINAL RESEARCH ARTICLE

Unveiling the mechanism of *Buddleja officinalis* against esophageal squamous cell carcinoma through network pharmacology and molecular docking approaches

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Abstract

In this research, we aim to explore the underlying mechanism of *Buddleja officinalis* (BO) in inhibiting esophageal squamous cell carcinoma (ESCC) by means of network pharmacology and molecular docking approaches. First, BO component targets were determined from the Traditional Chinese Medicine Systematic Pharmacology and HERB databases (known as BenCaoZuJian in Chinese transliteration), and ESCC disease targets were identified from GeneCards and DisGeNET databases. Second, the Venny 2.1 online tool was utilized to visualize the intersection targets, and shared potential targets between BO and ESCC were identified using the STRING database. Third, the component-target-pathway networks were constructed using Cytoscape software. Gene Ontology and Kyoto Encyclopedia of Genes and Genomes were utilized for further analyzing the mechanism of BO in inhibiting ESCC. Finally, molecular docking technique was employed to delineate the docking profiles of BO and determine the optimal active component, which is threonine protein kinase (AKT1). We screened six active components and 227 targets from BO, of which 24 were shared targets of ESCC and BO. The network pharmacology analysis indicated core targets with high degrees, namely, serum albumin, insulin-like growth factor 1 receptor, AKT1, estrogen receptor, and basic fibroblast growth factor receptor 1, which are the most likely binding sites for the active components in BO. The related signaling pathways underpinning the inhibition of ESCC by BO encompass MAPK signaling pathway, adhesion junction pathway, and gastric cancer pathway. Moreover, linarin was recognized as the most suitable component for AKT1. Our results revealed that BO exhibits multicomponent, multi-target, and multi-pathway characteristics, which offer a scientific foundation for elucidating its therapeutic mechanism in ESCC and present novel insights for future investigations.

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Keywords: Network pharmacology; Molecular docking; *Buddleja officinalis*; Esophageal squamous cell carcinoma; Threonine protein kinase

1. Introduction

The incidence of esophageal cancer (EC), one of the most common malignancies worldwide, has dramatically increased over the past 30 years, especially in developed countries.¹⁻³ The primary pathological variants of EC are esophageal squamous cell carcinoma (ESCC) and adenocarcinoma, of which ESCC accounts for the vast majority (90%) of the EC cases.^{4,5} Although various factors have been identified to contribute to the development of ESCC, the specific molecular mechanism of the disease remains elusive. Since obvious symptoms are hardly discoverable in the early stage, most of the ESCC cases are usually diagnosed at an advanced stage,^{6,7} prompting the urgent need for effective treatments.

Buddleja officinalis (BO) is an indigenous traditional Chinese medicine (TCM) used in traditional formulations to treat inflammation, vascular disease, conjunctivitis, headache, and stroke.⁸⁻¹⁰ For example, some studies have extensively reported the pharmacologic profiles and usage of BO in traditional recipes and demonstrated that flavonoids in BO flower buds possess anti-inflammatory and antioxidant activities.¹¹ In addition, aqueous extracts of BO can inhibit high glucose-induced matrix metalloproteinase activity in human umbilical vein endothelial cells.⁹ Given that TCM plays a regulatory and synergistic role systematically through multi-target and multi-channel intervention strategies, it holds distinct advantages in terms of prevention and personalized treatment of ESCC. Nevertheless, the underlying mechanism by which BO exerts its effects on ESCC remains obscure.

Network pharmacology integrates multiple disciplines to establish network relationships between drugs, targets, and diseases, expanding the study of drugs from single-target research to the entire biological network, thus providing a more comprehensive understanding of drug mechanisms. This field can assist in discovering new drug candidates, optimizing drug design, and predicting drug efficacy.^{12,13} The research methodology of network pharmacology conforms to the basic requirements of the holistic view of TCM, providing novel ideas and methods for TCM research.¹⁴⁻¹⁶

Herein, we used the approach of network pharmacology to investigate primary bioactive constituents, targets, and putative mechanisms of BO in inhibiting ESCC. The research flowchart is presented in Figure 1.

2. Materials and methods

2.1. Identification of dominant ingredients in BO

The dominant ingredients of BO were determined from the TCM systematic pharmacology database (TCMSP, <http://tcmsp.com/tcmsp.php>) and HERB database (<http://herb.ac.cn>). The parameters were set as follows: oral bioavailability (OB) $\geq 30\%$ and drug-likeness ≥ 0.18 .

2.2. Establishment of BO active component database

The targets were identified from the PharmMapper database (<http://www.lilab-ecust.cn/pharmmapper/>). The species in the PharmMapper database was set as “*Homo sapiens*” and filtered by ≥ 0.6 of the norm fit. The BO-associated targets were converted to standardized names shown in the UniProt database (<https://www.uniprot.org/>), with the status set to “reviewed” and the species set to “human.”

2.3. Identification of ESCC-associated targets

The search term “esophageal squamous cell carcinoma” was used to acquire ESCC-related targets in GeneCards (<https://www.genecards.org/>) and DisGeNET (<https://www.disgenet.org/home/>).

2.4. Determination of BO-ESCC shared targets

After obtaining BO component targets (pharmmapper: <http://www.lilab-ecust.cn/pharmmapper/>), Venny 2.1 (<https://bioinfogp.cnb.csic.es/tools/venny/index.html>) was used to determine the common target of disease and active ingredients.

2.5. Establishing the BO-ESCC protein-protein interaction (PPI) network

The PPI network for BO-ESCC shared targets was constructed using the interaction database platform STRING v11.0 (<https://cn.string-db.org/>). The parameters are set as follows: the species was set to “*Homo sapiens*,” with the 0.4 of confidence score cutoff.

2.6. Visualization of network analysis results

The determined BO-ESCC targets were further visualized using Cytoscape v3.9.1, and the network analysis plugin was utilized in the software, employing its statistical nodes to analyze the core targets within the network.

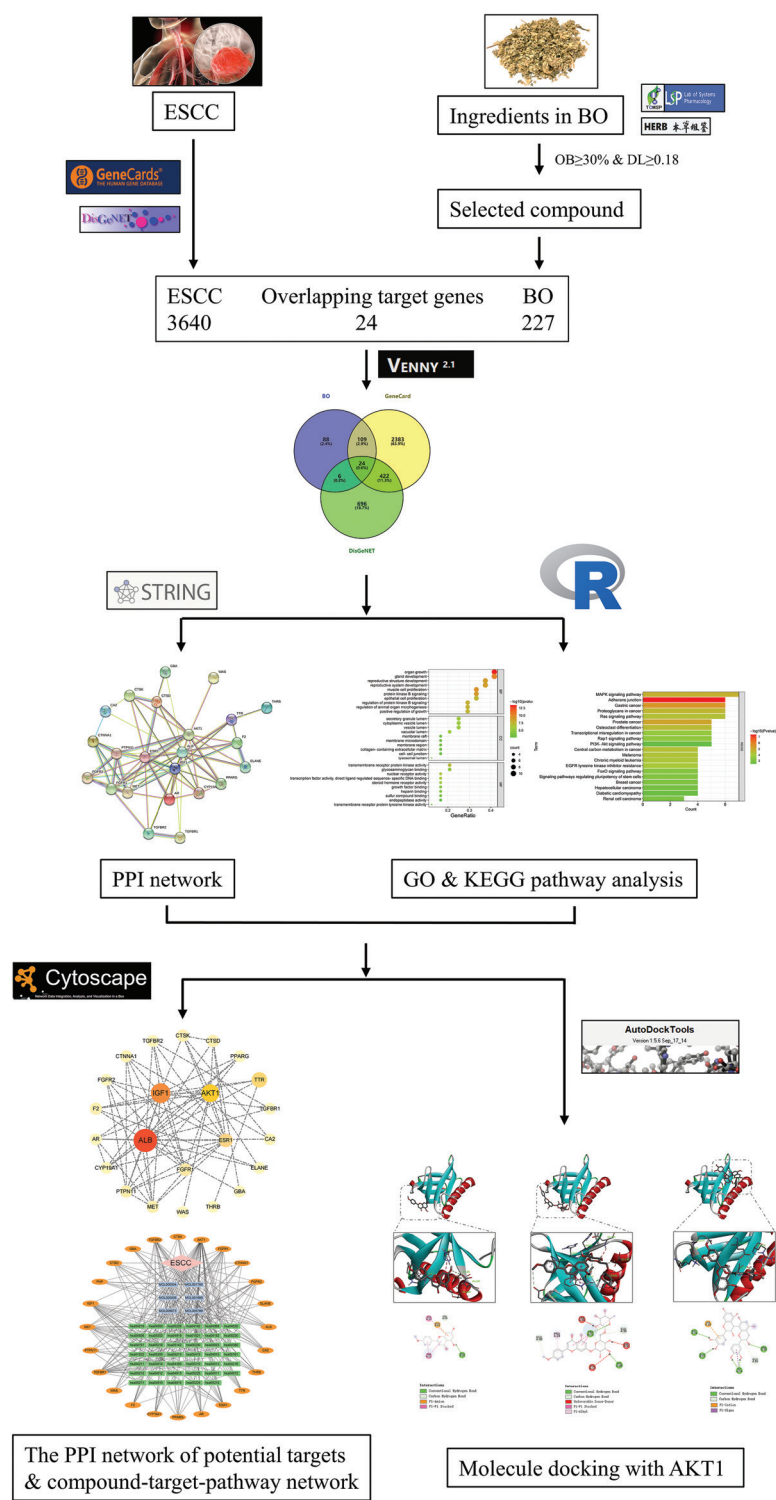


Figure 1. Flow chart of creating networks based on putative mechanisms of BO in inhibiting ESCC. Abbreviations: BO: *Buddleja officinalis*; ESCC: Esophageal squamous cell carcinoma; GO: Gene ontology; KEGG: Kyoto encyclopedia of genes and genomes; PPI: Protein-protein interaction.

2.7. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis

Initially, the potential target genes were converted to Entrez IDs using the R package (“org.Hs.eg.db, version = 3.8”). Subsequently, GO biological functions and KEGG pathway analysis were visualized by the R packages (“DOSE,” “clusterProfiler,” and “Pathview”).

2.8. Docking for dominant ingredients

We investigated the docking of selected active components identified from the BO-ESCC PPI network onto the receptors of threonine protein kinase (AKT1) using AutoDock software. The names, molecular weights, and three-dimensional structures of BO ingredients were initially acquired from PubChem (<https://pubchem.ncbi.nlm.nih.gov/>). Next, the three-dimensional structure of ingredients was derived from the RCSB protein data bank (<http://www.rcsb.org/>). Finally, the AutoDock software was utilized to facilitate the preparation of ligands and targets essential for docking. Afterward, for the target proteins, the water molecules of the crystal structures were removed, hydrogenation was performed, amino acids were modified, energy was optimized, and the force field parameters were adjusted to satisfy the low-energy conformations of the ligand structures. Subsequently, the AKT1 and key active components were molecularly docked, with the affinity (kcal/mol) value representing the binding ability between targets and ligands. Finally, the docking results were observed and analyzed using Discovery Studio software.

3. Results

3.1. Establishment of the BO active ingredients database

Six dominant ingredients of BO were retrieved from the TCMSP and HERB databases, including acacetin, linarin, luteolin, butyrospermol acetate, procyanidin B1, and neobakangelicol (Table 1).

3.2. Potential targets of BO for inhibiting ESCC and visualization of PPI network

By searching the GeneCard and DisGeNET databases, a total of 3640 ESCC target genes were generated after excluding duplicates. The results of the Venny 2.1 online tool showed that 24 shared BO-ESCC targets were obtained (Figure 2A and Table 2). To further clarify the relationships among these overlapping target genes, PPI network analysis was performed, indicating that these

target gene networks had 24 nodes and 73 edges, as shown in Figure 2B. The PPI network files were subsequently visualized by Cytoscape 3.9.1. NetworkAnalyzer utilized the “degrees” to highlight the importance of nodes, and the top five nodes with high degrees were considered core targets, *that is*, serum albumin (ALB), AKT1, insulin-like growth factor 1 receptor (IGF1), estrogen receptor (ESR1), and fibroblast growth factor receptor 1 (FGFR1) (Figure 2C and Table 3).

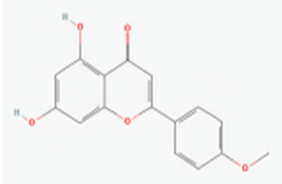
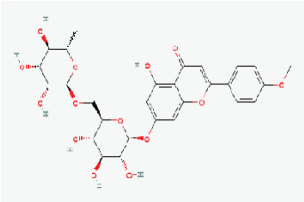
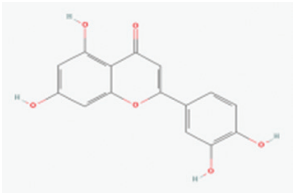
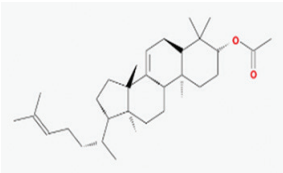
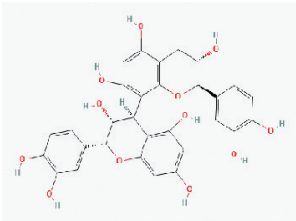
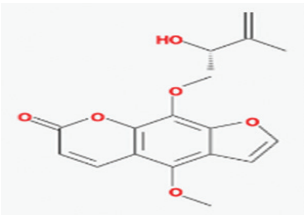
3.3. GO and KEGG analyses

To further examine whether the biological functions of the candidates are associated with ESCC, the terms subdivided in three distinct ontologies, *that is*, biological process (BP), cellular component (CC), and molecular function (MF), were enriched in GO and KEGG analyses by R programming language. In the BP group (Figure 3A), the results suggested that BO might regulate ESCC-related BPs, such as organ growth, gland development, and reproductive structure development. Subsequently, the majority of the GO terms are associated with secretory granule lumen, cytoplasmic vesicle lumen, and vesicle lumen (Figure 3B). In the BP group (Figure 3C), the GO terms mainly included transmembrane receptor protein kinase activity, glycosaminoglycan binding, and nuclear receptor activity. In addition, 41 signaling pathways were identified through KEGG pathway analysis as significantly enriched ($P < 0.05$). Figure 4 displays a bar plot showcasing the top 20 important pathways. The signaling pathways that are closely related to ESCC include MAPK signaling pathway, adhesion junction pathway, and gastric cancer pathway. The gastric cancer pathway is closely associated with the MAPK signaling pathway and is involved in processes such as activation of oncogenes, inactivation of tumor suppressor genes, dysregulation of cell cycle control, and impairment of apoptosis in gastric cancer. In addition, MAPK signaling pathway, the most relevant signaling pathway according to counting numbers, is illustrated in Figure 5. The network revealed that BO is associated with the treatment of ESCC through multiple targets and multiple pathways. Figure 6 shows a compound-target-pathway network centered on the inhibition of ESCC.

3.4. Docking of BO dominant ingredients to AKT1

The six dominant ingredients of BO can bind to AKT1 receptor proteins to varying degrees as ligands, and the lower vina scores indicate stronger and more stable binding capacity between ligands and receptors (Table 4). The vina scores of linarin, procyanidin B1, acacetin,

Table 1. Main active ingredients in BO

No.	ID	Ingredient	Molecular weight	Oral bioavailability (%)	Drug likeness	Chemical structure
1	MOL001689	Acacetin	284.26	34.97	0.24	
2	MOL001790	Linarin	592.5	39.84	0.71	
3	MOL000006	Luteolin	286.24	36.16	0.25	
4	MOL006673	Butyrospermyl acetate	468.84	46.04	0.83	
5	MOL000004	Procyanidin B1	578.5	67.87	0.66	
6	MOL005789	Neobyakangelicol	316.33	36.18	0.31	

Abbreviation: BO: *Buddleja officinalis*.

luteolin, butyrospermyl acetate, and neobyakangelicol increased sequentially, with linarin expressing a strong affinity with AKT1 and engaging in the most stable binding with AKT1. It is speculated that linarin may be the most suitable natural component of AKT1 in BO. Figure 7 shows three-dimensional and two-dimensional

maps of the binding of the above six components to AKT1.

4. Discussion

The mechanism of TCM treatment is complex, involving many components and targets. It is a challenge in

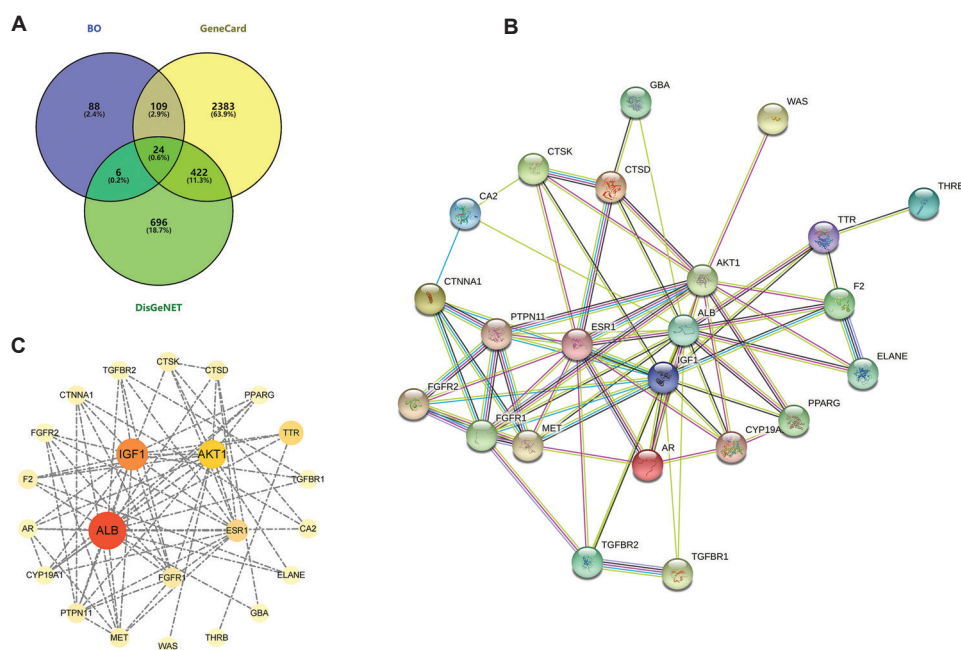


Figure 2. Putative target genes of BO for inhibiting ESCC, and PPI network. (A) The putative target genes of BO for inhibiting ESCC are shown in a Venn diagram. (B) The associations among 24 shared target genes are illustrated by a PPI network. (C) The core targets in the PPI network are visualized using Cytoscape 3.9.1 software. The node size from large to small represents the degree value from large to small. The width of the line between two nodes indicates the strength of their interaction, and the darker color indicates higher significance.

Abbreviations: BO: *Buddleja officinalis*; ESCC: Esophageal squamous cell carcinoma; PPI: Protein–protein interaction.

exploring its mechanism in treating diseases, especially when the pathogenesis remains to be clarified. Network pharmacology, an approach unifying the advantages of systematic network analysis and pharmacology, enables the comprehensive investigation for targets and the underlying pathways of drugs at the molecular level, and enhances our insights into drug mechanisms.^{17,18}

According to the component–target network established in this study, six ingredients in BO were found to have multiple targets in the network, suggesting their potential therapeutic effects in the treatment of ESCC, which merit further investigation. The cancer-inhibiting effect of luteolin has been vastly reported in the literature. According to a previously published review,¹⁹ the suppression effects of luteolin on cancer progression are mediated by inhibiting tumor cell proliferation, promoting cell cycle arrest, safeguarding cells against oncogenic stimuli, and triggering apoptosis through diverse signaling pathways. Furthermore, luteolin possesses the capability to reverse epithelial–mesenchymal transition by upregulating the expression of the epithelial biomarker E-cadherin and downregulating

the mesenchymal biomarkers N-cadherin, Snail, and vimentin. Moreover, the anticancer potential of acacetin stems from multiple potential mechanisms, *for example*, inducing cytochrome c release and formation of reactive oxygen species by targeting mitochondria, inducing apoptosis, reducing matrix metalloproteinases (MMPs), inhibiting invasion and migration, and promoting cell cycle arrest and autophagy.²⁰ Linarin can induce apoptosis,²⁰ inhibit epithelial cell proliferation²¹ and inhibit NF- κ B activation in cancer cells to downregulate MMP-9 expression.²² In addition, linarin can reduce phagocytosis, inhibit pro-inflammatory cytokine production, and downregulate activation marker expression in macrophages.²³ Procyanidin B1 and neobakangelicol have rarely been covered in cancer research. Procyanidin B1 has antioxidant properties and can suppress fat accumulation^{24,25} and even inhibit the migration and proliferation of liver cancer cells.²⁶ As one of the first furocoumarins isolated from *Angelica dahuricae*,²⁷ neobakangelicol may be associated with antioxidant activity. Despite the first investigation on this compound initiated in 1999, further explorations on its effects on cancer have been in slow progress.

Table 2. Potential targets for ESCC inhibition by BO

No.	Target	Gene symbol	Entrez ID
1	Serum albumin	ALB	213
2	Carbonic anhydrase 2	CA2	760
3	Thyroid hormone receptor beta	THRB	7068
4	Transthyretin	TTR	7276
5	Estrogen receptor	ESR1	2099
6	Androgen receptor	AR	367
7	Peroxisome proliferator-activated receptor gamma	PPARG	5468
8	Cytochrome P450 19A1	CYP19A1	1588
9	Prothrombin	F2	2147
10	Wiskott-Aldrich syndrome protein	WAS	7454
11	TGF-beta receptor type-1	TGFBR1	7046
12	Tyrosine-protein phosphatase non-receptor type 11	PTPN11	5781
13	Hepatocyte growth factor receptor	MET	4233
14	Insulin-like growth factor 1 receptor	IGF1	3479
15	Cathepsin D	CTSD	1509
16	Glucosylceramidase	GBA	2629
17	TGF-beta receptor type-2	TGFBR2	7048
18	Calmodulin	CTSK	1513
19	Threonine-protein kinase	AKT1	207
20	Basic fibroblast growth factor receptor 1	FGFR1	2260
21	Catenin alpha-1	CTNNA1	1495
22	Fibroblast growth factor receptor 2	FGFR2	2263
23	Leukocyte elastase	ELANE	1991

Abbreviations: BO: *Buddleja officinalis*; ESCC: Esophageal squamous cell carcinoma.

Table 3. Top five core targets with high degree

No.	Target	Gene symbol	Degree
1	Serum albumin	ALB	17
2	Insulin-like growth factor 1 receptor	IGF1	16
3	Threonine-protein kinase	AKT1	13
4	Estrogen receptor	ESR1	12
5	Basic fibroblast growth factor receptor 1	FGFR1	9

Among the 23 PPI networks of BO-ESCC interaction, the top five based on degree were ALB, IGF1, AKT1, ESR1, and FGFR1. These proteins were considered as core proteins and could potentially contribute significantly to BO's therapeutic effect on ESCC. A low ALB level is a predictor of poor survival outcomes in patients with ESCC,²⁸ and the status of ESR1s in ESCC is closely associated with poor prognosis and may be involved in regulating the proliferation of cancer cells.²⁹

Clinically, high FGFR1 amplification serves as an independent adverse prognostic factor solely for stage I–II ESCC patients,³⁰ and fibroblast growth factor-2-mediated FGFR1 signaling is involved in the migration and survival of ESCC cells *in vitro*.³¹ The IGF1 and its receptor (insulin-like growth factor-1 receptor, IGF-1R) promote cell proliferation and inhibit apoptosis.³² The IGF-1R can activate MAPK and PI3K/AKT signaling pathways and play an essential role in various cancer types.^{33–36} The AKT is differentially expressed in various cancers and can be involved in various BPs, including cell proliferation, apoptosis, transcription, migration, invasion, and metastasis.^{37–40}

To predict the underlying mechanism of therapeutic effects of BO on ESCC, we performed GO enrichment on 23 putative targets. The top 10 targets in BP was mainly enriched in organ growth, gland development, and reproductive structure development, indicating that associated targets were ESR1, AR, TGFBR1, *etc.* In

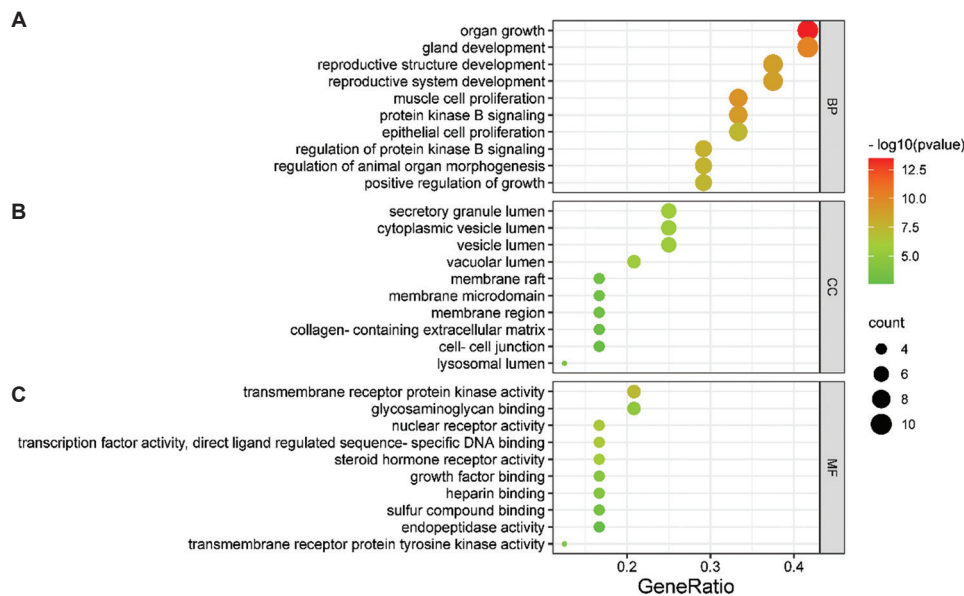


Figure 3. GO enrichment analysis of treating ESCC targets. GO analysis (including BP, CC, and MF) was visualized using the R packages “DOSE”. (A) BP analysis of treating ESCC targets. (B) CC analysis of treating ESCC targets. (C) MF analysis of treating ESCC targets. Abbreviations: ESCC: Esophageal squamous cell carcinoma; GO: Gene Ontology; BP: Biological process; CC: Cellular component; MF: Molecular function.

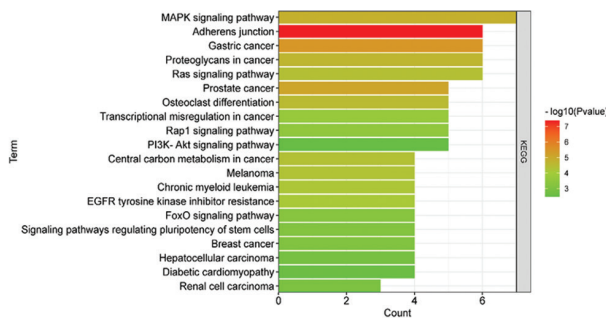


Figure 4. KEGG enrichment analysis for the signaling pathways. KEGG pathway enrichment was analyzed using the R packages “clusterProfiler”. Abbreviation: KEGG: Kyoto Encyclopedia of genes and genomes.

addition, the target enrichment of CC unveiled secretory granule lumen, cytoplasmic vesicle lumen, and vesicle lumen as the potential pathways, and revealed ALB, TTR, and IGF1 as the relevant targets. The target enrichment of MF yielded transmembrane receptor protein kinase activity and glycosaminoglycan binding, and nuclear receptor activity, and the involved targets mainly encompass TGFBR2, FGFR1, and FGFR2. Overall, these findings illuminate the complexity of ESCC pathology. To further examine the underlying mechanism by which BO acts on ESCC, we performed KEGG analysis on the targets and found that BO inhibited ESCC-related pathways, such as MAPK signaling pathway, RAS signaling pathway, and Rap1 signaling pathway.

Here, we selected the most pertinent pathway to discuss the mechanism of BO in treating ESCC. The MAPK signaling pathway is a widely prevalent cellular signaling pathway in living organisms that plays a crucial role in regulating cellular processes, *that is*, cell growth, differentiation, migration, and apoptosis. It also plays a vital role in various biological systems including neural signal transmission, inflammatory responses, and tumor development. Specifically, the MAPK signaling pathway is involved in regulating the expressions of multiple ESCC-related genes⁴¹ and includes several regulatory components, such as extracellular signal-related kinases (ERK1/2), Jun N-terminal kinases (JNK1/2/3), p38-MAPK, and ERK5.⁴² Therefore, MAPK signaling pathway is closely related to the malignant behavior of tumors and plays an indispensable role in the occurrence and development of various cancers.⁴³ For example, BO can inhibit high glucose-induced atherosclerosis by inhibiting p38, JNK, NF- κ B and MMP signaling pathways in human aortic smooth muscle cells.⁴⁴ In the study by Oh *et al.*,⁴⁵ BO inhibited the synthesis and release of NO, iNOS, and pro-inflammatory factors by blocking ERK1/2 and NF- κ B in BV-2 microglia. These findings shed light on an underlying mechanism for the anticancer effects of BO.

For the sake of further exploring the potential mechanism of BO in inhibiting ESCC, we carried out a molecular docking study on AKT1, a closely related target of ESCC, based on KEGG screening and using

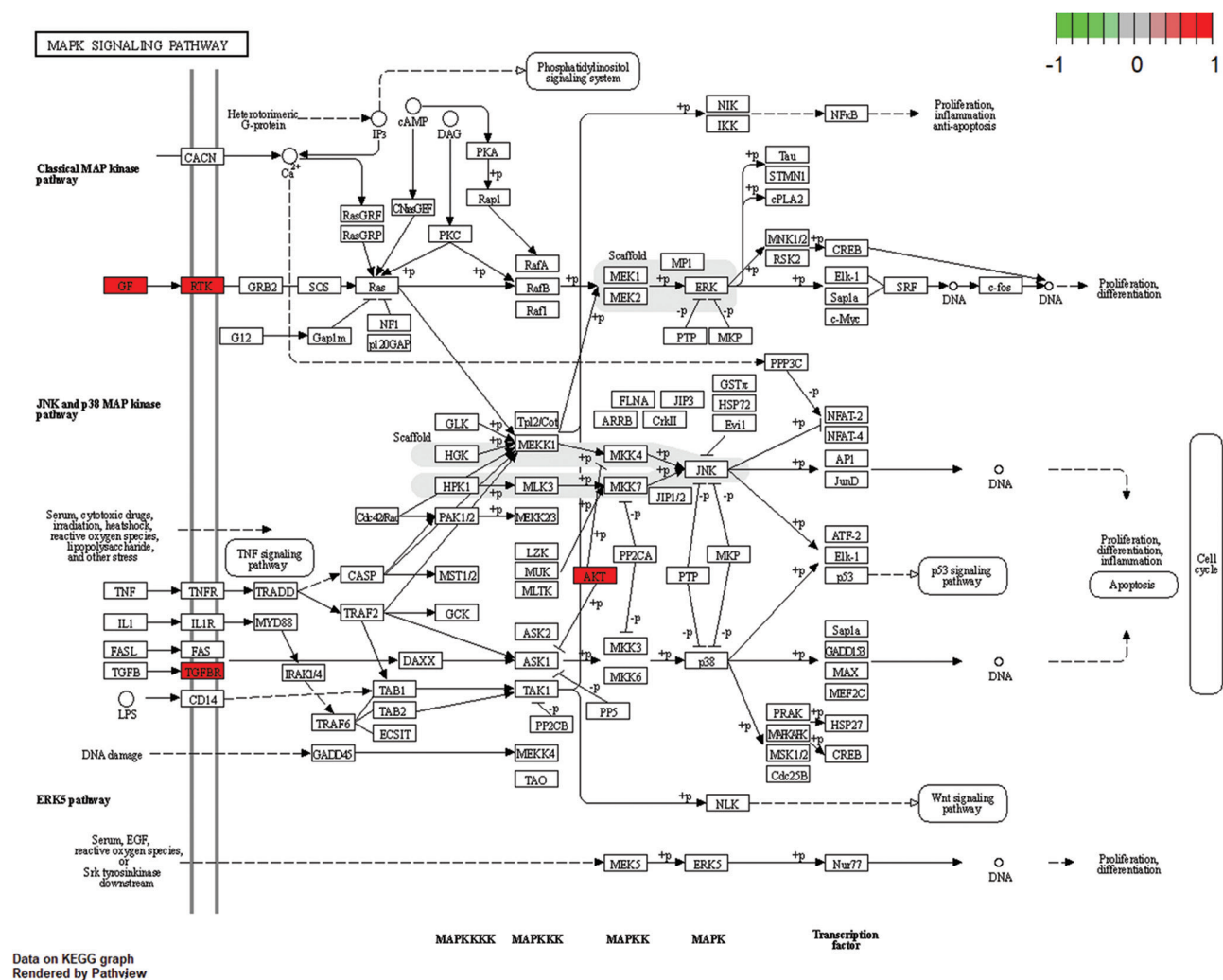


Figure 5. MAPK signaling pathway underpinning the suppression of ESCC by BO. The KEGG pathway enrichment was analyzed using the R packages “Pathview”. The arrows represent the direction of regulation in which upstream genes regulate downstream genes. Targets dyed red indicate targets in the signaling network that are regulated by BO.
Abbreviations: BO: *Buddleja officinalis*; ESCC: Esophageal squamous cell carcinoma; KEGG: Kyoto encyclopedia of genes and genomes.

Table 4. Results of docking six active ingredients in BO to AKT1

No.	ID	Ingredient	Vina scores (kcal/mol)	Center*			Size*		
				x	y	z	x	y	z
1	MOL001689	Acacetin	−6.3	22.4029	15.3988	9.9840	42.8067	39.7904	49.2565
2	MOL001790	Linarin	−8.0	16.7471	15.0165	9.1125	53.3138	42.1770	50.9995
3	MOL000006	Luteolin	−6.2	16.7471	15.0165	9.1125	53.3138	42.1770	50.9995
4	MOL006673	Butyrospermil acetate	−6.2	16.7002	15.8043	9.9840	51.0469	40.6014	49.2565
5	MOL000004	Procyanidin B1	−7.2	16.7471	15.0165	9.1125	53.3138	42.1770	50.9995
6	MOL005789	Neobakangelicol	−6.0	16.5953	15.8043	9.1125	53.0102	40.0614	50.9995

*The center coordinates of the docking pocket between the ingredients and the target AKT1. #The size of the ingredients of the pocket with AKT1 in the x, y and z directions.
Abbreviation: BO: *Buddleja officinalis*; AKT1: Threonine protein kinase.

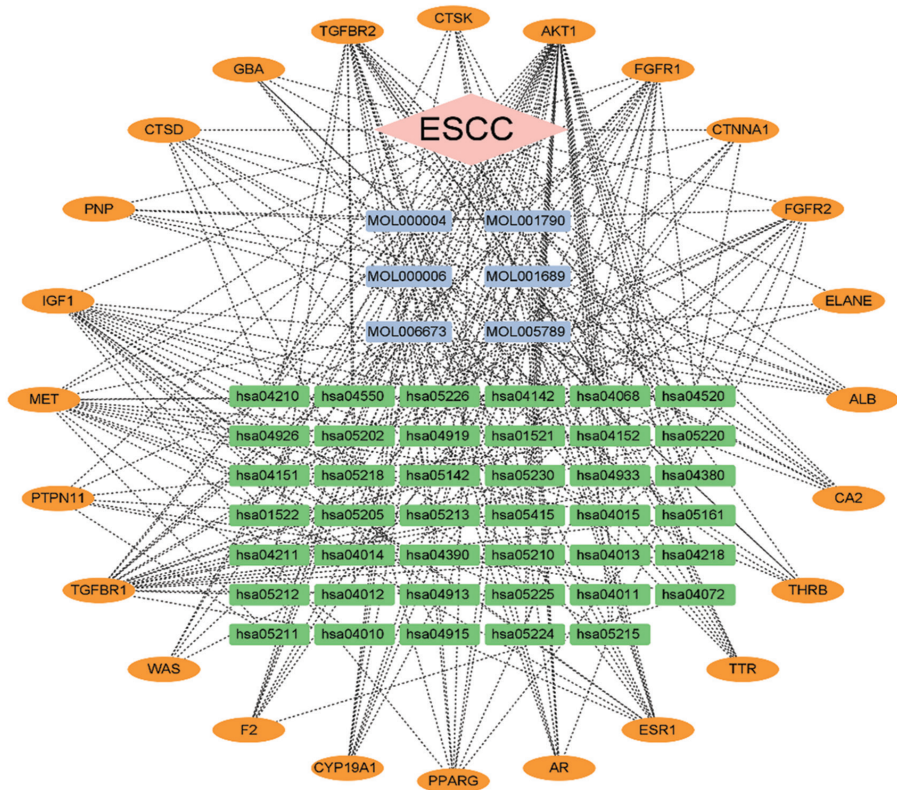


Figure 6. Compound-target-pathway network underlying the inhibition of ESCC. The map of compound-target-pathway was visualized using Cytoscape 3.9.1 software.
Abbreviation: ESCC: Esophageal squamous cell carcinoma.

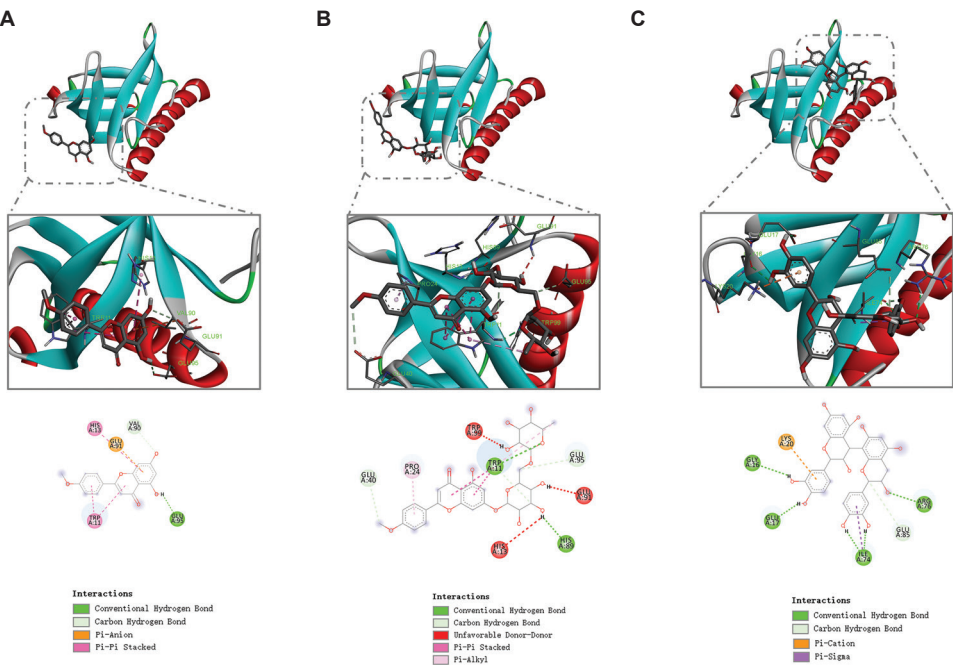


Figure 7. Three-dimensional and two-dimensional maps of the binding of acacetin, linarin, and procyanidin B1 to AKT1. AutoDock software was used to dock (A) acacetin, (B) linarin, and (C) procyanidin B1 to AKT1.
Abbreviation: AKT1: Threonine protein kinase.

key components acacetin, linarin, and procyanidin B1 as ligands. The results showed that linarin had a good binding ability to AKT1, and its interaction with AKT1 was more stable than that of acacetin or procyanidin B1. In the current study, we used a network pharmacology approach to systematically uncover the inhibitory effect of a TCM medicine on ESCC. However, several noteworthy limitations in this study are worth highlighting. For example, we did not validate target genes experimentally to eliminate irrelevant targets. Moreover, experimental validation analysis of the mechanisms was not integrated into this study; therefore, further basic or clinical studies are required to validate the molecular mechanisms by which BO inhibits ESCC.

5. Conclusion

Acacetin, linarin, luteolin, butyrospermol acetate, procyanidin B1, and neobyanangelicol are potential active components of BO. In addition, AKT1 is a potential therapeutic target of BO, and its effects on ESCC may be mediated by MAPK signaling pathway.

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Conflict of interest

The authors declare no conflicts of interest.

Author contributions

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Data analysis: Tian-Li Fan

All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

Data used in this work are available from the corresponding author on reasonable request.

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