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Identification of breast cancer molecular targets interacting with molecules present in the fruits of Antidesma bunius: In silico network pharmacology - based analysis

Identificación de dianas moleculares del cáncer de mama que interactúan con moléculas presentes en los frutos de Antidesma bunius: análisis basado en farmacología de red in silico

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ABSTRACT

The fruit of *Antidesma bunius* has both medicinal and edible properties. In previous studies, the fruit extract of *A. bunius* showed anti-proliferation activity on breast cancer cells, but its functional components and anti-tumor mechanism are still unclear. In this research, the main active components of *A. bunius* fruits (detected by UHPLC-MS/MS) and the corresponding targets were analyzed by network pharmacology method, and its interactions were verified by molecular docking to explore the possible tumor suppressor mechanisms. A total of 24 active chemical components were screened from fruits extract of *A. bunius*, and 44 targets genes were intersected with breast cancer, among them, AKT1, ESR1, EGFR, EP300, ERBB2 and AR were the top core targets. The GO enrichment of target genes mainly involved processes of cellular lipid metabolism, response to hormones, tube development, and KEGG pathway analysis centers in cancer pathways present in breast, pancreatic and non-small cell lung cancer. The flavonoids in the fruits of *A. bunius* showed strong binding to the core targets by molecular docking analysis. These results strongly suggest that the flavonoids in the fruit of *A. bunius* can inhibit proliferation of breast cancer through multiple targets, mainly by ERK and PI3K-AKT pathways.

Keywords: *Antidesma bunius, breast cancer, network pharmacology, target of action, molecular docking.*

RESUMEN

El fruto de *Antidesma bunius* tiene propiedades medicinales y comestibles. En estudios anteriores, el extracto de fruta de *A. bunius* mostró actividad antiproliferativa en las células de cáncer de mama, pero sus componentes funcionales y mecanismo antitumoral aún no están claros. En esta investigación se analizaron los principales componentes activos de los frutos de *A. bunius* (detectados por UHPLC-MS / MS) y las dianas correspondientes mediante el método de farmacología en red, y se

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verificaron sus interacciones mediante acoplamiento molecular para explorar los posibles mecanismos supresores de tumores. Se seleccionaron un total de 24 componentes químicos activos del extracto de frutas de *A. bunius*, y 44 genes diana se cruzaron con el cáncer de mama, entre ellos, AKT1, ESR1, EGFR, EP300, ERBB2 y AR fueron los principales objetivos principales. de los genes diana involucraban principalmente procesos de metabolismo de lípidos celulares, respuesta a hormonas, desarrollo de tubos y centros de análisis de la vía KEGG en las vías del cáncer presentes en el cáncer de mama, páncreas y pulmón de células no pequeñas. Los flavonoides en los frutos de *A. bunius* mostraron fuertes unión a los objetivos centrales mediante análisis de acoplamiento molecular. Estos resultados sugieren fuertemente que los flavonoides en la fruta de *A. bunius* pueden inhibir la proliferación del cáncer de mama a través de múltiples dianas, principalmente por las vías ERK y PI3K-AKT.

Palabras clave: *Antidesma bunius, cáncer de mama, farmacología de la red, objetivo de la acción, acoplamiento molecular.*

I. Introduction

Antidesma bunius, also known as five-flavored leaf, is a sour tree in China, belonging to the Euphorbiaceae family (B. Li & Hoffmann, 2008). *A. bunius* plants are native to Southeast Asia. Currently, its habitat is mainly distributed in Jiangxi, Hunan, Guangdong, Guangxi, Fujian, Guizhou, Yunnan, Tibet and Hainan in China and in tropical and subtropical regions of Asia, Africa and America. There are about 170 species of Antidesma. Among them, *A. bunius*, *A. ghaesembilla, A. montanous* and *A. pseudomicrophyllum* are considered to have medicinal properties (Wei, Gao, 2015).

After the fruit of *A. bunius* matures, the color also changes from red to dark purple. The taste is sweet and sour, and can be eaten raw, or used to prepare jam, fruit juice and fruit wine products. In traditional Chinese medicine the roots, leaves and fruits of *A. bunius* are used as medicine: to relieve coughing and diarrhea, and promoting blood circulation, as well as improving sores and malignant syphilis (Shanghai Science and Technology Press, 1999). Traditionally, an *A. bunius* tree planted in the Ming Dynasty in Guangdong is used to formulate medicinal materials to treat various diseases of the villagers (Dai, 2016).

It has been reported that the fruit of *A. bunius* contains several important molecules for our health, such as organic acids, vitamins, minerals, anthocyanins, flavonoids and phenolic acids (J. Li, 1995). Breast cancer is the malignant disease tumor with the highest incidence in women. Our previous study found that the ethanol extract of the fruit of *A. bunius* and the ethyl acetate extract of the leaves of *A. mentounes* inhibit breast cancer cell proliferation and invasion (Krongyut & Sutthanut, 2019; Zhaohui, 2011). In the present study, the main active components in the fruit of *A. bunius* were used to identify key targets to inhibit breast cancer growth through network pharmacology and molecular docking methods.

II. Materials and Methods

Screening of active components and target prediction of *A. bunius* fruit extract

Compounds of *A. bunius* fruit extract were obtained by UHPLC-MS/MS and were sorted by peak area, and the top 100 chemical components with MS2 score >0.9 were selected using PubChem (https://pubchem.ncbi.nlm.nih.gov/). The database queried its Simplified Molecular Input Line Entry System (SMILES) chemical formula, entered the stitch (http://stitch.embl.de/cgi/) database in groups, and screened the active ingredients with homology coexpression > 0.5, and developed a network map of the active ingredients of *A. bunius*. To predict the target of the compounds, the SEA (http://sea.bkslab.org/) and Chemmapper (http://lilab-ecust.cn/chemmapper/) database were used. Then the Uniprot (www.uniprot.org/) database was used to transform the target name into a standard human gene.

Breast cancer related targets prediction and protein interaction network (PPI) construction

The keyword "Breast cancer" was searched in NCBI-gene (https://www.ncbi.nlm.nih.gov/gene), Genecards (https://www.genecards.org/) and Uniprot (https://www.uniprot.org). Breast cancer (BC)-related targets were obtained and combined by setting the species as human "Homo sapiens" and with screening threshold score> 10 in Genecards.

The intersection targets of *A. bunius* active ingredients and BC were solved by Tbtools software, and the results were applied to construct the protein-protein interaction (PPI) with common targets of *A. bunius* and BC, by setting the lowest interaction threshold "highest confidence" to 0.9 in the String database (https://string-db.org/). The CytoHubba plug-in of Cytoscape software was used to rank network data nodes and screen out key target proteins.

GO biological function analysis, KEGG pathway enrichment

The intersection targets of active ingredients of *A. bunius* and BC were loaded into Webgestalt (http://www.webgestalt.org/) database for GO function enrichment (P≤0.01) and KEGG pathway enrichment to analyze the main pathways involved in inhibiting BC.

A. bunius fruit components-Disease Target-Pathway Network Construction

The key active ingredients of *A. bunius* fruit, the intersection targets of active ingredients of *A. bunius* and BC and the top 10 KEGG pathways interactions were analyzed by Cytoscape software, and use Merge function to construct a network diagram.

Molecular docking for target verification

The protein crystal structure of the key target and the 3D structure of the key active ingredients were downloaded from Uniprot (www.uniprot.org/), the PDB

(https://www.rcsb.org/) and the Pubchem websites (https://pubchem.ncbi.nlm.nih.gov/). AutoDock Vina (http://autodock.scripps.edu/) were used to molecularly dock the key targets with higher Degree values active ingredients, and finally Pymol (https://pymol .org/) software was used to visualize the molecular docking view.

III. Results

Key active compounds and targets of A.bunius

The 100 chemical components of *A. bunius* were grouped into the Stitch (http://stitch.embl.de/cgi/) website, and the compounds with a score > 0.5 were combined and screened as active ingredients. A total of 24 active ingredients were obtained as shown in Table 1. Among them, phenylpropane and polyketone compounds accounted for the highest proportions, with a total of 11 ingredients, followed by benzoic acid esters with 6 types, and organic acids and their derivatives with 4 types.

Table 1.
Active compounds of *A. bunius*. mz = mass-to-charge ratio, OB = oral bioavailability, MOL ID = Molecular identification.

No.	Molecule name	mz	Compound type	OB/%	MOL ID	Type
1	4-hydroxycinnamic acid	163.0396	Phenylpropane and polyketone compounds	43.29	MOL000771	-
2	Gallic acid	169.0134	Parabens	31.69	MOL000513	-
3	luteolin-7-O-g.	447.0927	Phenylpropane and polyketone compounds	7.29	MOL000009	-
4	3-hydroxybenzo.	137.0235	Parabens	22.19	MOL011957	-
5	fumarate	115.0031	Organic acid	29.03	MOL004211	-
6	choline	104.1071	Organic nitrogen	0.47	MOL000394	+
7	cosmosiin	431.0987	Phenylpropane and polyketone compounds	9.68	MOL000007	+
8	pyrogallol	125.0236	Parabens	22.98	MOL000106	-
9	Vanillic acid	167.0345	Parabens	35.47	MOL000114	-
10	Aspirin	179.0344	Parabens			-
11	benzoic acid	121.0287	Parabens	30.15	MOL000103	-
12	malonate	103.0028	Organic acid	21.69	MOL001738	-
13	succinic semia.	101.0236	Lipids and lipid-like molecules			-
14	naringenin	271.0606	Phenylpropane and polyketone compounds	43.29	MOL000771	-
15	quercitrin	447.0926	Phenylpropane and polyketone compounds	4.04	MOL000701	-
16	biochanin A	285.0754	Phenylpropane and polyketone compounds	25.21	MOL000510	-
17	Diosmetin	301.0697	Phenylpropane and polyketone compounds	31.14	MOL002881	+
18	gamma-amin.yri.	104.0708	Organic acids and their derivatives	24.09	MOL000388	+
19	daidzin	417.1153	Phenylpropane and polyketone compounds	14.32	MOL009720	+
20	Luteolin	285.0401	Phenylpropane and polyketone compounds	36.16	MOL000006	-
21	Kaempferol	287.055	Phenylpropane and polyketone compounds	41.88	MOL000422	+
22	Adenine	136.0616	Organic heterocyclic compound	62.81	MOL001788	-
23	betaine	118.0863	Organic acids and their derivatives	40.92	MOL000430	+
24	genistein	269.0451	Phenylpropane and polyketone compounds	17.93	MOL000481	-

From the active ingredient relationship diagram analyzed on the Stitch website, it can be seen that flavonoids (narigenin, kaempferol, luteolin and genistein) and organic acids (gallic acid, vanillic acid, benzoic acid, coumaric acid) are the ingredients centered with more interactions (Figure 1). At the center of the network, the main targets are ESR2 and ESR1. Aspirin, choline and adenine are connected to the center through PTGS1, PTGS2, and BCHE targets.

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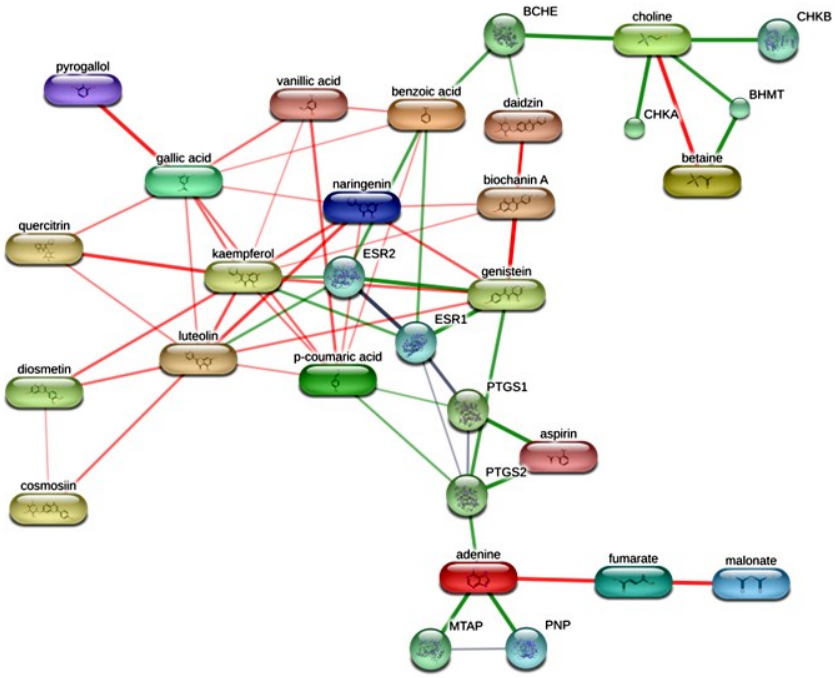

Figure 1.
Target network of effective chemical components of *A. bunius* fruit. Circles and rounded rectangles represent BC molecular targets and *A. bunius* molecules, respectively. Small nodes: protein of unknown 3D structure, large nodes: some 3D structure is known or predicted; colored nodes: query proteins and first shell of interactors. The thickness of the connecting lines indicates the level of confidence between the connected molecules.

Common targets for breast cancer and compounds

The 24 key compounds of *A. bunius* were queried in the SEA database to obtain 261 target points, and 538 target points were obtained from the Chemmapper database. After integrating the targets of the Stitch database a total of 792 targets for the fruit components of *A. bunius* were obtained.

By searching the NCBI-gene database, 4696 potential targets related to breast cancer were retrieved. The Genecards database (score>10) screened 1670 targets related to breast cancer and 1881 related targets in the Uniprot database. Using Tbtools to draw a Venn diagram with the results of the above three databases and the compound targets of *A. bunius* (Figure 2), 44 target genes of *A. bunius* in breast cancer were obtained (Table 2), including RAC-αserine/threonine RAC-alpha serine/threonine-protein kinase (AKT1), estrogen receptor (ESR1), epidermal growth factor receptor (EGFR), histone acetyltransferase (Histone acetyltransferase p300, EP300), receptor tyrosine-protein kinase erbB-2 (Receptor tyrosine-protein kinase erbB-2, ERBB2), androgen receptor (Androgen Receptor, AR) and other enzymes and factors (Table 2).

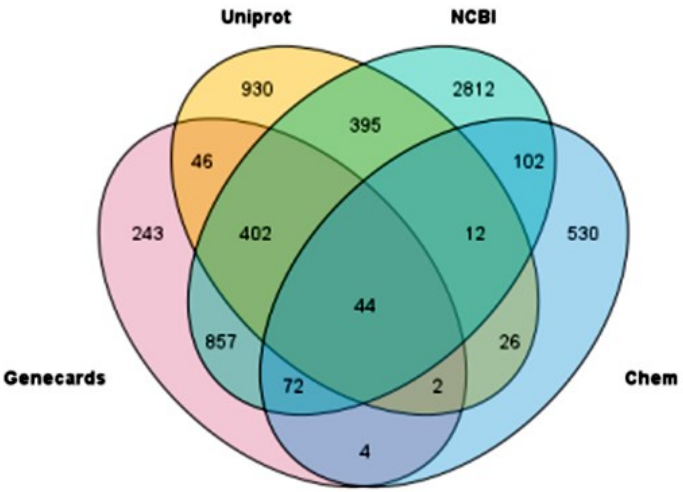

Figure 2.
Wayne map of the targets related to breast cancer. This map was obtained by TBtools software using shared targets of breast cancer from NCBI, Genecards and Uniprot database with the compound targets of *A. bunius* fruits.

https://doi.org/10.46908/tayacaja.v4i2.171

Table 2.
Common targets of effective chemical components of *A. bunius* in breast cancer.

No.	Target	Name	Family	No.	Target	Name	Family
1	AKT1	RAC-alpha serine/threonine-protein kinase	Kinase	23	ABCG2	Broad substrate specificity ATP-binding cassette transporter ABCG2	Transporter
2	ESR1	Estrogen receptor	Nuclear receptor	24	IDH1	Isocitrate dehydrogenase [NADP] cytoplasmic	Enzyme
3	EGFR	Epidermal growth factor receptor	Kinase	25	HDAC5	Histone deacetylase 5	Epigenetic
4	EP300	Histone acetyltransferase p300	Epigenetic	26	NOS2	Nitric oxide synthase, inducible	Enzyme
5	ERBB2	Receptor tyrosine-protein kinase erbB-2	Kinase	27	FABP4	Fatty acid-binding protein, adipocyte	
6	AR	Androgen receptor	Nuclear receptor	28	KLF5	Krueppel-like factor 5	Transcription factor
7	PPARG	Peroxisome proliferator-activated receptor gamma	Nuclear receptor	29	PIN1	Peptidyl-prolyl cis-trans isomerase NIMA-interacting 1	Enzyme
8	HDAC1	Histone deacetylase 1	Epigenetic	30	RET	Proto-oncogene tyrosine-protein kinase receptor Ret	Kinase
9	CDK4	Cyclin-dependent kinase 4	Kinase	31	DPP4	Dipeptidyl peptidase 4	Enzyme
10	SMAD3	Mothers against decapentaplegic homolog 3	Transcription factor	32	EDNRA	Endothelin-1 receptor	G protein coupled receptor
11	PARP1	Poly [ADP-ribose] polymerase 1	Enzyme	33	AURKB	Aurora kinase B	Kinase
12	RHOA	Transforming protein RhoA	Enzyme	34	KDM5B	Lysine-specific demethylase 5B	Transcription factor; epigenetic
13	CDK2	Cyclin-dependent kinase 2	Kinase	35	BRAF	Serine/threonine-protein kinase B-raf	Kinase
14	GSK3B	Glycogen synthase kinase-3 beta	Kinase	36	INSR	Insulin receptor	Kinase
15	STAT1	Signal transducer and activator of transcription 1-alpha/beta	Transcription factor	37	HMGB1	High mobility group protein B1	Transcription factor
16	NOS3	Nitric oxide synthase, endothelial	Enzyme	38	FOLH1	Glutamate carboxypeptidase 2	Enzyme
17	PTPRC	Receptor-type tyrosine-protein phosphatase C	Enzyme	39	RARB	Retinoic acid receptor beta	Nuclear receptor
18	AURKA	Aurora kinase A	Kinase	40	SDHD	Succinate dehydrogenase [ubiquinone] cytochrome b small subunit, mitochondrial	Enzyme
19	PRKACA	Protein kinase C alpha type	Kinase	41	GRM1	Metabotropic glutamate receptor 1	G protein coupled receptor
20	ESR2	Estrogen receptor beta	Nuclear receptor	42	EPHB4	Ephrin type-B receptor 4	Kinase
21	TOP1	DNA topoisomerase 1	Enzyme	43	CA2	Carbonic anhydrase 2	Enzyme
22	HDAC6	Histone deacetylase 6	Epigenetic	44	LEPREL1	Prolyl 3-hydroxylase 2	Enzyme

Protein interaction network (PPI)

Import 44 common targets into the String database to obtain protein interaction information (Figure 3). Cytoscape's cytoHubbs was used to select the top 10 targets as AKT1, ESR1, EGFR, EP300, ERBB2, AR, PPARG, HDAC1, SMAD3, CDK4. The Degree and Closeness values of the first 6 targets of Degree are shown in Table 3.

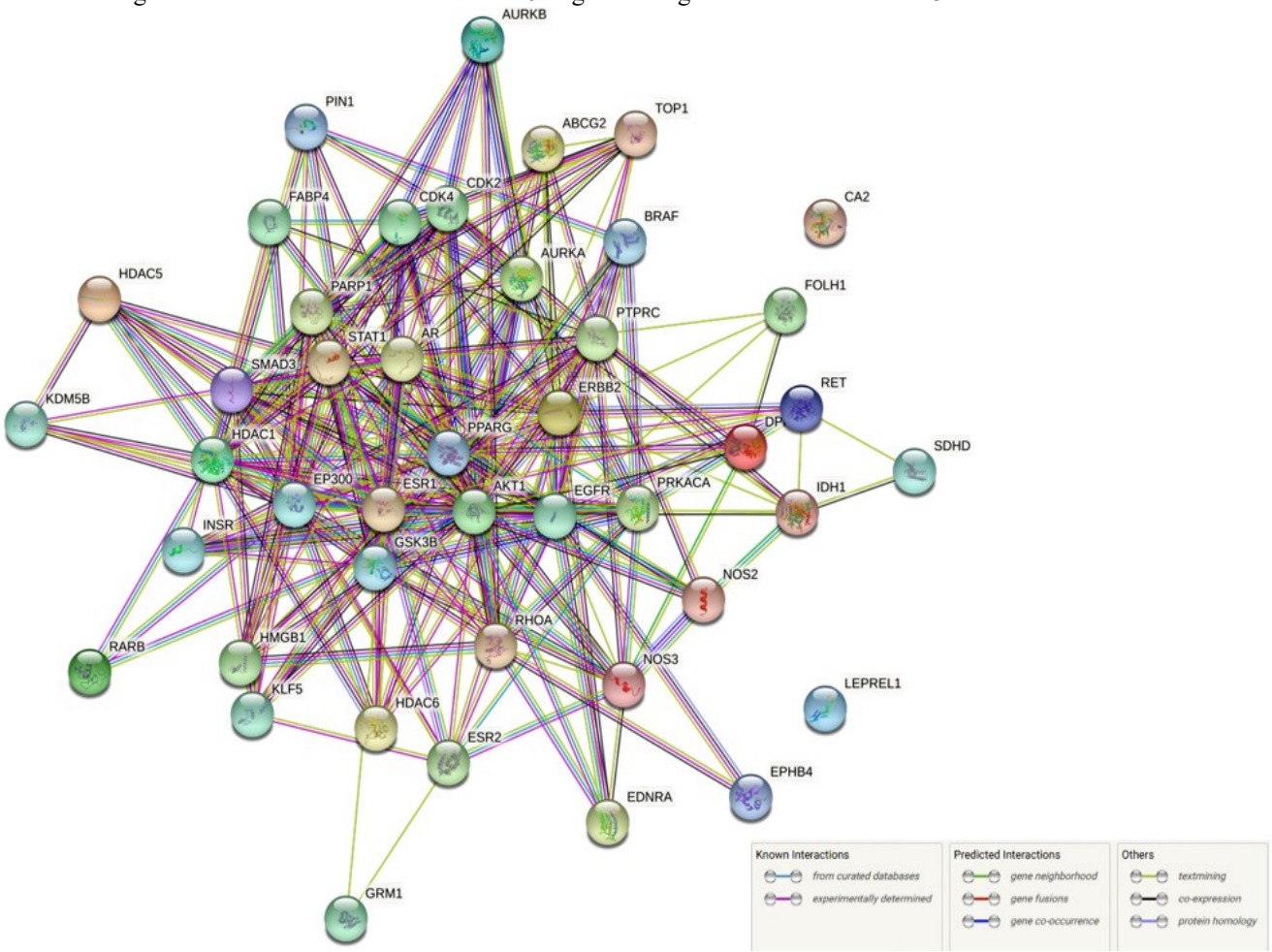

Figure 3.
PPI network of targets of *A. bunius* fruit effective chemical components in breast cancer context.

Table 3. Degree values of core targets.

No.	Target	Degree	Protein	Protein name	Protein receptor	Closeness
1	AKT1	32	P31749	RAC-alpha serine/threonine-protein kinase	6CCY	37
2	ESR1	24	P03372	Estrogen receptor	3OS8	33
3	EGFR	24	P00533	Epidermal growth factor receptor	5UGA	33
4	EP300	23	Q09472	Histone acetyltransferase p300	5XXH	32.3
5	ERBB2	23	P04626	Receptor tyrosine-protein kinase erbB-2	3PPO	32.5
6	AR	20	P10275	Androgen receptor	4OEA	30.3

GO enrichment and KEGG pathway

The key targets were enriched and analyzed on the Metascape website, and GO was enriched in biological processes such as cellular response lipid, response to hormone, and tube development. The KEGG pathway was mainly enriched in pancreatic cancer, no-small cell lung cancer, endocrine resistance, prostate cancer, hypoxia inducible factor (HIF-1) and Fork head transcription factor O (FoxO) signaling pathway, breast cancer, gastric cancer, pathways in cancer, human papillomavirus infection and other pathways.

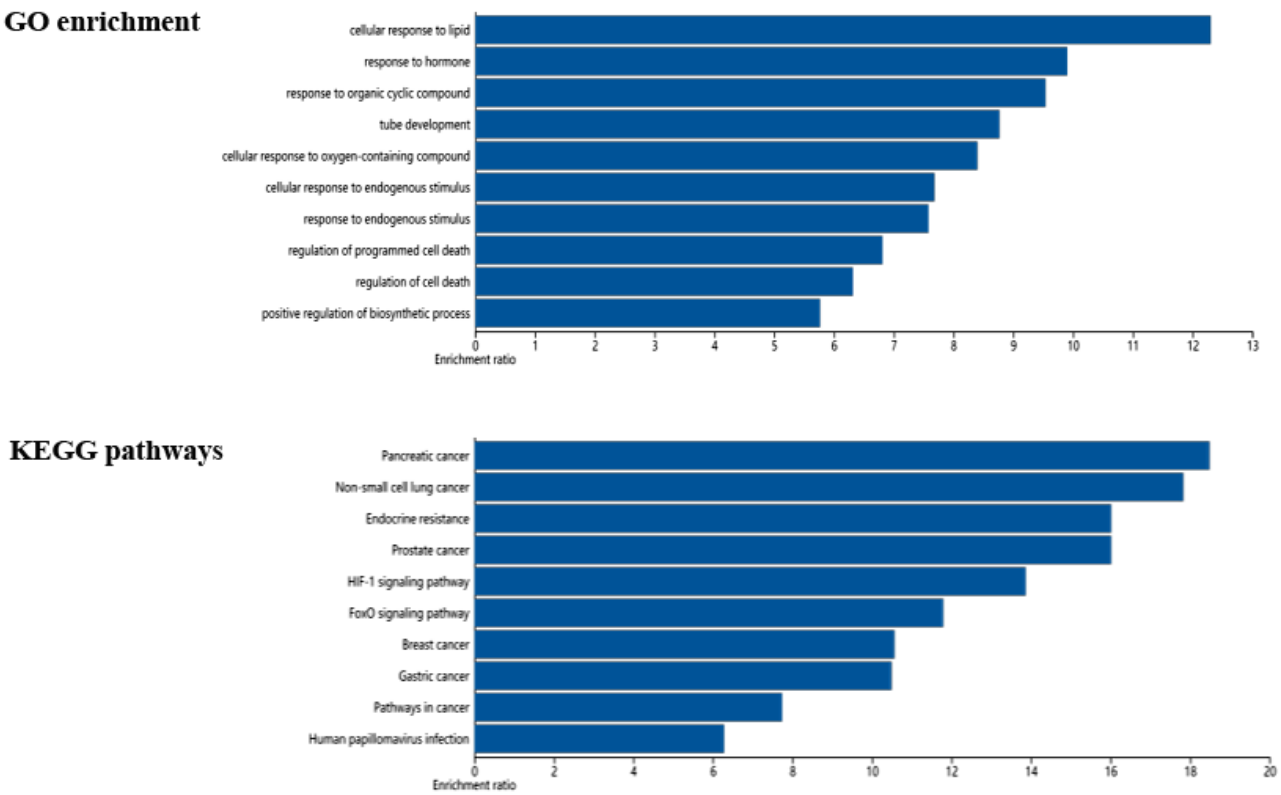

Figure 4.
Diagram of GO enrichment and KEGG pathway of *A. bunius* inhibition of BC.

KEGG network diagram of active ingredients in *A. bunius* interacting with breast cancer targets

Cytoscape software was used to design a network diagram of *A. bunius* active compounds-key targets-KEGG pathway (Figure 5). The active compounds of *A. bunius* ranked in the top 10 according to degree are kaempferol, luteolin, p-coumaric acid, gallic acid, genistein Genistein, benzoic acid, narigenin, choline, vanillic acid and quercetin. From this pathway network diagram, it can be found that the active components of *A. bunius* interact with a variety of cancer pathways, such as estrogen, thyroid hormone and HIF-1 signaling pathways. In addition to breast cancer, the active ingredients of *A. bunius* interacted with other types of cancer like prostate and pancreatic cancers

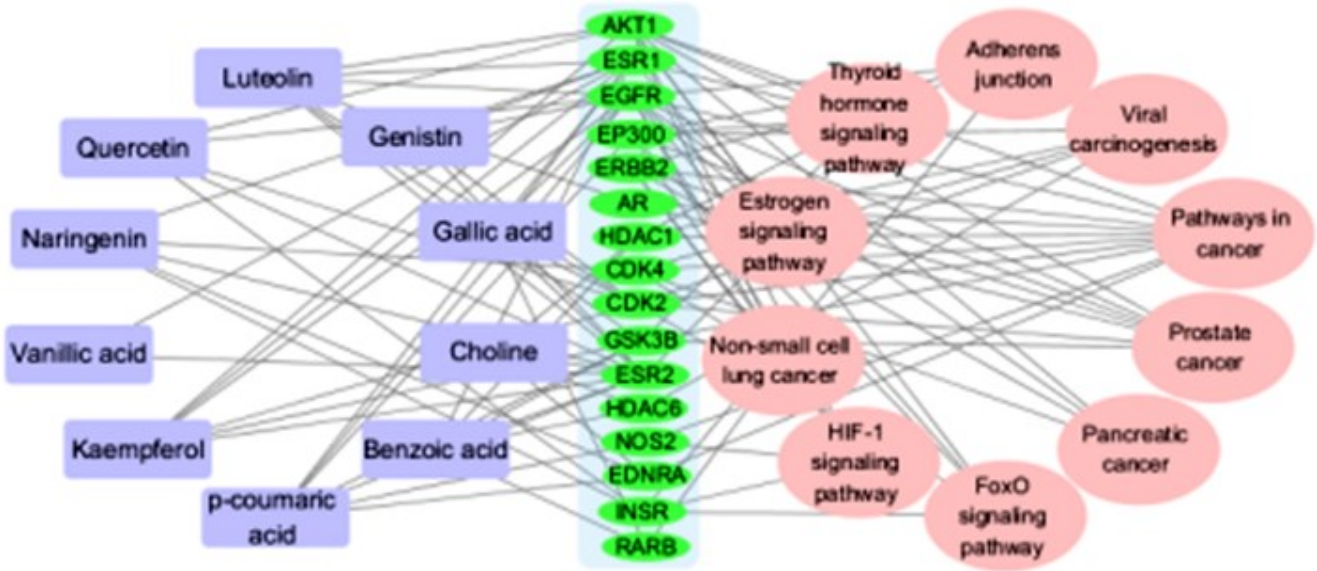

Figure 5.
Network of *A. bunius* active compounds - Breast cancer molecular key targets - KEGG pathways.

From the breast cancer KEGG pathway diagram (Figure 6), it can be seen that 4 of the 6 core targets (ER, HER, EGFR, AKT, marked in red) enrich this pathway. For luminal type A breast cancer, the estrogen receptor (ER) that acts on the cytoplasm or nucleus could affect the cell cycle regulator CCND1, thereby contributing to cell proliferation. For other types of breast cancer, the active ingredients of *A. bunius* could affect the cell cycle and block the proliferation of tumor cells by acting on the receptors EGFR, HER, ERK, and PI3K-AKT on the cell membrane.

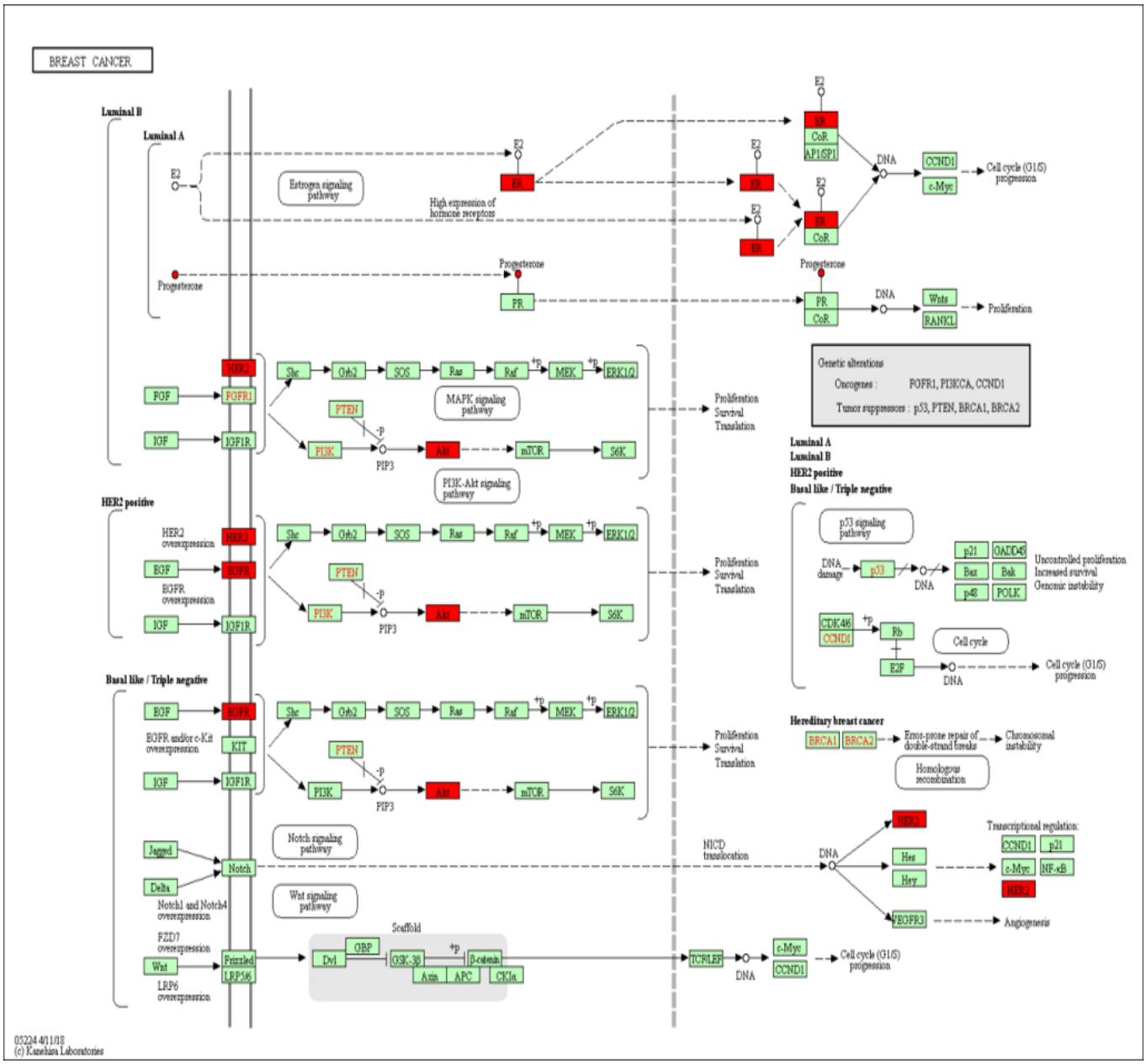

Figure 6.
Diagram of breast cancer KEGG pathway.

Molecular docking

The top 10 degrees of active ingredients of *A. bunius*, one diflavonoid (amentoflavone) and one flavonoid glycoside (luteolin 7-galactoside), and 6 core target proteins: AKT1 (6CCY), ESR1 (3OS8), EGFR (5UGA), EP300 (5XXH), ERBB2 (3PPO), AR (40EA) were selected for molecular docking. The molecular docking binding energy is based on -7kJ·mol-1 as the affinity threshold, and those smaller than this value are considered to have better binding properties (Cai et al., 2021). From the docking binding energy score, it can be seen that the score values of kaempferol, luteolin, genistein, naringenin binding to AKT1, ESR1, ERBB2, and AR are all less than -7kJ·mol-1 (Figure 7). The score values of coumaric acid, gallic acid, benzoic acid and vanillic acid are in the range of -7 to -4, and the score value of choline is greater than -4kJ·mol-1. Although amentoflavone and luteolin 7-galactoside are not among the key compounds of *A. bunius*, the score values of the binding of amentoflavone and luteolin 7-galactoside with the 6 core target proteins are all less than -7kJ·mol-1.

	AKT1	ESR1	EGFR	EP300	ERBB2	AR
Kaempfeol	-7.4	-8.2	-7.7	-6.8	-8.9	-7.2
Luteolin	-7.7	-8.6	-7.7	-6.4	-9.5	-8
Coumaric acid	-5.4	-5.9	-6	-4.8	-6.1	-6.3
Gallic acid	-5.5	-5.7	-5.5	-4.6	-6.2	-6.1
Genistin	-7.3	-8.7	-7.4	-6.6	-8.2	-8.5
Naringenin	-8	-8.1	-7.7	-6.5	-8.9	-9.1
Benzoic acid	-5.3	-5.6	-5.3	-4.4	-5.9	-6
Choline	-3.4	-3.5	-3.3	-2.9	-3.6	-3.8
Vanllic acid	-5.3	-5.6	-5.3	-4.5	-6.2	-6
Quercitrin	-7.8	-6.4	-8.5	-6.6	-7.9	-7
Amentoflavone	-10.3	-8.7	-9.9	-8.1	-9.1	-8.7
Luteolin 7-galactoside	-9.3	-8.2	-8.7	-7.1	-9.4	-8.8

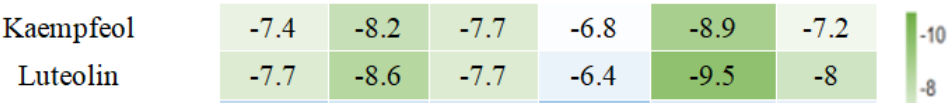

Figure 7.
Docking results of key active components of *A. bunius* with target protein. Heat maps shows molecular docking binding energy less than -7kJ·mol-1 in dark green color and above -7kJ·mol-1 in dark blue color, respectively.

From the schematic diagram of the molecular docking 3D and hydrogen bonding (Figure 8), it can be seen that several major flavonoids of *A. bunius* bind to the amino acids in the docking pocket of the key target protein through hydrogen bonding. The binding energy of genistein and ESR1 protein is -8.7kJ·mol-1 through asparagine (ASN-1705), glutamine (GLN-711) and arginine. Compared with several other key compounds of *A. bunius*, the binding energy of amentoflavone with AKT1, EGFR, and EP300 is the lowest, implying a strong binding. The chemical structures of luteolin and naringenin are very similar with only one hydroxyl difference on the B-benzene ring (Figure 9). The hydrogen bond sites of luteolin and ERBB2 are arginine, lysine and leucine. Naringenin and AR protein are combined with arginine, histidine (HIS-524) and phenylalanine (PHE-404). The docking results of flavonoids and organic acid compounds with the key target proteins further proved that the flavonoids in the fruit of *A. bunius* could be important functional components for inhibition of breast cancer growth.

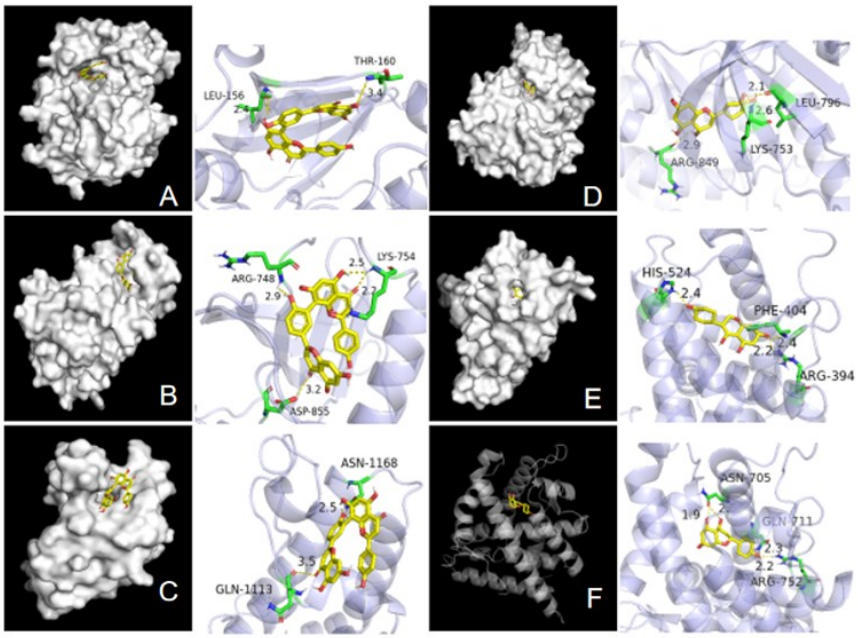

Figure 8.
Schematic diagrams of the docking and hydrogen bonding of active components of *A. bunius* with target protein A: amentoflavone-AKT1, B: amentoflavone-EGFR, C: amentoflavone-EP300, D: luteolin-ERBB, E: Naringenin-AR, F:Genistin-ESR1.

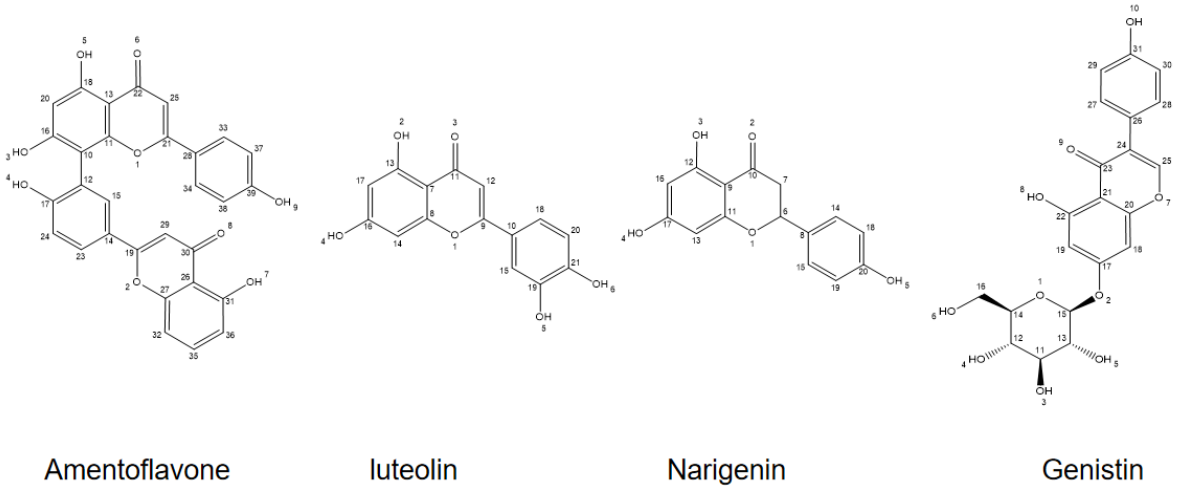

Figure 9.
The chemical structure of flavonoids present in the fruit of *A.bunius*.

IV. DISCUSSION

From the network analysis of *A. bunius* fruit components and breast cancer targets, flavonoids and organic acid compounds were identified as the main functional substances. Organic acids are the main substances that make up the sourness of fruits, and flavonoids are the largest type of polyphenols, which are involved in the production of plant pigments, the regulation of hormone activity, and the resistance to biological and abiotic stresses. Flavonoids are also an important member of many medicinal active ingredients of plants, with anti-oxidation, antibacterial, anti-tumor, hypoglycemic and other medicinal biological activities (Russo et al., 2020). The fruit of *A. bunius* is rich in polyphenols and phenolic acid compounds, especially gallic acid, epicatechin pyrocatechin and cyanidin-3-O-glucoside (Butkhup & Samappito, 2008). Previously, it has been reported that the crude methanol extract of leaves and fruits of *A. bunius* could effectively reduce the hatching rate of Artemia salina with significant cytotoxic activity (Micor et al., 2005). In addition, our group has reported that the fruit of *A. bunius* contains anti-proliferative compounds against breast cancer cells (Ma et al., 2020, 2021).

Amentoflavone is a kind of diflavonoids with a relatively high content in the total components of the fruit of *A. bunius* (Ma et al., 2020). Amentoflavone was first discovered from Selaginellae Moellendorfii (Pei et al., 2012), also discovered in *Ginkgo Folium, Ginkgo Semen, Forsythiae Fructus, Platycladi Cacumen* and other plants. Studies have shown that amentoflavone has anti-inflammatory, anti-viral, anti-oxidant, hypoglycemic and anti-tumor *biological activities (Islam et al., 2018), also showed inhibitory activity on breast* cancer cells (Cao et al., 2017). The oral bioavailability (OB%) of amentoflavone is as low as 2.95 by checking the TCMSP website (Ru et al., 2014). Although amentoflavone is not included in the key functional ingredients of *A. bunius* in the present study, its various biological activities have been confirmed by other studies that deserve more attention.

AKT kinase (RAC-αserine/threonine-protein kinase) regulates cell metabolism, proliferation and angiogenesis through a series of downstream substrates' serine or threonine phosphorylation. AKT1 may play an anti-TNBC (triple negative breast cancer) effect by acting on the PI3K-Akt signaling pathway (Jiang et al., 2020). Hormone signaling is closely related to the occurrence and development of breast cancer. Estrogen receptor (ESR) is a nuclear receptor that is involved in regulating eukaryotic gene expression and affecting cell proliferation and differentiation in target tissues. There are two types of estrogen receptors: ESR1 and ESR2 are the main targets of luminal A breast cancer (Abula et al., 2019). Epidermal growth factor receptor (EGFR) and ERBB2 (HER2) are both cell membrane receptors. EGFR is over-expressed in a variety of malignant tumors. Activating EGFR will speed up tumor reproduction and metastasis. EGFR inhibitors can block signal transduction. Receptor tyrosine protein kinase erbB-2 (ERBB2) is a cell membrane receptor encoded by the proto-oncogene erbB-2. It belongs to the receptor tyrosine kinase family and is also a member of the EGFR family including HER1 HER2, HER3 and HER4 are the basic component of the neuregulin-receptor complex, which regulates the growth and stability of peripheral microtubules. The overexpression of EGFR and ERBB2 affects the ERK pathway and the PI3K-AKt pathway in HER2-positive and TNBC breast cancer (Park et al., 2020). Histone acetyltransferase P300 (EP300) is a nuclear receptor and a co-activator of HIF1A (hypoxia inducible factor 1α). It mainly acts on the vascular endothelial growth factor (VEGF) signaling pathway affect vascular proliferation (H. Li et al., 2020). Androgen receptor (AR) belongs to the steroid hormone receptor, which affects cell proliferation by interacting with the downstream prostate specific antigen PSA (prostate specific antigen) (Hickey et al., 2021). The flavonoids in the fruit of *A. bunius* have a strong combination with core targets such as AKT1, ESR1, EGFR, EP300, ERBB2, and AR, further verifying the anti-cancer activity of *A. bunius.*

In this study, network pharmacology and molecular

docking analysis were performed on the active ingredients of *A. bunius* fruit and the targets of breast cancer, and the main active ingredients and key targets were comprehensively screened. Flavonoids of *A. bunius* fruit could act on multiple relevant signaling pathways in breast cancer. Therefore, fruit of *A. bunius* has the potential for developing anti-cancer functional foods or nutraceuticals for adjuvant cancer treatment.

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