## RESEARCH

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### Abstract

**Aim** To explore, using network pharmacology and RNA-seq technologies, potential active targets and mechanisms underpinning *Radix Bupleuri's* effectiveness during sepsis treatment.

**Methods** Following the Sepsis-3.0 criteria, the research cohort, comprising 23 sepsis patients and 10 healthy participants, was obtained from public databases. Peripheral blood samples were collected and subjected to RNA-seq analysis. Active ingredients and potential targets of *Radix Bupleuri* were identified using the Bioinformatics Analysis Tool for Molecular mechANism of Traditional Chinese Medicine 2.0 (BATMAN-TCM 2.0) database and TCMSP database. Subsequently, protein-protein interaction (PPI) network construction, Gene Ontology (GO) analysis, and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were conducted to explore cross-targets between disease and drugs. Survival analysis of key targets was performed using the GSE65682 dataset, and single-cell RNA-seq was employed for cellular localization analysis of key genes. Finally, molecular docking and Molecular dynamics simulation of the core target was conducted.

**Results** Differential expression analysis revealed 4253 genes associated with sepsis. Seventy-six active components and 1030 potential targets of *Radix Bupleuri* were identified. PPI, GO, and pathway enrichment analyses indicated involvement in the regulation of transmembrane transport, monatomic ion transport, and MAPK signaling. Survival curve analysis identified *PIK3CD*, *ARRB2*, *SUCLG1*, and *SPI1* as key targets associated with lower mortality in the high expression group, while higher mortality was observed in the high *PNP* and *FURIN* expression groups. Single-cell RNA sequencing unveiled the cellular localization of PIK3CD, PNP, SPI1, and FURIN within macrophages, while ARRB2 and SUCLG1 exhibited localization in both macrophages and T-cells. Subsequent molecular docking and Molecular dynamics simulation indicated a potential binding interaction for Carvone-PIK3CD, Encecalin-ARRB2, Lauric Acid-SUCLG1, Pulegone-FURIN, Nootkatone-SPI1, and Saikogenin F-PNP.

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**Conclusion** *Radix Bupleuri* could modulate immune function by affecting PIK3CD, ARRB2, SUCLG1, FURIN, SPI1, and PNP, thereby potentially improving the prognosis of sepsis.

Keywords Radix Bupleuri, Network pharmacology, RNA-seq, Molecular docking, Sepsis

#### Introduction

Sepsis represents a life-threatening organ dysfunction resulting from a dysregulated host response to infection [1]. Its intricate development involves complex pathophysiological mechanisms, including inflammatory imbalance, immune system dysfunction, mitochondrial impairment, coagulopathy, abnormal neuroendocrine immune network, endoplasmic reticulum stress, and autophagy [2]. Currently, specific and effective treatments for sepsis are lacking. Nevertheless, research on Traditional Chinese Medicine (TCM) in sepsis treatment has shown promising results, indicating that TCM plays a significant role in reducing mortality, inflammatory indicators, and coagulation indicators in sepsis patients [3]. Radix Bupleuri, the main component of Baidu Powder, has been employed in TCM for millennia [4], demonstrating various biological activities, including hepatoprotective, neuroprotective, antiviral, antibacterial, antipyretic, anti-inflammatory, and immunomodulatory effects [5]. Notable active ingredients in Radix Bupleuri include Carvone, Encecalin, and Lauric Acid, among others.

The emerging field of network pharmacology involves constructing multi-layered networks encompassing disease-phenotype-gene-drug interactions. This holistic approach aids in predicting drug targets and enhancing drug discovery efficiency [6]. The " multiple targets and multiple pathways" advantage of network pharmacology provides a framework for exploring the mechanism of action of traditional Chinese medicines [7]. Given the unclear underlying mechanisms of *Radix Bupleuri* in improving sepsis prognosis, this study aims to elucidate potential targets and mechanisms using network pharmacology, RNA-seq technology, and extensive public databases.The flow chart of the study is shown in Fig. 1.

#### Methods

#### Data source

The raw sepsis data (Data No.: CNP0002611) was obtained from the China National GeneBank Data-Base (CNGBdb) (https://db.cngb.org/search/project/ CNP0002611/). In the Emergency Intensive Care Unit of Southwest Medical University Hospital, venous blood samples were taken from 23 patients who were inpatients due to sepsis between February 2019 and December 2020. Additionally, venous blood samples from 10 healthy participants served as the control group. This dataset adheres to the sepsis-3.0 criteria (infection+ $\Delta$ SOFA score $\geq$ 2) jointly published by the Society of Critical Care Medicine (SCCM) and the Intensive Care Medicine Society of Europe (ESICM) in 2016. GSE65682 and prognostic data were downloaded from the Gene Expression Omnibus (GEO) public database for survival analysis. This study followed the Declaration of Helsinki and was approved by the Ethics Committee of the Affiliated Hospital of Southwest Medical University (ky2018029),Clinical Trial No: ChiCTR1900021261, Date of Registration:4 February 2019.

#### Screening for differentially expressed RNA

Using the online tool iDEP96ADDIN (http://bioinformatics.sdstate.edu/idep96/) [8], a rigorous data quality control process was implemented. Boxplots were employed to verify dataset comparability and reliability. Principal component analysis (PCA) identified and excluded outlier samples. Differential expression analysis, using the DEseq2 method, involved a minimal fold change (FC) of 2 and a false discovery rate (FDR) below 0.05.

#### Selecting active ingredients and targets of Radix Bupleuri

The Bioinformatics Analysis Tool for Molecular mechA-Nism of Traditional Chinese Medicine 2.0 (BATMAN-TCM 2.0) database [9] (http://bionet.ncpsb.org.cn/ batman-tcm/index.php/) was utilized to identify active components and potential targets of *Radix Bupleuri*, screening for a component score greater than 20 and a p-value less than 0.05. In addition, the identification of active ingredients and potential targets of *Radix Bupleuri* from the TCMSP database according to Lipinski rules [8]. Subsequently, after removing the duplicate targets of *Radix Bupleuri* and sepsis, intersection genes for diseases and drugs were obtained. A Venn plot (http://www.liuxiaoyuyuan.cn/) was generated to illustrate the intersected genes.

#### Protein-protein interaction (PPI) analysis

PPI networks enhance our comprehension of protein interactions within cells, revealing mechanisms and modes of regulation. The Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database (https:// www.string-db.org), built on public databases and literature [10], was utilized for PPI analysis. The organism selected was "homo", and the minimum interaction value was set to 0.7, with concealed disconnected nodes. The resulting intersection genes were imported into the web platform to construct PPI networks.



Fig. 1 Flowchart of this study. This figure illustrates the study's workflow, employing network pharmacology and RNA-seq technology to explore *Radix Bupleuri's* potential active targets and mechanisms in the context of sepsis treatment

# Construction of "active ingredient - target - disease" network

To further elucidate the mechanism of *Radix Bupleuri* in treating sepsis, network maps of active ingredient targets for sepsis treatment were constructed using Cytoscape 3.7.2. Freedom analysis was performed using its plugin Network Analyzer. The node size in the network was positively correlated with degrees of freedom, and more connections of nodes indicated higher degree values.

#### **Functional enrichment analysis**

Metascape (http://metascape.org/) [11] was employed as a gene function annotation analysis tool for Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis. GO annotation was divided into three categories: molecular function, cellular component, and biological process. KEGG pathway information for different species was also included. Screening was performed with a significance level of  $P \le 0.01$ , and critical gene-disease-signaling pathway networks were constructed with Cytoscape 3.7.2 for visualization.

#### Survival curve

Survival curve analysis was employed to assess the clinical significance of key targets, exploring their potential prognostic value. The public dataset GSE65682 [12], containing peripheral blood samples from 478 sepsis patients, along with gene expression profiling and clinical 28-day outcome data, was downloaded. Gene expression values were ranked from high to low, dividing the cohort into a low-expression group (n=293) and a high-expression group. The log-rank test was used for statistical analysis, and p-values of less than 0.05 were regarded as statistically significant.

#### Single-cell sequencing

To further elucidate the cell lineage localization of key genes in peripheral blood mononuclear cells (PBMCs), Five peripheral blood mononuclear cells (PBMCs) were obtained for 10× single-cell RNA sequencing from two healthy participants, one patient with systemic inflammatory response syndrome, and two with sepsis. Data quality control was performed using the Cell Ranger online platform, integrating the Spliced Transcripts Alignment to a Reference (STAR) software for data comparison with the reference genome. Single-cell transcriptome sequencing provided absolute values for each transcribed molecule within a single cell using Unique Molecular Identifiers (UMIs) and cell barcodes. Dimensionality reduction techniques, including the Mutual Nearest Neighbors (MNN) algorithm with the t-distributed Stochastic Neighbor Embedding (t-SNE) technique, were applied. t-SNE was used to display the MNN-based downscaling results and determine the ideal cell population [13].

#### Molecular docking

Critical proteins identified through the "active ingredient-target-disease" network, PPI analysis, and survival curve analysis were selected for molecular docking with their corresponding active components. An adapted version of the protein-ligand docking software, Autodock Vina 1.2.2, was used to evaluate binding energies and interaction sequences. The three-dimensional conformation of the drug or small molecule was retrieved from the PubChem database [14] (https://pubchem.ncbi.nlm.nih. gov/), while the Protein Data Bank (PDB) database [15] (https://www.rcsb.org/) was used to get protein structures. Both protein and ligand files were prepared and converted to PDBQT format, involving the removal of water molecules and the introduction of polar hydrogen atoms. Each protein's domain was enclosed by a grid box, allowing free movement of the molecule. The docking pocket, characterized by a 30 A  $\times$  30 A  $\times$  30 A square pocket with grid points spaced 0.05 nm apart, facilitated molecular docking studies using Autodock Vina 1.2.2 (http://autodock.scripps.edu/) to determine the binding energy.

#### Molecular dynamics simulation

The molecular dynamics simulations were performed using AMBER 20 software [16]. Prior to the simulation, the system was subjected to energy optimization, including the steepest descent method with 2500 steps and the conjugate gradient method with 2500 steps. After the system energy optimization was completed, the system was warmed using 200 ps at a fixed volume and constant rate of warming to slowly increase the system temperature from 0 K to 298.15 K. The NVT (isothermal isobaric) system synthesis simulation was carried out for 500 ps to further distribute the solvent molecules homogeneously in the solvent box at a maintained system temperature of 298.15 K. The system was then simulated using the NVT (isothermal isobaric) system synthesis simulation for 500 ps to further homogeneously distribute the solvent molecules in the solvent box. Finally, the NPT (isothermal isobaric) case was performed for 500 ps of equilibrium simulation for the whole system. Finally, the composite system was subjected to NPT (isothermal isobaric) tethered simulations for 100 ns under periodic boundedness conditions. For the simulations, the non-bond truncation distance was set to 10 Å. The Particle mesh Ewald (PME) method was used to calculate long-range electrostatic interactions [17], the SHAKE method was used for the limitation of the bond lengths of the hydrogen atoms, and Langevin's algorithm was used for the temperature control [18]. The collision frequency  $\gamma$  was set to 2 ps<sup>-1</sup>. The system pressure was 1 atm, and the integration step was 2 fs, with trajectories saved at intervals of 10 ps were used to save trajectories for subsequent analysis.

#### Results

#### **Differential screening results**

Figure 1 depicts the study's flow chart. Boxplots, volcano plots, and PCA were performed to ensure the reliability and comparability of the dataset. In Fig. 2A-C, we identified 4501 differentially expressed genes, with 2447 RNAs highly expressed and 2054 RNAs lowly expressed in the sepsis group. After removing duplicate and irregular genes, a set of 4253 disease-related genes was obtained.

## Exploration of active ingredients and targets of *Radix Bupleuri*

We extracted 67 active ingredients of *Radix Bupleuri* and 938 potential targets from the BATMAN-TCM 2.0 database and 38 active ingredients and 143 potential targets



Fig. 2 Performing data quality control, screening differentially expressed genes as well as intersection genes. A: The box plots demonstrate uniform data distribution across each sample, ensuring comparability. B: PCA reveals significant distinctions between the experimental and control groups, excluding outlier samples. C: The volcano plot illustrates downregulated (blue) and up-regulated (red) genes. D: Blue represents 1030 drug targets, yellow represents 4253 disease targets, and the central area consists of 309 intersecting genes

of *Radix Bupleuri* from the TCMSP database. After deduplication, a total of 76 active components and 1030 potential targets of *Radix Bupleuri* were identified. A Venn diagram (Fig. 2D) was generated to visualize the overlap between 4253 sepsis targets and 1030 targets associated with the active ingredients of *Radix Bupleuri*, yielding 309 intersected genes. Table 1 depicts these intersected genes along with their corresponding active components and properties.

# Construction of "active ingredient - target – disease" network

A network with 309 cross-targets and drug components was generated (Fig. 3) using Cytoscape 3.7.2. Circular nodes represent cross-targets, square nodes represent active pharmaceutical ingredients, and inverted triangle nodes represent diseases. Lines indicate interactions of components with targets. Degrees of freedom analysis using the Network Analyzer plugin revealed that Carvone and Nootkatone exhibited higher values than others among the active compounds, suggesting their potential importance in sepsis treatment.

### Table 1 Active ingredients and partial target of Radix Bupleurum

Alpha-Linolenic Add         SLRM         SLRM <thslrm< th=""> <thslrm< th="">         SLRM<th>Ingredients</th><th>Molecule formula</th><th>Molecule weight</th><th>Target</th></thslrm<></thslrm<>	Ingredients	Molecule formula	Molecule weight	Target
CRM GG         CGG           Menthyl Acetate         C3H602         74.08         MRR AR           Salkoaponin         C42/180013         781         GBAN           Tetradecane         C14B3         198.39         CAT           Merthyl Anethe         C1719402         27045         MPR           Bethyl Palmitate         C1719402         27045         MRD           B-Nonenoic Acid         C1719402         27043         APON2.183           Li         C4         MPR         MPR           B-Nonenoic Acid         C3H800         120.15         APON2.185           Catopic Acid         C41800         120.15         APON2.185           Catopic Acid         C3H800         144.25         CARSSOMIN           Nethonal         C3186024         144.25         CARSSOMIN           Catopic Acid         Callifo.2         144.25         CARSSOMIN           Catopic Acid         Callifo.2         144.25         CARSSOMIN           Catopic Acid         Callifo.2         144.21         CHRM CHRMA           Salkopopin V         CS3H86024         11072         HDAC TGAL           D-Imonene         C10416         230.48604         CARSI         CDNN	Alpha-Linolenic Acid	C18H30O2	278.4	SLC8A1
CR8         CR8         CR8           Salkotaponin         24062013         781         CIA8.3           Salkotaponin         C14180         781         CIA8.3           Salkotaponin         C14180         781         CIA8.3           Salkotaponin         C14180         98.39         CIA8.3           Salkotaponin         C14180         98.39         MP           Methy Palmitate         C1719402         270.45         MPRITYR           Selvoneic Acid         C184800         200.5         CIA8.3           Nonanol         C184800         201.5         CIA8.3           Salkotaponin V         C184800         201.5         CIA8.3           Cayophyllene         C19120         144.21         CIA8.3           Cayophyllene         C1914         201.5         CIA8.1           Cayophyllene         C1914         160.2         MPR           Cayophyllene         C1914         160.2         MPR           Cayophyllene         C19140         160.2         MPR           Cayophyllene         C19140         160.2         MPR           Cayophyllene         C19140         20.4         MPR           Cayophylene         C191400 </td <td></td> <td></td> <td></td> <td>CKM</td>				CKM
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Sakosponin     C12408013     781     CRB33 CARPB3       Tetradecane     C14430     98.39     CRB33       Wethy Flaminite     C1743002     770.45     NRE 178       Abonencic Acid     C1743002     770.45     NRE 178       Boloncic Acid     C1813002     726.43     NRE 178       Boloncic Acid     CB18002     156.22     CB       Bata Elemene     CB18002     120.15     CABERG 20KT       Nohomanol     C61900     144.21     CABERG 20KT       Caryophyllene     C19170     144.21     CHMU 104M22       Sakosponin V     CB180024     130.22     CRB100       Octamolc Acid     CB1100     136.23     CRB100/CTMPM22       Sakosponin V     CB1100     136.23     CRMI 104/CT       Dumonene     C10116     136.23     CRMI 104/CT       Putsatilic Acid     CG1116     136.23     CRMI 104/CT       Sakosponin V     CS111006     286.24     CMI 104/CT       Sakosponin V     CS111006     286.24     CRMI 104/CT       Sakosponin V     CS111006     286.24     CRMI 104/CT       Sakosponin C     CI311006     286.34     CRMI 104/CT       Sakosponin C     CI311006     286.34     CRMI 104/CT       Cabehin     CO	Menthyl Acetate	C3H6O2	/4.08	NPR1
Antioapidum         Christoria         Pail         Christoria           Tetradecane         Cl4H30         198.39         CAT           Wethyl Falmitate         Cl/H3402         2/045         MPB TYR           8-Noneroic Acid         Cl/H3402         2/045         AP0A2 INS           11         Linolenic Acid         Cl/H3002         2/84.3         AP0A2 INS           8-Noneroic Acid         Cl/H3002         2/84.3         AP0A2 INS           8-therene         Cl/H3002         2/84.3         AP0A2 INS           8-therene         Cl/H3002         2/84.3         AP0A2 INS           8-therene         Cl/H3002         120.15         DA21P CAT           Nonanol         Cl/H302         144.25         CARR53 DMTN           Octanoic Acid         Cl/H302         144.21         CH/M104 PME           Octanoic Acid         Cl/H105         130.23         MBC 2 (FGL           Pulmorene         Cl/H116         130.23         MBC 2 (FGL           Pulmorene         Cl/H116         130.23         MBC 2 (FGL           Pulmorene         Cl/H116         130.23         MBC 2 (FGL           Pulmorene         Cl/H11603         730.27         RMA53 YMHAF           Sainfur	Saikacapanin	C42U69O12	701	AK
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London         Lindon         Name         Main           Methyl Palmitate         C17H3402         270.45         NPR1 TYR           Bronenoic Add         C9H1602         155.22         APOA2 L13           Lin         Lin         Lin         APOA2 L13           Lindenic Add         C18H3002         278.43         APOA2 NS           Mark         C18H3002         120.15         DABJP CAT           N=Nonanol         C19H20         144.25         CABRG3 DMTN           Garyophyllene         C19H20         144.21         CHMI CHM2           Octanoic Add         C8H1602         144.21         CHMI CHM2           Sakosponin V         C3H80024         1107.2         HOAC2 (CAL           D-Linonene         C10116         136.23         EDNI           Pentanol         CGH1405         286.28         SREBT POR           PTTR2         CARA         CARA         CARA           Sahfuran         C14H1603         232.27         NNA63 WM4E           CALCA         CSH1006         891.5         CARA           Stearin         C14H1603         232.27         NA63 SWM4E           CALCA         CSH1006         891.5         CARA	Tetradecane	C14H30	198 39	CAT
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Linolenic AcidC18H30022/843APOA2 INS APPBeta-ElemeneC8H80120.15DA82/P CATNNonanolC9H200144.25GABRG3 DMTN CaryophylleneC18H1602144.21Octanoic AcidC8H1602144.21CHRM1 CHRM2 Sakosaporin VHDAC2 ITGALD-LimoneneC19H16136.23HDAC2 ITGALPentanolC3H1860241107.2HDAC2 ITGALD-LimoneneC10H1636.23HDN1Pulsatilic AcidC3H4604470.7PTGS1 COX1PrifscrC16H1405286.28SREEPI PORPrifscrC16H1405230.27RLASES YWHAESainfuranC16H1403232.27RLASES YWHAECaccalinC14H1603232.27RLASES YWHAECaccalinC14H1603230.27RLASES YWHAECatcalinC3H1006S86.94KFI 4 PRKCDCatcalinC3H1006S86.94KFI 4 PRKCDCatcalinC14H1603291.5NR2Lauric AcidC14H202200.32COX411 COX411StearinC14H202200.32COX411 COX411StearinC14H2006356.37TD2PA TUBBCapylic AcidC14H200S61.37TD2PA TUBBCapylic AcidC14H200S61.44S10.44Capylic AcidC14H202248.5COX411 COX411Capylic AcidC14H202S61.87TD2PA TUBBCapylic AcidC14H202S61.87TD2PA TUBBCapylic AcidC14H202S61.86PDAT11<				IL1
AMP         AMP           Ni-Nonanol         C8H80         120.15         D&82/I CAT           Ni-Nonanol         C9H200         144.25         GABRG3 DMTN           Canyophyllene         C15H24         204.35         MIP           Octanoic Acid         C8H1602         144.21         CHRM1 CHRM2           Sakosaponin V         C5H80224         1107.2         HDAC2TIGAL           D-Limonene         C10H16         136.23         NB3C2 IGF1           Pottanol         C3H4604         470.7         PTGEN           Putastilic Acid         C30H4604         470.7         PTGEN           Sainfuran         C16H1405         286.28         SREBF1 POR           PTGER         CTATO         PTGEN         PTGEN           Sainfuran         C14H1603         232.27         CALC           CALC         CALC         CALC         CALC           Stearin         C36H5806         S86.94         KF14 PRKCD           CH404         C12H1006         891.5         MRAS 23/WHAE           CALC         CALC         CALC         CALC           Stearin         CS/H1006         891.5         MRA           Stearin         CALC         CALC <td>Linolenic Acid</td> <td>C18H30O2</td> <td>278.43</td> <td>APOA2 INS</td>	Linolenic Acid	C18H30O2	278.43	APOA2 INS
Beta-Filemene         Lahabo         12.01         DASA/P CAT           Nonanal         G9H200         144.25         GABG3 DMTN           Caryophyllene         C15H24         204.35         MP           Octanoic Acid         G8H1602         144.21         CHRMI CHRMI CR           Saikosaponin V         C33H86024         1107.2         H0AC2 (TGAL           D-Limonene         C10H16         136.23         EDN1           Pentanol         C10H16         136.23         EDN1           Pentanol         C30H4604         470.7         PTGS1 COX1           Prissi COX1         PTGS1 COX1         PTGS1 COX1         PTGS1 COX1           Prissi COX1         PTGS1 COX1         PTGS1 COX1         PTGS1 COX1           Prissi COX1         C14H1603         232.27         RNASE31 WHAE           Caccalin         C14H1603         232.27         RNASE31 WHAE           Cacla Cac         Stearin         CACCA         RASE3           Stearin         C57H11006         891.5         AR           Lauric Acid         C14H202         20.32         COX4II COX4II COX4II COX4II SCNA           Cubebin         Cacla         Stearic Acid         Stearic Acid         Stearic Acid         Stearic Acid		60110.0	100.15	AVP
NNN0100         CM200         M42.5         GABRAS UNIN           Caryophyllene         204.35         MP           Octanoic Acid         C8H1602         144.21         CHRM1 CHRM2           Salkosaponin V         C53H86024         1107.2         HAC2 ITGAL           D-Limonene         C10H16         136.23         NB32 (GF1 EDN1           Pentanol         C3H4004         470.7         PTGS1 C0X1           Putsatilic Acid         C3H4004         470.7         PTGS1 C0X1           Salinfuran         C16H1405         286.28         SPEBF1 P0R           PTRA2         CALFA         SPEBF1 P0R         PTRA2           Encecalin         C14H1603         232.27         RNASE3 YWHAE           CALCA         CALCA         CALCA         CALCA           Stearin         C15H1006         891.5         NPR2           Lauric Acid         C12H2402         20.32         COX4II CXX4II SCMAA           Casto Acid         C491000         284.5         CC2S1 CXX4II SCMAA           Casto Acid         C4910200         356.37         T0P2A TUBB           Capylic Acid         C4910200         356.37         C0X1I CXX4II SCMAA           Capylic Acid         C4910200         36	Beta-Elemene	C8H8O	120.15	DAB2IP CAI
Caryophylene         C151/24         204.35         ONT MIP           Octanoic Acid         ORH ICO2         144.21         CHRMI CHRM2           Saikosaponin V         OSH66024         1107.2         HDAC2 ITGAL           D-Limonene         C10H16         136.23         HRI CHRM2           D-Limonene         C10H16         136.23         HRI CHRMA4           KCN3         KCN3         HDAC2 ITGAL         HDAC2 ITGAL           Pentanol         CSH120         88.15         KCN3           Pulsatillic Acid         C30H4604         470.7         PTGS1 COXIS           Sainfuran         C16H1405         286.28         SREBF1 POR           PTPN2         Encecalin         C14H1603         232.27         RNASE3 YMHAE           CALCA         CALCA         CALCA         CALCA         CALCA           Stearin         C3H5806         S86.94         KIF1 4 PR/CD         CALCA           Stearic Acid         C13H2402         20.32         COX411 COX411         SCN4A           Capylic Acid         Caluebin         Caluebin         Caluebin         Caluebin         SCOX2         COX411 COX411           Capylic Acid         Caluebin         Caluebin         SCOX12         SCOX42	N-Nonanol	C9H20O	144.25	GABRG3 DMIN
Actanoic AcidCBH1602144.21CHRM1 CHRM2Saikosaponin VG34860241107.2H0AC2 TGALD-LimoneneC10H16136.23HDAC2 TGALPentanolCSH12088.15CNR1 CHRNA4RockalC30H4604470.7PTGS1 COX1Pulsatilic AcidC16H1405286.28PTPN2SainfuranC16H1405286.28PTPN2EncecalinC14H163232.27RNAES TWAFAE20-HexadecanoylingenolC36H5806586.94KIF14 PRKCDStarinC57H11006891.5ARLauric AcidC12H240220.32COX411 COX411StarinC19H206CH356.37TDPA TUBEStarinC19H206CH356.37TDPA TUBELauric AcidC19H206CH356.37TDPA TUBEStarin AcidC19H206CH356.37TDPA TUBEStarin AcidC30H206CH356.37TDPA TUBEStarin AcidC19H206CH356.37TDPA TUBELauric AcidC19H206CH356.37TDPA TUBEStakosaponin CC48H7801727.12DIMTI TGF1Saikosaponin CC30H502135.14SIRT2 CNTFLinaj/ AcetateC30H502135.14SIRT2 CNTFLinaj/ AcetateC19H202264.36DPP4Saikosaponin DC19H20122048013780.96DNMT1 POLESaikosaponin DC19H20122048013780.96DNMT1 POLESaikosaponin DC19H20122048013780.96DNMT1 POLE<	Caryophyllene	CI5H24	204.35	OX I MID
Octation Acid         Of 1002         Path         Of 1002         Path         Of 1002           D-Limonene         C39H86024         1107.2         HDAC2 TGAL           D-Limonene         C10H16         136.23         NB3C2 LGF1           EDN1         Phranol         CSH12O         88.15         NB3C2 LGF1           Pentanol         C30H4604         470.7         PTGS1 COX1         PTGS1           Sainfuran         C16H1405         286.28         SRBF1 POR         PTFN2           Encecalin         C14H1603         232.27         RNASE3 YWARE         CALCA           20-Hexadecanoylingenol         C3F111006         891.5         NPR2         AR           21-Luvir Acid         C12H2402         20.032         COX411 COX411         SCNAA           Stearin         C3F111006         891.5         NPR2         AR           Lauric Acid         C12H2402         20.032         COX411 COX411         SCNAA           CCES1         CGA11 COX411         SCNAA         CCES1         COX2           Cubebin         Calca         SCNAB         SCNAB         SCNAB           Caprylic Acid         Calla COX411         SCNAB         SCNAB         SCNAB           Capryl	Octanoic Acid	C8H16O2	144 21	
Jakka Sakolini YDi Nobel 10221072 </td <td>Saikosaponin V</td> <td>C53H86O24</td> <td>144.21</td> <td></td>	Saikosaponin V	C53H86O24	144.21	
Pentanol Calification (Second Pertanol (Second Pe		C10H16	136.23	NB3C2 IGE1
PentanolSH12088.15CNR1 CHRNA4 KCNJ3Pulsatilit AcidC30H460470.7PTGS1 COX1 PTGS1 COX1 PTGS1 COX1SainfuranC16H140S286.28SEEB1 POR PTPN2EncecalinC14H1603232.27SR523 WHAE CALCA20-HexadecanoylingenolC36H5806Se6.4KIF14 PRKCD CHKCD21-HexadecanoylingenolC37H11006891.5NPR2 AR21-HexadecanoylingenolC37H11006991.5NPR2 AR21-Lauric AcidC12H240220.32C0X411 COX411 CX1121-Lauric AcidC12H240220.32C0X411 COX411 		Clotho	150.25	EDN1
Pulsatillic AcidKCNU3KCNU3Pulsatillic AcidC30H4604470.7PTGS1 COX1SainfuranC16H1405286.28SREBF1 POR PTPN2EncecalinC14H1603232.27CALCA20-HexadecanoylingenolC36H5806586.94KIF14 PRKCD CHGA20-HexadecanoylingenolC37H11006891.5MR2StearinC57H11006891.5ARLauric AcidC12H240220.32COX411 COX411 SXMAA CES1Stearin AcidC14H1603266.37COX411 COX411 SXMAA CES1Stearin AcidC14H200CH356.37COX411 COX411 SXMAA CES1Stearin AcidC1912060H356.37TOP2A TUBB COX2Cubebin Capylic AcidC3H1602144.21SCM48 CES1LongifoleneC15H24204.35EDN1 IGF1 EDN1Longispinogenin 3-0-Beta-D-GlucuronopyranosideC36H5809634.94NR3C1 PTR2Longispinogenin 3-0-Beta-D-GlucuronopyranosideC36H5809634.94NR3C1 PTR2Longispinogenin 3-0-Beta-D-GlucuronopyranosideC36H5809634.94NR3C1 PTR2Longispinogenin 3-0-Beta-D-GlucuronopyranosideC36H5809634.94NR3C1 PTR2Longispinogenin 3-0-Beta-D-GlucuronopyranosideC36H5809634.94NR3C1 PTR2Longispinogenin 3-0-Beta-D-GlucuronopyranosideC36H5809634.94NR3C1 PTR2Longispinogenin 3-0-Beta-D-GlucuronopyranosideC36H5809634.94NR3C1 PTR2Longispinogenin 3-0-Beta-D-GlucuronopyranosideC36H	Pentanol	C5H12O	88.15	CNR1 CHRNA4
Pulsatilic AcidC30H4604470.7PTGS1 COX1 PTGS1 COX1				KCNJ3
SainfuranC16H1405286.28PTGR1 PTPN2EncecalinC14H1603232.27RNASE3 YWHAE CALCAEncecalinC14H1603232.27RNASE3 YWHAE CALCA20-HexadecanoylingenolC36H5806896.94CHCAStearinC37H11006891.5NPR2 ARLauric AcidC12H240220.32COX411 COX411 SCNAA CES1Lauric AcidCH3(CH2)16COOH284.5SCC3A1 CES1 COX411 COX411 SCNAA CES1Stearin AcidCH3(CH2)16COOH356.37TOP2A TUBB SCNAA CES1CubebinC20H2006356.37TOP2A TUBB SCNABCupifoleneC15H2420436COX11 CIS1 COX41LongifoleneC15H2420.15COX41 CIS1Longifologenin 3-0-Beta-D-GlucuronopyranosideC48H7801727.12DNMT1 POLB DNMT1 POLE DNMT1 POLELongispinogenin 3-0-Beta-D-GlucuronopyranosideC48H7801726.35RRS12 CMTELongispinogenin 3-0-Beta-D-GlucuronopyranosideC41H20226.436NRS12 FADS1 FSN1 FADS1Longispinogenin 3-0-Beta-D-GlucuronopyranosideC15H24N20226.436NRT1 POLE FADS1 FSN1 FADS1CutalupineC15H24N20226.436DPM4Saikosaponin DC15H24N20226.436DPM4Saikosaponin DC15H24N20226.436DPM1TPOLE FADS1CutalupineC15H24N20226.436DPM1TPOLE FADS1Saikosaponin DC16H80FADS1FADS1CutalupineCutalupineFADS1FADS1	Pulsatillic Acid	C30H46O4	470.7	PTGS1 COX1
SainfuranC16H140S286.28SEBEF1 POR PTIN2EncecalinC14H1603232.27RNASE3 WHAE CALCA20-HexadecanoylingenolC36H5806S86.94KIF14 PRKCD CALCA20-HexadecanoylingenolC57H11006891.5NPR2 ARStearinC57H11006891.5NPR2 ARLauric AcidC12H240220.32COX411 COX411 COX411 COX411 CES1Stearic AcidC14120COH284.5SIC.25A1 CES1 COX2CubebinC2042006356.37TOP2A TUBB SCNABCapylic AcidC20H2006144.21COX71 PLA2G1B SCNABLongifoleneC15H24204.35CDN1 KF1 EDN1 KF1 EDN1 KF1Saikosaponin CC36H5809634.94NRS11 NRS11Longispinogenin 3-0-Beta-D-GlucuronopyranosideG6H5809308.5ANS12Chalphinogenin 2-Detar-D-GlucuronopyranosideC36H5809308.5ADS1 ESR1 FADS1Chalphinogenin DC15H24N202264.36PP4Saikosaponin DC15H24N202264.36PP4Saikosaponin DC15H24N202264.36DNMT1POLB FADS1Chalphinogenin DC15H24N202264.36DNMT1POLBSaikosaponin DC15H24N202264.36DNMT1POLBCalupino EntroC15H24N202264.36DNMT1POLBSaikosaponin DCALUPINACALUPINACALUPINACalupino EntroC15H24N202264.36DNMT1POLBSaikosaponin DCALUPINACALUPINASAINTPOLBCalupino EntroCalupino Entro </td <td></td> <td></td> <td></td> <td>PTGER1</td>				PTGER1
FreecalinPTFN2EncecalinC14H1603232.27CALCA20-HexadecanoylingenolC36H5806586.94CALCA20-HexadecanoylingenolC57H11006891.5CHGAStearinC37H11006891.5CALCALauric AcidC12H240220.32COX411 COX411ScruarC12H240220.32COX411 COX411ScruarC12H2402284.5COX21CubebinC4H6021HCOOH284.5COX21CubebinC19H206356.37T0P2A TUBBCapylic AcidC15H24244.35CDN11LongifoleneC15H24244.35EDN116F1LongifoleneC36H5809634.94NR3C1Longispinogenin 3-0-Beta-D-GlucuronopyranosideC36H5809634.94NR3C1Phenylacetic AcidC15H24N202308.5FAD51 ESR1Linalyl AcetateC15H24N202264.36DNNT1 POLBCotalupineC15H24N202760.88DNNT1 POLBChapter AcidC15H24N202264.36DPP4Linalyl AcetateC15H24N202760.98DNNT1 POLBCotalupineC15H24N202760.98DNNT1 POLBChapter AcidC15H24N202FA0.51FAD51 ESR1Chapter AcidC15H24N202FA0.56FAD51 ESR1CotalupineC15H24N202FA0.56FAD51 ESR1CotalupineC15H24N202FA0.56FA0.51CotalupineC10H180FA0.51FA0.51CotalupineC10H180FA0.51FA0.51	Sainfuran	C16H14O5	286.28	SREBF1 POR
Encecalin     C14H1603     232.27     RNASE3 YMHAE CALCA       20-Hexadecanoylingenol     C36H5806     \$86.94     KF14 PRKD CHGA       21-Hexadecanoylingenol     C57H11006     891.5     NPR2 AR       21-uric Acid     C57H11006     891.5     CALCA       21-uric Acid     C14H2402     200.32     C0X411 COX411 SCN4A       21-bit Acid     C141/2020     200.32     COX411 COX411 SCN4A       21-bit Acid     C12H2402     284.5     COX2       Cubebin     C20H2006H     356.37     T0P2A TUBB       Capylic Acid     C3H1602     144.21     COX1       Longifolene     C15H24     244.35     EDN1 IGF1 EDN1       Saikosaponin C     C48H78017     27.12     DNMT1 POLB DNMT1       Longispinogenin 3-0-Beta-D-Glucuronopyranoside     C36H5809     634.94     NR3C1       Phenylacetic Acid     CATO2     35.14     SRT2 CNTF       Linalyl Acetate     C20H3602     264.36     DP4       Octalupine     C15H24N202     264.36     DP4       Saikosaponin D     C42H68013     780.98     DMMT1POLB       Octalupine     C4140202     264.36     DP4       Saikosaponin D     C42H68013     780.98     DMMT1POLB       Octalupine     C42H68013     780.98				PTPN2
20-Hexadecanoylingenol Calca 20-Hexadecanoylingenol Calca Stearin Calca Steari	Encecalin	C14H16O3	232.27	RNASE3 YWHAE
20-Hexadecandylingenol         Consistor         Seo.94         NH 4 PARCD (HGA           Stearin         C57H11006         891.5         NPR2 AR           Lauric Acid         C12H2402         200.32         C0X411 COX411 SCN4A           Stearic Acid         C12H2402         200.32         C0X411 COX411 SCN4A           Stearic Acid         C13(CH2)16COOH         284.5         Sc.25A1 CES1 COX2           Cubebin         C20H2006         356.37         T0P2A TUBB           Caprylic Acid         C3H1602         144.21         OXCT1 PLA2G1B SCN4B           Caprylic Acid         C15H24         204.35         EDN1 IGF1 EDN1           Longifolene         C15H24         204.35         EDN1 IGF1 EDN1           Longispinogenin 3-0-Beta-D-Glucuronopyranoside         C48H78017         927.12         DNMT1 POLB DNMT1           Longispinogenin 3-0-Beta-D-Glucuronopyranoside         C48H78017         308.5         SIR12 CNTF           Linalyl Acetate         C15H24N202         264.36         DPV4           Sakosaponin D         C15H24N202         264.36         DPV4           Sakosaponin D         C15H24N202         264.36         DPV4           Sakosaponin D         C15H24N202         264.36         DNMT1POLB           Sak		C2(1)50C(	506.04	
Stearin         C57H11006         891.5         NPR2 AR           Lauric Acid         C12H2402         200.32         C0X4I1 COX4I1 SCN4A           Stearic Acid         CH3(CH2)16COOH         284.5         COX2           Stearic Acid         C19H200         356.37         T0P2A TUBB           Coxe         COX2         COX2         COX2           Cubebin         C20H2006         356.37         T0P2A TUBB           Caprylic Acid         C8H1602         144.21         OXCT1 PLA2G1B SCN4B           Longifolene         C19424         C8H1602         144.21         DNI IGF1 EDNI           Saikosaponin C         C48H78017         204.35         DNI IGF1 EDNI           Longispinogenin 3-0-Beta-D-Glucuronopyranoside         C36H5809         634.94         NR3C1           Phenylacetic Acid         C8H702-         135.14         SRT2 CNTF           Linalyl Acetate         C19H202         264.36         DPP4           Saikosaponin D         C15H24N202         264.36         DPP4           Saikosaponin D         C15H24N202         264.36         DNMT1 POLB           Cotaupine         C15H24N202         264.36         DPP4           Saikosaponin D         C15H24N202         264.36 <t< td=""><td>20-Hexadecanoylingenol</td><td>C36H58U6</td><td>586.94</td><td>CHGA</td></t<>	20-Hexadecanoylingenol	C36H58U6	586.94	CHGA
JedamChrinocoOne of some and arrest ar	Stearin	C57H110O6	801 5	NPR2
Lauric AcidC12H2402200.32COX411 COX411 SCNAA CES1Stearic AcidCH3(CH2)16COOH284.5SC.25A1 CES1 COX2CubebinC20H2006356.37TOP2A TUBB COX11 PLA2G1BCaprylic AcidC8H1602144.21OXCT1 PLA2G1B SCNABLongifoleneC15H24204.35EDN1 IGF1 EDN1Longispinogenin 3-0-Beta-D-GlucuronopyranosideC48H78017204.35EDN1 IGF1 EDN1Longispinogenin 3-0-Beta-D-GlucuronopyranosideC36H5809634.94NR3C1 NMT1Longispinogenin 3-0-Beta-D-GlucuronopyranosideC36H5809634.94NR3C1 SCNABLongispinogenin 3-0-Beta-D-GlucuronopyranosideC36H5809634.94NR3C1 SCNAFCotalupineC15H24N202264.36SIR12 CNTF FADS1CotalupineC15H24N202264.36DPP4Saikosaponin DC42H68013780.98DNMT1POLB FADS1CotalupineC15H24N202264.36DNMT1POLB FADS1Saikosaponin DC15H24N202264.36DNMT1POLB FADS1CotalupineC15H24N202264.36DNMT1POLB FADS1Saikosaponin DC15H24N202264.36DNMT1POLB FADS1CotalupineC15H24N202264.36CESaikosaponin DC15H24N202264.36CECotalupineC15H24N202780.98SINT1Saikosaponin DC15H180FADS1SINT1CotalupineC15H180FADS1SINT1Saikosaponin DC15H180FADS1SINT1<	Steam	Commode	071.5	AR
Schuka     Schuka     Schuka     Schuka       Stearic Acid     Schuka     Schuka     Schuka       Cubebin     Cohlococh     Schuka     Schuka       Garylic Acid     Schuka     Schuka     Schuka       Garylic Acid     Schuka     Schuka     Schuka       Congifolene     Cibebin     Schuka     Schuka       Longifolene     Cibebin     Schuka     Schuka       Saikosaponin C     Schuka     Schuka     Schuka       Longispinogenin 3-O-Beta-D-Glucuronopyranoside     Schuka     Schuka     Schuka       Phenylacetic Acid     Schuka     Schuka     Schuka       Longispinogenin 3-O-Beta-D-Glucuronopyranoside     Schuka     Schuka     Schuka       Schuka     Schuka     Schuka     Schuka     Schuka       Longispinogenin 3-O-Beta-D-Glucuronopyranoside     Schuka     Schuka     Schuka       Schuka     Schuka     Schuka     Schuka     Schuka       Longispinogenin 3-O-Beta-D-Glucuronopyranoside     Schuka     Schuka     Schuka       Schuka     Schuka     Schuka     Schuka     Schuka       Schuka     Schuka     Schuka     Schuka     Schuka       Schuka     Schuka     Schuka     Schuka     Schuka       Schuka	Lauric Acid	C12H24O2	200.32	COX4I1 COX4I1
Stearic Acid     CB1       Stearic Acid     C20H200CH     284.5       Cubebin     C20H200C     356.37       Caprylic Acid     CB11602     144.21       Caprylic Acid     CB11602     144.21       Caprylic Acid     CB1602     204.35       Longifolene     C15H24     204.35       Saikosaponin C     C48H78017     201.32       Longispinogenin 3-O-Beta-D-Glucuronopyranoside     C36H5809     634.94       Phenylacetic Acid     C8H702-     135.14       Linalyl Acetate     C20H3602     204.36       Cotclupine     C15H24N202     264.36       Saikosaponin D     C15H24N202     264.36       Octalupine     C15H24N202     264.36       Saikosaponin D     C10H180     FADS1 ESR1       Saikosaponin D     C10H180     FADS1       Sa				SCN4A
Stearic Acid     CH3(CH2)16COOH     284.5     SLC2SA1 CES1 COX2       Cubebin     C20H2006     356.37     TOP2A TUBB       Caprylic Acid     C8H16O2     144.21     OXCT1 PLA2G1B SCN4B       Longifolene     C15H24     204.35     EDN1 IGF1 EDN1       Saikosaponin C     C48H78017     27.12     DNMT1 POLB DNMT1       Longispinogenin 3-O-Beta-D-Glucuronopyranoside     C36H5809     634.94     NR3C1       Phenylacetic Acid     C8H702-     135.14     SIRT2 CNTF       Linalyl Acetate     C20H3602     308.5     FADS1 ESR1 FADS1       Cotalupine     C15H24N202     264.36     DPP4       Saikosaponin D     C15H24N202     264.36     DPP4       Saikosaponin D     C15H24N202     264.36     DPMT1       Saikosaponin D     C15H24N202     264.36     DPMT1       Saikosaponin D     C10H180     154.25     KL GF11 GF11 GF11       Your     C10H180     154.25     KL GF11				CES1
COX2CubebinC20H2O06356.37TOP2A TUBBCaprylic AcidC8H16O2144.21OXCT1 PLA2G1B SCNABLongifoleneC15H24204.35EDN1 IGF1 EDN1Saikosaponin CC48H78017927.12DNMT1 POLB DNMT1 POLB DNMT1Longispinogenin 3-O-Beta-D-GlucuronopyranosideC36H5809634.94NR3C1Phenylacetic AcidC8H702-135.14SIRT2 CNTFLinalyl AcetateC20H3602308.5FADS1 ESR1 FADS1OctalupineC15H24N202264.36DPP4Saikosaponin DC15H24N202264.36DNMT1 POLB FADS17-Octen-4-OlC10H180154.25KL FADS1	Stearic Acid	CH3(CH2)16COOH	284.5	SLC25A1 CES1
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Octalupine         C15H24N2O2         264.36         DPP4           Saikosaponin D         C42H68O13         780.98         DNMT1POLB           7-Octen-4-Ol         C10H18O         154.25         KL				FADS1
Saikosaponin D         C42H68013         780.98         DNMT1POLB           7-Octen-4-Ol         C10H180         154.25         KL	Octalupine	C15H24N2O2	264.36	DPP4
7-Octen-4-Ol C10H18O 154.25 KL GFI1	Saikosaponin D	C42H68O13	780.98	DNMT1POLB
GFI1	7-Octen-4-Ol	C10H18O	154.25	KL
				GFI1 Bax

### Table 1 (continued)

Ingredients	Molecule formula	Molecule weight	Target
Longispinogenin	C30H50O3	458.72	PGR
			ADA
Dulagana	C10U1CO	1	TFAP2C
Pulegone	C10H160	152.23	AIM KCNO1
sugmasterol	C29H48O	412.7	ADH1C
Alpha-Spinasterol-Beta-D-Glucoside	C35H58O6	560.9	ATP1A1
Angelicin	C11H6O3	186.16	B4GALT1
-			GC
Myrtenol	C10H16O	152.23	DHRS4
			DHRS4
Iridecanoic Acid	C13H26O2	214.34	BBOX1
Myrtenal	C10H14O	150.22	CRP
wytendi	clothio	150.22	TSPO
Kaempferol	C15H10O6	286.24	NQO1
Adonitol	C5H12O5	152.15	CHRNA2
			KCNJ3
2 Mathulauslatridasan 1 Ona	C14U2CO	210.26	GABRE
3-Methylcyclothdecan-1-One	C14H20U	210.30	ו ז ג ג חפרז
Quercetin	CISITION	302.24	DRD3 DRD2
Geraniol	C10H18O	154.25	HSD17B7
Beta-Humulene	C15H24	204.35	KCNQ1
			CRLF1
Saikosaponin B	C42H68O13	780.98	GLRA3
Saikosaponin K	C54H88O22	1089.3	GLRA3
Naatkatana	C15U000	210.22	GABRB3
NOORALOITE	CISHZZO	210.55	PHR
			CRP
			TNF
Delta-Terpineol	C10H18O	154.25	TRPV3
Nachthalana	C10U0	100.17	PGR
Naphthalene	CIUH8	128.17	ITA
Spinasterol	C29H48O	412.7	TRIM24
			RXRA
Guaiacol	C7H8O2	124.14	SEC14L3
			TRPV3
Myrtanol	C10H18O	154.25	VCAM1
Saikogenin F	C30H48O4	172.7	DCR
Sakogeniin	00014004	4/2./	PNP
Carvone	C10H14O	150.22	NR3C1
			PIK3CD
Saikosaponin A	C42H68O13	780.98	POLB
Myricadiol	C30H50O2	442.72	VDR
Nononaia Asid	C0111000	150.24	PGR
Nonanoic Acid	C9H18O2	158.24	CES1
			COX2
Saikosaponin T	C43H72O14	813.15	POLB
			DNMT1
Fuend	C10U12O2	164.2	ADORA2B
Eugenoi	CIUHIZUZ	104.2	OPKKI CNR1

Ingredients	Molecule formula	Molecule weight	Target
Saikogenin E	C30H48O3	456.7	MIF ADORA1 ITFG2
Linalool	C10H18O	154.25	TFAP2C PGR TOX3
Undecanoic Acid	C11H22O2	186.29	TRPA1 OPRK1
Myrcene	C10H16	136.23	FBP1 GRIN2A SPARC
Alpha-Limonene	C10H16	136.23	LEP CAT
Thymol	C10H14O	150.22	HRC PDE4B COLQ
(+)-Anomalin	C24H26O7	426.5	KCNH2 STX3
3,5,6,7-tetramethoxy-2-chromone	-	432.46	PTGS2 PTGS1
Areapillin	C18H16O8	360.34	ESR1 CCNA2
Baicalin	C21H18O11	446.39	AR MAOB
Isorhamnetin	C16H12O7	316.28	ACHE RELA
Longikaurin A	C20H28O5	348.48	ESR2 OLR1
Petunidin	C16H13O7+	317.29	NOS2 CHRM2
Troxerutin	C27H30O16	346.56	PTGS1 PGR
α-spinasterol	C29H48O	412.77	CCNA2 NR3C2

#### **PPI analysis**

After removing irrelevant data, the PPI network was modified to include 104 nodes. *PIK3CD*, *ARRB2*, *SUCLG1*, *FURIN*, *SPI1*, and *PNP* were situated at the core of the network (Fig. 4A). These genes were associated with mast cell differentiation, small molecule binding, multicellular biological process regulation, and response to organic substances, indicating their potential as important targets for *Radix Bupleuri* in improving the prognosis of sepsis. A heatmap of the expression of these key genes was generated (Fig. 4B).

#### GO and KEGG enrichment analysis

A comprehensive compilation of 7826 functional gene profiles was obtained based on GO annotation of crosstargets. In this collection, 6217 genes were enhanced in biological processes, 508 in cellular components, and 1101 in molecular functions. GO enrichment analysis revealed that the target was primarily engaged in transmembrane transport regulation, monatomic ion transport, and redox (Fig. 5A). Target genes were primarily engaged in cancer pathways, MAPK signaling pathways, and vascular smooth muscle contraction, according to KEGG pathway analysis (Fig. 5C). To further elaborate the relationship between them, GO and KEGG enrichment analysis results were presented as network graphs, and the networks were displayed by Cytoscape3.7.2, with each node in the GO analysis network representing a term and first colored by its cluster ID (Fig. 5B), with polygons representing key genes, inverted triangles representing signaling pathways(Fig. 6).

#### Survival curve analysis

To evaluate the association between patient prognosis and the 309 cross-targets, a prognostic analysis was conducted by integrating the public database GSE65682 [12]. The log-rank test demonstrated a significant correlation between prognosis and the expression of *PIK3CD*, *ARRB2*, *SUCLG1*, *FURIN*, *SPI1*, and *PNP* (Fig. 7A-F). The larger the gap between the survival curves, the



Fig. 3 Drug component target network diagram: In this network, circular nodes represent intersected genes, square nodes indicate active compounds of *Radix Bupleuri*, and inverted triangle nodes indicate diseases. Lines indicate drug interactions with ingredients and targets

more pronounced the change in patient prognosis. High expression of *PIK3CD*, *ARRB2*, *SUCLG1*, and *SPI1* was associated with lower mortality rates, while low expression of *PNP* and *FURIN* indicated a better prognosis. Therefore, *PIK3CD*, *ARRB2*, *SUCLG1*, *FURIN*, *SPI1*, and *ARRB2* are potential core targets for *Radix Bupleuri* in sepsis treatment. Box plots were generated to illustrate the expression levels of these six key genes in normal and sepsis groups (Fig. 8A-F).

#### Single-cell RNA sequencing

Transcriptome sequencing analysis of the five cell samples (Fig. 9A) incorporated the six key genes identified in this study—*PIK3CD*, *ARRB2*, *SUCLG1*, *FURIN*, *SPI1*, and *PNP*—into the single-cell libraries for cell line localization. The results showed that PIK3CD, PNP, SPI1, and FURIN were predominantly situated in macrophages, while ARRB2 and SUCLG1 was predominantly located in macrophages and T cells (Fig. 9B-I).

#### Molecular docking

Based on the above analysis, Carvone, Encecalin, Lauric Acid, Pulegone, Nootkatone, and Saikogenin F, the active components of *Radix Bupleuri*, underwent molecular docking studies with PIK3CD, ARRB2, SUCLG1, FURIN, SPI1, and PNP, respectively (Fig. 10A-F). Affinity values below -4.25 kcal·mol<sup>-1</sup> indicate binding activity, those below -5.0 kcal·mol<sup>-1</sup> indicate binding solid activity, and values below -7.0 kcal·mol<sup>-1</sup> indicate significant docking interactions [19]. The results of docking the active components to the targets are summarized in Table 2, with Saikogenin F showing the best docking activity with PNP.

#### Molecular dynamics simulations

Molecular dynamics results showed that Carvone-PIK3CD, Encecalin-ARRB2, Lauric Acid-SUCLG1, Pulegone-FURIN, Saikogenin F-PNP, and Nootkatone-SPI1 six systems of RMSD, RMSF, RoG, SASA, and hydrogen bonding changes. Except for Nootkatone-SPI1, the RMSD of the five systems showed good convergence during the simulation period, indicating that these



Fig. 4 PPI Analysis and intersected gene analysis. A: The interaction network between proteins. PIK3CD, ARRB2, SUCLG1, FURIN, SPI1, and PNP are located at the center of this network. B: Heatmap of the six key genes at the center of the PPI network (*PIK3CD, ARRB2, SUCLG1, FURIN, SPI1, PNP*). Blue represents higher expression, and yellow represents lower expression

complexes were dynamically stable and did not undergo major conformational changes; whereas the RMSD of the Nootkatone-SPI1 complex fluctuated considerably during the first 50 ns second simulation period(Fig. 11). The RMSF results showed that the RMSF of the protein after binding small molecules was low except for the ends, indicating that the core structure of the protein possesses better rigidity(Fig. 12A-F). The trend of radius of gyration (RoG) showed that the Carvone-PIK3CD, Lauric Acid-SUCLG1, Pulegone-FURIN, and Saikogenin F-PNP systems had less fluctuation in compactness, and Encecalin-ARRB2, Nootkatone-SPI1 systems fluctuated more(Fig. 13).SASA analysis showed that the fluctuation of solvent-accessible and surface area of the



Fig. 5 GO and KEGG enrichment analysis of cross-targets. A: GO enrichment analysis revealed that the target was primarily involved in biological processes such as transmembrane transport regulation, monatomic ion transport regulation, and redox. B: Different colors of each node correspond to different biological processes. (Cluster ID) C: KEGG pathway analysis showed that target genes were primarily associated with various signaling pathways such as cancer pathway, MAPK signaling pathway, and vascular smooth muscle contraction

six systems was smooth, indicating that the complexes existed stably in aqueous solution(Fig. 14). Hydrogen bonding analysis showed that the number of hydrogen bonds of Lauric Acid-SUCLG1 complexes stabilized at 2-3 at the late stage of the simulation, Pulegone-FURIN at 1-2, and the rest of the systems at 0-1, suggesting that hydrogen bonding contributes to the Lauric

# Acid- SUCLG1 binding contributes the most, followed by Pulegone-FURIN(Fig. 15A-F).

#### Discussion

Sepsis, characterized by a systemic inflammatory response to infection, remains a leading cause of global mortality [20]. With the lack of specific effective



Fig. 6 target-disease-signaling pathway network. Polygons in the "target-disease-signaling pathway" network represent key genes, inverted triangles represent signaling pathways, and rectangles represent diseases

treatments, TCM shows promise in reducing sepsisinduced organ dysfunction through anti-inflammatory actions, oxidative stress reduction, immunity enhancement, and cellular homeostasis maintenance [21]. This study utilized a network pharmacology approach to reveal how the active ingredients of *Radix Bupleuri* modulate immune responses and signaling processes in sepsis patients by interacting with core targets, thereby improving the prognosis of sepsis patients. The resulting PPI network identified 104 targets, with six potential targets selected for further investigation: PIK3CD, ARRB2, SUCLG1, FURIN, SPI1, and PNP. These findings lay the groundwork for future studies on *Radix Bupleuri's* therapeutic mechanism against sepsis.

ARRB2, a member of the G protein-coupled receptor adaptor family [22], promotes the anti-apoptotic Akt signaling pathway and prevents apoptosis by inhibiting pro-apoptotic ERK1/2 and p38 MAPKs [23]. It also enhances EPC-mediated neovascularization via ERK and Akt signaling pathways [24]. Survival curve analysis indicated higher ARRB2 expression in the group with a better prognosis of sepsis, associated with increased survival. RNA-seq analysis revealed ARRB2's predominant localization in macrophages and T-cell lines. Network pharmacological analysis identified Encecalin, an active component of *Radix Bupleuri*, as a potential antibacterial agent targeting ARRB2.

FURIN, a significant mammalian proprotein convertase, plays a substantial role in the pathophysiology of neurodegenerative diseases and neuropsychiatric disorders [25]. Additionally, FURIN is responsible for the processing of transforming growth factor-beta, elevated in bronchoalveolar lavage fluid of PWCF, associated with neutrophilic inflammation and decreased lung function [26]. In the present study, survival analysis demonstrated reduced FURIN expression in the sepsis group with a better prognosis, leading to increased survival. Accordingly, downregulated FURIN expression may be advantageous in sepsis, and RNA-seq analysis revealed its predominant localization in macrophage cell lines. Network pharmacological analysis highlighted Pulegone, an active ingredient in Radix Bupleuri, targeting FURIN and exhibiting antibacterial properties.

Purine nucleoside phosphorylase (PNP) is a vital enzyme involved in purine nucleoside degradation, and PNP deficiency leads to progressive T-cell



Fig. 7 Survival curve analysis of six key targets. The plot shows survival time in days on the horizontal axis and survival on the vertical axis. Green lines correspond to low mRNA samples, while red lines represent high mRNA samples. **A** – **F**: The lower mortality rates were observed in the high-expression groups of PIK3CD, ARRB2, SUCLG1, and SP11, and the low-expression groups of PNP and FURIN, indicating a better prognosis (*P* < 0.05)

immunodeficiency, increased susceptibility to infections, autoimmunity, and neurological abnormalities [27]. Mutations in the *PNP* gene can result in decreased T lymphocyte numbers, causing immunodeficiency [28]. In this study, survival analysis indicated decreased PNP expression in the sepsis group with a better prognosis, contributing to increased survival. Reduced PNP expression may favor sepsis, and RNA-seq analysis showed predominant localization in the macrophage lineage. Network pharmacological analysis revealed Saikogenin F, an active ingredient in *Radix Bupleuri*, targeting PNP and exhibiting antibacterial properties.

*SUCLG1* is a gene encoding a protein crucial for maintaining mitochondrial nucleotide pool balance [29], and its mutations cause mitochondrial encephalomyopathy [30]. Survival analysis showed increased SUCLG1 expression in the sepsis group with a better prognosis, leading to increased survival. Elevated SUCLG1 expression may



Fig. 8 Expression levels of six key genes. FURIN, ARRB2, SUCLG1, SPI1, and PNP were highly expressed in the sepsis group. PIK3CD expression was low in the sepsis group (P < 0.05)

be favorable in sepsis, and scRNA-seq analysis identified its primary location in macrophages and T cells. Network pharmacological analysis uncovered Lauric Acid, an active component in *Radix Bupleuri*, with the potential to target SUCLG1 and exhibit antibacterial properties.

PIK3CD, closely tied to the immune function of the human body, can influence T cell activation, differentiation, and trafficking [31]. Mutations in PIK3CD may result in symptoms such as immunodeficiency and autoimmunity [32]. Survival curve analysis demonstrated increased PIK3CD expression in the sepsis group with a better prognosis, contributing to increased survival. Elevated PIK3CD expression may favor sepsis, and RNAseq analysis indicated its predominant location in macrophage cell lines. Network pharmacology analysis revealed that Carvone, an active component in *Radix Bupleuri*, targets PIK3CD and exhibits antibacterial properties. SPI1, a transcription factor, may play a crucial role in leukemogenesis when abnormally regulated [33]. Studies have shown that SPI1 regulates microglia/macrophage orientation and maturation and may affect monocyte autophagy in a mouse sepsis model by regulating ANXA [34, 35]. Survival curve analysis indicated higher SPI1 expression in the sepsis group with a better prognosis, resulting in increased survival. Increased SPI1 expression may be advantageous in sepsis, and RNA-seq analysis revealed its predominant location in macrophage cell lines. Network pharmacological analysis highlighted Nootkatone, an active compound in *Radix Bupleuri*, targeting SPI1 and exhibiting antibacterial properties.

Unlike previous studies on sepsis, this study used single-cell sequencing to better understand the individual differences and pathological mechanisms of sepsis patients. Based on this information, personalized



Fig. 9 Cell line localization of key genes. A: Groups 3 and 5 represent macrophages, Group 4 represents natural killer cells, and Groups 1, 2, 6, and 8 represent T cells. B cells represent Group 7, and Group 9 represents platelets. B-H: PIK3CD, PNP, SPI1, and FURIN were primarily situated in macrophages, SUCLG1and ARRB2 was primarily situated in macrophages and T cells. I: Bubble plots show the expression abundance values and proportions of each hub gene in different cell populations



Fig. 10 Molecular docking results. A: The binding affinity between Carvone and PIK3CD is -6.258 kcal·mol<sup>-1</sup>. B: The binding affinity between Encecalin and ARRB2 is -6.954 kcal·mol<sup>-1</sup>. C: The binding affinity between Lauric Acid and SUCLG1 is Lauric Acid-SUCLG1. D: The binding affinity between Nootkatone and SPI1 is -5.546 kcal·mol<sup>-1</sup>. E: The binding affinity between Saikogenin F and PNP is -8.86 kcal·mol<sup>-1</sup>. F: The binding affinity between Pulegone and FURIN is -6.348 kcal·mol<sup>-1</sup>.

Page 16 of 19

Table 2 Molecular docking results

Compounds	Targets	Bind Energy
Encecalin	ARRB2	-6.954kcal·mol <sup>-1</sup>
Pulegone	FURIN	-6.348kcal·mol <sup>-1</sup>
Saikogenin F	PNP	-8.86 kcal·mol <sup>-1</sup>
Lauric Acid	SUCLG1	-5.009kcal·mol <sup>-1</sup>
Carvone	PIK3CD	-6.258kcal·mol <sup>-1</sup>
Nootkatone	SPI1	-5.546kcal·mol <sup>-1</sup>



**Fig. 11** Root mean square deviation (RMSD) of the complexes during molecular dynamics simulation with time. The root mean square deviation of the molecular dynamics simulation can reflect the movement process of the complex, the larger RMSD as well as the more violent fluctuation indicates the violent movement, and on the contrary, the movement is smooth

therapeutic strategies can be developed to improve treatment efficacy and prognosis. In addition, potential targets of action and signaling pathways of *Radix Bupleuri* were analyzed to support its clinical translation. Molecular docking and molecular dynamics simulations were used to analyze the binding of *Radix Bupleuri* to its targets, revealing new ideas on the pathogenesis of sepsis and providing new avenues for the treatment and prevention of the disease. In summary, this study integrates multiple advanced technologies and has broad prospects for future research and clinical applications.

Based on previous studies, Pulegone has been shown to exert anti-inflammatory effects on LPS-induced sepsis in mice by inhibiting NLRP3 expression [36]. We speculated that Pulegone may modulate FURIN expression in sepsis through a similar mechanism, but whether Pulegone exerts its antisepsis effect by down-regulating FURIN remains unclear. Saikogenin F increases the expression of Bax, cleaved-caspase-3, cleaved-caspase-9, and cleavedpoly ADP-ribose polymerase (PARP) and decreases the expression of Bcl-2, resulting in a significant inhibitory effect on A549 cells, which can be considered as a potential anticancer drug [37]. Similarly, Lauric Acid promotes the expression of mitochondrial biogenesisregulated genes such as TFAM, PGC-1a, and PPAR-y, thereby improving insulin sensitivity [38]. Carvone has been shown to express antioxidant and anti-inflammatory capacity by promoting the expression of TNF- $\alpha$ , IL-1β, IL-6 and NF-κB mRNA in rats [39]. Nootkatone was found to attenuate asthmatic airway inflammation by reducing the production of Th2 inflammatory cytokines (IL-4, IL-5, and IL-13) in reduced serum levels of BALF and IgE, and by inhibiting ROS-triggered NLRP3 activation [40]. In addition, Encecalin has also been shown to play a role in controlling blood glucose levels, thus playing a very important role in the therapeutic response of diabetic patients [41]. In summary, these active ingredients have shown positive pharmacological activity in other disease models by acting on targets associated with them. This provides new ideas and targets for the clinical treatment of sepsis. Although we have analyzed through previous studies that these active ingredients (Pulegone, Saikogenin F, Lauric Acid, Carvone, Nootkatone, and Encecalin) may exert their antisepsis effects through the modulation of the relevant genes, direct evidence is still insufficient and the specific mechanisms by which these active ingredients regulate the target genes (PIK3CD, ARRB2, SUCLG1, FURIN, SPI1, and PNP) remains unclear, and the regulatory mechanisms of these genes in different pathological states may have similarities in sepsis, but this hypothesis needs to be verified by further studies.

The study has certain limitations that merit consideration. Firstly, the bioinformatic data available from the public databases relied upon for this study are limited. The sample size involved in this study kind is small, if we want to fully understand the pharmacological effects of *Radix Bupleuri*. A large number of clinical trial validation and evidence-based medical studies are also needed, in addition, the inhibitory or promoting effects of small molecules binding to key targets need to be verified by further mechanistic cellular experiments, which will be carried out in our subsequent studies.



Fig. 12 Root Mean Square Fluctuation (RMSF) calculated based on molecular dynamics simulation trajectory. A-F: RMSF can respond to the protein flexibility during molecular dynamics simulation. Usually, the protein flexibility decreases after the drug binds to the protein, which in turn stabilizes the protein while exerting the enzyme activation effect





**Fig. 13** Radius of gyration of the six systems during molecular dynamics simulations. The radius of gyration reflects the embodied compactness and can reflect the degree of compactness of the system. The above figure shows the variation of RoG with time for six complex systems during molecular dynamics simulation, and the size of the fluctuation can be very intuitively judged from the degree of densification or the convergence of the system

**Fig. 14** Solvent accessible surface area (SASA) of individual complexes during molecular dynamics simulations. Based on the fluctuation analysis of SASA, we can see that the fluctuation of the six systems of Carvone-PIK3CD, Encecalin-ARRB2, Lauric Acid-SUCLG1, Pulegone-FURIN, Saiko-genin F-PNP, and Nootkatone-SPI1 is smooth, which indicates that the complexes' exposed and buried regions of the surface undergo little change, and the complexes are stable in aqueous solution, providing a basis for the relative stability of small molecules and proteins



Fig. 15 Change in the number of hydrogen bonds between small molecules and proteins during molecular dynamics simulations. Hydrogen bonding is one of the strongest non-covalent binding interactions, and a higher number indicates better binding

#### Conclusion

Carvone, Encecalin, Lauric Acid, Pulegone, Nootkatone, and Saikogenin F exhibit the potential to improve survival outcomes and confer antimicrobial characteristics in individuals with sepsis.

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Not applicable.

#### Author contributions

 $\rm H.W$  and W.X assisted with data analysis and paper writing, Y.L and L.H drew pictures and tables, and Y.H and W.Z were responsible for the final review of the article.

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#### Data availability

The CNGBdb repository contains the datasets that were analyzed for this study, (https://db.cngb.org/search/project/CNP0002611/).

#### Declarations

#### Ethics approval and consent to participate

Each patient and their family members voluntarily participated in this study and signed an informed consent form. Approval for the study was granted

by the Ethics Committee at the Affiliated Hospital of Southwest Medical University (No.1. ky2018029), Clinical Trial No: ChiCTR1900021261,Registration Date: February 4, 2019.

#### **Consent for publication**

Not applicable.

#### Disclosure

The authors declare no conflicting interests in this study.

#### **Competing interests**

The authors declare no competing interests.

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