ORIGINAL ARTICLE

Exploring the anticancer potential of *Eleutherine bulbosa*: A systematic network pharmacology study on lung cancer

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ABSTRACT

Chemotherapy application in lung cancer patients has several side effects and shows lower effectiveness due to chemoresistance. Although *Eleutherine bulbosa* (Mill.) Urb. (EBE) elicit anticancer properties, yet the exact profile of its active compounds and lung cancer inhibition mechanisms were not fully understood. This study aimed to identify suggestive compounds from EBE extract and explain the molecular mechanisms of EBE against lung cancer. Identification of the compound from the EBE extract was confirmed using liquid chromatography–tandem mass spectrophotometry (LC–MS/MS). The bioavailability profile of three major metabolites was identified using absorption, distribution, metabolism, excretion, toxicity software. The anticancer molecular mechanism prediction of the drugs was ascertained by network pharmacology using Cytoscape 3.9.1 and the protein–protein interaction network technique with STRING 11.0. Interaction between resveratrol and extracellular growth factor receptor (EGFR) was analyzed using site-specific molecular docking with erlotinib as the control using PyRx Autodock Vina 9.0 and BIOVIA Discovery Studio. A total of 16 active compounds were identified from LC-MS/MS. Only resveratrol showed anticancer properties by its interaction with 13 genes and 6 signaling pathways related to lung cancer. The molecular docking result supports the network pharmacology finding. The binding affinity of resveratrol with EGFR, important receptor in lung cancer, was more negative (−6.9 kcal/mol) than erlotinib (−6.2 kcal/mol) as the control. Evidence suggested that resveratrol in EBE exhibits anticancer effects by modulating lung cancer cell proliferation and apoptosis through EGFR binding.

Key words: *Eleutherine bulbosa,* lung cancer, molecular docking, network pharmacology, resveratrol

INTRODUCTION

The leading cause of cancer mortality globally is caused by lung cancer.[1] Previous studies showed a significant number of deaths as a result of drug treatment failure and recurrence.[2] In addition, chemotherapy-based treatment for lung cancer often has side effects and resistance problems.[3,4] Pleiotropic active ingredients in natural compounds showed

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multitarget and safety in long-term use, and thus become a new promising candidate for cancer therapeutic strategy.[5]

One of the medicinal plants that showed potency to be developed as a treatment for lung cancer is *Eleutherine bulbosa* (Mill.) Urb. (EBE), from the Iridaceae family.[6] This bulb has main active compounds such as naphthalene, anthraquinone, and naphthoquinone that have beneficial effects against several metabolic disorders and cancer in several reports.[6,7] However, the main secondary metabolites and antilung cancer activity of EBE remain elusive.

The highlight of this study was to discover the active chemicals of EBE and to explore their potential inhibition mechanism on signaling transduction pathways related to lung cancer using a combination strategy of metabolite profiling and network pharmacology. Therefore, this study provided important insights for the development of novel candidates for lung cancer treatment.

MATERIALS AND METHODS

Preparation of *Eleutherine bulbosa* extract

The EBE roots with collection number 074/348/102.7/2021 were collected from East Borneo at an altitude of 29 m; the average temp was 26.4, and the average rainfall was 2376 mm. The ultrasound-assisted extraction method was used to extract simplicial powder at a ratio of 1:20 with 96% ethanol for 30 min at 25°C. Ethanol extract was prepared for further testing after being kept in an oven at 40°C for 5 h.

Investigation of EBE's metabolite profiling

The analysis was conducted using ultra-high performance liquid chromatography-mass spectrophotometry (UPLC-MS) systems with quadrupole time-of-flight as the analyzer and positive Electrospray Ionization (ESI) as the ionization source with the acuity C18 column 1.8 µm; 2.1 mm × 150 mm. The eluent applied was a combination of (a) Water (HPLC [High Performance Liquid Chromatography] grade)/formic acid (Merck, Darmstadt, Germany) 99.9/0.1 (v/v); (b) Acetonitrile (Merck, Darmstadt, Germany)/formic acid 99.9/0.1 (v/v) and the system of gradient elution. 100 and 350°C were the source and desolation temperatures, respectively. In a 10 mL volumetric flask containing absolute methanol, 10 mg of the extract was dissolved, and 5 µL was then introduced into the UPLC-MS apparatus. The MassLynx 4.1 software (Waters, Massachusetts, USA) and PubChem (https://pubchem.ncbi.nlm.nih.gov/) were used to process the chromatogram and then identify the compound. The matching of MS/MS and an error of <5 ppm were used to verify a compound's accuracy.

Oral bioavailability screening

Absorption, distribution, metabolism, excretion, toxicity (ADMET) database (https://admetmesh.scbdd.com/) was used to test important metrics of the selected compounds, including Caco-2 cell permeability assay, OB limits F (*F*-20%, *F*-30%), and human intestinal absorption.[8]

Identification of potential lung cancer targets

One of the most important aspects of drug research is predicting whether a chemical interacts with the target's protein or gene. The gene targets of each EBS's compound based on liquid chromatography-MS/MS results were identified from the GeneCards database (cutoff >0.7) (https://www.genecards.org/). On the other side, the gene targets associated with lung cancer were explored using the DisGeNET database (https://www.disgenet.org). Subsequently, the network pharmacology was analyzed using Cytoscape software 3.9.1 to get an overview of the interaction between active compound-gene targets and disease-gene targets.[9]

Construction of the target protein–protein interaction and enrichment analysis

The intersection genes between active compounds and disease targets were selected for further analysis using STRING 11.0 (https://string-db.org/). Common target proteins with a minimal required interaction score of 0.4 were used to construct the protein–protein interaction (PPI) network. The result was then utilized to look into biological activities by examining the gene ontology and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment of proteins and their functions.[10]

Molecular docking analysis

The structure of extracellular growth factor receptor (EGFR) as an important receptor in lung cancer progression was downloaded from https://www.rcsb.org/. Subsequently, the water and native ligands were removed. The ligand resveratrol was downloaded from Pubchem and PyRx Autodock Vina 9.0 was used to minimize the energy. The EGFR active sites (LYS 721, MET 742, CYS 751, LEU 764, MET 765, THR 766, GLN 767, and MET 769) were tagged after the receptor was signed as macromolecules. After making adjustments using a grid box with the active site as the reference, the center (X: 27.8391; Y:-2.9946; and Z: 55.2511) dimensions (*X*: 19.7139; *Y*: 11.8022; and *Z*: 17.8568) of the pocket was determined and the docking process was carried out. three-dimensional and two-dimensional visualization were further used to analyze the interaction between ligand and receptor.[11]

RESULTS

Suggested secondary metabolites of EBE based on liquid chromatography-mass spectrophotometry/mass spectrophotometry analysis

The total ion chromatogram obtained from mass spectroscopy is presented in Figure 1, resulting in the identification of 16 compounds [Table 1].

Secondary metabolite data are described in Table 1. The major compound, eleutherol, had the largest percentage area (18.87%) with a retention time (Rt) of 7.94, followed by resveratrol (18.49%; Rt 10.5).

The pharmacokinetic profile of identified compound of EBE

The components found in EBE must have good bioavailability to deliver pharmacological effects. Table 2 describes the

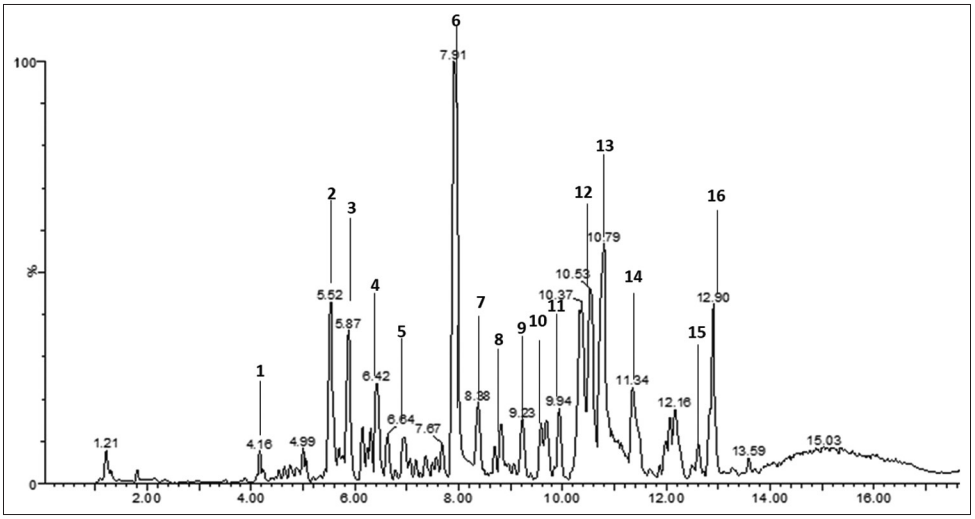

Figure 1: The chromatogram of EBE root extract. Each peak on the chromatogram indicated the presence of a single compound

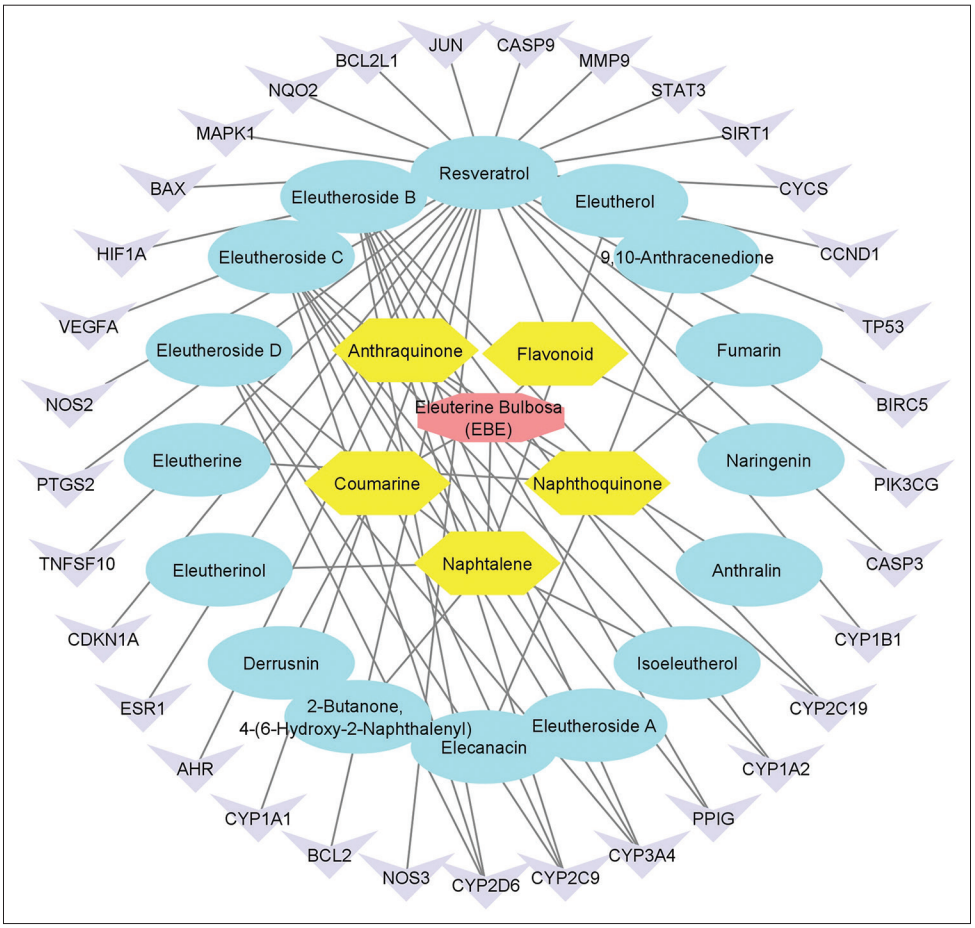

Figure 2: Network topology of compounds in EBE in lung cancer. The pink hexagons, yellow elliptical, blue ellipses, and purple triangles represented the plant, group of compounds, active components, and gene target of cancer which also related to lung cancer, respectively

Table 1: Prediction of compounds in *Eleutherine bulbosa* from liquid chromatography tandem mass spectrophotometry analysis

RT	Percentage area	Measured mass	Calculated mass	Formula	Compound	Group
4.178	0.81	208.2104	208.2105	$C_8H_{16}O_6$	Eleutheroside C	Naphthalene
5.542	7.01	419.1345	419.1342	$C_{21}H_{22}O_9$	Naringenin	Flavonoid
5.872	5.2	245.0813	245.0814	$C_{14}H_{12}O_4$	Isoeleutherol	Naphthalene
6.421	6.3	742.7121	742.7121	$C_{34}H_{46}O_{18}$	Eleutheroside D	Naphthalene
6.969	1.44	215.1072	215.1072	$C_{14}H_{14}O_2$	2-butanone, 4-(6-hydroxy-2-naphthalenyl)	Naphthalene
7.94	18.87	257.0808	257.0812	$C_{15}H_{12}O_4$	Eleutherinol	Naphthalene
8.375	2.07	272.2991	272.2997	$C_{16}H_{16}O_4$	Eleutherine	Naphthoquinone
8.818	1.89	372.4221	372.4225	$C_{17}H_{24}O_9$	Eleutheroside B	Naphthalene
9.212	1.75	244.2435	244.2440	$C_{14}H_{12}O_4$	Eleutherol	Naphthalene
9.647	3.53	268.1010	268.1015	$C_{16}H_{13}NO_3$	9,10-anthracenedione	Naphthoquinone
9.936	2.27	299.0928	299.0923	$C_{17}H_{14}O_5$	Fumarin	Naphthoquinone
10.505	18.49	229.0868	229.0865	$C_{14}H_{12}O_3$	Resveratrol	Flavonoid
10.773	17.13	227.0709	227.0708	$C_{14}H_{10}O_3$	Anthralin	Anthraquinone
11.363	5.99	576.4542	576.4533	$C_{35}H_{60}O_6$	Eleutheroside A	Naphtalene
12.614	0.94	272.2963	272.2968	$C_{16}H_{16}O_4$	Elecanacin	Naphthoquinone
12.903	6.29	357.0976	357.0974	$C_{19}H_{16}O_7$	Derrusnin	Coumarine

RT: Retention time

Table 2: Bioavailability profile of *Eleutherine bulbosa* compounds

Compounds	Caco-2	HIA	F20	F30
Eleutheroside C	−5.260	0.843	0.014	0.599
Naringenin	−4.803	0.018	0.972	0.997
Isoeleutherol	−4.818	0.006	0.002	0.004
Eleutheroside D	−6.464	0.89	0.01	0.817
2-butanone, 4-(6-hydroxy-2-naphthalenyl)	−4.638	0.01	0.025	0.002
Eleutherinol	−4.864	0.017	0.095	0.992
Eleutherine	−4.867	0.01	0.011	0.338
Eleutheroside B	−5.421	0.47	0.009	0.508
Eleutherol	−4.818	0	0.002	0.003
9,10-anthracenedione	−4.969	0.85	0.909	0.994
Fumarin	−4.755	0.005	0.003	0.003
Resveratrol	−4.916	0.012	0.264	0.055
Anthralin	−4.996	0.73	0.919	0.997
Eleutheroside A	−4.793	0.01	0.901	0.038
Elecanacin	−4.761	0.01	0.015	0.988
Derrusnin	−4.751	0.003	0.003	0.020

HIA: Human intestinal absorption

result of the bioavailability test. Most of the substances demonstrated good absorption characteristics.

Network pharmacology of EBE compound on lung cancer

Four compounds (Eleutheroside B, C, D, and resveratrol) were utilized to make the network pharmacology because they showed a similarity score >7. Figure 2 displays the interaction of secondary metabolites, lung cancer, gene targets associated with the disease, and the EBE compound. There was a total of 55 nodes, 65 edges,

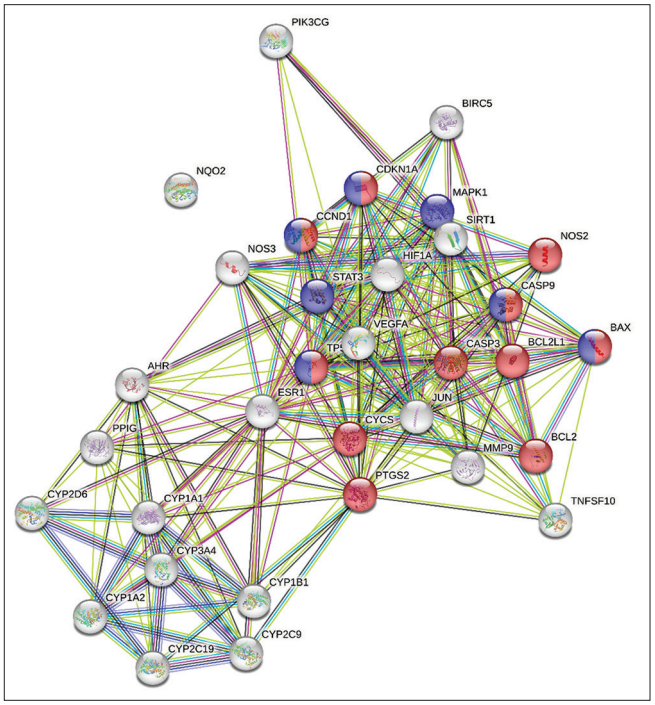

Figure 3: Protein–protein interaction network between lung cancer gene targets and resveratrol. The red node represents the small-cell lung cancer pathway, while the blue node represents the nonsmall-cell lung cancer pathway

an average number of 2291 neighbors, a characteristic path length of 1835, and a density of 0.021 [Figure 3]. Interestingly, only resveratrol interacted with 10 genes involved in lung cancer as follows: *CASP3, BCL2, VEGFA, BAX, JUN, MMP9, MAPK1, CASP9, CCND1, and PIK3CG*.

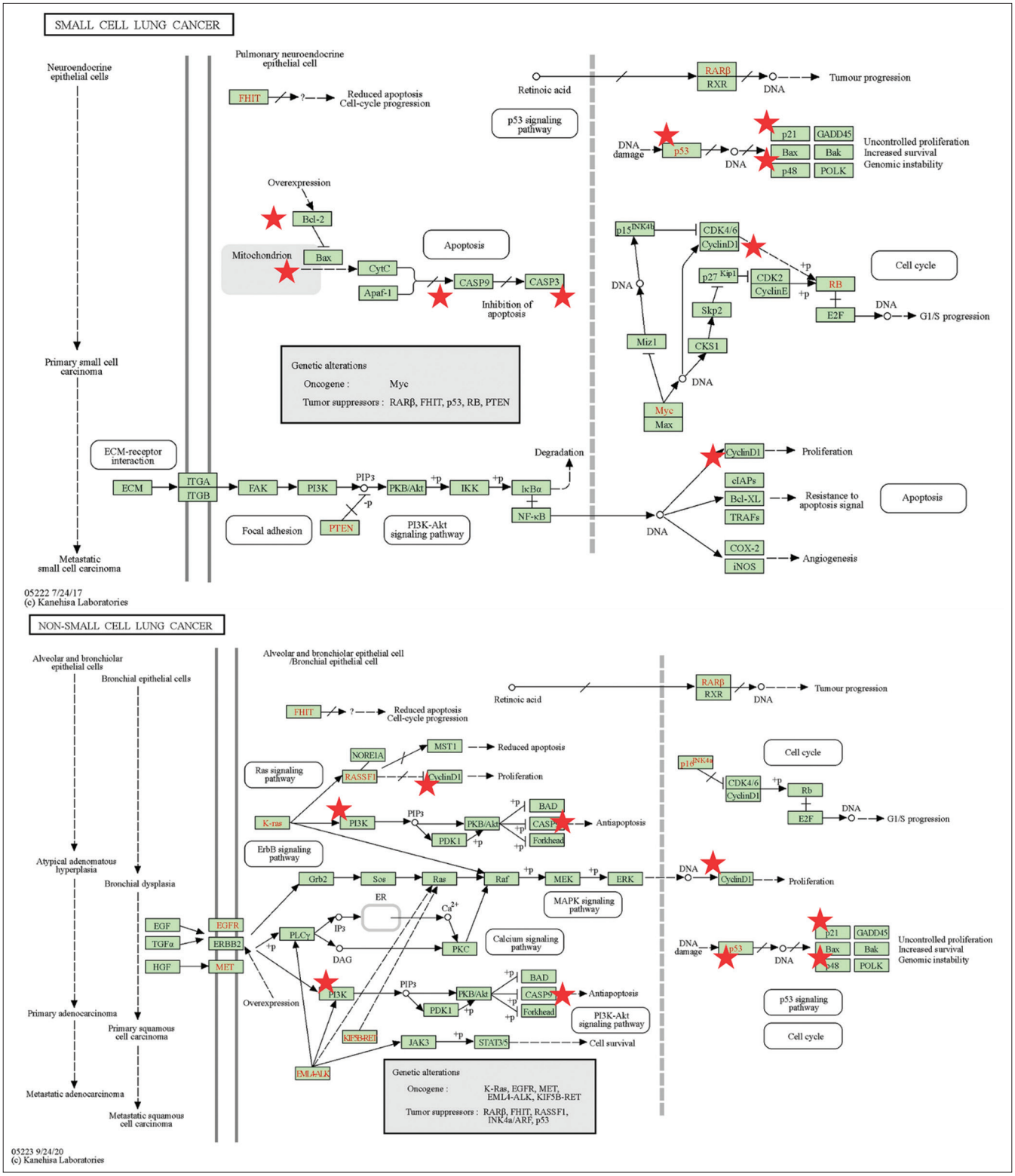

Figure 4: Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway of SCLC (hsa05222) NSCLC (hsa05223). Asterisks indicate EBE compound target as a part of the pathway. The molecular mechanisms altered in SCLC and NSCLC are overexpression of oncogene, loss of tumor suppressor genes, which turn induce rapid proliferation, loss of terminal differentiation, and reduced apoptosis. The predictive target of Resveratrol in SCLC and NSCLC inhibition based on network pharmacology analysis was to target proteins marked with red star symbol including in apoptosis (Bcl-2, Bax, CASP9, CASP3), cell cycle, proliferation (p53, p21, cyclinD1), and oncogene pathway (PI3K). Abbreviation: Bcl-2, B-cell lymphoma 2; Bax, Bcl-2-associated X protein; CASP, caspase; PI3K, Phosphoinositide 3 kinase (PI3K), small-cell lung cancer (SCLC), non small-cell lung cancer (NSCLC)

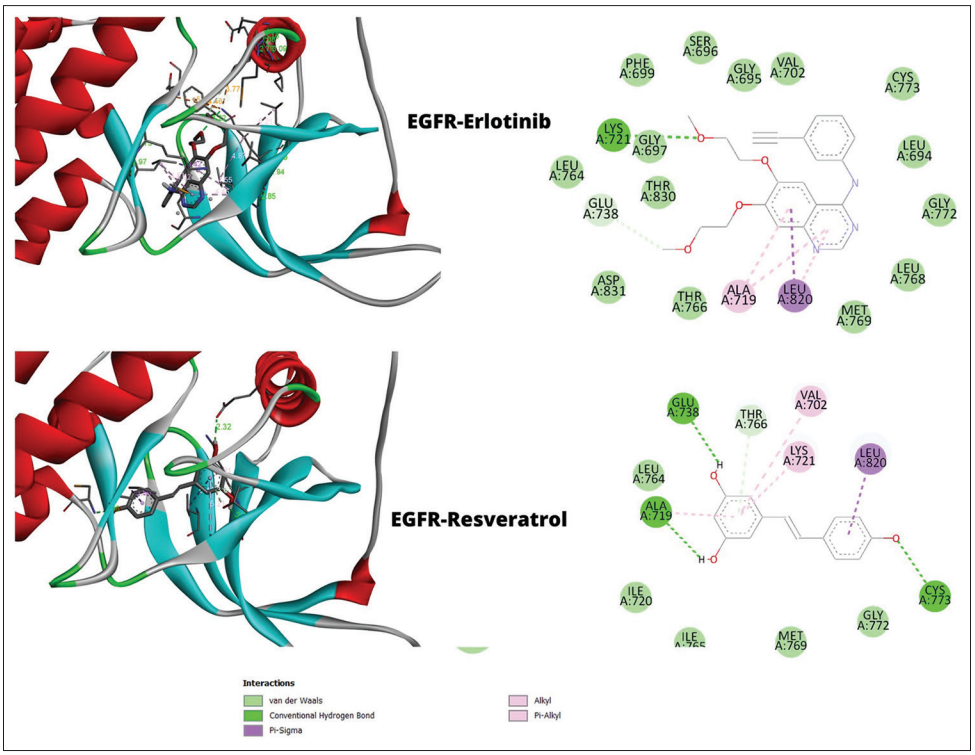

Figure 5: Interaction between extracellular growth factor receptor with resveratrol. The receptor displayed as ribbon model and completed with the ligand and line. Visualization of two dimensional showed the type of ligand-receptor interaction and the amino acid residue of the receptor that responsible for the binding. EGFR: Extracellular growth factor receptor

Construction of target protein–protein interaction, pathways, and enrichment analysis

From PPI analysis, a total of 36 proteins were obtained. The target nodes are connected by 253 edges, with an average node degree and local clustering of 15.3 and 0.764, respectively. It was found that there were 11 target proteins of the compound in the EBE that were involved in lung cancer mechanism, including *PTGS2, CYCS, CASP3, BCL2, BCL2 L1, BAX, CASP9, CCND1, CDKNIA, NOS2, TP53, MAPK1, and STAT3*.

Among 153 pathways obtained from the KEGG pathway, there were six pathways closely related to the pathophysiology of lung cancer where each pathway crosstalk to others, including the P53 signaling pathway (Hsa04115), small-cell lung cancer (Hsa05222), nonsmall-cell lung cancer (Hsa05223), Chemical carcinogenesis (Hsa05204), MicroRNAs in cancer (Hsa05206), and Apoptosis (Hsa04210) [Figure 4].

Molecular interaction of extracellular growth factor receptor and resveratrol

The binding affinity value of resveratrol with EGFR (−6.9) was higher than erlotinib (−6.2). Figure 5 showed the conventional hydrogen bond of resveratrol was found in the EGFR binding site with ALA 719, GLLU 738, and CYS 773. The hydrophobic binding was observed in EGFR VAL 702, LYS 721, and LEU 820. The similarity of amino acid residues was LEU 820.

DISCUSSION

Natural ingredients proven to have anticancer properties include resveratrol.[12,13] Our findings showed that resveratrol had good bioavailability and interacted with genes associated with lung cancer. Consistently, resveratrol might be involved in the biological process of lung cancer either the P53 signaling pathway (Hsa04115), MicroRNAs in cancer (Hsa05206), and Apoptosis (Hsa04210) either in small-cell lung cancer (Hsa05222) or nonsmall-cell lung cancer (Hsa05223) from network pharmacology followed by PPI network analysis. The P53 signaling pathway (Hsa04115) regulates cell proliferation, apoptosis (cell death), and deoxyribonucleic acid damage.[14] Some studies supported our findings that resveratrol is involved in the cancer-p53 signaling pathway by increasing p53 activity and thus causing cancer cell death, as well as inhibiting cancer cell proliferation through various mechanisms, including inhibition of the PI3K/Akt activity that leads to decreased p53 activity.[15]

Interestingly, network pharmacology and PPI results showed resveratrol might also work in the apoptosis pathway (Hsa04210), either targeting gene or protein: BAX, BCL2, CASP3, and CASP9. These results were supported by previous studies focusing on lung cancer inhibition that resveratrol activated proapoptosis proteins such as BAX and Caspase-3, as well as decreased the

antiapoptosis protein expression like Bcl-2.[16] In addition, resveratrol modulated the myc and mTOR through MAPK and PI3K/Akt pathways.[17] The nonsmall-cell lung cancer pathway (Hsa05223) from enrichment analysis also targeted PI3K, which plays a vital role in apoptosis.[18]

The microRNAs in the cancer pathway (Hsa05206) are involved in gene expression regulation in cancer, including lung cancer.[19] Resveratrol was known to enhance the expression of miR-200b and miR-34a, which are typical miRNAs that inhibit the growth and metastasis of lung cancer cells.[20] In addition, resveratrol lowered miR-21, which role as an oncogene.[19,21] The importance of EGFR was also highlighted as the main receptor that regulates cancer proliferation in lung cancer. In addition, the binding affinity of resveratrol showed more negative compared to erlotinib. Thus, resveratrol was suggested to have potency to suppress cancer cell proliferation through its interaction with EGFR.

CONCLUSION

Resveratrol might be the main active ingredient of EBE that had a crucial role in lung cancer by cancer cell proliferation inhibition and apoptosis stimulation based on network pharmacological, which become a novelty of this study. However, further wet laboratory experiments are needed to verify our findings in understanding the EBE's effects on lung cancer.

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Conflicts of interest

There are no conflicts of interest.

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