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Heca Journal of Applied Sciences

Vol. 3, No. 1, 2025

Network Pharmacology Insights into Broccoli Microgreens for Prostate Cancer

Puspita Wijaya [1], Trina Ekawati Tallei [2,3,*], Lydia Estelina Naomi Tendean [2], Fatimawali Fatimawali [4,5], Grace Lendawati Amelia Turalaki [2] and Diana Shintawati Purwanto [6]

[1] Medical Education Study Program, Faculty of Medicine, Sam Ratulangi University, Manado 95115, Indonesia; puspitawijaya011@student.unsrat.ac.id (P.W)
[2] Department of Biology, Faculty of Medicine, Sam Ratulangi University, Manado 95115, Indonesia; trina_tallei@unsrat.ac.id (T.E.T); tendeanlydia@unsrat.ac.id (L.E.N.T); gracelat@unsrat.ac.id (G.L.A.T)
[3] Department of Biology, Faculty of Mathematics and Natural Sciences, Sam Ratulangi University, Manado 95115, Indonesia
[4] Department of Chemistry, Faculty of Medicine, Sam Ratulangi University, Manado 95115, Indonesia; fatimawali@unsrat.ac.id (F)
[5] Pharmacy Study Program, Faculty of Mathematics and Natural Sciences, Sam Ratulangi University, Manado 95115, Indonesia
[6] Department of Biochemistry, Faculty of Medicine, Sam Ratulangi University, Manado 95115, Indonesia; dianashintapurwanto@unsrat.ac.id (D.S.P)

***** Correspondence: trina_tallei@unsrat.ac.id

Article History

Received 15 December 2024
Revised 13 February 2024
Accepted 20 February
Available Online 26 February 2024

Keywords:
Prostate cancer
Network pharmacology
Broccoli microgreens
Anticancer therapy
Bioactive compound

Abstract

Prostate cancer is a leading malignancy in men, ranking fourth globally and fifth in Indonesia (GLOBOCAN 2020). Conventional therapies, though available, are limited by high costs, side effects, and resistance, highlighting the need for accessible alternatives. Broccoli microgreens, rich in bioactive compounds, have shown potential in preventing and treating various cancers. This study hypothesized that bioactive compounds in broccoli microgreens interact with molecular targets involved in prostate cancer progression. To test this hypothesis, we employed a network pharmacology-based in silico approach to systematically explore these interactions and identify potential therapeutic mechanisms. Bioactive compounds in broccoli microgreens were identified using liquid chromatography-mass spectrometry (LC-MS) and analyzed via the PubChem database. The biological activities of these compounds were predicted using PASS Online, focusing on their capacity to modulate TP53 gene expression. Pharmacokinetic and toxicity evaluations were performed using ADMETLab 3.0 and Protox 3.0 to assess their safety and drug-like properties. Target proteins were identified through SwissTargetPrediction and GeneCards, while protein-protein interaction networks were constructed using STRING. The pharmacological network was visualized using Cytoscape to elucidate the molecular mechanisms of action. The analysis identified 528 relevant target proteins, with key roles attributed to SRC and EGFR, both critical in resistance to EGFR tyrosine kinase inhibitors and in regulating processes such as cell proliferation, apoptosis resistance, and metastatic potential. Through network pharmacology, bioactive compounds such as kaempferol and polydatin were identified as potential inhibitors of these targets, demonstrating their ability to modulate pathways essential to prostate cancer progression. In conclusion, broccoli microgreens contain bioactive compounds with potential pharmacological relevance for prostate cancer, particularly through their interaction with SRC and EGFR pathways, warranting further experimental validation.

1. Introduction

Prostate cancer is one of the most prevalent malignancies of the urogenital system in men globally. It originates in the prostate gland, an organ responsible for producing ejaculatory fluid, and often presents with symptoms such as urinary difficulties. According to 2022 Global Cancer Observatory (GLOBOCAN) data, prostate cancer ranks as the fourth most common cancer worldwide and the second most common among men, with 1,467,854 new cases and over 390,000 deaths. The highest prevalence in the past five years was observed in Europe (38.1%), followed by Asia and North America at 22.2%. In Indonesia, prostate cancer ranks fifth in terms of cancer incidence among men, with an incidence rate of 11.6 cases per 100,000 men and a mortality rate of 4.5 per 100,000 men [1].

At the molecular level, prostate cancer progression is driven by a complex interplay of genetic and epigenetic alterations, including mutations in tumor suppressor genes (e.g., TP53, PTEN), oncogene activation (e.g., MYC, AR), and dysregulation of cell cycle pathways (e.g., cyclin D1, CDK4/6). Additionally, oxidative stress and chronic inflammation play critical roles in prostate carcinogenesis by promoting DNA damage, cell proliferation, and resistance to apoptosis [2, 3]. These molecular mechanisms underscore the need for therapeutic strategies to simultaneously target multiple pathways to effectively suppress tumor growth and progression.

Prostate cancer treatment encompasses active surveillance, surgery, radiation therapy, hormone therapy, and chemotherapy, with the choice of approach depending on tumor characteristics, disease stage, patient age, and recurrence risk [4, 5]. However, these therapies are associated with high costs and may result in severe side effects, including fatigue, hair loss, neurological disorders, incontinence, erectile dysfunction, and drug resistance. Moreover, they are often not curative or sufficiently effective when used as monotherapies [6].

In recent decades, research into the potential of bioactive compounds derived from natural materials as anticancer agents has garnered significant attention from the global scientific community [7]. Identifying natural sources with active compounds exhibiting cytotoxic effects may offer a viable approach to suppress prostate cancer progression while minimizing side effects and treatment resistance [6]. One promising natural source is broccoli microgreens (Brassica oleracea var. italica), the young plants of broccoli. Studies have demonstrated that broccoli microgreens contain significantly higher concentrations of bioactive compounds compared to mature broccoli plants, including vitamins (such as C, E, and K), pigments (chlorophyll and carotenoids), beta-carotene, phenolics, and glucosinolates, all of which contribute to their antioxidant capacity [8–11]. Notably, glucosinolates, such as sulforaphane, have been extensively studied for their ability to modulate key cancer-related pathways, including the induction of phase II detoxification enzymes, inhibition of histone deacetylases, and suppression of NF-κB signaling, which collectively contribute to their antiproliferative and pro-apoptotic effects [12, 13]. Previous studies have demonstrated the efficacy of broccoli microgreens in inhibiting the growth of various cancer cell types, including colon cancer, through mechanisms involving oxidative stress reduction, cell cycle arrest, and apoptosis induction [14]. These findings provide a strong rationale for investigating their potential in prostate cancer, given the shared molecular pathways between prostate and colon cancers, such as dysregulated Wnt/β-catenin signaling, oxidative stress response, and cell cycle control [15, 16].

This study aimed to employ an in silico pharmacological network approach to systematically explore the therapeutic potential of broccoli microgreens. Network pharmacology is a powerful methodology integrating bioinformatics, systems biology, and pharmacology to elucidate the complex interactions between bioactive compounds and molecular targets within a biological network. This approach is particularly well-suited for studying natural compounds, as it identifies multi-target effects, predicts synergistic interactions, and explores underlying mechanisms at a systems level [17–19]. By mapping the interactions between the bioactive compounds of broccoli microgreens and molecular targets involved in prostate cancer, this study aims to provide a comprehensive understanding of their potential therapeutic effects and inform the development of more effective, multi-targeted therapies for prostate cancer.

2. Materials and Methods

2.1. Analytical Tools and Validation in Silico Predictions

This study utilized a range of online database-driven tools, including WAY2DRUG PASS (version 2.0), ADMET Lab 3.0, Protox 3.0, the Similarity Ensemble Approach (SEA) database (https://sea.bkslab.org/), SwissTargetPrediction (http://www.swisstargetprediction.ch/), Draw Venn Diagram (https://bioinformatics.psb.ugent.be/webtools/Venn/), STRING (version 12.0), and Cytoscape (version 3.10.3). A multi-step validation approach was employed to ensure the reliability of the in-silico predictions. First, the predicted biological activities, pharmacokinetic

properties, and toxicity profiles of the identified compounds were cross-referenced with existing experimental data from reputable databases such as PubChem (https://pubchem.ncbi.nlm.nih.gov/), Dr. Duke's Phytochemical and Ethnobotanical Databases (https://phytochem.nal.usda.gov/), and COCONUT (version 2.0). For instance, the predicted anticancer activities of the compounds were compared with literature reports on their known pharmacological effects. Second, the results from WAY2DRUG PASS (version 2.0) were validated using alternative prediction tools, including SwissTargetPrediction (http://swisstargetprediction.ch/), and the Similarity Ensemble Approach (SEA) database (https://sea.bkslab.org/), to ensure consistency across different computational platforms. Third, the identified protein targets and pathways were evaluated for their biological relevance to prostate cancer using GeneCards (version 5.23) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) version 113.0. This comprehensive validation strategy enhances the confidence in the computational predictions and aligns them with established biological knowledge.

2.2. Preparation and Metabolite Extraction of Broccoli Microgreens

The study focused on bioactive compounds extracted from fresh broccoli microgreens, cultivated under controlled conditions to ensure reproducibility. Broccoli seeds were soaked in deionized water for 6 hours to initiate germination and then sown onto seedling trays lined with plastic mesh. The seeds were incubated in darkness for three days to promote germination. Subsequently, the sprouted broccoli was grown into microgreens under artificial LED lighting with an intensity of approximately 7000 lux, an air humidity range of 52-60%, and a temperature of 26-26.5°C. After 12 days of growth, the microgreens were harvested and subjected to drying treatments using either air-fryer drying at 160°C for 10 minutes or microwave drying at 600W for 3 minutes. The dried microgreens were processed for metabolite extraction using a solvent mixture of methanol, acetonitrile, and water (2:2:1 vol ratio), followed by homogenization, sonication, and centrifugation. The extracted metabolites were identified using Liquid Chromatography-Mass Spectrometry (LC-MS) [20]. Filtration of the identified compounds was performed to remove probable non-natural contaminants using reference databases such as PubChem (https://pubchem.ncbi.nlm.nih.gov/), Dr. Duke's Phytochemical and Ethnobotanical Databases (https://phytochem.nal.usda.gov/), and COCONUT (version 2.0). The filtering process involved the application of specific criteria: compounds were excluded if they were not listed in natural product databases or if their molecular structures were inconsistent with known phytochemical profiles. Additionally, compounds were filtered out if they lacked key functional groups commonly found in natural metabolites or exhibited synthetic-like structural features, as determined through cheminformatics analysis. This ensured that only compounds with a high likelihood of being natural metabolites were retained for further analysis [21].

2.3. Bioactive Compound Profiling and Activity Prediction of Broccoli Microgreens

The bioactive compounds of broccoli microgreens were profiled using the Simplified Molecular-Input Line-Entry System (SMILES) data retrieved from PubChem [22]. Their potential as prostate anticancer agents was analyzed using WAY2DRUG PASS (version 2.0), which employs Structure Activity Relationship (SAR) analysis to compare the structures of the identified compounds with those of known active compounds.

2.4. Pharmacokinetic and Toxicity Analysis

Pharmacokinetic properties, encompassing Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET), were assessed using ADMETLab 3.0 and Protox 3.0. Lipinski's Rule of Five was also applied to evaluate the drug-likeness and feasibility of the identified compounds as potential therapeutic agents [23].

2.5. Protein Target Identification, Pathway Annotation, and Network Pharmacology Analysis

Protein targets were identified using SwissTargetPrediction and the Similarity Ensemble Approach (SEA), with SMILES notation as input. Prostate cancer-associated proteins were obtained from GeneCards (version 5.23), and shared targets between diseases and compounds were mapped using Venn diagrams. Molecular pathways were analyzed using the Kyoto Encyclopedia of Genes and Genomes (KEGG) version 113.0, while biological processes were annotated via ShinyGO version 0.81. The interactions among target proteins were assessed using STRING version 12.0, and the resulting data were processed and visualized as protein interaction networks using Cytoscape version 3.10.3.

3. Results and Discussion

3.1. Bioactivity Profiling of Broccoli Microgreens Metabolites

After filtration, 232 natural product compounds were identified, including compounds derived from broccoli microgreens and others from various plant sources.

These compounds were analyzed using PubChem to obtain their canonical Simplified Molecular-Input Line-Entry System (SMILES) notations, which were utilized to predict their biological activity. The bioactivity analysis, based on Probability Active (Pa) and Probability Inactive (Pi) values, indicated that compounds with a Pa > 0.7 exhibited very high biological activity. At the same time, those with 0.5 < Pa < 0.7 demonstrated moderately high biological activity and potential for further development [24].

Among the 232 identified compounds, 159 demonstrated potential as TP53 expression enhancers, a key regulatory pathway in cancer cell proliferation, with a Pa > 0.5 (Table 1). The compounds were filtered based on their predicted biological activities using the PASS Online tool, which provides a Probability Active (Pa) score indicating the likelihood of a compound exhibiting a specific biological activity. This threshold was chosen because a Pa score > 0.5 indicates a sufficiently high probability of biological activity. These compounds are suitable for in vitro and in vivo testing and potential development as novel therapeutic agents. From these, the 20 compounds with the highest Pa values were selected for further detailed analysis (Table 2). These findings highlight the promising potential of broccoli microgreen-derived compounds as therapeutic candidates for prostate cancer. However, it is equally important to note that 73 compounds exhibited low bioactivity (Pa < 0.5), suggesting limited potential as TP53 enhancers. While not prioritized in this study, these compounds may still hold value for other therapeutic applications or as leads for structural optimization in future research.

Among the top 20 compounds identified as TP53 expression enhancers, these can be grouped into several key categories based on their structure and function. The nucleoside group, which consists of compounds such as thymidine, 5-methylcytidine, 2'-deoxyadenosine, adenosine, isoguanosine, and guanosine, plays a vital role in DNA and RNA replication due to the presence of nitrogenous bases attached to ribose or deoxyribose sugars [25, 26]. Interestingly, nucleoside analogs such as gemcitabine and 5-fluorouracil (5-FU), which are widely used in chemotherapy, also function by interfering with nucleic acid metabolism, leading to apoptosis in cancer cells. This suggests that the identified nucleosides may exhibit a similar mechanism of action [27–29]. The flavonoids, such as kaempferol, kaempferol derivatives (C193, C189, C207), rutin, luteolin-3',7-diglucoside, and polydatin, are known for their antioxidant and anti-inflammatory properties, which contribute to their anticancer potential [30]. Kaempferol, for instance, has been reported to exert antiproliferative effects through multiple mechanisms, including the induction of apoptosis and inhibition of PI3K/AKT and MAPK signaling pathways—mechanisms that overlap with the activity of clinically used kinase inhibitors such as erlotinib (EGFR inhibitor) and sorafenib (multi-kinase inhibitor) [31, 32]. Similarly, curcumin, a well-known flavonoid with anticancer properties, shares structural and functional similarities with several identified flavonoids, highlighting their potential as natural alternatives or adjuncts to current therapies [33]. Additionally, esculin, a coumarin derivative, exhibits biological activities, including anticoagulation and protection against oxidative stress [34]. Several coumarin-based compounds have been explored as anticancer agents, with some showing inhibitory activity against topoisomerase enzymes, similar to etoposide and other clinically used topoisomerase inhibitors [35, 36].

Compounds such as 13(s)-HPOT and 13(s)-HPODE are categorized as oxygenated fatty acids (oxylipins), which play significant roles in cellular signaling and inflammatory processes [37]. Their involvement in lipid peroxidation pathways suggests possible synergy with lipid metabolism-targeting drugs such as cerulenin or orlistat, which have been explored for cancer therapy [38]. Glycitin, an isoflavone, is recognized for its protective effects against hormone-sensitive cancers. It exhibits structural similarities to genistein, a known phytoestrogen with anti-proliferative effects in prostate and breast cancer [39, 40]. Rhaponticin, a stilbenoid phenolic compound, exhibits strong antioxidant properties similar to resveratrol, a compound extensively studied for its ability to modulate apoptotic pathways in various cancers [41–43]. In contrast, the compounds Np-020521 and Lmpk12110619 have not yet been fully classified and require further investigation.

The identified compounds were primarily flavonoids, nucleosides, and other phenolic compounds, all exhibiting notable bioactivity and offering multitargeting capabilities that distinguish them from single-target synthetic drugs, potentially reducing the likelihood of drug resistance. Unlike conventional chemotherapy drugs, which often exhibit high toxicity and limited target specificity, the compounds identified in broccoli microgreens may provide a safer therapeutic alternative with fewer side effects. Their ability to enhance TP53 expression offers a strong basis for further development as therapeutic agents for prostate cancer. This potential is attributed to their role in upregulating TP53, a crucial tumor suppressor gene involved in the regulation of cancer cell growth and proliferation. Moreover, the broad-spectrum bioactivity of these compounds suggests their possible application in combination therapies,

Table 1. List of identified bioactive compounds of broccoli microgreens with potential TP53 expression enhancers.

Compounds Code	Compound's Name	TP53 Expression Enhancer
C1	2-c-methyl-d-erythritol 4-phosphate	0.550
C2	Agmatine	0.316
C3	Threonine	0.682
C4	D-(+)-maltose	0.757
C5	Uracil	0.606
C6	D-a-hydroxyglutaric acid	0.767
C7	L-pyroglutamic acid	0.403
C8	Hypoxanthine	0.423
C9	Pyridoxine	0.444
C10	Guanine	0.438
C11	Adenosine	0.934
C12	Thymidine	0.969
C13	4-hydroxybutyric acid (ghb)	0.665
C14	2'-deoxyadenosine	0.942
C15	Thymine	0.728
C16	4-methyl-5-thiazoleethanol	
C17	9,10,18-trihydroxystearate	0.707
C18	(9z,11e)-(13s)-13-hydroperoxyoctadeca-9,11-dienoic acid	0.927
C19	13(s)-hpot	0.863
C20	Pentadecanoic acid	0.740
C21	Palmitoleic acid	0.791
C22	1-palmitoylglycerol 3-phosphate	0.591
C23	Pulegone	0.414
C24	A-linolenic acid	0.731
C25	Cis-11-eicosenoic acid	0.791
C26	Docosahexaenoic acid	0.726
C27	Oleic acid	0.791
C28	5'-s-methyl-5'-thioadenosine	0.733
C29	Gentisic acid	0.727
C30	1-o-sinapoyl-beta-d-glucose	0.786
C31	5-hydroxyindole-3-acetic acid	0.583
C32	Riboflavin	0.511
C33	4-coumaric acid	0.772
C34	Indole-3-acetyl-beta-1-d-glucoside	0.554
C35	3-coumaric acid	0.772
C36	Rutin	0.893
C37	Kaempferol	0.931
C38	Sinapinic acid	0.787
C39	Indole-3-acetic acid	0.525
C40	A-d-mannose 1-phosphate	0.721
C41	D-(+)-pipecolinic acid	0.283
C42	L-citrulline	0.528
C43	N-ethylglycine	0.470
C44	D-(-)-arabinose	0.580
C45	L-glutamic acid	0.703
C46	5-hydroxyectoine	0.256
C47	L-methionine (s)-s-oxide	0.554
C48	Sedoheptulose	0.741
C49	Gnh	0.338
C50	N-acetylaspartic acid	0.495
C51	5,6-dimethoxy-2-(2-methoxyphenyl)-4h-chromen-4-one	0.783
C52	2-oxoglutaric acid	0.615
C53	Trans-aconitic acid	0.679
C54	5-methylcytidine	0.944
C55	Malonic acid	0.671
C56	N(omega)-methyl-l-arginine	0.675
C57	2-furoic acid	0.492
C58	Pipecolic acid	0.283
C59	N-acetylputrescine	0.327
C60	Diaminopimelic acid	0.685
C61	L-(-)-malic acid	0.699

Compounds Code	Compound's Name	TP53 Expression Enhancer
C62	N-acetylornithine	0.333
C63	Isoguanosine	0.880
C64	Miserotoxin	0.589
C65	N-a-acetyl-l-lysine	0.340
C66	Adenine	0.511
C67	Progoitrin	
C68	Sinigrin	0.272
C69	Citric acid	0.613
C70	Itaconic acid	0.589
C71	4-guanidinobutyric acid	0.517
C72	7-(1-pyrrolidinyl)pyrimido[4,5-d]pyrimidin-4-amine	
C73	(2s,4s)-pinnatanine	0.488
C74	O-acetyl-l-carnitine	0.313
C75	Xanthine	0.457
C76	N-acetylalanine	0.523
C77	3-hydroxy-3-(methoxycarbonyl)pentanedioic acid	0.614
C78	3-hydroxy-3-methylglutaric acid	0.687
C79	L-tyrosine	0.640
C80	Succinic acid	0.723
C81	2-hydroxycinnamic acid	0.714
C82	Guanosine	0.850
C83	Inosine	0.811
C84	Citroflex 2	0.632
C85	Leucine	0.706
C86	Pilocarpine	0.255
C87	6-hydroxynicotinic acid	0.358
C88	Glycyl-l-leucine	0.381
C89	L-phenylalanine	0.578
C90	Epinephrine	0.493
C91	Valylproline	
C92	Dihydrocoumarin	0.518
C93	Valerophenone	0.697
C94	Benzoylpaeoniflorin	
C95	Ipsdienone	0.550
C96	(15z)-9,12,13-trihydroxy-15-octadecenoic acid	0.765
C97	3-hydroxydecanoic acid	0.778
C98	2,3-dinor-8-epi-prostaglandin f2a	0.543
C99	Butyl benzoate	0.521
C100	Solasodine	0.423
C101	11-deoxy prostaglandin f1b	0.617
C102	Linoelaidic acid	0.764
C103	4-ethoxy ethylbenzoate	0.482
C104	2-linoleoyl glycerol	0.762
C105	12-hydroxydodecanoic acid	0.671
C106	Dehydrojuvabione	0.269
C107	2-{(2s,3r,4s,5r)-5-[(4-{[benzyl(methyl)amino]methyl}-1h-1,2,3-triazol-1-yl)methyl]-3,4-dihydroxytetrahydro-2-furanyl}-1-[4-(2-methoxyphenyl)-1-piperazinyl]ethanone	
C108	Montanol	0.489
C109	3-hydroxy-4-methoxy-9h-xanthen-9-one	0.795
C110	(3e,5s,8r,11e,13s,16r)-5,13-dihydroxy-8,16-dimethyl-1,9-dioxacyclohexadeca-3,11-diene-2,10-dione	0.655
C111	Polydatin	0.874
C112	2-hydroxymyristic acid	0.765
C113	Pg(18:3(6z,9z,12z)/0:0)	0.673
C114	12-oxo phytodienoic acid	0.528
C115	16-oxopalmitate	0.729
C116	Methyl hexadecanoate	0.709
C117	N-[(1s,2s,8s,8as)-8-hydroxy-7-{(2s)-1-[(2r)-2-(methoxymethyl)-1-pyrrolidinyl]-1-oxo-2-propanyl}-1,4a-dimethyldecahydro-2-naphthalenyl]-5-pyrimidinecarboxamide	
C118	Stearolic acid	0.627
C119	Tetrahydrofurfuryl methacrylate	0.363

Compounds Code	Compound's Name	TP53 Expression Enhancer
C120	Pe(18:2(9z,12z)/0:0)	0.481
C121	7-oxotetradecanoic acid	0.744
C122	Lmpk12110619	0.923
C123	A-eleostearic acid	0.756
C124	Pg(16:0/0:0)	0.638
C125	Curdione	0.380
C126	(+/-)9(10)-epome	0.725
C127	Stearic acid	0.740
C128	4-n-hexylphenol	0.717
C129	N-butylbenzene	0.624
C130	Perillene	0.508
C131	(-)-caryophyllene oxide	0.653
C132	Octadec-9-ynoic acid	0.627
C133	Jasmone	0.587
C134	Alpha-irone	0.352
C135	Leupeptin	
C136	Oleamide	0.658
C137	Heptadecatrienal	0.656
C138	9-oxo-10(e),12(e)-octadecadienoic acid	0.739
C139	3-hydroxy-palmitic acid methyl ester	0.775
C140	Palmitelaidic acid methyl ester	0.764
C141	Sphingosine (d18:1)	0.780
C142	Heptadecanoic acid, methyl ester	0.709
C143	Clareolide	0.457
C144	1-hexadecanoyl-sn-glycero-3-phosphate	0.591
C145	5a-dihydrotestosterone	0.669
C146	Labdanolic acid	0.386
C147	11(z),14(z),17(z)-eicosatrienoic acid	0.731
C148	Trans-nerolidol	0.703
C149	(r)-3-hydroxy myristic acid	0.778
C150	(3z,6z,9z)-3,6,9-dodecatrien-1-ol	0.597
C151	Myristoleic acid methyl ester	0.764
C152	Delta-decalactone	0.503
C153	Crucigasterin 277	0.579
C154	(13z,16z)-docosadienoic acid	0.764
C155	Linoleoyl ethanolamide	0.554
C156	Linalyl oxide	0.482
C157	Icosadienoic acid	0.764
C158	9(z),11(e),13(e)-octadecatrienoic acid methyl ester	0.788
C159	Octadecanal	0.670
C160	Avocadyne 1-acetate	0.457
C161	Methyl linoleate	0.735
C162	N-[2-(dimethylamino)ethyl]-2-[(1s,4s,5s)-5-isopropyl-2-methyl-4-{[(phenylcarbamoyl)amino]methyl}-2-cyclohexen-1-yl]acetamide	
C163	Messagenin	0.305
C164	Jervine	0.459
C165	1-linoleoyl glycerol	0.785
C166	Np-020521	0.918
C167	Palmitoyl ethanolamide	0.524
C168	Arachidic acid	0.740
C169	5-ethoxy-10-gingerol	0.797
C170	1-palmitoylglycerol	0.762
C171	Betulin	
C172	20(s)-protopanaxadiol	
C173	Monoolein	0.813
C174	8-hydroxyquinoline	0.563
C175	Trigonelline hcl	
C176	Epigoitrin	0.360
C177	Dl-4-hydroxyphenyllactic acid	0.720
C178	Pheophorbide a	
C179	Erucic acid	0.791
C180	Np-021292	
C181	Artemether	

Compounds Code	Compound's Name	TP53 Expression Enhancer
C182	Erucamide	0.658
C183	Hexadecanamide	0.584
C184	Dl-tryptophan	0.371
C185	(+)-7-isojasmonic acid	0.422
C186	5-hydroxyindoleacetylglycine	
C187	Hydroxysenkirkine	
C188	Kaempferol 3-sophorotrioside	0.946
C189	4-methoxyglucobrassicin	
C190	Feruloylputrescine	0.446
C191	2-hydroxycaproic acid	0.765
C192	Kaempferol-7-o-b-d-glucopyranoside	0.969
C193	Luteolin-3',7-diglucoside	0.939
C194	N-acetyl-l-leucine	0.505
C195	Sinapine	
C196	Citrinin	0.442
C197	3-phenyllactic acid	0.660
C198	3-(4-hydroxy-3-methoxyphenyl)propanoic acid	0.715
C199	Indole-3-acetyl-myo-inositol	0.439
C200	Scutellarioside ii	0.493
C201	7-keto-8-aminopelargonic acid	0.601
C202	2,4,6-trihydroxyacetophenone	0.647
C203	Quinaldic acid	0.457
C204	Esculin	0.873
C205	Lawsone	0.773
C206	Kaempferol 3-(6''-sinapylglucosyl)-(1->2)-galactoside	0.920
C207	Cynaropicrin	0.417
C208	Indole-2-carboxylic acid	0.411
C209	4-indolecarbaldehyde	
C210	3-hydroxybenzoic acid	0.692
C211	2,4-quinolinediol	0.510
C212	Azelaic acid	0.728
C213	Lumichrome	0.481
C214	Methyl 4-hydroxy-3-methoxycinnamate	0.712
C215	Limonin	
C216	Rhaponticin	0.899
C217	Encelin	0.724
C218	3-hydroxy-3,4-bis[(4-hydroxy-3-methoxyphenyl)methyl]oxolan-2-one	0.602
C219	Diffractic acid	0.622
C220	Isoliquiritin	0.776
C221	Glycitin	0.857
C222	Equol	0.663
C223	Tetradecanoyl-coa	
C224	N-(p-coumaroyl) serotonin	0.294
C225	Salidroside	0.710
C226	Brassicanal a	
C227	Np-002999	0.778
C228	Diisobutyl adipate	0.652
C229	9,12,13-trihome	0.803
C230	Corchorifatty acid f	0.764
C231	Sedanolide	0.600
C232	2,4-dichlorobenzoic acid	0.572

where they may enhance the efficacy of existing anticancer drugs while minimizing adverse effects. Future studies should focus on comparative analyses of these natural compounds with standard chemotherapy agents to establish their therapeutic viability and clinical relevance.

3.2. Pharmacokinetic Analysis and Drug-Likeness Assessment

Additionally, compounds were evaluated for their pharmacokinetic properties and toxicity profiles using ADMETLab 3.0 and Protox 3.0. Compounds with unfavorable ADMET properties (e.g., poor bioavailability, high toxicity) were excluded to ensure the selection of compounds with drug-like characteristics and minimal

Table 2. SAR Profile of TP53 expression enhancers for top 20 broccoli microgreen bioactive compounds with Pa Scores > 0.5.

Compounds Code	Compound's Name	TP53 Expression Enhancer Score
C12	Thymidine	0.969
C193	Kaempferol-7-o-b-d-glucopyranoside	0.969
C189	Kaempferol 3-sophorotrioside	0.946
C54	5-methylcytidine	0.944
C14	2'-deoxyadenosine	0.942
C194	Luteolin-3',7-diglucoside	0.939
C11	Adenosine	0.934
C37	Kaempferol	0.931
C18	13(S)-Hpode	0.927
C123	Lmpk12110619	0.923
C207	Kaempferol 3-(6''-sinapylglucosyl)-(1->2)-galactoside	0.920
C167	Np-020521	0.918
C217	Rhaponticin	0.899
C36	Rutin	0.893
C63	Isoguanosine	0.880
C112	Polydatin	0.874
C205	Esculin	0.873
C19	13(s)-hpot	0.863
C222	Glycitin	0.857
C82	Guanosine	0.850

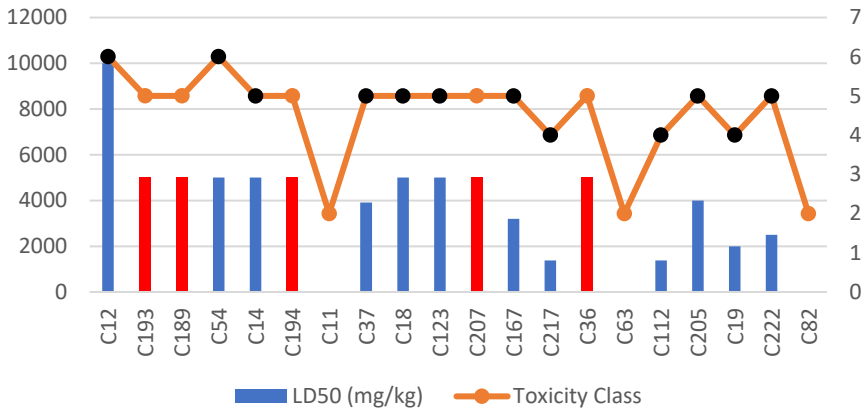

Figure 1. Prediction of toxicity based on LD50 values and toxicity classification of the compounds identified in broccoli microgreens.

safety concerns. This step was crucial to prioritize compounds with the highest potential for therapeutic application.

Pharmacokinetic and drug-likeness evaluations identified compounds in toxicity classes IV to VI as safe for further development. The graphical analysis (Figure 1) shows the twelve selected compounds as black dots, indicating favorable pharmacokinetic profiles. In contrast, the red bars represent compounds that did not meet Lipinski's Rule of 5 (Ro5) criteria, suggesting they are unsuitable for further development due to poor drug-like properties.

The analysis showed that most of the 20 selected bioactive compounds adhered to Lipinski's Rule of Five (Ro5), suggesting their potential as drug candidates with favorable toxicity profiles. Based on this evaluation, 12 compounds—C12, C54, C14, C37, C18, C123, C167, C217, C112, C205, C19, and C222—were prioritized for further investigation due to their safe LD50 values and classification within low-toxicity categories. However, certain compounds require more detailed assessment due to possible toxic effects, such as hepatotoxicity, drug-induced liver injury, mutagenicity, carcinogenicity, cytotoxicity, and immunotoxicity, especially when considered standalone therapeutic agents. Despite these concerns, the overall analysis indicated that these compounds exhibit low toxicity and hold considerable promise for further development as drug candidates [44].

3.3. Target Proteins of Broccoli Microgreen Compounds in Prostate Cancer

The final selection of compounds, including Thymidine, 5-methylcytidine, 2'-deoxyadenosine, Kaempferol, 13(S)-Hpode, Lmpk12110619, Np-020521, Rhaponticin, Polydatin, Esculin, 13(s)-hpot, and Glycitin was based on their relevance to prostate cancer pathways, as identified through SwissTargetPrediction and GeneCards.

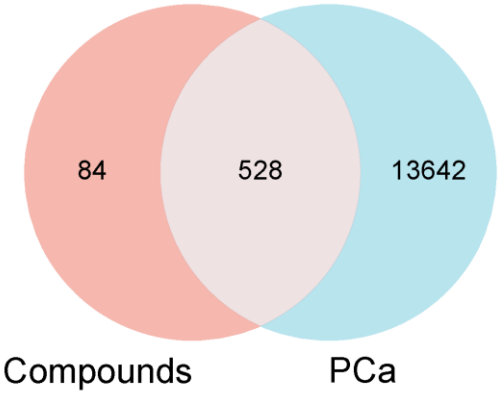

Figure 2. Intersection of target proteins associated with broccoli microgreen compounds and prostate cancer, revealing 528 relevant targets.

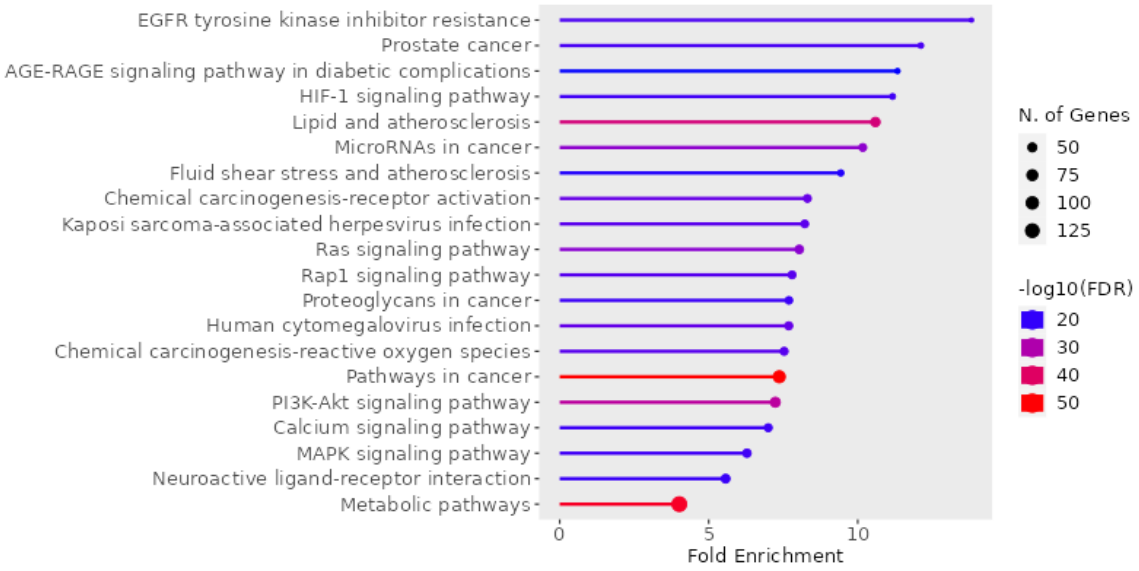

Figure 3. Annotation of the top 20 KEGG pathways targeted by broccoli microgreens using ShinyGO website.

The subsequent phase focused on identifying the target proteins associated with the bioactive compounds in broccoli microgreens. Target identification revealed 619 proteins predicted to interact with the secondary metabolites of broccoli microgreens alongside 14,170 proteins linked to prostate cancer. The intersection of these datasets identified 528 relevant target proteins, as depicted in Figure 2.

Identifying 528 target proteins highlights the multi-target potential of bioactive compounds in broccoli microgreens, particularly in modulating pathways critical to prostate cancer progression. However, the lack of experimental validation for these targets represents a significant limitation of this study. Future studies should validate these target proteins using experimental approaches such as in vitro binding assays or transcriptomic/proteomic analyses in prostate cancer mode.

The analysis of the selected target proteins using the KEGG pathway database revealed their involvement in several critical biological pathways, including the cancer pathway, PI3K-Akt signaling, resistance to EGFR tyrosine kinase inhibitors, HIF-1 signaling, prostate cancer pathway, and MAPK signaling. These pathways are well-established in the progression of prostate cancer. The KEGG pathway analysis results are presented in Figure 3, where the size of each circle corresponds to the number of genes associated with a specific pathway, and the fold enrichment level is displayed along the horizontal axis. The color gradient, from red to blue, indicates biological activity levels, with red representing high activity and blue signifying low activity. This analysis underscores the potential of broccoli microgreen compounds to influence key biological pathways linked to prostate cancer, supporting their therapeutic relevance.

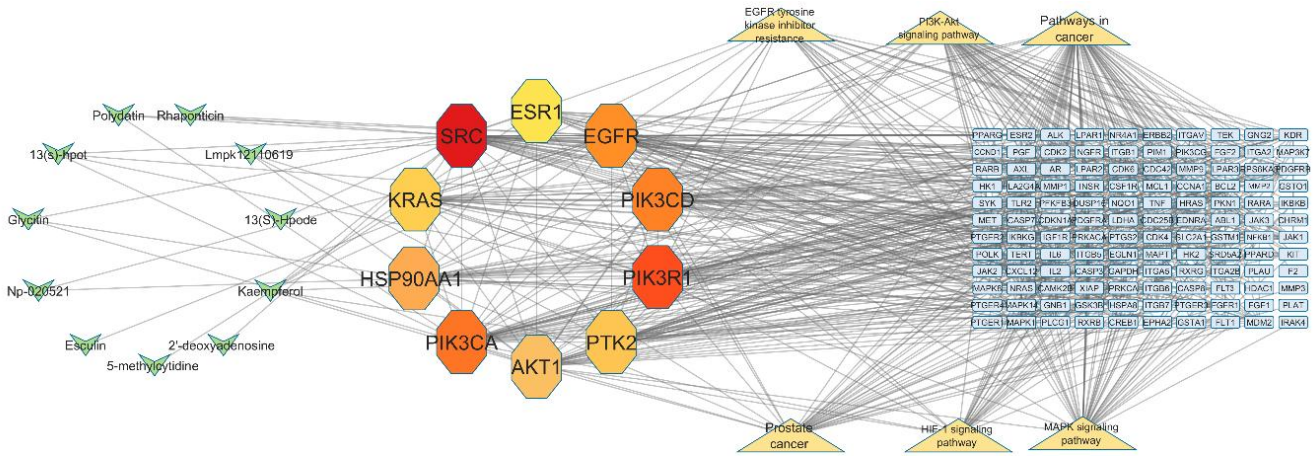

Figure 4. A topological network visualization that examines interactions between compounds, proteins, and biological pathways.

Notably, several of these pathways exhibit significant overlap and crosstalk, crucial for understanding the overall biological impact of the identified compounds. For instance, the PI3K-Akt signaling pathway, which plays a central role in cell survival and proliferation, intersects with the MAPK signaling pathway, both implicated in prostate cancer progression [45]. Additionally, the HIF-1 signaling pathway, which regulates cellular responses to hypoxia, interacts with the EGFR tyrosine kinase inhibitor resistance pathway, suggesting a potential mechanism by which cancer cells evade therapeutic interventions [46]. This crosstalk highlights the importance of multitargeting strategies, as compounds that simultaneously modulate multiple pathways may offer enhanced therapeutic efficacy by disrupting redundant signaling networks.

Furthermore, the involvement of pathways such as lipid and atherosclerosis, fluid shear stress, and chemical carcinogenesis—reactive oxygen species—suggests a broader biological impact beyond prostate cancer, potentially linking metabolic and inflammatory processes to cancer progression [47]. These interconnected pathways underscore the complexity of prostate cancer biology and the need for a systems-level approach to therapeutic development.

This analysis underscores the potential of broccoli microgreen compounds to influence key biological pathways linked to prostate cancer, supporting their therapeutic relevance. Future studies should explore the combinatorial effects of these compounds on overlapping pathways to optimize their therapeutic potential and address prostate cancer's multifaceted nature.

3.4. Pharmacology Network Analysis

Protein-protein interaction (PPI) network analysis, using a confidence score threshold of >0.900, identified ten key proteins—SRC, PIK3R1, PIK3CA, PIK3CD, EGFR, HSP90AA1, AKT1, PTK2, KRAS, and ESR1—based on degree value, connectivity centrality, and proximity centrality. The topological network, presented in Figure 4, depicts interactions between broccoli microgreen bioactive compounds (green V-shaped nodes), target proteins (octagonal nodes), and biological pathways (yellow triangular nodes). Hub proteins such as SRC and EGFR emerged as critical targets due to their extensive interactions and central roles in molecular networks. These proteins are integral to pathways, including those mediating resistance to EGFR tyrosine kinase inhibitors, underscoring their potential as pivotal therapeutic targets in prostate cancer management.

Broccoli microgreens harbor several bioactive compounds with notable therapeutic potential to overcome resistance to EGFR tyrosine kinase inhibitors (EGFR-TKIs) by modulating signaling pathways involving SRC and EGFR proteins. Compounds such as 2'-deoxyadenosine, kaempferol, 13(S)-Hpode, and 13(S)-Hpoot have demonstrated selective interaction with SRC as their primary target. Additionally, compounds including kaempferol, Lmpk12110619, rhaponticin, and polydatin exhibit broader activity by targeting both SRC and EGFR, while glycitin has been identified as a potential SRC inhibitor (Table 3). SRC, a proto-oncogene encoding a non-receptor tyrosine kinase, is pivotal in regulating critical cellular processes such as proliferation, migration, adhesion, and survival. In cancer progression, elevated SRC activity is frequently associated with tumor growth and resistance to therapy [48, 49].

Topological network and KEGG pathway analyses further validate the involvement of SRC in resistance pathways to EGFR-TKIs, underscoring its central role in cancer progression. These findings highlight the potential of broccoli microgreens as a natural source of therapeutic compounds that target key molecular pathways involving SRC and EGFR. For instance, kaempferol and rhaponticin,

Table 3. Analytical results linking broccoli microgreens' bioactive compounds targeting their proteins through resistance to EGFR tyrosine kinase inhibitors.

Compounds	Target Protein
2'-deoxyadenosine	SRC
Kaempferol	SRC dan EGFR
13(S)-Hpode	SRC
Lmpk12110619	SRC dan EGFR
Rhaponticin	SRC dan EGFR
Polydatin	SRC dan EGFR
13(s)-hpot	SRC
Glycitin	SRC

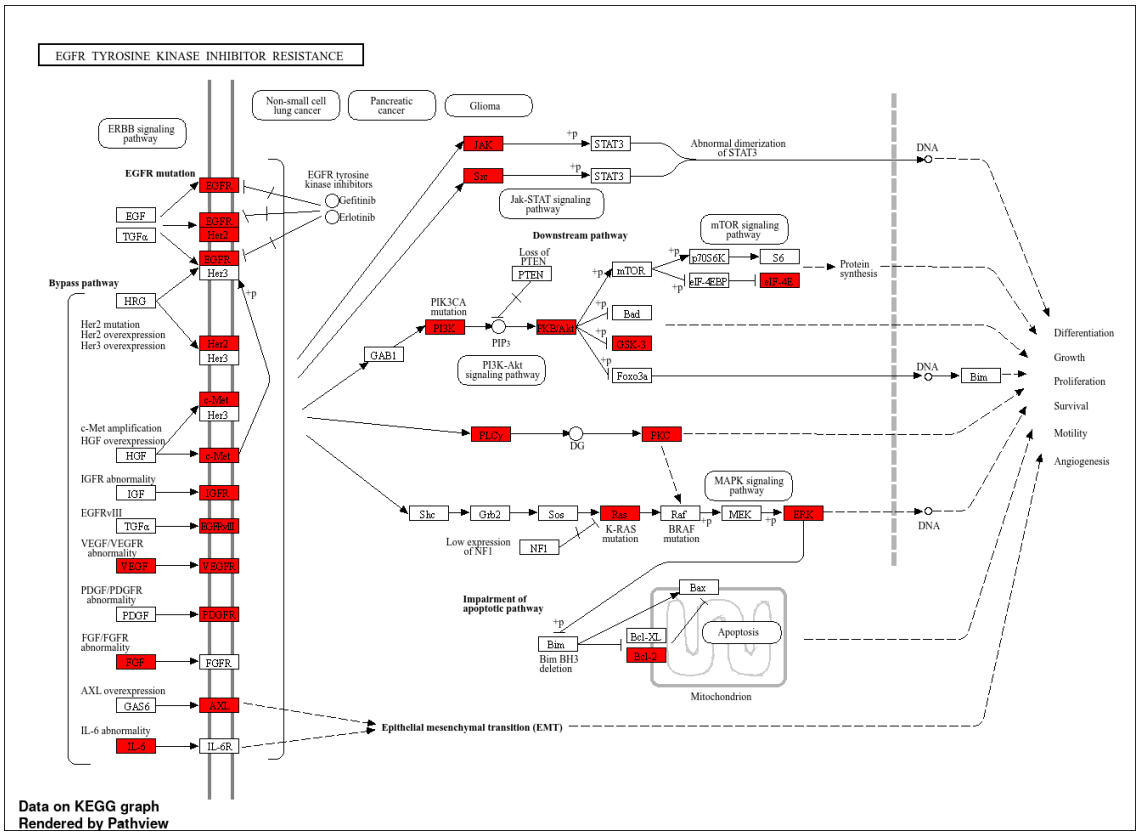

Figure 5. KEGG Pathway of resistance to EGFR tyrosine kinase inhibitors. Proteins identified through network pharmacology are highlighted in red. Adapted from [50].

which target SRC and EGFR, may disrupt redundant signaling pathways, thereby overcoming resistance mechanisms more effectively than single-target therapies [51, 52]. However, the potential for antagonistic interactions between compounds cannot be ruled out and warrants further investigation. For example, competition for binding sites or overlapping downstream effects might sometimes reduce overall efficacy. Future studies should explore the combinatorial effects of these compounds to optimize their therapeutic potential.

The identified compounds, particularly those targeting SRC and EGFR, demonstrated potential mechanisms of action similar to those of established anticancer drugs. For example, kaempferol and rhaponticin, which target both SRC and EGFR, share mechanistic similarities with dasatinib (an SRC inhibitor) and gefitinib (an EGFR inhibitor), respectively [53, 54]. However, unlike these synthetic drugs, the natural compounds from broccoli microgreens may offer additional benefits, such as reduced toxicity and multitargeting capabilities, which could help overcome drug resistance commonly observed with single-target therapies [55]. Further comparative studies are needed to evaluate the efficacy and safety of these compounds relative to existing drugs.

As illustrated in Figure 5, the activation of SRC plays a pivotal role in resistance mechanisms by amplifying downstream signaling pathways such as PI3K/AKT and STAT3, enabling cancer cells to evade the apoptotic effects of EGFR-TKIs. Furthermore, the interaction between SRC and androgen receptors (AR) has been

linked to increased tumor aggressiveness and resistance to therapy in prostate cancer [56].

Given these underlying mechanisms, a combinatorial therapeutic strategy targeting SRC alongside EGFR-TKIs or AR inhibitors presents a promising avenue for overcoming resistance and improving treatment outcomes. This dual-targeting approach offers the potential for more effective tumor control by disrupting redundancies in molecular signaling pathways that drive prostate cancer progression and resistance to therapy [52].

The findings of this study highlight the potential of bioactive compounds in broccoli microgreens, such as 2'-deoxyadenosine, kaempferol, 13(S)-Hpode, Lmpk12110619, rhaponticin, polydatin, 13(S)-Hpot, and glycitin to modulate key molecular targets involved in prostate cancer progression, particularly SRC and EGFR pathways. Through network pharmacology, we identified multiple interactions between these compounds and critical proteins regulating cell proliferation, apoptosis resistance, and metastatic potential. These results suggest that broccoli microgreens could serve as a promising source of multi-targeted therapeutic agents for prostate cancer.

However, it is important to acknowledge the limitations of this study. While in silico methods provide valuable insights into potential molecular interactions and mechanisms, they are inherently predictive and rely on computational models that may not fully capture the complexity of biological systems. For instance, the predicted binding affinities and interactions between compounds and target proteins may differ under physiological conditions due to bioavailability, metabolic stability, and cellular microenvironment factors. Therefore, the therapeutic potential of these compounds, as suggested by our in silico analysis, requires experimental validation to confirm their efficacy and safety.

Several key steps are necessary to advance the therapeutic potential of broccoli microgreen compounds. Preclinical validation should be conducted in vitro and in vivo to assess efficacy, bioavailability, and toxicity in prostate cancer models [57, 58]. Additionally, investigating the synergy between these compounds and existing therapies, such as EGFR-TKIs or AR inhibitors, could enhance treatment efficacy and reduce resistance. Further mechanistic studies are needed to elucidate how these compounds modulate SRC/EGFR and downstream pathways like PI3K/AKT and STAT3 [59]. Early-phase clinical trials will be crucial to evaluate their safety, tolerability, and therapeutic potential in patients resistant to current treatments [60]. Optimizing formulation strategies, such as nanoparticles or liposomes, can improve compound stability and targeted tumor delivery [61, 62]. These steps will help bridge preclinical findings to clinical applications, paving the way for novel prostate cancer therapies.

4. Conclusions

This study demonstrates that broccoli microgreens contain bioactive compounds, including 2'-deoxyadenosine, kaempferol, 13(S)-Hpode, Lmpk12110619, rhaponticin, polydatin, 13(S)-Hpot, and glycitin, which exhibit potential to target SRC and EGFR proteins in silico. These proteins are critically involved in developing resistance to EGFR tyrosine kinase inhibitors, a major contributor to prostate cancer progression and therapeutic resistance. SRC and EGFR were identified as primary targets with the highest scores in the analysis, playing key roles in several significant molecular pathways, including cancer pathways, PI3K-Akt signaling, prostate cancer, MAPK signaling, resistance to EGFR tyrosine kinase inhibitors, and HIF-1 signaling. These findings suggest that broccoli microgreens may modulate complex molecular mechanisms relevant to prostate cancer management.

The findings from this study underscore the potential of broccoli microgreens as a source of nutrient-based therapeutic agents for prostate cancer treatment. While in silico predictions offer valuable insights into the inhibitory effects of identified compounds on SRC and EGFR, further experimental validation is essential to confirm their biological relevance and therapeutic potential. Future research should prioritize in vitro validation using prostate cancer cell lines, in vivo studies in animal models to assess bioavailability and efficacy, mechanistic investigations into downstream signaling pathways, and the design of early-phase clinical trials to evaluate safety and preliminary efficacy in patients, particularly those resistant to current therapies. Despite the inherent limitations of computational approaches, such as incomplete capture of biological complexity and challenges in translation to real-world applications, this study provides a robust foundation for advancing these compounds toward clinical use. By integrating computational predictions with rigorous experimental validation, this work paves the way for developing more effective treatment strategies for prostate cancer, especially in cases of advanced or resistant disease.

Author Contributions: Conceptualization, T.E.T. and P.W.; methodology, T.E.T.; software, P.W.; validation, T.E.T.; formal analysis, P.W.; investigation, T.E.T. and P.W.; resources, T.E.T.; data curation, T.E.T. and P.W.; writing—original draft preparation, T.E.T. and P.W.; writing—review and editing, T.E.T.

and P.W.; supervision, T.E.T., L.E.N.T, F., G.L.A.T., D.S.P.; project administration, T.E.T.; funding acquisition, T.E.T. All authors have read and agreed to the published version of the manuscript.

Funding: This study does not receive external funding.

Ethical Clearance: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data supporting this study can be accessed by contacting the corresponding author, subject to applicable data protection and privacy policies.

Acknowledgements: The authors would also like to thank the author's team for their contributions in data collection and administrative support, which helped the study run smoothly.

Conflicts of Interest: All the authors declare no conflicts of interest.

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