Network Pharmacology-Based Study to Uncover Potential Pharmacological Mechanisms of Korean Thistle (Cirsium japonicum var. maackii (Maxim.) Matsum.) Flower against Cancer

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Abstract: Cirsium japonicum var. maackii (Maxim.) Matsum. or Korean thistle flower is a herbal plant used to treat tumors in Korean folk remedies, but its essential bioactives and pharmacological mechanisms against cancer have remained unexplored. This study identified the main compounds(s) and mechanism(s) of the C. maackii flower against cancer via network pharmacology. The bioactives from the C. maackii flower were revealed by gas chromatography-mass spectrum (GC-MS), and SwissADME evaluated their physicochemical properties. Next, target(s) associated with the obtained bioactives or cancer-related targets were retrieved by public databases, and the Venn diagram selected the overlapping targets. The networks between overlapping targets and bioactives were visualized, constructed, and analyzed by RPackage. Finally, we implemented a molecular docking test (MDT) to explore key target(s) and compound(s) on AutoDockVina and LigPlot+. GC-MS detected a total of 34 bioactives and all were accepted by Lipinski’s rules and therefore classified as drug-like compounds (DLCs). A total of 597 bioactive-related targets and 4245 cancer-related targets were identified from public databases. The final 51 overlapping targets were selected between the bioactive targets network and cancer-related targets. With Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment, a total of 20 signaling pathways were manifested, and a hub signaling pathway (PI3K-Akt signaling pathway), a key target (Akt1), and a key compound (Urs-12-en-24-oic acid, 3-oxo, methyl ester) were selected among the 20 signaling pathways via MDT. Overall, Urs-12-en-24-oic acid, 3-oxo, methyl ester from the C. maackii flower has potent anti-cancer efficacy by inactivating Akt1 on the PI3K-Akt signaling pathway.

Keywords: Akt1; cancer; C. maackii flower; network pharmacology; PI3K-Akt signaling pathway; Urs-12-en-24-oic acid; 3-oxo; methyl ester

1. Introduction

The definition of cancer is that normal cells are damaged via an aberrant endogenous process such as an abnormality during DNA replication or instability of the DNA sequence, which transforms into malignancy [1]. The DNA damage responses represent chronic inflammation via the immune signaling pathway, which results in accelerating tumorigenesis [2]. The damaged normal cells undergo cellular senescence, triggering secretion in the inflammatory cytokines leading to cellular mechanical disruption [3,4]. Because inflammation is a leading factor in causing pathological symptoms such as unknown severe pain, fatigue, and comorbidity in cancer patients, anti-inflammation strategies are thus key therapeutics [5,6]. Current anti-inflammatory agents for cancer treatment NSAIDs, including Cox-2, due to fewer adverse effects and a lower mortality rate [7]. Most commonly, anti-cancer agents aim to inhibit DNA replication and induce cancer cell death; however, cancer chemotherapeutics also attack healthy cells resulting in serious side effects.
such as nausea, vomiting, hair loss, and fatigue [8–10]. On the other hand, traditional herbal plants with innovative bioactives and secondary metabolites play an essential role as effective anti-inflammatory, anti-oxidant, or anti-cancer agents [11]. For instance, plant extracts (Urtica membranaceae, Artemisia monosperma, and Origanum dayi Post) in combination with anti-cancer drugs showed enhanced potency against specific cancer cell lines (lung, breast, colon, and prostate cancer) without exposing normal lymphocytes and fibroblasts to cytotoxicity [12]. Herbal-derived bioactives possess fewer unwanted side effects than chemotherapy, and have led to new clinical drugs such as taxol from Taxus brevifolia L., vincristine from Catharanthus roseus G. Don, and Epipodophyllotoxin from Podophyllum peltatum L. [12,13].

C. maackii is a perennial herbal plant, belonging to the family of Compositae, and is widely distributed in the mountainous areas of Korea, Japan, and China [14]. Furthermore, Cirsium species have been reported to have diverse pharmacological activities such as anti-oxidant, anti-inflammation, anti-cancer, and hepatoprotection effects [15–18]. Specifically, a C. maackii extract at a concentration of 200 µg/mL showed 36.89% inhibition against a breast cancer cell line (MDA-MB-231) [19]. Another study demonstrated that HepG2 cells treated with a MeOH extract of C. maackii have potent antioxidant efficacy against severe oxidant conditions [20]. Anti-inflammatory agents assist in protection against cancer development, thereby preventing cytokine storms [21]. It implies that anti-inflammatory compounds are important agonists to protect normal cells adjacent to tumor cells because they can block the overflow of cytokines. Until now, C. maackii flower compounds were identified by HPLC and had only been reported for anti-Alzheimer efficacy by inhibiting BACE1 [22]. Generally, the identification of polar and mid-polar compounds from extracts is based on HPLC due to its good separation capability [23,24]. From a different perspective, we utilized GC-MS analysis to discover lipophilic bioactives, which mainly act as drug-like compounds and uptake efficiently into the cells. Lipophilicity is a significant physicochemical parameter that influences membrane permeability and affinity [25]. More importantly, GC-MS, along with the molecular docking test (MDT) and ADME (Absorption, Distribution, Metabolism, and Excretion) study, is an optimal analytical method to determine drug-like compounds [26]. At present, the bioactives and mechanisms of the C. maackii flower against cancer remain unknown. Hence, we aimed to uncover its potential bioactives with their fundamental mechanisms through network pharmacology.

Network pharmacology (NP) as a systemic method can analyze holistic bioactive–target–disease relationships [27]. It can decipher the unknown mechanism(s) with “multiple targets”, “multiple bioactives”, instead of “one target”, “one bioactive” [28]. This approach is a very effective method to recognize the mechanism of action for lead compounds discovered from herbal plants [29]. An existing drug may be re-modelled to bind on multiple targets with the concept of NP, thus it can be a guide for drug repurposing [30]. The NP application is a powerful tool to elucidate novel targets and bioactives from natural products, and is especially effective for anti-cancer research to investigate multi-target activity in biological pathways and interaction networks [31]. Here, we implemented NP to provide key bioactive(s), uppermost target(s), and potential mechanism(s) of the C. maackii flower against cancer. Mainly, the flower part of herbal plants are the most underexplored part compared to other parts such as leaves, roots, and stems. During the growing season of the C. maackii plant, the flowering part might be utilized as a source of essential bioactives or be taken as a functional food. In this study, we suggest that C. maackii flowers are valuable parts with potential anti-cancer compounds.

To prove their therapeutic value, we performed a GC-MS analysis, protein network investigation, and a molecular docking test (MDT). Its brief processes are discussed below.

Firstly, bioactives from the C. maackii flower were identified by GC-MS analysis and screened to find drug-likeness compounds via an in silico tool. Then, targets associated with bioactives or cancer were identified through public databases, and final overlapping targets were utilized to analyze protein–protein interaction (PPI) and the highest degree of a target. Thirdly, a signaling pathway–target protein–bioactive (S–T–B) relationship against
cancer was identified by networking analysis. Lastly, we found the most potent bioactive and a hub target to alleviate cancer severity by exploring the molecular mechanism of the *C. maackii* flower based on MDT. The workflow diagram is displayed in Figure 1.

**Figure 1.** Workflow diagram of network pharmacology analysis of the *C. maackii* flower against cancer.

2. Results

2.1. Bioactives from *C. maackii* Flower

A total of 34 bioactives in the *C. maackii* flower were identified by GC-MS analysis (Figure 2), and the name of compounds, PubChem ID, retention time (mins), and peak area (%) are listed in Table 1. All 34 compounds were accepted by Lipinski’s rules (Molecular Weight ≤ 500 g/mol; Moriguchi octanol-water partition coefficient ≤ 4.15; Number of Nitrogen or Oxygen ≤ 10; Number of NH or OH ≤ 5), and all bioactives corresponded with the standard of “Abbott Bioavailability Score (>0.1)” through SwissADME. The TPSA (Topological Polar Surface Area) value of all bioactives was also accepted (Table 2).

**Figure 2.** GC-MS peak of the *C. maackii* flower MeOH extract and an indication of the uppermost bioactive.
Table 1. A list of the 34 bioactives identified from *C. maackii* through GC-MS and profiling of bioactivities.

| No. | Compounds                                      | Pubchem ID | RT (mins) | Area (%) | Pharmacological Activities (References)                  |
|-----|------------------------------------------------|------------|-----------|----------|----------------------------------------------------------|
| 1   | Glyceraldehyde                                 | 751        | 3.510     | 0.91     | No reported                                              |
| 2   | 6-Methyluracil                                 | 12283      | 4.279     | 0.88     | No reported                                              |
| 3   | 3-Hydroxy-2,3-dihydromaltol                    | 119838     | 4.789     | 5.19     | No reported                                              |
| 4   | Pyranopyridine                                 | 534033     | 5.414     | 0.69     | No reported                                              |
| 5   | Formicin                                        | 69365      | 6.096, 6.212 | 0.58 | No reported                                              |
| 6   | 4-Nitro-2-picoline N-oxide                     | 95291      | 6.721     | 0.6      | No reported                                              |
| 7   | 2-Vinyl-9-[beta-d-ribofuranosyl]hypoxanthine   | 135493011  | 6.933     | 1.35     | Antibacterial and antifungal activity [32]              |
| 8   | 2-Chloromethyl-3,3-dichloropropene              | 43492      | 7.058     | 0.51     | No reported                                              |
| 9   | d-Lyxo-d-manno-nononic-1,4-lactone              | 535556     | 7.183     | 0.43     | No reported                                              |
| 10  | Pentyl isobutyrate                             | 75554      | 7.327     | 0.26     | Antimicrobial activity [33]                              |
| 11  | 2-Methyl-3-nitrosooxazolidine                  | 38357      | 7.673     | 0.23     | No reported                                              |
| 12  | N-Nitrosomethylethanolamine                    | 33646      | 7.808     | 2.71     | No reported                                              |
| 13  | 1,4,7,10-Tetraoxacyclododecan-2-one            | 533646     | 8.145     | 0.15     | No reported                                              |
| 14  | 4-methylpyrimidin-2-ol                         | 407091     | 8.231     | 0.58     | No reported                                              |
| 15  | Diethyl malonate                               | 7761       | 8.750     | 0.46     | No reported                                              |
| 16  | Palmitic acid                                  | 985        | 8.943, 9.443, 9.760 | 4.03 | Anti-inflammation [34]                                   |
| 17  | Methyl 3,6-anhydrohexopyranoside #             | 91691384   | 9.154     | 0.65     | No reported                                              |
| 18  | 3-Hydroxypropionic acid                        | 68152      | 9.625     | 1.17     | Antibacterial activity [35]                              |
| 19  | 2,2,4-Trichloro-1,3-cyclopentenedione          | 150757     | 9.846     | 0.65     | No reported                                              |
| 20  | Isopropyl palmitate                            | 8907       | 10.673    | 0.54     | Antibacterial activity [36]                              |
| 21  | Stearic acid                                   | 5281       | 10.933    | 0.72     | Antibacterial activity [37]                              |
| 22  | Pregn-5,7-diene-3-ol-20-one                    | 21117403   | 11.068    | 0.25     | No reported                                              |
| 23  | 9-Heptadecanone                                | 10887      | 11.173    | 0.95     | Antibacterial activity [38]                              |
| 24  | Allyl stearate                                 | 80500      | 12.087    | 1.05     | Anti-oxidant [39]                                        |
| 25  | Squalene                                       | 638072     | 12.250    | 0.54     | Anti-oxidant [39]                                        |
| 26  | Prexanthoperol                                 | 628742     | 13.952    | 13.65    | No reported                                              |
| 27  | α-Tocopherol                                   | 14985      | 14.664    | 0.94     | Anticancer [40]                                          |
| 28  | β-Stigmasterol                                 | 6432745    | 16.289    | 1.68     | Anti-inflammation [39]                                   |
| 29  | 4,4-Dimethylcholest-7-en-3-ol                  | 5460076    | 17.077    | 1.06     | No reported                                              |
| 30  | Aristol-9-en-8-one #                           | 6432651    | 17.837    | 1.11     | No reported                                              |
| 31  | α-Amyrin                                       | 73170      | 18.587    | 2.53     | Anti-obesity [41]                                        |
| 32  | Urs-12-en-24-oic acid, 3-oxo-, methyl ester    | 612822     | 19.125    | 9.04     | Anti-inflammation [42]                                   |
| 33  | Lupenyl acetate                                | 323074     | 20.019    | 24.42    | Antibacterial activity [43]                              |
| 34  | Cholesterol                                    | 5997       | 21.693, 21.952 | 15.19 | Protective cell against pathogens [44]                  |

PCIDB: PhytoChemical Interactions DB.
### Table 2. Physicochemical properties of bioactives for good oral bioavailability and cell membrane permeability.

| Compounds                          | Lipinski Rules | Lipinski’s Violations | Bioavailability Score | TPSA (Å²) |
|------------------------------------|----------------|-----------------------|-----------------------|-----------|
|                                   | MW  | HBA | HBD | MLog P | ≤5 | ≤4.15 | ≤1 | >0.1 | <140 |
| 1. Glyceraldehyde                  | 90.08 | 3   | 2   | −1.66 | 0  | 0     | 0.55 | 57.53 |
| 2. 6-Methyluracil                  | 126.11 | 2   | 2   | −0.39 | 0  | 0     | 0.55 | 65.72 |
| 3. 3-Hydroxy-2,3-dihydromaltol     | 144.13 | 4   | 2   | −1.77 | 0  | 0.85  | 22.12 |
| 4. Pyranopyridine                  | 133.15 | 2   | 0   | 0.73  | 0  | 0.55  | 49.33 |
| 5. Formicin                         | 89.09  | 2   | 2   | −0.85 | 0  | 0.55  | 49.33 |
| 6. 4-Nitro-2-picoline N-oxide      | 154.12 | 3   | 0   | −0.15 | 0  | 0     | 0.55 | 71.28 |
| 2.2. Overlapping Targets between SEA and STP Associated with Bioactives |

A total of 309 targets from SEA and 396 targets from STP connected to 34 bioactives were identified (Supplementary Table S1). The Venn diagram showed that 108 targets overlapped between the two public databases (Supplementary Table S1) (Figure 3A).
Figure 3. (A) Overlapping targets (108 targets) between SEA (309 targets) and STP (396 targets). (B) Overlapping targets (51 targets) between 108 overlapping targets from two databases (SEA and STP) and cancer associated with targets (4245 targets).
2.3. Overlapping Targets between Cancer-Associated Targets and the Final 51 Overlapping Targets

A total of 4245 targets related to cancer were selected via retrieval from TTD and OMIM databases (Supplementary Table S2). The Venn diagram results revealed 51 overlapping targets that were selected between 4245 targets associated with cancer and 108 overlapping targets (Figure 3B) (Supplementary Table S3).

2.4. Acquisition of a Hub Target from PPI Networks

From STRING analysis, 46 out of 51 overlapping targets were directly related to cancer occurrence and development, indicating 46 nodes and 145 edges (Figure 4). The five targets removed (PAM, EPHX1, PPARD, KDM5C, and BCHE) had no connectivity to the overlapping 51 targets. In PPI networks, the Akt1 target was the highest degree (29) and was considered a hub target (Table 3).

Figure 4. PPI networks (46 nodes, 145 edges). The size of circles represents the degree of values.
Table 3. The degree value of PPI networks.

| No. | Target  | Degree | No. | Target  | Degree |
|-----|---------|--------|-----|---------|--------|
| 1   | AKT1    | 29     | 24  | MGLL    | 5      |
| 2   | VEGFA   | 21     | 25  | S1PR1   | 5      |
| 3   | ESR1    | 16     | 26  | SHH     | 5      |
| 4   | AR      | 13     | 27  | VDR     | 5      |
| 5   | CYP19A1 | 11     | 28  | TOP2A   | 5      |
| 6   | PPARG   | 11     | 29  | ADORA2B | 4      |
| 7   | IL1B    | 10     | 30  | PTGER2  | 4      |
| 8   | CNR1    | 9      | 31  | S1PR3   | 4      |
| 9   | TRPV1   | 9      | 32  | ADORA2A | 3      |
| 10  | PPARA   | 9      | 33  | CYP24A1 | 3      |
| 11  | PTPN1   | 9      | 34  | FOLH1   | 3      |
| 12  | HMGCR   | 8      | 35  | PTPN6   | 3      |
| 13  | ESR2    | 7      | 36  | CA2     | 2      |
| 14  | PRKCA   | 7      | 37  | FABP3   | 2      |
| 15  | ABCB1   | 6      | 38  | NR1H3   | 2      |
| 16  | CDC25B  | 6      | 39  | PHLP1   | 2      |
| 17  | PTGER4  | 6      | 40  | PTPFR   | 2      |
| 18  | SHBG    | 6      | 41  | PRKCE   | 2      |
| 19  | CYP17A1 | 6      | 42  | PTGFR   | 2      |
| 20  | SRD5A2  | 6      | 43  | CA1     | 1      |
| 21  | ADORA3  | 5      | 44  | FNTB    | 1      |
| 22  | AKB1    | 5      | 45  | NR1H4   | 1      |
| 23  | CDC25A  | 5      | 46  | IMPDH2  | 1      |

2.5. Identification of a Hub Signaling from Bubble Chart

The output of KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway enrichment analysis revealed that 20 signaling pathways were associated with 24 targets (False Discovery Rate < 0.05). The 20 signaling pathways were directly connected to cancer, suggesting that these 20 signaling pathways might be the noteworthy pathways of the *C. maackii* flower against cancer. The descriptions of 20 signaling pathways are displayed in Table 4. Additionally, a bubble chart suggested that the PI3K-Akt signaling pathway might be a hub signaling pathway of the *C. maackii* flower against cancer (Figure 5). Among the 20 signaling pathways, the Akt1 target was associated with 18 signaling pathways, representing the highest degree of value. Most importantly, Akt1 is directly related to the PI3K-Akt signaling pathway whereas both the PPAR signaling pathway and the Calcium signaling pathway are not correlated with Akt1.

Table 4. The degree value of S–T–B networks.

| No. | Target  | Degree | Bioactive                                               | Degree | No. | Target  | Degree | Bioactive                                               | Degree |
|-----|---------|--------|--------------------------------------------------------|--------|-----|---------|--------|--------------------------------------------------------|--------|
| 1   | AKT1    | 18     | Urs-12-en-24-oic acid,3-oxo-, methyl ester             | 36     | 13  | S1PR1   | 1      | Cholesterol                                            | 14     |
| 2   | PRKCA   | 12     | Palmitic acid                                          | 27     | 14  | S1PR3   | 1      | Pregn-5,7-diene-3-ol-20-one                            | 10     |
| 3   | VEGFA   | 7      | Stearic acid                                           | 26     | 15  | PTPN6   | 1      | Methyl 3,6-anhydrohexopyranoside #                     | 7      |
| 4   | PPARA   | 3      | α-Tocopherol                                           | 26     | 16  | CNR1    | 1      | Aristol-9-en-8-one #                                   | 7      |
| 5   | ESR1    | 3      | Isopropyl palmitate                                    | 26     | 17  | HMGCR   | 1      | Squalene                                              | 3      |
| 6   | PPARG   | 2      | Allyl stearate                                         | 25     | 18  | PTPN1   | 1      | Lupenyl acetate                                        | 3      |
| 7   | ESR2    | 2      | α-Amyrin                                              | 21     | 19  | PTGFR   | 1      | [β-d-ribofuranosyl] hypoxanthine                      | 1      |
| 8   | PRKCE   | 2      | 9-Heptadecanone                                        | 21     | 20  | CDC25B  | 1      |                                                         |        |
| 9   | IL1B    | 2      | β-Stigmasterol                                         | 20     | 21  | PTGFR   | 1      |                                                         |        |
Table 4. Cont.

| No. | Target | Degree | Bioactive                          | Degree | No. | Target | Degree |
|-----|--------|--------|------------------------------------|--------|-----|--------|--------|
| 10  | NR1H3  | 1      | 4,4-Dimethylcholest-7-en-3-ol     |        | 20  | ADORA2B| 1      |
| 11  | FABP3  | 1      | Pentyl isobutyrate               | 17     | 23  | PTGER2 | 1      |
| 12  | CYP17A1| 1      | d-Lyxo-d-mannonoronic-1,4-lactone | 15     | 24  | PHLPP1 | 1      |

Figure 5. Bubble chart of 20 signaling pathways connected to cancer.

2.6. S–T–B Network Analysis of C. maackii Flower against Cancer

The S–T–B network of the C. maackii flower is exhibited in Figure 6. There were 20 pathways, 24 targets, and 19 bioactives (63 nodes and 204 edges). The nodes represented a total of three elements: Signaling pathways-Target protein-Bioactive (S-T-B). The edges represented relationships of a total number of three elements. The S–T–B relationship suggested that the network might interact with therapeutic efficacy against cancer. Regarding the 20 signaling pathways, the highest degree among targets was “Akt1” with 18 degrees, and the highest degree among bioactives was “Urs-12-en-24-oic acid, 3-oxo-, methyl ester” with 36 degrees (Table 5).
Figure 6. S–T–B networks (63 nodes, 204 edges). Green rectangle: Signaling pathway; pink triangle: Target protein; orange circle: Bioactive.

Table 5. Targets in 20 signaling pathways’ enrichment related to cancer.

| KEGG ID & Description                                                                 | Targets                                                                 | False Discovery Rate |
|--------------------------------------------------------------------------------------|------------------------------------------------------------------------|----------------------|
| hsa03320: PPAR signaling pathway                                                    | PPARA, PPARD, PPARG, NR1H3, FABP3                                       | 0.0000695            |
| hsa04917: Prolactin signaling pathway                                               | AKT1, ESR1, ESR2, CYP17A1                                              | 0.00063              |
| hsa04933: AGE-RAGE signaling pathway in diabetic complications                      | AKT1, VEGFA, PRKCA, PRKCE, IL1B                                         | 0.00018              |
| hsa04370: VEGF signaling pathway                                                    | AKT1, VEGFA, PRKCA                                                     | 0.0068               |
| hsa04066: HIF-1 signaling pathway                                                   | AKT1, VEGFA, PRKCA                                                     | 0.00027              |
| hsa04664: Fc epsilon RI signaling pathway                                            | AKT1, PRKCA                                                           | 0.0492               |
| hsa04920: Adipocytokine signaling pathway                                           | AKT1, PPARA                                                            | 0.0492               |
| hsa04662: B cell receptor signaling pathway                                         | AKT1, ESR1, PRKCA                                                     | 0.0236               |
| hsa04015: Rap1 signaling pathway                                                   | AKT1, VEGFA, PRKCA, VEGFA, ADORA2B                                    | 0.03                  |
| hsa04152: AMPK signaling pathway                                                   | AKT1, PPARG, HMGCR                                                    | 0.025                |
| hsa04915: Estrogen signaling pathway                                               | AKT1, ESR1, ESR2                                                      | 0.0278               |
| hsa04910: Insulin signaling pathway                                                | AKT1, PTPN1, PTPF                                                     | 0.0279               |
| hsa04072: Phospholipase D signaling pathway                                         | AKT1, PRKCA                                                           | 0.0324               |
| hsa04100: MAPK signaling pathway                                                   | AKT1, VEGFA, PRKCA, IL1B                                               | 0.013                |
| hsa0420: Calcium signaling pathway                                                | PRKCA, ADORA2B                                                        | 0.0457               |
| hsa04204: cAMP signaling pathway                                                  | AKT1, PPARA, PTGER2                                                  | 0.0492               |
| hsa04151: PI3K-Akt signaling pathway                                              | AKT1, VEGFA, PRKCA, PHLPP1                                             | 0.0487               |

2.7. MDT of 3 Targets and 10 Bioactives Connected to PI3K-Akt Signaling Pathway

The Akt1 protein was related to two bioactives (Urs-12-en-24-oic acid, 3-oxo-, methyl ester and α-Tocopherol), the VEGFA target to three bioactives (Allyl stearate, Isopropyl palmitate, and Methyl 3,6-anhydro hexopyranoside #), and the PRKCA target with seven
bioactives (Allyl stearate, Isopropyl palmitate, Pentyl isobutyrate, 9-Heptadecanone, Palmitic acid, Stearic acid, and d-Lyxo-d-manno-nononic-1,4-lactone). MDT was performed to verify the affinity of target protein(s) and bioactive(s), which displayed the docking figure of a hub target—the uppermost bioactive (Figure 7). The Akt1 protein (PDB ID: 5KCV) connected to two compounds on the PI3K-Akt signaling pathway was subjected to MDT. It was observed that Urs-12-en-24-oic acid, 3-oxo-, methyl ester (−12.8 kcal/mol) docked on the Akt1 protein (PDB ID: 5KCV) manifested the highest binding energy, followed by α-Tocopherol (−5.8 kcal/mol). The docking detail results of two bioactives are shown in Table 6. The MDT score of three bioactives on the VEGFA protein (PDB ID: 3V2A) was analyzed in the “Homo Sapiens” mode. It was revealed that Methyl 3,6-anhydro hexopyranoside # (−5.2 kcal/mol) docked on the VEGFA protein (PDB ID: 3V2A) exhibited the highest binding energy followed by Allyl stearate (−5.1 kcal/mol) and Isopropyl palmitate (−5.1 kcal/mol). The docking detail results of three bioactives are shown in Table 7. The MDT score of seven bioactives on the PRKCA protein (PDB ID: 3IW4) was analyzed in the “Homo Sapiens” mode. It was exposed that d-Lyxo-d-manno-nononic-1,4-lactone (−6.9 kcal/mol) docked on the PRKCA protein (PDB ID: 3IW4) demonstrated the highest binding energy, followed by Isopropyl palmitate (−6.3 kcal/mol), Stearic acid (−6.2 kcal/mol), Allyl stearate (−6.2 kcal/mol), 9-Heptadecanone (−5.4 kcal/mol), Pentyl isobutyrate (−5.0 kcal/mol), and Palmitic acid (−4.8 kcal/mol). The docking detail results of seven bioactives are shown in Table 8. Collectively, both VEGFA (PDB ID: 3V2A) and PRKCA proteins (PDB ID: 3IW4) showed that the affinity of each bioactive did not give a valid binding score (>−7.0 kcal/mol) [45].

**Figure 7.** MDT of Urs-12-en-24-oic acid, methyl ester on Akt1 (PDB ID: 5KCV).
Table 6. Binding energy of potential bioactives on Akt1 (PDB ID: 5KCV).

| Protein | Ligand | PubChem ID | Binding Energy (kcal/mol) | Amino Acid Residue | R Group(s) Involved in Hydrogen Bonding | Distance (Å) | Amino Acid Residue |
|---------|--------|------------|---------------------------|--------------------|----------------------------------------|--------------|-------------------|
| 5KCV    | Urs-12-en-24-oic acid, 3-oxo-, methyl ester | 612822 | −12.8 | N/A | N/A | N/A | Gln59, Lys268, Asn53 |
|         | α-Tocopherol | 14985 | −5.8 | Leu78 | R-OH | 2.71 | |

Table 7. Binding energy of potential bioactives on VEGFA (PDB ID: 3V2A).

| Protein | Ligand | PubChem ID | Binding Energy (kcal/mol) | Amino Acid Residue | R Group(s) Involved in Hydrogen Bonding | Distance (Å) | Amino Acid Residue |
|---------|--------|------------|---------------------------|--------------------|----------------------------------------|--------------|-------------------|
| 3V2A    | Allyl stearate | 80500 | −5.1 | Phe47 | RCOOR′ | 3.18 | Ile46, Lys48, Lys286, Asp276, Arg275, Pro40, Phe36 |
|         | Isopropyl palmitate | 8907 | −5.1 | N/A | N/A | N/A | Asp276, Asp34, Ile46, Phe47, Lys48, Lys286, Phe36, Pro40 |
|         | Methyl 3,6-anhydro hexopyranoside # | 91691384 | −5.2 | Ser310, Pro85 | R-O-R′, R-OH | 2.98, 3.23, 2.82 | Lys84, Gln87, Gly312, Asp257, Gly255, Ile256, Glu44 |
Table 8. Binding energy of potential bioactives on PRKCA (PDB ID: 3IW4).

| Protein   | Ligand                  | PubChem ID | Binding Energy (kcal/mol) | Amino Acid Residue | R Group(s) Involved in Hydrogen Bonding | Distance (Å) | Amino Acid Residue |
|-----------|-------------------------|------------|---------------------------|--------------------|----------------------------------------|--------------|--------------------|
| 3IW4      | Allyl stearate          | 80500      | −6.2                      | Lys 396            | RCOOR′                                | 3.21         | Asp395, Leu393, Pro397 Arg608, Pro398, Val664 Pro666, Ser473, Glu474 His665, Lys478, Ile667 Gln402, Asn660 |}
| Isopropyl palmitate | 8907  | −6.3          | Asn660                    | RCOOR′              | 2.92                                   | Asp395, Leu393, Pro397 Lys396, Gln402, Pro398 Arg608, Ile667, Pro666 Val664, His665, Lys478 Pro502, Gln650, Ile645 Gly540, Asp542, Asp503 Asp539, Gln642, Leu546 Glu543 |}
| Pentyl isobutyrate | 75554 | −5.0          | N/A                       | N/A                | N/A                                    | N/A          | N/A                |}
| 9-Heptadecanone | 10887 | −5.4          | Lys478                    | RCOR′              | 2.80                                   | Asn607, Gln548, Pro398 Arg608, Ile667, Tyr419 Asn421, Ser473, Pro666 Glu18, Asp472, His665 Val664, Gln402, Glu552 Val664, Pro398, Glu552 Gln662, Asp395, Leu393 Leu394, Gln402, Pro397 Leu394, Gln402, Pro398 Lys478, His665, Ile667 Pro666, Val664, Arg608 Pro397, Asn660 |}
| Palmitic acid | 985    | −4.8          | Asn660, Lys396            | RCOR′, R-OH        | 2.93, 2.95                             | N/A          | N/A                |}
| Stearic acid | 5281   | −6.2          | Lys396, Leu393           | R-OH               | 3.04, 2.95                             | N/A          | N/A                |}
| d-Lyxo-d-manno-nononic-1,4-lactone | 535556 | −6.9          | Leu393, Lys396, Pro397, Gln402 | RCOOR′, R-OH      | 3.30, 2.73, 3.31                       | Leu394, Asp395, Gln662 |}
|           |           |             |                           |                    |                                        |               | Asn660, Pro398      |
2.8. Comparative Analysis of MDT against Positive Controls on a Hub Target

A key bioactive (Urs-12-en-24-oic acid, 3-oxo-, methyl ester) associated with the PI3K-Akt signaling pathway revealed the greatest binding score (−12.8 kcal/mol) on Akt1 (PDB ID: 5KCV). The 13 positive controls on Akt1 (PDB ID: 5KCV) are as follows. The MDT score of BAY1125976 (PubChem ID: 70817911) was the highest score with −9.1 kcal/mol, followed by Miransertib (PubChem ID: 53262401; −9.0 kcal/mol), Akti-1/2 (PubChem ID: 135398501; −8.9 kcal/mol), Oridonin (PubChem ID: 5321010; −8.3 kcal/mol), MK-2206 dihydrochloride (PubChem ID: 46930998; −7.8 kcal/mol), AT7867 (PubChem ID: 11175137; −7.6 kcal/mol), A-674563 (PubChem ID: 11314340; −7.6 kcal/mol), Gsk-690693 (PubChem ID: 16725726; −7.4 kcal/mol), Ipatasertib (PubChem ID: 24788740; −7.3 kcal/mol), Capivasertib (PubChem ID: 25227436; −7.1 kcal/mol), AT13148 (PubChem ID: 24905401; −6.9 kcal/mol), Afuresertib (PubChem ID: 46843057; −6.9 kcal/mol), and Uprosertib (PubChem ID: 51042438; −6.8 kcal/mol), respectively. In particular, the MDT score of Urs-12-en-24-oic acid, 3-oxo-, methyl ester on Akt1 (PDB ID: 5KCV) had a higher affinity (−12.8 kcal/mol) compared to the 13 positive controls. Detailed information is listed in Table 9.

Table 9. Binding energy of the positive controls on Akt1 (PDB ID: 5KCV).

| Protein   | Ligand                  | PubChem ID | Binding Energy (kcal/mol) | Amino Acid Residue Involved in Hydrogen Bonding | Distance (Å) | Amino Acid Residue |
|-----------|-------------------------|------------|---------------------------|-------------------------------------------------|--------------|-------------------|
| 5KCV      | MK-2206 dihydrochloride | 46930998   | −7.8                      | N/A                                             | N/A          | Leu78, Leu202, Gln203 |
|           |                         |            |                           |                                                  |              | Lys268, Trp80, Ala58, Gln59 |
|           |                         |            |                           |                                                  |              | Glu85, Phe161, Val83 |
|           |                         |            |                           |                                                  |              | Cys296, Gly294, Phe293 |
|           |                         |            |                           |                                                  |              | Lys276, Leu316, Pro313 |
|           |                         |            |                           |                                                  |              | Arg273, Leu295, Ile84 |
| Gsk-690693|                         | 16725726   | −7.4                      | Tyr18, Asp274                                   | 3.18, 3.06   | Lys268, Asn53, Gln79 |
|           |                         |            |                           |                                                  |              | Ala58, Phe225, Leu223 |
|           |                         |            |                           |                                                  |              | Leu202, Leu78, Trp80 |
|           |                         |            |                           |                                                  |              | Ile84, Arg273, Asn279 |
|           |                         |            |                           |                                                  |              | Asp274, Thr291, Glu278 |
|           |                         |            |                           |                                                  |              | Phe293, Gly294, Cys296 |
|           |                         |            |                           |                                                  |              | Glu85, Glu17 |
|           |                         |            |                           |                                                  |              | Lys268, Trp80, Asn53 |
|           |                         |            |                           |                                                  |              | Leu78, Cys60, Gln79 |
|           |                         |            |                           |                                                  |              | Ala58, Cys77, Val101 |
| Ipatasertib|                       | 24788740   | −7.3                      | N/A                                             | N/A          | Leu202, Gln203 |
|           |                         |            |                           |                                                  |              | Lys268, Trp80, Asn53 |
|           |                         |            |                           |                                                  |              | Leu78, Cys60, Gln79 |
|           |                         |            |                           |                                                  |              | Ala58, Cys77, Val101 |
| Capivasertib|                     | 25227436   | −7.1                      | Val83                                           | 2.95         | Lys268, Trp80, Asn53 |
|           |                         |            |                           |                                                  |              | Leu78, Cys60, Gln79 |
|           |                         |            |                           |                                                  |              | Ala58, Cys77, Val101 |
| AT7867    |                         | 11175137   | −7.6                      | Ser56                                           | 3.04         | Lys268, Trp80, Asn53 |
|           |                         |            |                           |                                                  |              | Leu78, Cys60, Gln79 |
|           |                         |            |                           |                                                  |              | Ala58, Cys77, Val101 |
Table 9. Cont.

| Protein | Ligand | PubChem ID | Binding Energy (kcal/mol) | Amino Acid Residue | R Group(s) Involved in Hydrogen Bonding | Distance (Å) | Amino Acid Residue |
|---------|--------|------------|---------------------------|--------------------|----------------------------------------|-------------|--------------------|
| A-674563 | 11314340 | −7.6 | Gln79, Gln203 | RNHH, RNH | 3.16, 3.20 | Ser56, Trp80, Leu202 Lys268, Asn53, Ala58 Leu78 |
| Miransertib | 53262401 | −9.0 | Gln203, Leu78 | RNHH | 3.19, 3.22 | Lys268, Trp80, Leu202 Phe225, Ser216, Val201 Gln59, Gln79, Ala58 |
| BAY1125976 | 70817911 | −9.1 | Asp274 | RNHH | 3.29 | Gly294, Thr291, Glu278 Asn279, Tyr229, Leu156 Glu234, Phe293, Lys158 Cys296 |
| Akti-1/2 | 135398501 | −8.9 | Cys296, Arg15 | RCOR′, RNR′ | 3.03, 3.15, 3.19 | Gly278, Gly294, Leu295 Val83, Gln85, Glu17 Tyr18, Ile84, Phe293 Asn279 |
| Uprosertib | 51042438 | −6.8 | Cys296, Leu295, Glu278 | RCOR′, RCOR′, RNHH | 2.93, 2.86, 2.93 | Tyr18, Arg273, Asp274 Lys276, Thr291, Phe293 Asn279, Gly294, Ile84 Thr82 |
| Afuresertib | 46843057 | −6.9 | Gly394, Gly395 | RNHH | 3.10, 2.95 | Ala50, Arg328, Lys389 Pro388, Ala329, Asp325 Gly327, Tyr326, Phe55 Ile36, Leu52, Pro51 |
| AT13148 | 24905401 | −6.9 | Glu341, Tyr315, His354 | ROR′, ROR′, RNR′ | 3.01, 2.70, 3.14 | Phe236, Glu278, Leu347 Pro313, Tyr350, Glu314 |
| Oridonin | 5321010 | −8.3 | Asp325, Ala50, Arg328 | RCOR′, ROH, ROH, ROH | 2.67, 2.71, 2.80, 3.24 | Phe55, Ile36, Leu52 Gly394, Ala329, Gly327 Tyr326 |
2.9. Toxicological Properties of a Selected Key Bioactive

Additionally, the toxicological properties of Urs-12-en-24-oic acid, 3-oxo-, methyl ester were predicted by the admetSAR online tool. Our result suggested that the bioactive did not disclose Ames toxicity, carcinogenic properties, acute oral toxicity, or rat acute toxicity properties (Table 10).

Table 10. Toxicological properties of the uppermost bioactive on AKT1 (PDB ID: 5KCV) in MDT.

| Parameters       | Compound Name                     |
|------------------|-----------------------------------|
| Ames toxicity    | NAT                               |
| Carcinogens      | NC                                |
| Acute oral toxicity | III                           |
| Rat acute toxicity | 2.1675                         |

AT: Ames toxic; NAT: Non-Ames toxic; NC: Non-carcinogenic; Category-II means (50 mg/kg > LD50 < 500 mg/kg); Category-III means (500 mg/kg > LD50 < 5000 mg/kg).

3. Discussion

The S–T–B network suggested that the therapeutic efficacy of the C. maackii flower against cancer was directly associated with 20 signaling pathways, 24 targets, and 19 bioactives. Through the network, we identified the most significant protein (Akt1) associated with the occurrence and development of cancer and a bioactive (Urs-12-en-24-oic acid, 3-oxo-, methyl ester) from the C. maackii flower. From a bubble chart, we identified a hub signaling pathway (PI3K-Akt signaling pathway) connected to the Akt1 target, indicating the lowest rich factor among 20 signaling pathways. A report demonstrated that the activated PI3K-Akt signaling pathway accelerates tumor cell proliferation, invasion, and metastasis, inhibiting apoptosis [46]. Furthermore, it was found that the Akt1 target was overexpressed in 15 out of 24 human hepatocellular carcinomas (63.3%) confirmed using PCR analysis through Northern blot [47]. Another research study suggested that the PI3K-Akt signaling pathway’s antagonists are potential anti-cancer candidates to regulate acute and chronic inflammatory responses [48].

In the S–T–B network, Urs-12-en-24-oic acid, 3-oxo-, methyl ester had the highest degree of value and was considered the uppermost bioactive of the C. maackii flower against cancer. Urs-12-en-24-oic acid, 3-oxo-, methyl ester is categorized into boswellic acids used widely as anti-inflammatory agents, including as an anti-cancer treatment. Additionally, the boswellic acids have potent anti-cancer efficacy against diverse malignant cancers [49,50]. The KEGG pathway enrichment analysis of 24 targets suggested that a total of 20 signaling pathways were involved in cancer occurrence and development. The relationships of the 20 signaling pathways with cancer are concisely discussed as follows. In the Peroxisome Proliferator-Activated Receptor (PPAR) signaling pathway, the activation of the PPAR signaling pathway functions as an anti-inflammatory agent, which can overwhelm the metabolic energy balance of cancer cells by inhibiting the fatty acid synthesis and accelerating fatty acid oxidation [51,52]. In the Mitogen-Activated Protein Kinase (MAPK) signaling pathway, MAPK inhibitors are efficient blockers to reduce pro-inflammatory cytokines and enhance the anti-cancer effect, particularly on human pancreatic cancer cells [53,54]. In the Rap1 (Ras-associated protein-1) signaling pathway, Rap1 promotes cytokine production during the inflammatory condition, which leads to tumor progression in human colorectal cancer [55,56]. Regarding the alcium signaling pathway, calcium is a significant second messenger to regulate inflammation; hence, blockers of the calcium channel can induce cancer cell death [57,58]. In the Cyclic AMP (cAMP) signaling pathway, an increased cAMP level has an anti-inflammatory effect, where the increased level can regulate DNA damage, DNA repair, and apoptosis of cancer cells [59,60]. Concerning the Hypoxia-Inducible Factor-1 (HIF-1) signaling pathway, HIF-1 is a central regulator to stimulate the production of inflammation. HIF-1 overexpression is related to increased tumor growth [61,62]. In the sphingolipid signaling pathway, sphingolipid
is implicated in the inflammatory response, which has been involved in cancer cell proliferation [63,64]. In the Phospholipase D (PLD) signaling pathway, PLD inhibition can induce two functions, namely anti-inflammation and anti-cancer. Mainly, the blocking of PLD during chemotherapy can sensitize one to chemotherapeutics [65,66]. Regarding the Phosphoinositide 3-kinase-Akt (PI3K-Akt) signaling pathway, inhibition of the PI3K-Akt signaling pathway reduces the severity of inflammation in mice. This strategy is a promising mechanism for the treatment of cancers such as lung cancer, colorectal cancer, renal cancer, prostate cancer, triple-negative breast cancer, mucinous adenocarcinoma of the ovary, and skin cancer [67,68]. In the AMP-activated protein kinase (AMPK) signaling pathway, AMPK activation suppresses inflammatory responses and dampens cancer growth with cell metabolism and the cell cycle [69,70]. Regarding the Vascular Endothelial Growth Factor (VEGF) signaling pathway, VEGF is a core mediator in the formation of new blood vessels for cancer cells; thereby, cancer cells can survive, grow, and metastasize [71]. In the B cell receptor (BCR) signaling pathway, B cells are involved in the inflammatory T cell receptor, which is implicated in antibody production [72]. B cells or BCR-associated kinases may function with anti-cancer activity via B cells activation [73]. In the Fc epsilon RI signaling pathway, the expression of Fc epsilon on mast cells stimulates immunoglobulin E, leading to type 1 hypersensitivity-induced local inflammatory responses at the tumor sites [74]. In the insulin signaling pathway, insulin-resistant patients undergo excessive production of reactive oxygen species (ROS) that can harm DNA attributed to carcinogenesis [75]. In the estrogen signaling pathway, estrogen exposure to chronic inflammatory disease activity is a key risk in breast cancer progression [76]. With regards to the prolactin signaling pathway, prolactin functions as a cytokine immune system, especially in breast cancer, which has the strongest correlation with an increased expression level of prolactin and prolactin receptors [77,78]. In the thyroid signaling pathway, the optimal regulation of the cellular thyroid hormone is essential for an adequate role of immune cells during inflammation [79]. In terms of the adipocytokine signaling pathway, adipocytokine is an inflammatory mediator during immune-associated diseases, which accelerates cancer progression and metastasizes from organ to organ [80]. In the Relaxin signaling pathway, Relaxin alleviates the inflammatory severity and diminishes the amount of leucocytes and the expression level of cytokines [81]. In terms of the Advanced Glycation End-product (AGE)-Advanced Glycation End-product Receptor (RAGE) signaling pathway in diabetic complications, RAGE can stimulate inflammatory responses by binding with AGEs [82].

The inhibitor (papaverine) of RAGE is a promising target for anti-cancer activity, which blocks nuclear factor kappa B (NF-κB) [83].

4. Materials and Methods

4.1. Plant Material Collection and Identification

The C. maackii flowers were collected from Mihogil of Bomunmyeon (Latitude: 36.666149, Longitude: 128.511759), Kyeongsang-bukdo, Republic of Korea, in October 2020, and the plant was identified by Dr. Dong Ha Cho, Plant biologist and Professor, Department of Bio-Health Convergence, College of Biomedical Science, Kangwon National University. A voucher number (CNB 015) has been deposited at the Kenaf Corporation in the Department of Bio-Health Convergence, and the material can only be used as research.

4.2. Plant Preparation, Extraction

The C. maackii flower was dried in a shady area at room temperature (20–22 °C) for 7 days, and dried leaves were powdered using an electric blender (Shinil, Cheonan, Korea). Approximately 50 g of C. maackii flower powder was soaked in 800 mL of 100% methanol (Daejung, Seohaean, Korea) for 5 days and repeated 3 times to collect extraction. The solvent extract was collected, filtered, and evaporated using a vacuum evaporator (RV8, IKA, Staufen, German). The evaporated sample was dried under a boiling water bath (HB10, IKA, Staufen, German) at 40 °C to obtain the extract.
4.3. GC-MS Analysis Condition

Agilent 7890A (Agilent, Santa Clara, CA, USA) was used to carry out the GC-MS analysis. GC was equipped with a DB-5 (30 m × 0.25 mm × 0.25 µm) capillary column (Agilent, Santa Clara, CA, USA). Initially, the instrument was maintained at a temperature of 100 °C for 2.1 min. The temperature rose to 300 °C at a rate of 25 °C/min and was maintained for 20 min. The injection port temperature and helium flow rate were sustained at 250 °C and 1.5 mL/min, respectively. The ionization voltage was 70 eV. The samples were injected in the split mode at 10:1. The MS scan range was set at 35–900 (m/z). The fragmentation patterns of mass spectra were compared with those stored in the W8N05ST Library MS database. The percentage of each compound was calculated from the relative peak area of each compound in the chromatogram. The concept of integration was used with the ChemStation integrator (Agilent, Santa Clara, CA, USA) algorithms (analyzed 19 May 2021) [84].

4.4. Bioactives Database Construction and Drug-Likeness Property

The bioactives from the C. maackii flower were identified by utilizing GC-MS analysis. Then, the GC-MS-detected bioactives were filtered in accordance with Lipinski’s rules through SwissADME (http://www.swissadme.ch/) (accessed on 3 June 2021) to confirm the “Drug-likeness” physicochemical properties. PubChem (https://pubchem.ncbi.nlm.nih.gov/) (accessed on 3 June 2021) was utilized to select the SMILES (Simplified Molecular Input Line Entry System) bioactives.

4.5. Target Targets Related to Selected Bioactives or Cancer

Targets connected to the bioactives were selected through both the Similarity Ensemble Approach (SEA) (http://sea.bkslab.org/) (accessed on 7 June 2021) [85] and Swiss Target Prediction (STP) (http://www.swisstargetprediction.ch/) (accessed on 9 June 2021) [86] with the “Homo Sapiens” setting, both of which are based on SMILES. The cancer-related targets in humans were obtained with keywords (cancer/tumor/neoplasia/carcinoma) from TTD (http://db.idrblab.net/TTD/) (accessed on 12 June 2021) and OMIM (https://www.omim.org/) (accessed on 13 June 2021). The overlapping targets between compounds of the C. maackii flower and cancer targets were illustrated by VENNY 2.1 (https://bioinfogp.cnb.csic.es/tools/venny/) (accessed on 14 June 2021).

4.6. Construction of PPI Networks and Bubble Chart

For the final overlapping targets, STRING (https://string-db.org/) (accessed on 16 June 2021) [87] was utilized to analyze the PPI network. Thereby, RPackage was used to identify the degree of value. Then, signaling pathways on STRING were visualized by RPackage, a hub signaling pathway (lowest rich factor) related to a hub target (highest degree of value from PPI).

4.7. Construction of a Size Map on S-T-B Network

Both the hub target (the highest degree of value among 20 signaling pathways) and the uppermost bioactive were identified via the S–T–B network. The S–T–B networks were utilized to construct a size map, based on the degree of values. In this size map, green rectangles (nodes) represented signaling pathways, pink triangles (nodes) represented targets, and orange circles (nodes) represented bioactives. The circle size represented the degree value, the size of pink triangles represented the number of connectivity with signaling pathways, and the size of orange circles represented the number of connections with targets. The merged networks were constructed using RPackage.

4.8. Preparation for MDT of Targets

Four targets of a hub signaling pathway, i.e., Akt1 (PDB ID: 5KCV), VEGFA (PDB ID: 3V2A), PRKCA (PDB ID: 3IW4), and PHLP1 (PDB ID: N/A), were selected on STRING via RCSB PDB (https://www.rcsb.org/) (accessed on 19 June 2021). Specifically, the PHLP1
protein structure was not determined; thereby, the PDB ID was not uploaded in the .pdb format. Thus, the final three proteins selected in the .pdb format were converted into the .pdbqt format via Autodock (http://autodock.scripps.edu/) (accessed on 22 June 2021) [88].

4.9. Preparation for MDT of Positive Standard Ligands

A total of 13 positive control compounds on Akt1 antagonists, i.e., MK-2206 dihydrochloride (PubChem ID: 46930998), Gsk-690693 (PubChem ID: 16725726), Ipatasertib (PubChem ID: 24788740), Capivasertib (PubChem ID: 25227436), AT7867 (PubChem ID: 11175137), A-674563 (PubChem ID: 11314340), Miransertib (PubChem ID: 53262401), BAY1125976 (PubChem ID: 70817911), Akti-1/2 (PubChem ID: 135398501), Uprosertib (PubChem ID: 51042438), Aferesertib (PubChem ID: 46843057), AT13148 (PubChem ID: 24905401), and Oridonin (PubChem ID: 5321010), were selected to verify the docking score.

4.10. Preparation for MDT of Ligand Molecules

The ligand molecules were converted from sdf in PubChem into the .pdb format using Pymol, and the ligand molecules were converted into the .pdbqt format through Autodock.

4.11. Ligand-Protein Docking

The ligand molecules were docked with targets utilizing autodock4 by setting up an energy range of 4 and exhaustiveness at 8 as the default to obtain 10 different positions of ligand molecules [89]. The center (the position of the middle coordinate point) in the target was X: −12.677, Y: 2.931, Z: −13.145 on AKT1 (PDB ID: 5KCV). The grid box size was set to 40 Å × 40 Å × 40 Å. The 2D binding interactions were used with LigPlot+ v.2.2 (https://www.ebi.ac.uk/thornton-srv/software/LigPlus/) (accessed on 23 June 2021) [90]. After docking, ligands of the lowest binding energy (highest affinity) were selected to visualize the ligand–protein interaction in Pymol.

4.12. Toxicological Properties Prediction by admetSAR

Toxicological properties of the key bioactive were established using the admetSAR web-service tool (http://lmmd.ecust.edu.cn/admetsar1/predict/) (accessed on 24 June 2021) [91] because toxicity is an essential factor in developing new drugs. Hence, Ames toxicity, carcinogenic properties, acute oral toxicity, and rat acute toxicity were predicted by admetSAR.

5. Conclusions

The bioactives and mechanisms of C. maackii flowers against cancer were firstly uncovered through network pharmacology. The findings suggested that 20 signaling pathways, 24 targets, and 19 bioactives are connected to cancer. Of these, the PI3K-Akt signaling pathway, Akt1, and Urs-12-en-24-oic acid, 3-oxo-, methyl ester were the hub signaling pathway, hub target, and key bioactive of C. maackii flowers against cancer, respectively. Furthermore, Urs-12-en-24-oic acid, 3-oxo-, methyl ester has the most potent efficacy on the Akt1 target protein than 13 other standard ligands. This study suggests that the mechanism of the C. maackii flower against cancer might strengthen anti-inflammatory responses by inactivating the PI3K-Akt signaling pathway, bound to Urs-12-en-24-oic acid, 3-oxo-, methyl ester on Akt1. From this viewpoint, we propose that C. maackii flowers can be utilized as functional or medicinal resources against cancer.

Supplementary Materials: The following are available online, Table S1: The 309, 396, and 108 targets from SEA, STP, and overlapping targets between SEA and STP, respectively. Table S2: The 4245 cancer-related targets from TTD and OMIM databases, Table S3: The final 51 targets against cancer.

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**Abbreviations**

| Abbreviation | Description |
|--------------|-------------|
| ADME         | Absorption; Distribution, Metabolism, Excretion; |
| AGE          | Advanced Glycation End-product; |
| AMPK         | AMP-activated protein kinase; |
| cAMP         | Cyclic AMP; |
| C. maackii   | Cirsium japonicum var. maackii (Maxim.) Matsum.; C. maackii; |
| DLCs         | Drug-Like Compounds; |
| BCR          | B cell receptor; |
| GC-MS        | Gas Chromatography Mass Spectrum; |
| HIF-1        | Hypoxia Inducible Factor-1; |
| KEGG         | Kyoto Encyclopedia of Genes and Genomes; |
| MAPK         | Mitogen Activated Protein Kinase; |
| MDT          | Molecular Docking Test; |
| NF-κB        | Nuclear Factor Kappa B; |
| OMIM         | Online Mendelian Inheritance in Man; |
| PI3K-Akt     | Phosphoinositide 3-kinase-Akt; |
| PLD          | Phospholipase D; |
| PPAR         | Peroxisome Proliferator Activated Receptor; |
| PPI          | Protein-protein interaction; |
| RAGE         | Advanced Glycation End-product Receptor; |
| Rap1         | Ras-associated protein-1; |
| ROS          | Reactive Oxygen Species; |
| SEA          | Similarity Ensemble Approach; |
| SMILES       | Simplified Molecular Input Line Entry System; |
| S-T-B        | Signaling pathway-Target protein-Bioactive; |
| STP          | SwissTargetPrediction; |
| t-BHP        | Tert-butyl hydroperoxide; |
| TTD          | Therapeutic Target Database; |
| VEGF         | Vascular Endothelial Growth Factor |

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