Elucidation of significant pathways of Korean Thistle (*Cirsium japonicum var. maackii* (Maxim.) Matsum.) flower against cancer through network pharmacology

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Introduction

The definition of cancer is that normal cell damaged via an aberrant endogenous process such as abnormality of DNA replication, instability of DNA sequence transformed to malignancy. The DNA damage responses represent chronic inflammation via the immune signaling pathway, which results in accelerating tumorigenesis. The damaged normal cells undergo cellular senescence, triggering secretion in the inflammatory cytokines leads to cellular mechanical disruption. Because inflammation is a leading factor to cause pathological symptoms such as unknown severe pain, fatigue, and comorbidity in cancer patients, and thus anti-inflammation strategy is a key therapeutics. Anti-inflammatory agents for cancer treatment are currently NSAIDs, including Cox-2, due to fewer adverse effects and mortality rate. Most commonly, anti-cancer agents target to inhibit DNA replication and induce cancer cell death; however, cancer chemotherapeutics attack even healthy cells results in serious side effects such as nausea, vomiting, hair loss, and fatigue. On the other hand, traditional herbal plants with innovative bioactives and secondary metabolites play an essential role as an effective anti-inflammatory, anti-oxidant or anti-cancer agents. For instance, the plant extracts (*Urtica membranaceae*, *Artemesia monosperma*, and *Origanum dayi Post*) with a combination of anti-cancer drugs showed enhanced potency against specific cancer cell lines (lung, breast, colon, and prostate cancer) without exposing cytotoxicity on normal lymphocytes and fibroblasts. Herbal-derived bioactives possess fewer unwanted side effects than chemotherapy, which 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.. *C. maackii* is a perennial herbal plant, belong to the family of *Compositae*, and widely distributed in the mountainous areas of Korea, Japan, and China. Furthermore, *Cirsium* species have been reported to have diverse pharmacological activities such as anti-oxidant, anti-inflammation, anti-cancer, and hepatoprotection effects. Specifically, *C. maackii* extract at 200㎍/㎖ concentration showed 36.89% inhibition against breast cancer cell line (MDA-MB-231). Another study demonstrated that HepG2 cells treated with MeOH extract of *C. maackii* have potent antioxidant efficacy against severe oxidant condition. Anti-inflammatory agents assist protection against cancer development, thereby acting on preventing cytokines storm. It implies that anti-inflammatory compounds are important agonists to protect normal cell adjacent to tumor cell because they can block the overflowing of cytokines. Until now, *C. maackii* flower compounds were identified by HPLC and reported for only anti-Alzheimer efficacy by inhibiting BACE1. Generally, the identification of polar and mid-polar compounds from extracts are based on HPLC due to its good separation capability. In a different perspective, we utilized the GC-MS analysis to discover lipophilic bioactives, which mainly act as drug-like compounds and uptake efficiently into the cells. The lipophilicity is a significant physicochemical parameter that influences membrane permeability and affinity. More importantly, GC-MS along with molecular docking test (MDT) and ADME (Absorption, Distribution, Metabolism, and Excretion) study, are an optimal analytical method to determine drug-likeness compounds [26]. At present, the biactives and mechanisms of *C. maackii* flower against cancer remain unknown. Hence, we aimed to uncover its potential bioactives with their fundamental mechanisms through network pharmacology.

Materials and methods

GC-MS analysis condition

Agilent 7890A was used to carry out GC-MS analysis. GC was equipped with a DB-5 (30m×0.25mm×0.25μm) capillary column. Initially, the instrument maintained at a temperature of 100 °C for 2.1 minutes. The temperature rose to 300 °C at the rate of 25°C/min and maintained for 20 minutes. Injection port temperature and helium flow rate were ensured as 250 °C and 1.5 ml/min, respectively. The ionization voltage was 70 eV. The samples injected in split mode at 10:1. MS scan range was set at 35-900 (m/z). The fragmentation patterns of mass spectra were compared with those stored in the using 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 the ChemStation integrater algorithms (analyzed 19 May 2021)

Bioactives database construction and drug-likeness property

The biactives from *C. maackii* flower was identified by utilizing GC-MS analysis. Then, GC-MS detected bioactives filtered as reported by Lipinski's rule through SwissADME (http://www.swissadme.ch/) (accessed on 3 June 2021) to confirm the "Drug-likeness" physicochemical property. The 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.

Target targets related to selected bioactives or cancer

Targets connected to the bioactives were selected through both Similarity Ensemble Approach (SEA) (http://sea.bkslab.org/) (accessed on 7 June 2021) and SwissTargetPrediction (STP) (http://www.swisstargetprediction.ch/) (accessed on 9 June 2021) with "*Homo Sapiens*" setting, both of which is based on SMILES. The cancer-related targets on human 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 *C. maackii* flower and cancer targets illustrated by VENNY 2.1 (https://bioinfogp.cnb.csic.es/tools/venny/).

Construction of PPI networks and bubble chart

On the final overlapping targets, STRING (https://string-db.org/) (accessed on 16 June 2021) 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).

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Abstract

Cirsium japonicum var. maackii (Maxim.) Matsum. or Korean thistle flower, is a herbal plant used to treat tumor 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 *C. maackii* flower against cancer via network pharmacology. The bioactives from *C. maackii* flower were revealed by gas chromatography-mass spectrum (GC-MS), and SwissADME evaluated their physicochemical properties. Target(s) associated with 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 the Lipinski's rules and therefore decided as drug-like compounds (DLCs). A total of 597 bioactives related targets and 4,245 cancer-related targets were identified from public databases. The final 51 overlapping targets were selected between the bioactives-targets network and cancer-related targets. On Kyoto Encyclopedia of Genes and Genomes (KEGG), the number 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 *C. maackii* flower has potent anti-cancer efficacy by inactivating Akt1 on the PI3K-Akt signaling pathway.

Results

Graphical abstract

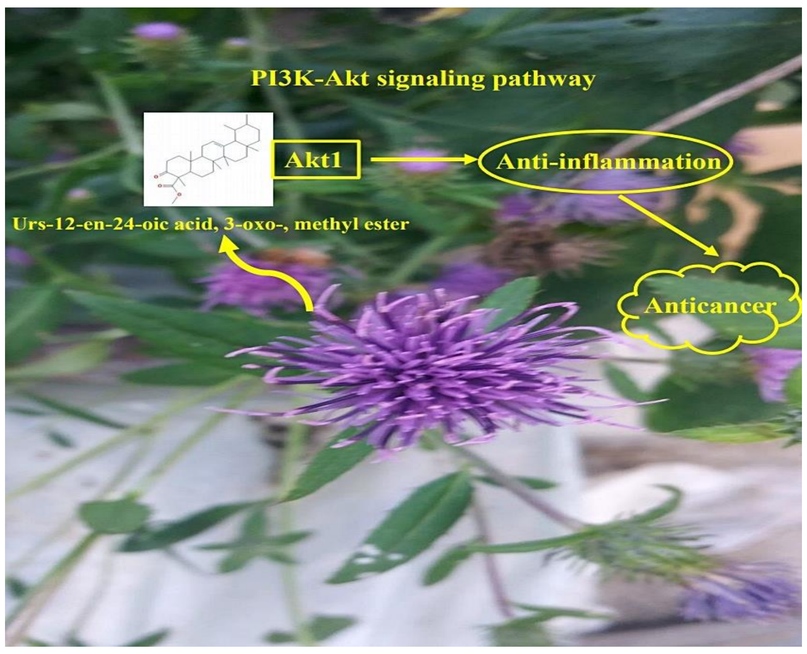


Figure.1 PPI networks (46 nodes, 145 edges)

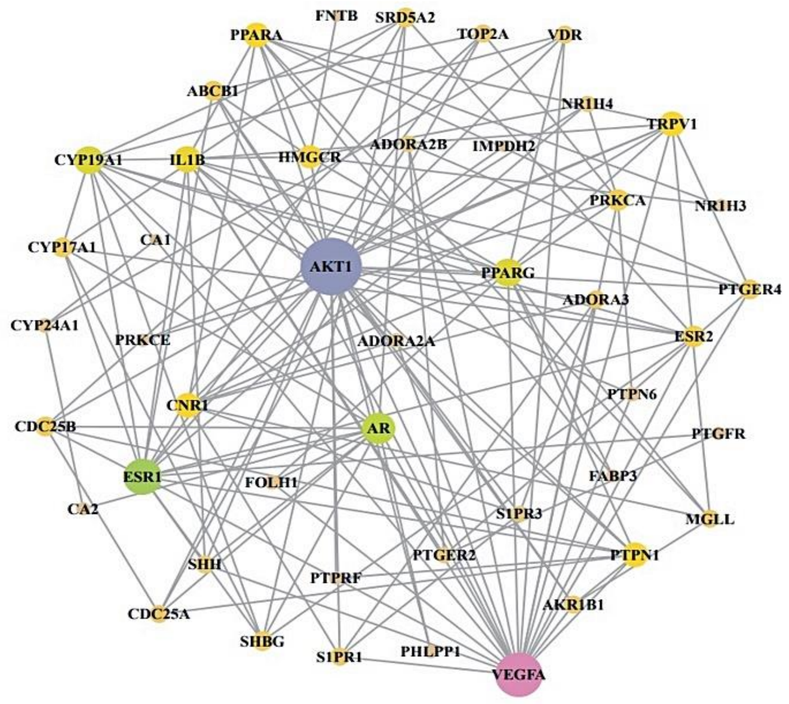
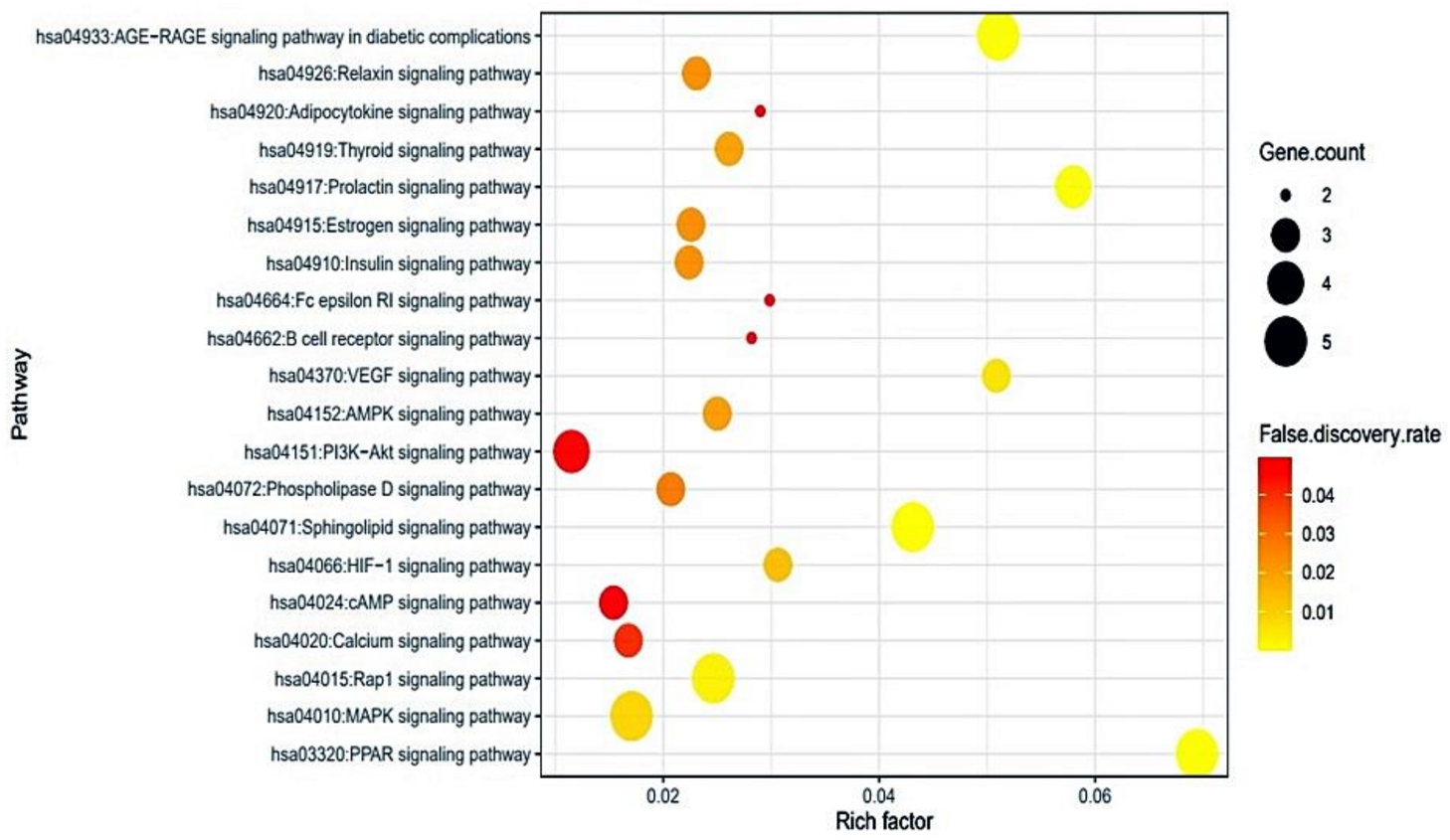


Figure.2 Bubble chart of 20 signaling pathways connected to cancer.



Conclusion

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, PI3K-Akt signaling pathway, Akt1, and Urs-12-en-24-oic acid, 3-oxo-, methyl ester were a hub signaling pathway, a hub target, and a key bioactive of *C. maackii* flowers against cancer, respectively. Also, Urs-12-en-24-oic acid, 3-oxo-, methyl ester has the most potent efficacy on Akt1 target protein than the number of 13 standard ligands. This study suggests that the mechanism of *C. maackii* flower against cancer might be to strengthen anti-inflammatory responses by inactivating PI3K-Akt signaling pathway, bound to Urs-12-en-24-oic acid, 3-oxo-, methyl ester on Akt1.

References

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