Uncovering of bioactives and mechanisms of garlic (*Allium sativum* L.) husk for the amelioration of type 2 diabetes mellitus via network pharmacology

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Introduction

Globally, diabetes mellitus (DM) is a major disease caused unexpected serious complications: retinopathy with loss of vision, nephropathy with failure of renal failure, neuropathy with foot ulcers, and hypertension and atherosclerosis. The dysfunction of lipid metabolism, defined as excessive accumulation of triglycerides, low-density lipoprotein, and mass of lipids in the cells, is the culprit to cause type 2 diabetes mellitus (T2DM), which is related directly to insulin resistance. Currently, anti-T2DM agents associated with lipid metabolism are Rosiglitazone and Pioglitazone, indicating that the two agents regulate peroxisome proliferation-activating receptor delta (PPARD). However, the two drugs in thiazolidinedione type have major side effects including hepatitis, heart failure, bladder carcinoma, water retention, and weight gain. Compared with traditional anti-T2DM drugs, natural products have been implemented critical roles in T2DM treatment. Moreover, the raising understanding of common people concerning food additive compounds has been triggered a demand for recognizing potential natural and cheaper sources of food against T2DM. The surrogate of traditional anti-T2DM drugs with natural organic compounds may be a replacement to diminish adverse effects. The natural-originated compounds have been produced over the last few years, rising research on natural extracts of medicinal plants, food processing technology, or agri-food discards. Allium sativum L. (ASL) has been used as a significant seasoning resource from olden times for both cuisine and health-promoting purposes. The ASL is a source of organosulfur compounds to inhibit inflammation and persistent diseases such as cancer, obesity, and neural disorder. The ASL bioactives are revealed in pharmacological functions including antioxidant, anti-bacterial, anti-fungal, anti-obesity, and even anti-diabetic properties. Throughout the gathering season of ASL bulb produces a great deal of husk, stem, and leaf which is not available for food additives. A report showed that the stem and leaf of ASL compose fewer allicin than garlic bulb. However, nutraceutical or therapeutic promising bioactives of Allium sativum L. husk (ASLH) has not been documented although it has been used as a reliever against T2DM in Korean traditional medicine. Therefore, studies on bioactives and pharmacological signalings of ASLH against T2DM should be demonstrated to decode biochemical evidence against T2DM.

The natural extracts are attributed to multi-compounds and multi-targets to make synergistic effects. Currently, multi-compounds and multitarget approaches have been identified to be a more effective approach and less side effects than traditional single bioactive-single target. The innovative development of bioinformatics and network pharmacology (NP) is considered as a promising tactic for drug discovery. Additionally, the NP has been focused on studying bioactives and signalings of natural herbal plants for the amelioration of T2DM due to viable bioactive-target–disease. The NP, a systemic methodology, can decipher the association between multiple components including drugs, targets, and diseases. It has been shown that NP is applicable to herbal compound, target and disease target prediction, decoding of synergistic efficacy of multi-compounds and bioactives. In this study, NP was employed to investigate the bioactives and signalings of ASLH against T2DM. First, we carried out GC–MS analysis on ASLH ethanol extraction. Then, we selected the identified bioactives by Lipinski's rule to obtain drug-like compounds (DLCs). The targets associated with DLCs were identified by public bioinformatics, the overlapping targets between DLCs and T2DM were adopted. Second, we depicted a protein–protein interaction (PPI) network to select a hub target of ASLH on T2DM. Thirdly, we analyzed signaling pathways linked directly to T2DM on a bubble chart. Fourthly, we constructed signaling pathways–targets–bioactives (STB) to prove the relationship between the three elements. Finally, we implemented a molecular docking assay (MDA) on a hub signaling pathway(s). This workflow is displayed in Figure 1.

Abstract

Allium sativum L. husk (ASLH) extracts have been utilized as an alleviator in the treatment for type 2 diabetes mellitus (T2DM). At present, its important signaling against T2DM has been unknown. Thus, the purpose of this study is to decode its key signaling pathways, targets, and bioactives. The bioactives in ASLH were detected by gas chromatography– mass spectrum (GC–MS) and accepted drug-like compounds (DLCs) in silico. Then, protein–protein interaction (PPI) networks and signaling pathways, targets, bioactives are described by utilizing R Package. Finally, we conducted a molecular docking assay (MDA) to understand the key signaling(s), target(s), and bioactive(s) of ASLH against T2DM. A total of 23 compounds in ASLH were detected by GC–MS, and all bioactives were confirmed by Lipinski's rule. The 23 bioactives were related to 521 targets and retrieved 4,736 T2DM-associated targets via Online Mendelian Inheritance in Man (OMIM) and DisGeNET. The final overlapping 87 targets were identified between bioactives –targets and T2DM-related targets. The number of 13 signaling pathways, 33 targets, and 19 bioactives of ASLH were associated with T2DM. Overall, MDA revealed four potential bioactives: (1) 9-hexacosene, (2) 2-(([2-ethylhexyl]oxy)car- bonyl)benzoic acid, (3) clionasterol, (4) 4-methyl-2-phenylpyrimidine on PPAR signaling pathway. Overall, the four bioactives from ASLH might exert an anti-T2DM synergistic efficacy by activating the PPAR signaling pathway or dampening the phospholipase D signaling pathway. In this study, we suggest that ASLH might be expected as nutraceutical or pharmaceutical resource to enhance our well-being.

Results

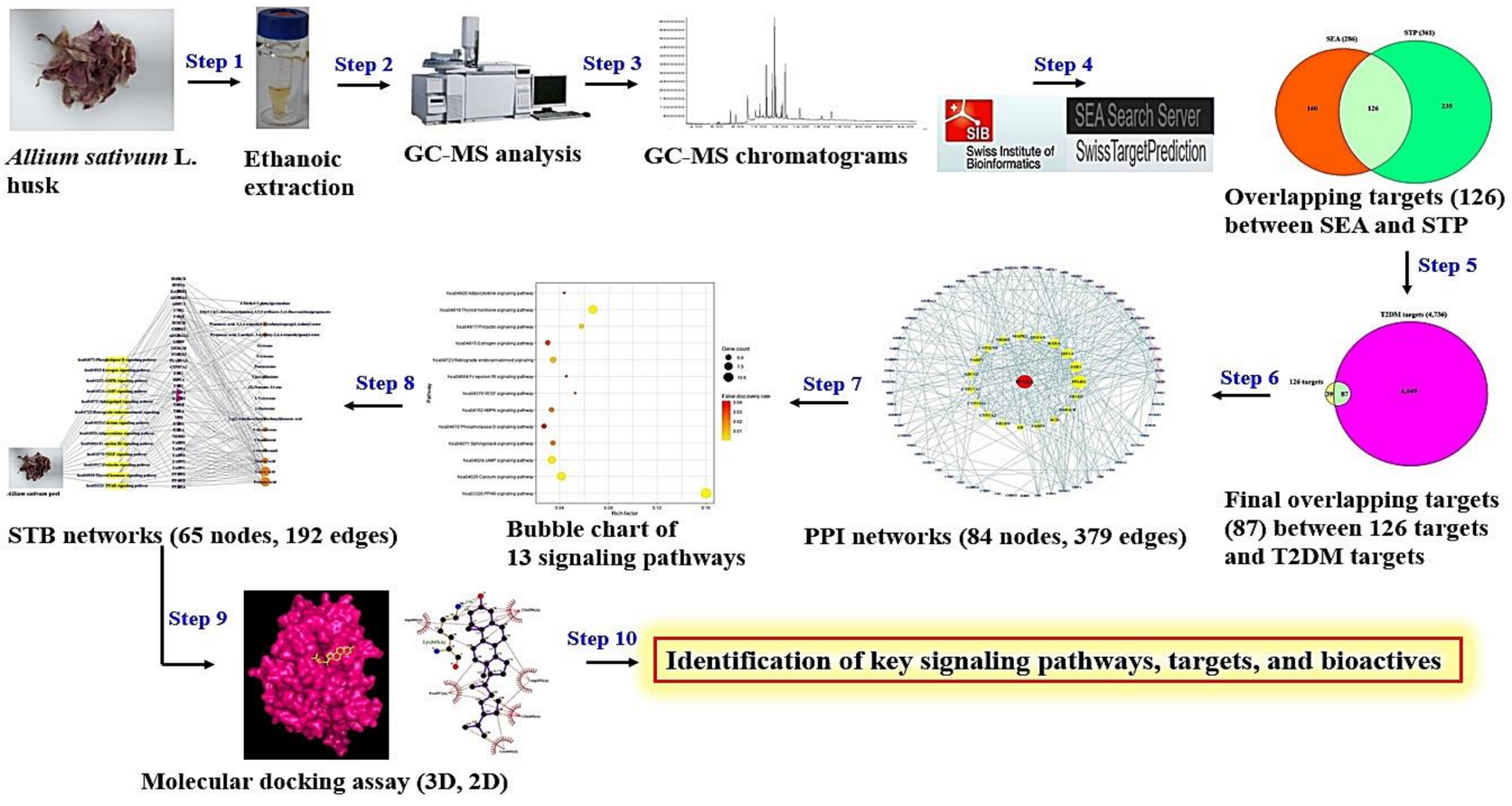

Figure 1. The workflow of this study.

Materials and methods

Identification of bioactives

The bioactives from ASLH were identified by GC–MS investigation. The identified bioactives evaluated "drug-likeness" physicochemical property and TPSA value (<140 Å2) on SwissADME (http://www.swissadme.ch/) (accessed on October 2, 2021). The accepted bioactives were transformed into simplified molecular input line entry system (SMILES) (accessed on October 2, 2021) format via PubChem (https://pubch em.ncbi.nlm.nih.gov/) (accessed on October 2, 2021).

Targets associated with bioactives from ASLH or diabetes mellitus

Targets linked to bioactives were browsed via both similarity ensemble approach (SEA) (http://sea.bkslab. org/) (accessed on October 3, 2021) and SwissTargetPrediction (STP) (http://www.swisstargetprediction. ch/) (accessed on October 3, 2021) with "Homo Sapiens" mode, both of which was identified on SMILES (accessed on May 14, 2021) format. The DM-associated targets on humans were selected by DisGeNET (https://www. disgenet.org/) (accessed on October 6, 2021), OMIM (https://www.omim.org/) (accessed on October 7, 2021), and literatures. The overlapping targets between bioactives of ASLH- and DM-related targets are represented by InteractiVenn (http://www.interactivenn.net/) (accessed on October 8, 2021). Then, we described it on Venn Diagram Plotter.

PPI networks and bubble chart

The final overlapping targets based on STRING (https://string-db. org/) (accessed on October 9, 2021) was used to construct PPI networks. The R Package software was utilized to recognize the degree of value on each target. Then, signaling pathways related to the occurrence and development of T2DM were depicted on a bubble chart by R Package. Thereby, a signaling pathway with the highest rich factor or related to a protein-coding gene with the highest degree value was regarded as a key signaling pathway.

Construction of a PTC network

The signaling pathways, targets, and bioactives were utilized to construct a pathway–target–bioactive (PTB) network. In this PTB network, yellow rectangles (nodes) represented signaling pathways, red triangles (nodes) stood for targets, and orange circles (nodes) indicated compounds, its size described the degree of values. The size of red triangles stood for the number of relationships with the signaling pathway, and the size of orange circles indicated the number of connectivity to targets. The integrated network was assembled by R Package software.

Bioactive–Protein docking test

The bioactives were docked with targets using autodock4 by setting up four energy ranges and eight exhaustiveness as default to obtain 10 different poses of bioactives (Khanal et al., 2020). The center of each target protein on the PPAR signaling pathway was PPARA (x = 8.006, y = −0.459, z = 23.392), PPARD (x = 39.265, y = −18.736, z = 119.392), PPARG (x = 2.075, y = 31.910, z = 18.503), FABP1 (x = 18.247, y = −1.610, z = −48.419), FABP2 (x = 9.272, y = −20.547, z = 45.960), FABP3 (x = −1.215, y = 46.730, z = −15.099), FABP4 (x = 7.693, y = 9.921, z = −14.698), FABP5 (x = 43.967, y = −12.214, z = 15.451), NR1H3 (x = 43.967, y = −12.214, z = 15.451), RXRA (x = 8.287, y = 46.448, z = −13.672), RXRG (x = − 1.383, y = 26.539, z = −13.577), and SCD (x = 18.806, y = 67.358, z = 40.111). The center of each target protein on the phospholipase D signaling pathway was MAPK1 (x = 20.844, y = 40.173, z = 21.018), PRKCA (x = −14.059, y = 38.224, z = 32.319), GRM5 (x = 18.296, y = −0.634, z = −4.180), and PLA2G4A (x = −0.058, y = 0.077, z = 0.285). The active site's grid box size was x = 40 Å, y = 40 Å, z = 40 Å. The 2D binding interactions were used with LigPlot+ v.2.2 (https://www.ebi.ac.uk/thornton-srv/software/LigPl us/) (accessed on October 15, 2021). The bioactives of the lowest binding energy (highest affinity) on each target were selected to visualize the ligand–protein interaction in Pymol (Schrödinger, New York, NY, USA).

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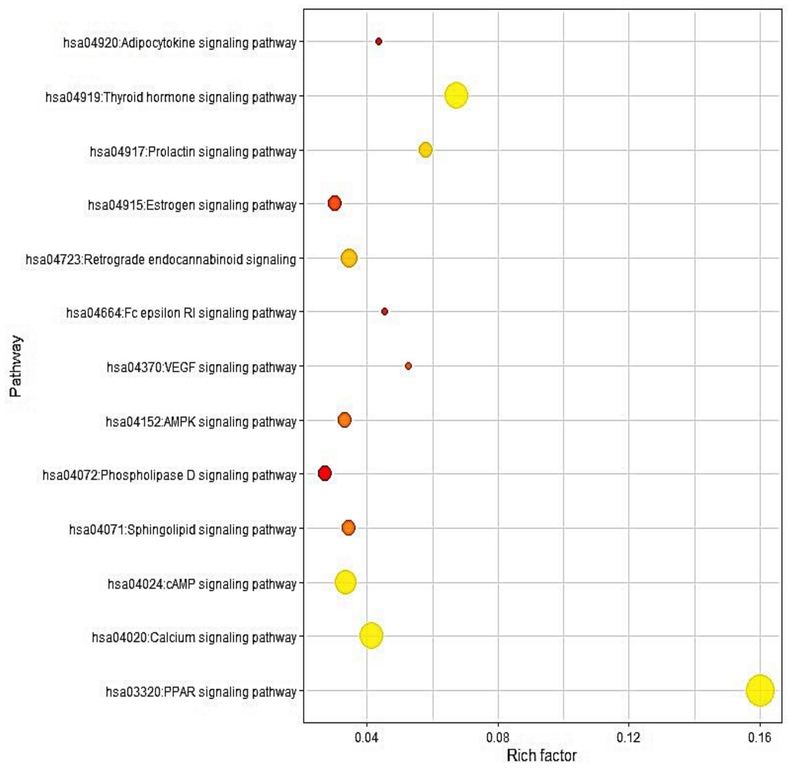

Figure 2. A bubble plot on 13 signaling pathways

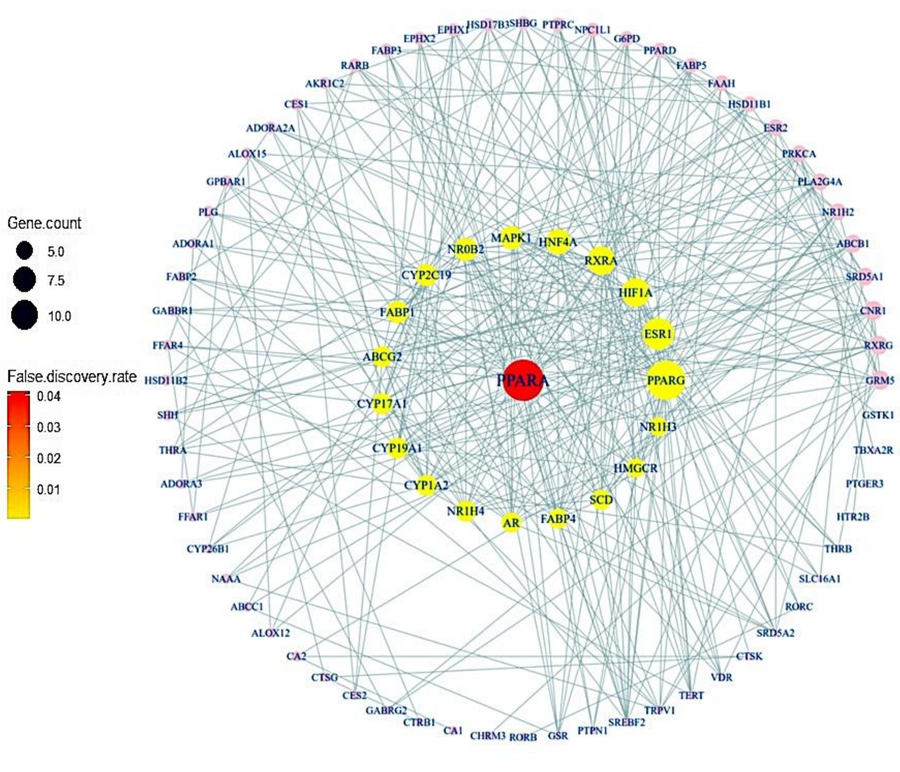

Figure 3. PPI networks

Conclusion

This study established the potential ASLH effector mechanism in ameliorating T2DM through the NP concept. We unraveled that 9-hexacosene, 2-(([2-ethylhexyl]oxy)carbonyl)benzoic acid, clionasterol, and 4-methyl-2-phenylpyrimidine played important role in T2DM by affecting the PPAR signaling pathway (PPARA, PPARD, PPARG, NR1H3, FABP1, FABP3, FABP4, and RXRG) or phospholipase D signaling pathway (MAPK1, PRKCA, and GRM5). In addition, the MDA test also demonstrated that the four key bioactives from ASLH could exert strong effects on the 11 targets, indicating crucial evidence for further investigation. Given the limitations of NP, the promising therapeutic signalings of ASLH against T2DM is identified by systemic biological analysis, and the efficacy of the key compounds from ASLH, PPI networks, and metabolism in vivo was not verified, which requires to be further confirmed through therapeutic and clinical trials. However, this work demonstrates nutraceutical and pharmacological value of ASLH wasted industrially, and a research approach for revealing the bioactives and mechanisms of ASLH in the treatment of T2DM.

References

1. Oh, K. K. (2022). A network pharmacology study to investigate bioactive compounds and signaling pathways of garlic (*Allium sativum* L.) husk against type 2 diabetes mellitus. Journal of Food Biochemistry, 00, e14106. https://doi.org/10.1111/jfbc.14106