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α-Glucosidase Inhibitors from *Polyscias serrata* Roots in a Parallel Study of Network Pharmacology

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Abstract: Nine triterpenoid saponins, one steroid and 4 simple phenolic compounds were isolated from the roots of *P. serrata*. All isolated compounds were examined for α- glucosidase inhibition effects. The simple phenolics 4-Hydroxy-4-methoxy-benzoic acid (**7**) and Protocatechuic acid (**9**) showed the greatest inhibition effects among the isolated compounds with IC_{50} values of 0.55 ± 0.01 and 0.84 ± 0.02 μg/mL, respectively. Network pharmacology approach was applied to investigate the molecular mechanisms of α- glucosidase inhibition activities of isolated compounds. In light of the data obtained in this study, it is evaluated that the insulin resistance pathway may be the primary mechanism of action.

Keywords: *Polyscias serrata*; α-glucosidase inhibitor; molecular docking; network pharmacology.

1. Plant Source

The plant materials were collected from Me Linh, Ha Noi, Viet Nam in January 2020. The species was identified as *Polyscias serrata* Balf by Nguyen Quoc Binh from Vietnam National Museum of Nature, which had already been deposited with a voucher specimen (HN 0000007753) at the Herbarium of Institute of Ecology and Biological Resources, VAST.

2. Previous Studies

P. serrata Balf. (see Figure S1) is a traditional medicinal plant in Vietnam used for diuretic and sedative treatments [1]. However, there are limited numbers of the phytochemical studies and biological activities on the species up to date. The chemical constituents from ethanol extract of *P. serrata* leaves showed the presence of saponins, ceramides, and glucoside derivatives [2]. There is no report about the chemical constituents and biological activity of *P. serrata* roots until now.

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3. Present Study

Although the pharmacological target of *P. serrata* Balf. for underlying the pathogenesis has not been investigated yet, some other members of this family such as *P. fulva* [3], *P. fruticosa* [4] is known to be effective in the treatment of diabetic patients.

Today, network pharmacology methodology has emerged as a pioneering paradigm for better understanding the molecular activity mechanisms of natural products and has found a strong place in this field [5]. This approach was used in this study to evaluate the structure and activity of secondary metabolites isolated from *P. serrata* roots.

This study reports an *in vitro* study evaluating the ability of medicinal plants to inhibit the alpha-glucosidase enzyme and the effect of the combination of secondary components obtained from the plant extract. Furthermore, *in silico* research based on secondary metabolites of solvent extracts in association with pharmacological targets were carried out to evaluate the practical applicability of *P. serrata* Balf plant extracts and isolated secondary compounds.

α-Glucosidase Inhibitory Activity of the Extracts from P. serrata: The roots of *P. serrata* Balt (5kg) were macerated and then ultrasonicated with 10L methanol (MeOH) for three times. Evaporation of the solvent under reduced pressure gave crude extract (1,0 kg). A crude extract of *P. serrata* Balt roots was suspended in H_2O and then partitioned sequentially with n-hexane, EtOAc (ethyl acetate), and MeOH (methanol) to give corresponding *n*-hexane, EtOAc, MeOH, and H_2O fractions after removed solvent under vacuum. These fractions were evaluated for their α-glucosidase inhibition effects at concentrations of 0.25, 4.0, 16.0, 64.0, and 256.0 μg/mL with Acarbose was used as a reference compound (see Figure S1). At the concentration to 256.0 μg/mL, n-hexan and EtOAc fractions exhibited the good inhibition effect on α-glucosidase enzyme.

α-Glucosidase Inhibitory Activity of the Isolated Compounds from P. serrata: Nine known compounds (**1**-**9**) were isolated from the ethyl acetate and water fractions using a combination of various chromatographic steps (see Figure 1). Their structures were identified based on the direct comparison of NMR data with those reported in previous studies (see supporting information).

The α-glucosidase inhibitory activities of isolated compounds (**1**-**9**) was evaluated according to the previously described method (see SI) Acarbose was used as a positive control with IC_{50} value of 206.98 ± 2.13 μg/mL. Efficiency of the compounds were examined at various concentrations ranging from 1.0 to 256.0 μg/mL and the 50% inhibitory concentration (IC_{50}) was calculated using a dose-dependent response curve (see Figure S2). The result showed that compounds **7** and **9** exhibited the most potent inhibitory effect, with IC_{50} of 0.55 ± 0.01 and 0.84 ± 0.02 μg/mL, respectively. Compounds **5** and **8** exhibited significant effect on α-glucosidase inhibitory activity with IC_{50} values of 17.10 ± 0.24 and 12.40 ± 0.25 μg/mL, and the other inhibited moderate or no activity.

Molecular Docking and Structural-activity Relationships: A molecular docking study was conducted to explore the interaction between active isolated compounds from *P. serrata* roots and α-glucosidase enzyme. Before performing molecular docking, it is essential to validate the docking protocol. The root-mean-square deviation (RMSD) value of the redocked ligand (α-glucose) with target protein was 0.70Å which reveals the validity of our method. Interactions between a redocked ligand with α-glucosidase were the same as the reported data [6] including hydrogen bonding with Asp69, Arg213, Glu277, His351, Asp342 and Arg442 (see Figure S3).

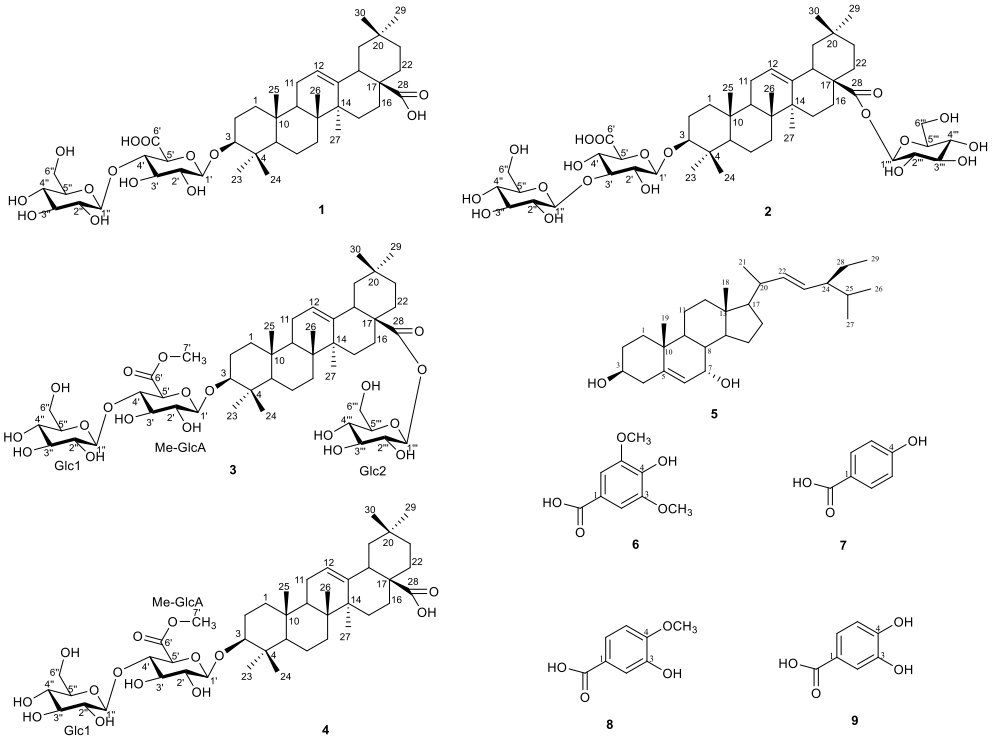

Figure 1. The chemical structure of isolated compounds from the *P. serrata* roots

Our molecular docking study revealed that bioactive compounds from *P. serrata* roots showed different binding behaviour with the active site of α-glucosidase enzyme. The structural similarity in compounds **7**, **8** and **9** may lead to having the same binding site with their binding energies at -4.25, -4.55 and -4.35 kcal mol^{-1}. The phenol -OH group showed hydrogen bonding with important residues in the active site including Asp215, Glu277 and Asp352. Carboxylic acid group exhibited a π-anion interaction with Arg442 while the phenyl ring formed an interaction with the target protein through π-π T-shaped with Tyr72 (see Figure S4). The 4-hydroxyl group in the phenyl ring might be crucial for bioactivity when comparing compound **9** (IC_{50} = 5.45μM) and compound **8** (IC_{50} = 73.74 μM). This is also supported by molecular docking study while 4-hydroxyl group in **9** showed hydrogen bonding with Asp215. The addition of the methoxy group in the 3,5 position could decrease the inhibition against α-glucosidase enzyme.

Hydrogen bondings were observed between compound **5** with Asp352 and Asp307. Meanwhile, compound **1** had interactions with Lys156, Tyr158, His280, Pro312 and Arg315 which are located at the entrance of the active site pocket as reported [6]. This might suggest that **1** played as a non-competitive inhibitor against α-glucosidase enzyme [6].

Screening of Potential Targets and the Construction of Compounds Targets Network: With our promising preliminary α-glucosidase inhibition data, we hypothesized whether our work could extend to develop an approach in the treatment of type 2 diabetes or not. Hence, we tried to investigate the underlying mechanism of *P. serrata* roots for the treatment of type 2 diabetes by using a network pharmacology approach. Research on the chemical composition of the *Polyscias* genus has attracted attention because of their usage in folk remedies in some Southeast Asian countries, such as Vietnam [4]. However, the intended effects of the ingredients in this plant are still unknown. The traditional method of researching one drug, one target is entirely inappropriate for evaluating and explaining the activities of some herbal medicines when their composition is too complex. So this method, the network pharmacology approach, demonstrates high applicability when exploring the overall correlation between medicinal ingredients and multiple targets acting on different metabolic pathways of the body.

Since the chemical constituents of *P. serrata* roots have not been reported, we used our results above for network pharmacology herein. The networks related to type 2 diabetes and these compounds

were built by targeting specific activities corresponding to the interaction of proteins through many biochemical pathways. 390 potential targets were obtained after inputting nine isolated compounds (**1-9**) into the Swiss Target Prediction database (SwissTargetPrediction: updated data and new features for efficient prediction of protein targets of small molecules | Nucleic Acids Research | Oxford Academic (oup.com)) [7] and SuperPred (http://prediction.charite.de/) [8]. A total of 2832 targets related to type 2 diabetes were retrieved from the DisGeNET database then 184 targets were obtained after overlapping with potential targets of nine secondary metabolites from *P. serrata* roots (see Figure S5). 184 overlapped targets (blue) and nine isolated compounds (orange) were used to establish the compounds-targets network. This network contained 192 nodes and 512 edges (see Figure S6).

The larger circles showed the nodes with fewer connections. Each circle was arranged according to the value of degree (k). Stigmasta-4,22-dien-3,7-diol **5** (k = 112) had the largest degree among all chemical constituents. Ranking details are available in the electronic support information (ESI). These results suggest that **5** might be one of the main targets for further study.

Construction of a Protein-Protein Interaction Network: After removing free targets, the protein-protein interaction (PPI) network was constructed with 151 nodes and 494 edges. Nodes represent the target proteins and edges represent the interactions between proteins. Greater node degrees indicate the importance of the core target. The top 10 core targets were obtained, including STAT3, ESR1, HSP90AA1, HIF1A, NFKB1, TLR4, MAPK1, PIK3CA, MMP9 and PPARG (see Figure S7 and Table S2). The identified pharmacological targets all demonstrate relationships with diabetes. Signal transducer and activator of transcription 3 protein, STAT3 is believed to participate in insulin resistance in skeletal muscle when continuously phosphorylated, increasing the amount of SOCS3 protein many times along with the progression of insulin resistance in muscle [9]. Meanwhile, estrogen receptor 1 (ESR1) gene variants are associated with diabetes risk [10] , HSF1 [11] regulates the transcription of cytoprotective stress response HSP70, or Toll-Like Receptor 4 (TLR4) [12] is involved in the regulation of innate immunity accompanied by anti-inflammatory effects and improved insulin sensitivity. A recent report by Lee *et al.* has shown reversal of hyperglycemia and improvement of insulin sensitivity in an insulin resistance model with long-term administration of HSP90 inhibitors in diet-induced obese mice [11]. HIF [13], PIK3CA via PI3K/Akt/mTOR channel, [14] Peroxisome proliferator-activated receptor gamma (PPARG) [15] is an effector of β-cell function and is directly related to diabetes. Nuclear factor-kappaB (NF-kappaB) family or stress-activated mitogen (SAPK/MAPK) family, Matrix metalloproteinase (MMP)-9 regulated by H-Ras [16], have also been shown to be indirect agents involved in the pathogenesis of diabetes.

GO and KEGG Enrichment Analysis: Immediately after the positive results of protein-protein interaction analysis in the network of pharmacological targets, the GO and KEGG analysis received attention to demonstrating several pathogenesis pathways related to diabetes and other diseases and other disorders of selected genes. The GO and KEGG methods demonstrate the ability to evaluate traditional treatment methods early and cheaply using folk remedies or Chinese oriental medicine. *P. fruticosa* and its parts have been used for a long time as folk remedies to enhance blood and nourish the body [4, 17]. Still, in a single form, their active ingredients often do not have a clear relationship with pathology. Another specie from Kenya traditional folk medicine, *P. fulva* (Heirn) is is reported for Diabetes Mellitus treatment in the diabetic SWISS mice [3]. Based on similar effect for diabetes of other *Polyscias* species, GO enrichment analysis should be carried out for *P. serrata* to reveal the direct involvement of the compounds' biological activity in improving the pathological state by mimicking specific targets without expensive experiments and ethical issues in mice as a keypoint of this work. GO enrichment analysis of 184 targets was performed using the DAVID database and filtered based on the P value ($P < 0.05$ as cutoff value). There were 399, 66 and 126 GO terms associated with biological process (BP), cellular components (CC) and molecular functions (MF) respectively. The top 10 GOs are shown in Figure S8.

Among the pharmacological targets in Figure S8, the intracellular receptor signaling pathway is the most notable. A number of intracellular pathways are involved in glucose metabolism, including the Janus kinase (JAK) signal transducer and activator of transcription (STAT) and mitogen cascade protein kinase (MAPK), extracellular signal regulation and p38 MAPK pathway can function as a

glucose metabolizer. Complex intracellular pathways are often interconnected in networks with long-distance interactions, often activated when cells respond to extracellular signals, such as kidney cells with high glucose concentrations. Therefore, the connection between compound structures in P. Serrata roots that inhibit intracellular pathways may be one of the promising research directions [17].

In addition, GO calculations suggest that positive gene regulation may serve as a potential therapeutic target through the binding process between insulin and plasma membrane receptors, which may influence a number of biochemical processes in cells. The author [16] mentioned that the variating expression of some genes after insulin manipulation also occurred when comparing the genes phosphoenolpyruvate carboxykinase, protein disulfide isomerase, and glyceraldehyde-3-phosphate dehydrogenase in the 5′-upstream regions. Besides, the author [18] and colleagues demonstrated that insulin treatment affects protein biosynthesis on Liver poly (A) RNA through enhancement of tyrosine aminotransferase activity. Regulation of gene transcription by insulin through receptors can improve the level of mRNA activity on the α2-microglobulin gene [19].

During the treatment of type 2 diabetes, the inflammatory process in some obese patients and the dysfunction of sugar metabolism in the body are thought to be systematically linked through the interaction between fat cells and immune cells [20]. Anti-inflammation can promote insulin resistance, leading to long-term hyperglycemia and other serious complications of diabetes. On the other hand, the TLR4 gene may also be related to type 2 diabetes, expressed as rs11536889 and rs4986790 SNPs in Saudi patients. In addition, the PPI network for the biological processes responding to xenobiotic stimulus and negative regulation of apoptotic processes have also been mentioned as the other results from GO calculation. These inhibitions of specific pharmacological targets over the biological processes above [21, 22], can be considered as the other potential treatment strategies for diabetes-related problems.

The KEGG analysis result of *P. serrata* roots in the treatment of type 2 diabetes was performed and top 25 pathways are shown in Figure S9. KEGG enrichment analysis results revealed that the insulin resistance pathway might play an important role in the pharmacological activities of chemical constituents from *P. serrata* roots against type 2 diabetes. The pathogenesis of insulin resistance is complex and difficult to explain by a single biochemical pathway. In addition, KEGG evaluation results also showed positive signs from the ability to inhibit various pathways related to diabetes, such as the AGE-Rage signaling pathway in diabetic complications, diabetic cardiomyopathy. However, these results were mainly based on prediction and computational study. In-depth studies in animal models would be required for further validation. The results from KEGG enrichment analysis also displayed noticeable pathways for cancer treatments.

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Supporting Information

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