Network Pharmacology Integrated Molecular Docking Based Prediction of Active Compounds and Potential Targets in *Tinospora crispa* Linn. as Insulin Sensitizer

Ummu Mastna Zuhri^{1¶}, Erni Hernawati Purwaningsih^{2¶}, Fadilah^{3,4¶*}, Nancy
Dewi Yuliana^{5¶}

¹ Doctoral Program in Biomedical Science, Faculty of Medicine, Universitas Indonesia,
Jakarta, 10430, Indonesia.

² Department of Medical Pharmacy, Faculty of Medicine, Universitas Indonesia,
Jakarta, 10430, Indonesia.

³ Department of Medical Chemistry, Faculty of Medicine, Universitas Indonesia,
Jakarta, 10430, Indonesia.

⁴ Bioinformatics Core Facilities, IMERI Faculty of Medicine Universitas Indonesia

⁵ Department of Food Science and Technology, Bogor Agricultural University, Bogor
16680, Indonesia

*Corresponding author :

Fadilah Fadilah

Email : fadilah.msi@ui.ac.id

¶ These authors contributed equally to this work.

Network Pharmacology Integrated Molecular Docking Based Prediction of Active Compounds and Potential Targets in *Tinospora crispa* Linn. as Insulin Sensitizer

Abstract

Insulin resistance is a metabolic disorder characterized by the decreased response to insulin in muscle, liver, and adipose cells. The normal insulin levels are unable to control glucose, lipids, and energy homeostasis. This condition remains a complex phenomenon that involves several genetic defects and environmental stresses, such as obesity. A full understanding is required to understand the entire itinerary and functional consequences of the occurrence of insulin resistance to develop a potent drug in diabetes management. In the present study, we investigated the mechanism of known phytochemical constituents of *Tinospora crispa* and its interaction with insulin resistant target proteins by using network pharmacology and molecular docking. The insulin sensitizer activities of *Tinospora crispa* may be associated with the inhibition of the activation of the inflammatory pathway and the activation of insulin signaling. Tinoscorside A, Makisterone C, Borapetoside A and B, and β sitosterol consider the main phytoconstituents of *Tinospora crispa* by its binding with active sites of main protein targets of insulin resistance potential therapy. In conclusion, *Tinospora crispa* was one of the promising therapeutic agent in type 2 diabetes mellitus management. Regulation in glucose homeostasis, adipolysis, cell proliferation, and antiapoptosis may be the critical mechanism of *Tinospora crispa* as an insulin sensitizer.

Keywords: *Tinospora crispa*; type 2 diabetes; network pharmacology; molecular docking; insulin resistance

Introduction

Insulin resistance is a metabolic disorder characterized by the decreased response to insulin in muscle, liver, and adipose cells. The normal insulin levels are unable to control glucose, lipids, and energy homeostasis. This condition remains a complex phenomenon

that involves several genetic defects and environmental stresses, such as obesity. A full understanding is required to understand the entire itinerary and functional consequences of the occurrence of insulin resistance to develop a potent drug in diabetes management¹. Furthermore, drug monotherapy is considered difficult to provide the desired effect in controlling blood glucose levels in type 2 diabetes patients. Combination therapy which has different mechanisms is needed for maintaining the requirement of therapeutic management. However, this strategy meets some disadvantages regarding the increasing drug side effects, toxicity, and interactions between the drugs. Another alternative is the search for a drug molecule that selectively modulates different targets and improving the balance of efficacy and safety compared to single target agents².

This approach is in line with the paradigm that has begun to change in natural product drug discovery, the "herbal shotgun" which utilizes the synergy of multi constituents which have varied targets³. Until now, research on antidiabetic drugs from *Tinospora crispa* (*T. crispa*) are still using the "silver bullet" approach which is oriented towards the single active principle with one target. As the result, the information obtained was not comprehensively explaining the activity of *T. crispa*, yet it closer to the activity of certain metabolites contained in *T. crispa*⁴⁻¹². Research on medicinal plants conducted by Wink *et al* also supports the herbal shotgun approach. It was reported that the combination of metabolite compounds in the medicinal plant had a better effect than the single isolate of metabolite compound¹³. This fact requires more recent research using the herbal shotgun approach so that the activity of medicinal plants can be comprehensively reveal³.

T. crispa as a multi-constituent preparation has not clearly known its action mechanism in insulin sensitization activity. It needs to be explored more extensively using a comprehensive approach such as *in silico* prediction using network pharmacology

analysis. The current development of bioinformatics technology allows researchers to predict the action mechanism of medicinal plant constituents as multicomponent simultaneously as a therapeutic activity.

In our study, the potential effect and mechanism of *T. crispa* as multi-compounds preparation were analyzed using network pharmacology to find out its activity as an insulin sensitizer. The most significant protein targets then verified its binding with *T. crispa* constituents by docking molecular strategy to verify its interactions (Fig 1).

Material and Methods

Constructing database of known *Tinospora crispa* chemical constituents

Data of chemical constituents contained in *T. crispa* was acquired from former researches related to *T. crispa* constituent identification⁴⁻¹². Database constructing of 2D structure was made in sdf format were collected from PubChem, ChemSpider (<http://www.chemspider.com/>), or drawn using MarvinSketch (<https://chemaxon.com/products/marvin>)¹⁴. Each 2D structure then changed to 3D structure using MarvinSketch and saved as pdb format¹⁵. The 2D structure was needed in druggability analysis in each component, besides the 3D structure was needed for molecular docking analysis. Its compound's drugability were predicted by SwissADME database (<http://www.swissadme.ch/>) including its drug likeness based on Lipinsky rules of five and its oral bioavailability prediction¹⁶.

Fig 1. General workflow of present work using network pharmacology and molecular docking analysis.

Constructing a database of protein target involved in insulin resistance

Target proteins related to insulin resistance were retrieved from DisGeNET, UniProt, OMIM, TTD, KEGG, and DrugBank database¹⁷⁻²¹. Data retrieval from DisGeNET were done with keyword "Insulin resistance" (CUI: C0021655) in diseases search engine column. The retrieved data was a summary of gene disease association that have been downloaded in xlsx format (80 genes). Data retrieval from UniProt was done using human disease option in supporting data and input keyword "Diabetes mellitus, non-insulin-dependent" (DI-02060). Data were downloaded as tab file format (23 genes). Data from both data sources were merging in Cytoscape 3.7.2 software (<https://cytoscape.org/>) using UniProt ID as their identity to eliminate double listed proteins^{18,22}.

Constructing Target Proteins of *Tinospora crispa* phytochemical Constituents

The simplified molecular input line entry specification (SMILES) information of 56 TC constituents were submitted in SWISS Target Prediction (<http://www.swisstargetprediction.ch/>) and PharmMapper to obtain its target prediction. Only human (*Homo sapiens*) target protein was set in the data retrieval^{23,24}. Protein ID were aligned using UniProt ID to synchronize protein ID and eliminate double listed protein¹⁸. The aligned target proteins ID were arranged in excel format to be imported to Cytoscape 3.7.2, be visualized, and analyzed its degree of connectivity²².

Prediction of significance target protein related insulin resistant

Protein-protein interaction (PPI) was generated by submitting all UniProt ID of the

proteins which have been constructed from the previous step into STRING database (<https://string-db.org/>) using multiple proteins option²⁵. *Homo sapiens* was set as the only organism and the interaction score confidence was set at the highest confidence (0.900). PPI data then downloaded in tsv format. Furthermore, the PPI network was analyzed using Cytoscape 3.7.2 software to discover the rank of significance protein based on the degree of connectivity score²².

Prediction of *Tinospora crispa* phytoconstituents target genes and their intersection on insulin resistant related target

All the phytochemical *T. crispa* compounds were predicted its target by SwissTargetPrediction and PharmMapper^{23,24}. To comprehensively understand its molecular mechanism, all compound-target data were visualized by Cytoscape 3.7.2 to reveal the interaction network between compound-target. Furthermore, the network then merged with PPI network to discover a new network, compound-target network of *T. crispa* targeted to insulin resistance.

Ligand and protein preparation for in silico molecular docking

Molecular docking was conducted towards the significant proteins in insulin resistance pathogenesis and was targeted by constituents of *T. crispa* at once. Target proteins that meet the criteria were PIK3R1, PTPN1, PPARG, INSR, EGFR, TNF, and AKT2. The 3D structure of *T. crispa* constituents act as ligands (file type .pdb) were opened using AutoDock Tools 1.5.6. software (Molecular Graphics Laboratory, the Scripps Research Institute)¹⁵. Water molecules were removed and chain contained active site was chosen.

The chosen structure then extracted from its native ligand. The 3D crystal structures of target proteins were selected from Protein Data Bank (PDB) (<http://www.rcsb.org/pdb/>) as shown in Fig 3A and Supplementary material 4²⁶. Protein structure with PDB accession number 3S2A were used for PIK3R1 target, 1LQF for PTPN1 target, 6O67 for PPARG target, 5E1S for INSR target, 6D8E for EGFR target, 5MU8 for TNF target, and 5D0E for AKT2 target. All protein models were prepared by the addition of polar hydrogen and the addition of Gasteiger charges. Ligands were prepared with torsion value less than 32. Both ligands and protein models were saved as pdbqt file. Preparation steps were done using AutoDock Tools 1.5.6 software.

Molecular docking

Molecular docking was generated using AutoDock Vina (Molecular Graphics Laboratory, the Scripps Research Institute) for initial screening of all *T. crista* constituents followed by AutoDock Tools 1.5.6 only for the top 3 constituents with the lowest binding energy^{15,27}. The ligands were set as rigid structures with active site was set visually at the center of the cavity docked by native ligand from each protein model. The grid box was adjusted at 40 x 40 x 40 Angstrom in x, y, and z axis respectively. Molecular docking was run in Lamarckian Genetic Algorithm after validation. Redocking of native ligand with its protein in the certain parameters with RMSD value less than 2 Amstrong remains as a valid docking parameter²⁸. The results were shown in value of its lowest binding energy (kcal/mol) calculated by total intermolecular energies including hydrogen bonds energy, Van der Walls energy, desolvation energy, and electrostatic energy. The more negative energy was, the better ligand-protein binding²⁹. The result of ligand-protein complexes were visualized using PyMOL (<https://pymol.org/2/>). The docking results were analyzed in LigPlot+ to evaluate the accurate binding interaction between ligands and proteins³⁰.

Results

Database of *Tinospora crispa* phytoconstituents

A total of 56 compounds were found in literatures related to *T. crispa*. 2D and 3D structures of all compounds were collected from PubChem, ChemSpider, or drawn using Marvin Sketch¹⁴. Its compounds druggability were predicted using SwissADME including its drug likeness based on Lipinsky rules of five and its oral bioavailability prediction (Table 1)¹⁶. The molecular weight range from 135.13 to 714.71 with Adenine as the lightest molecule and Borapetoside H as the heaviest molecule. Predicted gastrointestinal absorption showed 34 of 56 compounds classified as highly absorbed in the GI tract. Drug likeness was assessed through the Lipinski Rules of Five, with 37 compounds meet the 5 criteria required by Lipinski, while 19 other compounds have violations of Lipinski's rules of 1-3 violations.

Table 1. Prediction of *Tinospora crispa* know phytoconstituents and its druggability¹⁶

Molecule	Formula	MW (Dalton)	GI abs	Lipinski
(-)-Secoisolariciresinol	C ₂₀ H ₂₆ O ₆	362.42	High	0
N-Acetylnornuciferine	C ₂₀ H ₂₁ NO ₃	323.39	High	0
N-cis-Feruloyltyramine	C ₁₈ H ₁₉ NO ₄	313.35	High	0
N-Formylanonaine	C ₁₈ H ₁₅ NO ₃	293.32	High	0
N-trans-Feruloyltyramine	C ₁₈ H ₁₉ NO ₄	313.35	High	0
Tembetarine	C ₂₀ H ₂₆ NO ₄	344.42	High	0
Tinoscorside A	C ₃₀ H ₃₇ NO ₁₃	619.61	Low	3
(-)-Litcubinine	C ₁₈ H ₂₀ NO ₄	314.36	High	0

Molecule	Formula	MW (Dalton)	GI abs	Lipinski
Borapetol A	C20H24O7	376.4	High	0
Borapetol B	C21H26O7	390.43	High	0
Borapetoside A	C26H34O12	538.54	Low	2
Borapetoside B	C27H36O12	552.57	Low	2
Borapetoside C	C27H36O11	536.57	Low	2
Borapetoside D	C33H46O16	698.71	Low	3
Borapetoside E	C27H36O11	536.57	Low	2
Borapetoside F	C27H34O11	534.55	Low	2
Cycloeucalenone	C30H48O	424.7	Low	1
Dihydrodiscretamin	C19H18NO4	324.35	High	0
Luteolin 4'-methyl ether 7-glucoside	C22H22O11	462.4	Low	2
Makisterone C	C29H48O7	508.69	Low	2
N-acetylanonaine	C19H17NO3	307.34	High	0
N-acetylanornuciferine	C20H21NO3	323.39	High	0
N-formylanonaine	C18H15NO3	293.32	High	0
N-formylasimilobine 2-O- β -D-glucopyranoside	C24H29NO7	443.49	High	0
N-trans-caffeoyltyramine	C17H17NO4	299.32	High	0
Paprazine	C17H17NO3	283.32	High	0
Rumphioside A	C27H36O13	568.57	Low	2
Rumphioside B	C28H38O13	582.59	Low	2

Molecule	Formula	MW (Dalton)	GI abs	Lipinski
(-)-Litchubinine	C18H29NO4	340.56	High	0
Berberine	C20H29NO4	347.45	High	0
Beta sitosterol	C29H50O	414.71	Low	1
Uridine	C9H12N2O6	244.2	Low	0
Tyramine	C8H11NO	137.18	High	0
Tinocrispol A	C21H24O7	388.41	High	0
Syringin	C17H24O9	372.37	Low	0
Syringaresinol	C22H26O8	418.44	High	0
Stigmasterol	C29H48O	412.69	Low	1
Secoisolariciresinol	C20H26O6	362.42	High	0
Salsolinol	C10H13NO2	179.22	High	0
Palmatine	C21H22NO4	352.4	High	0
Rumphiol E	C20H24O6	360.4	High	0
N-demethyl-N-formyldehydronornuciferine	C19H17NO3	307.34	High	0
Magnoflorine	C20H24NO4	342.41	High	0
Lysicamine	C18H13NO3	291.3	High	0
Luteolin 4'-methyl ether 7-glucoside	C22H22O11	462.4	Low	2
Luteolin 4'-methyl ether 3'-glucoside	C22H22O11	462.4	Low	2
Jatrorrhizine	C20H20NO4	338.38	High	0

Molecule	Formula	MW (Dalton)	GI abs	Lipinski
Higenamine	C16H17NO3	271.31	High	0
Genkwanin	C16H12O5	284.26	High	0
Cycloeucalenol	C30H50O	426.72	Low	1
Columbamine	C20H20NO4	338.38	High	0
Borapetoside H	C33H46O17	714.71	Low	3
Borapetoside G	C26H32O12	536.53	Low	2
Apigenin	C15H10O5	270.24	High	0
Adenosine	C10H13N5O4	267.24	Low	0
Adenine	C5H5N5	135.13	High	0

191

192 From SWISS target prediction and DisGeNET, identified that 56 constituents of *T. crista*
193 were documented to have 5666 protein-specific targets to insulin resistance^{17,23}. Those
194 5666 target proteins then analyzed by Cytoscape 3.7.2 to find out its intersection with
195 insulin resistant target protein²².

196 Protein-protein interaction networks related to insulin 197 resistance

198 267 targets were documented to have relation to insulin resistance pathogenesis.
199 All targets were identified its interaction using STRING database²⁵. Those interactions
200 construct a PPI network which then assessed by Cytoscape 3.7.2 software for the degree
201 of connectivity value. The analysis results were displayed in Fig 2 in white to purple
202 gradation. The higher the degree of a node (gene) in the network is marked with a darker
203 purple color. The higher the degree value indicates that the protein has a greater role in

the pathogenesis of insulin resistance. The top degree ranks were INS, LEP, PIK3R1, PTPN1, IRS1, PPARG, IGF1, INSR, CAV1, EGFR, IRS2, TNF, and AKT2, respectively. The degree rating of the entire PPI network is shown in Fig 2. The detailed degree value was shown in Supplementary material 1.

Fig 2. PPI network of protein related insulin resistance in *Homo sapiens*.

The darker purple color and the bigger its nodes indicate higher degree of protein in the network.

Interaction of lead compounds of *Tinospora crispa* on insulin resistant related target proteins

To proof the underlying interactions between *T. crispa* predicted active phytoconstituents to insulin resistance, we conducted the molecular docking strategy. Those targets were PIK3R, PTPN1, PPARG, INSR, EGFR, TNF, and AKT2. The best docking interaction was PI3K with Tinoscorside A as a ligand (binding energy -11.64) as shown in Table 2. Its binding sketch map was shown in Fig 3 as well. The detail docking scores of *T. crispa* targeted to 7 significance proteins were shown in Supplementary material 3 and the docking sketch maps were shown in Supplementary material 4. The structures of main constituents of *T. crispa* were showed in Fig 4. Seven target proteins that meet the criteria to have a high degree of connectivity value, targeted by *T. crispa* constituents, and were involved in insulin resistance pathogenesis (Fig 5) were used in this docking analysis.

Fig 3. Docking sketch map of Tinoscorside A interaction with the crystal structure of PI3K.

(A) Pocket view of Tinoscorside A binding with PI3K active site. (B) 2D docking pattern of Tinoscorside A with amino acids hydrogen bonds LYS833 and ASP964.

Table 2. Docking scores of the lowest ligand-protein binding energy of *T. crispa* constituents and significant proteins in insulin resistance.

Target protein	Compound	Ki (nM)	The lowest binding energy (kcal/mol)
PI3K	Tinoscorside A	2.94	-11.64
INSR	Tinoscorside A	4.19	-11.43
AKT2	Makisterone C	5.17	-11.3
PPARG	Borapetoside A	51.96	-9.94
EGFR	Tinoscorside A	191.99	-9.16
PTPN1	Beta sitosterol	453.59	-8.65
TNF	Borapetoside B	1.62x10 ³	-7.90

Fig 4. Structure of active constituents prediction of *T. crispa* as insulin sensitizer.
 (A) Tinoscorside A (B) Makisterone C (C) Borapetoside A (D) β Sitosterol (E) Borapetoside B. All structures were drawn using MarvinSketch based on the previous studies⁶.

Fig 5. Systematic prediction of 7 significant protein targets and therapeutic pathways of *T. crispa* constituents on insulin resistant.

The highlighted nodes represent the significant targets of *T. crispa* and other nodes represent their related target on insulin resistant. All the presented pathways were summarized by KEGG pathway database²¹.

Compound-Target Network

To further discover the potential target and therapeutic pathway of *T. crispa* constituents in ameliorating insulin resistance, the targets of insulin resistance were merged with the target of *T. crispa* (Fig 6A). The compound-target network of *T. crispa* against insulin resistance was shown in Fig 6C. After the network merging using Cytoscape 3.7.2, 30 *T. crispa* constituents were reserved as the main active constituents in *T. crispa* treating insulin resistance. In addition, 22 targets were reserved as the main targets of TC in treating insulin resistance. Moreover, 7 targets were revealed to have significant protein in insulin resistance pathogenesis and targeted by *T. crispa* constituents (Fig 6B). The complete *T. crispa* constituents and their targets were shown in Supplementary material 2.

Fig 6. (A) Intersection Venn diagram of *T. crispa* constituents and target protein related insulin resistance. There were 30 constituents of *T. crispa* that were predicted to be targeted to insulin resistance target protein. **(B) Intersection Venn diagram between predicted target protein of *T. crispa* constituents, target proteins of insulin resistance pathogenesis, and high degree of connection value of target proteins of insulin resistance.** There were 7 target proteins that meet the three intersections. In addition there were 22 proteins targeted by *T. crispa* constituents that were shown visually in figure 6C. **(C) Compound-target network of *T. crispa* constituents targeted to insulin resistance pathogenesis.** The blue dots represent *T. crispa* constituents, the yellow dots represent protein targets, and the connecting lines represent compound-target interaction.

Discussion

The present study tried to collect and investigate clues from the known phytoconstituents

of *T. crispa* in ameliorating insulin resistance. Investigations were conducted using *in silico* approaches including network pharmacology and molecular docking. This analysis informed that the vital phytoconstituents of *T. crispa* as insulin sensitizer were Tinoscorside A, Makisterone C, Borapetoside A, Beta sitosterol, and Borapetoside B. In addition, we elucidated the potential therapeutic network of insulin resistance and intersect this network to the *T. crispa* phytoconstituents potential targets network. Both networks were complicated networks with 5666 and 267 nodes. The intersection showed us only the protein related insulin resistance targeted by *T. crispa* constituents. From 267 targets, there were only 22 target proteins that targeted by *T. crispa* constituents. KEGG pathways analysis had indicated several signaling pathways including insulin signaling, PI3K-Akt signaling, MAPK signaling, and TNF signaling pathway that play vital roles in the therapeutic mechanism of *T. crispa* in ameliorating insulin resistance. Those signaling pathways had close relative to glucose lowering activity through the regulation of glucose homeostasis, adipolysis, cell proliferation, and antiapoptosis. This showed that *T. crispa* with its various secondary metabolites had activity as an insulin sensitizer through different pathways. This finding supports Wink *et al* statement that the activity of a medicinal plant was due to synergistic interactions of several phytoconstituents.¹³. The network pharmacology approach provides an insight into prediction in the mode of action of a medicinal plant in a comprehensive manner with multiple target therapies³¹.

Based on former studies, Borapetoside A-C, and E may be the key of *T. crispa* constituents as hypoglycaemic agents^{7,11,12,32,33}. Lam *et al* reported, the isolated Borapetoside A and C had significantly reduced plasma glucose levels but unable to increase insulin secretion in MIN6 pancreatic beta cells³². It indicated that they had other mechanisms of hypoglycemic activity. This study predicts the mechanism of action of

Borapetoside A through activation of PPARG and Borapetoside B through inhibition of TNF cytokines to bind to their receptor.

Contrary to previous studies that indicated diterpenoids as active constituents, this study presents Tinoscorside A (N-formylasimilobine 2-O- β -D-glucopyranosyl-(1 $\rightarrow$ 2)- β -D-glucopyranoside) as a constituent of *T. crispa* which has the strongest binding with some protein targets including PI3K, INSR, and EGFR respectively. Tinoscorsida A was a member of aporphine alkaloid which has never been proven as part of active compounds of *T. crispa* in reducing blood sugar level. This finding indicated that Tinoscorside A could be a potential glucose lowering agent through multiple ways. In addition, this study also predicted that *T. crispa* had anti-inflammatory activity through the TNF signaling pathway by inhibit the binding between TNFA and TNFR.

Conclusion

The present study highlights the insulin sensitizing role of *T. crispa* on insulin resistance pathogenesis by network pharmacology and molecular docking study. The role of *T. crispa* phytoconstituents that served as plant extract with multiple constituents remains still undescribed. Our study provides functional clues to reveal the prediction of their possible targets and mechanism pathways of *T. crispa*. The result was showed that *T. crispa* was a promising plant-based drug to be developed as insulin sensitizer through multiple pathways including glucose homeostasis, adipolysis, cell proliferation, and antiapoptosis. However, there are several limitations in the current study, including the limitation of *T. crispa* phytoconstituents database that could be more phytoconstituents were not reveal yet and the limitation of the database that serves the activity of the constituents as a single compound neglecting the interaction of the multi compound itself. Further study on the insulin sensitizing role of *T. crispa* and the underlying mechanism

are still urgently to be investigated in other methods such as *in vitro*, *in vivo*, and clinical study evaluation to verify this *in silico* study.

Acknowledgments

This study was funded by Universitas Indonesia research grants for doctoral students through PUTI Grant with contract number NKB585/UN2.RST/HKP05.00/2020.

References

1. Pessin JE, Saltiel AR. Signaling pathways in insulin action: Molecular targets of insulin resistance. Vol. 106, Journal of Clinical Investigation. The American Society for Clinical Investigation; 2000. p. 165–9.
2. Artasensi A, Pedretti A, Vistoli G, Fumagalli L. Type 2 diabetes mellitus: A review of multi-target drugs. Molecules. 2020;25(8):1–20.
3. Ulrich-Merzenich G, Zeitler H, Jobst D, Panek D, Vetter H, Wagner H. Application of the “-Omic-” technologies in phytomedicine. Phytomedicine. 2007;14(1):70–82.
4. Fukuda N, Yonemitsu M KT. Isolation and structure elucidation of the five new furanoid diterpene glycosides borapetoside C–G. Liebigs Ann der Chemie. 1993;1993(5):491–5.
5. Lokman EF. The possible mechanisms by which borapetol B stimulates insulin release from rat islets. Int J Med Med Sci. 2014;47(1):1461–8.
6. Ahmad W, Jantan I, Bukhari SNA. *Tinospora crispa* (L.) Hook. f. & Thomson: A review of its ethnobotanical, phytochemical, and pharmacological aspects. Front

- 335 Pharmacol. 2016;7(MAR):1–19.
- 336 7. Ruan CT, Lam SH, Chi TC, Lee SS, Su MJ. Borapetoside C from *Tinospora*
337 *crispa* improves insulin sensitivity in diabetic mice. *Phytomedicine*. 2012;19(8–
338 9):719–24.
- 339 8. Thomas A, Rajesh EK, Kumar DS. The significance of *Tinospora crispa* in
340 treatment of diabetes mellitus. *Phyther Res*. 2016;30(3):357–66.
- 341 9. Cachet X, Langrand J, Riffault-Valois L, Bouzidi C, Colas C, Dugay A, Michel
342 S, Boucaud-Maitre D. Clerodane furanoditerpenoids as the probable cause of
343 toxic hepatitis induced by *Tinospora crispa*. *Sci Rep*. 2018;8(1):1–11.
- 344 10. Fukuda N, Yonemitsu M, Kimura T. Studies on the Constituents of the Stems of
345 *Tinospora tuberculata* BEUMEE. III. New Diterpenoids, Borapetoside B and
346 Borapetol B. *Chem Pharm Bull*. 1986;34(7):2868–72.
- 347 11. Xu Y, Niu Y, Gao Y, Wang F, Qin W, Lu Y, Hu J, Peng L, Liu J, Xiong W.
348 Borapetoside E, a clerodane diterpenoid extracted from *Tinospora crispa*,
349 improves hyperglycemia and hyperlipidemia in high-fat-diet-induced type 2
350 diabetes mice. *J Nat Prod*. 2017;80(8):2319–27.
- 351 12. Ruan CT, Lam SH, Lee SS, Su MJ. Hypoglycemic action of borapetoside A from
352 the plant *Tinospora crispa* in mice. *Phytomedicine*. 2013;20:667–75.
- 353 13. Wink M. Modes of Action of Herbal Medicines and Plant Secondary
354 Metabolites. *Medicines*. 2015;2(3):251–86.
- 355 14. Kim S, Chen J, Cheng T, Gindulyte A, He J, He S, Li Q, Shoemaker BA,
356 Thiessen PA, Yu B, Zaslavsky L, Zhang J, Bolton EE. PubChem 2019 update:

- 357 Improved access to chemical data. *Nucleic Acids Res.* 2019 Jan
358 8;47(D1):D1102–9.
- 359 15. Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, Olson
360 AJ. AutoDock4 and AutoDockTools4: Automated docking with selective
361 receptor flexibility. *J Comput Chem.* 2009 Dec;30(16):2785–91.
- 362 16. Daina A, Michielin O, Zoete V. SwissADME: A free web tool to evaluate
363 pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small
364 molecules. *Sci Rep.* 2017;7:42717.
- 365 17. Piñero J, Ramírez-Anguita JM, Saüch-Pitarch J, Ronzano F, Centeno E, Sanz F,
366 Furlong LI. The DisGeNET knowledge platform for disease genomics: 2019
367 update. *Nucleic Acids Res.* 2019 Nov 4;48(Database issue):D845–55.
- 368 18. UniProt Consortium T. UniProt: the universal protein knowledgebase. *Nucleic*
369 *Acids Res.* 2018 Mar 16;46(5):2699–2699.
- 370 19. Usman MS, Kusuma WA, Afendi FM, Heryanto R. Identification of significant
371 proteins associated with diabetes mellitus using network analysis of protein-
372 protein interactions. *Comput Eng Appl J.* 2019;8(1):41–52.
- 373 20. Li YH, Yu CY, Li XX, Zhang P, Tang J, Yang Q, Fu T, Zhang X, Cui X, Tu G,
374 Zhang Y, Li S, Yang F, Sun Q, Qin C, Zeng X, Chen Z, Chen YZ, Zhu F.
375 Therapeutic target database update 2018: Enriched resource for facilitating
376 bench-to-clinic research of targeted therapeutics. *Nucleic Acids Res.*
377 2017;46(D1):D1121–7.
- 378 21. Kanehisa M, Sato Y, Furumichi M, Morishima K, Tanabe M. New approach for

- 379 understanding genome variations in KEGG. *Nucleic Acids Res.*
380 2019;47(D1):D590–5.
- 381 22. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N,
382 Schwikowski B, Ideker T. Cytoscape: A Software Environment for Integrated
383 Models. *Genome Res.* 2003;13(22):2498–504.
- 384 23. Feller D, Grosdidier A, Wirth M, Daina A, Michielin O, Zoete V. Swiss Target
385 Prediction: A web server for target prediction of bioactive small molecules.
386 *Nucleic Acids Res.* 2014 Jul;42(Web Server issue):W32-8.
- 387 24. Wang X, Shen Y, Wang S, Li S, Zhang W, Liu X, Lai L, Pei J, Li H.
388 PharmMapper 2017 update: A web server for potential drug target identification
389 with a comprehensive target pharmacophore database. *Nucleic Acids Res.* 2017
390 Jul 3;45(W1):W356–60.
- 391 25. Szklarczyk D, Franceschini A, Wyder S, Forslund K, Heller D, Huerta-Cepas J,
392 Simonovic M, Roth A, Santos A, Tsafou KP, Kuhn M, Bork P, Jensen LJ,
393 von Mering C. STRING v10: protein–protein interaction networks, integrated
394 over the tree of life. *Nucleic Acids Res.* 2015;43(Database issue):D447.
- 395 26. Berman HM. The protein data bank. *Nucleic Acids Res.* 2000 Jan 1;28(1):235–
396 42.
- 397 27. Olson, Arthur J. OT. AutoDock Vina: improving the speed and accuracy of
398 docking with a new scoring function, efficient optimization and multithreading.
399 *NIH Pubic Access.* 2011;31(2):455–61.
- 400 28. Hernndez-Santoyo A, Yair A, Altuzar V, Vivanco-Cid H, Mendoza-Barrer C.

Protein-Protein and Protein-Ligand Docking. Protein Eng - Technol Appl.
2013;(May).

29. Fadilah F, Arsianti A, Kusmardi K, Tedjo A. Molecular docking studies of
opened-chain analogues of antimycin A3 as caspases inhibitors of apoptosis in
colorectal cancer. Asian J Pharm Clin Res. 2016;9(3):8–10.

30. Laskowski RA, Swindells MB. LigPlot+: Multiple ligand-protein interaction
diagrams for drug discovery. J Chem Inf Model. 2011 Oct 24;51(10):2778–86.

31. Hopkins AL. Network pharmacology: The next paradigm in drug discovery. Nat
Chem Biol. 2008;4(11):682–90.

32. Lam SH, Ruan CT, Hsieh PH, Su MJ, Lee SS. Hypoglycemic diterpenoids from
Tinospora crispa. J Nat Prod. 2012;75(2):153–9.

33. Lokman EF, Gu HF, Wan Mohamood W, Yusof M, Meng L, Östenson C-G.
Anti-diabetic effect of oral borapetol B, isolated from the plant *Tinospora crispa*,
by stimulating insulin resistance. Diabetologia. 2013;56:1–7.

Supporting Information

S1

Table S1. Degree ranking of insulin resistant related targets analyzed by

Cytoscape 3.7.2

Gene name	Uniprot ID	Degree	Gene name	Uniprot ID	Degree
INS	P01308	23	MC4R	P32245	2
LEP	P41159	13	ACACB	O00763	2
PIK3R1	P27986	12	HSD11B1	P28845	2
PTPN1	P18031	11	TCF7L2	Q9NQB0	2
IRS1	P35568	11	TRIB3	Q96RU7	2
PPARG	P37231	10	LIPC	P11150	2
IGF1	P05019	10	POLD1	P28340	2
INSR	P06213	9	GNAS	Q5JWF2	2
CAV1	Q03135	8	PTRF	Q6NZI2	2
EGFR	P00533	8	SOD2	P04179	2
IRS2	Q9Y4H2	8	PNPLA3	Q9NST1	2
TNF	P01375	7	LIPE	Q05469	2
AKT2	P31751	7	PDX1	P52945	2
ADIPOQ	Q15848	6	TBC1D4	O60343	2
GH1	P01241	5	IGFBP2	P18065	2
PRKAA2	P54646	5	FGF19	O95750	1
SIRT1	Q96EB6	5	NR4A1	P22736	1
FOS	P01100	5	CNR1	P21554	1
PTEN	P60484	5	STS	P08842	1
LEPR	P48357	5	XRCC4	Q13426	1

PRKAA1	Q13131	4		BSCL2	Q96G97	1
RETN	Q9HD89	4		CTF1	Q16619	1
AR	P10275	4		SCD	O00767	1
SLC2A4	P14672	4		HMOX1	P09601	1
NOS3	P29474	4		SREBF2	Q12772	1
SREBF1	P36956	3		INPPL1	O15357	1
PPARA	Q07869	3		NEUROD1	Q13562	1
CD36	P16671	3		WRN	Q14191	1
ADRB2	P07550	3		CPE	P16870	1
GCK	P35557	3		CYP19A1	P11511	1
HSD3B2	P26439	3		GPX3	P22352	1
C3	P01024	2		IGFALS	P35858	1
AGPAT2	O15120	2		ABCC8	Q09428	1
PPP1R3A	Q16821	2		KCNJ11	Q14654	1

424

425

426

427

428

429

430

431

432

433

434

435 **S2**

436 **Table S2. 30 *T. crista* phytoconstituents and its target proteins^{17,18,24}**

No	Compound	Target	UniProt ID
1	(-)-Litchubinine	X-box-binding protein 1	P17861
		Steryl-sulfatase	P08842
		Adrenergic receptor beta	P07550
2	(-)-Secoisolariciresinol	Cannabinoid receptor 1	P21554
		Androgen Receptor	P10275
		Steryl-sulfatase	P08842
		Cytochrome P450 19A1	P11511
3	Berberine	11-beta-hydroxysteroid dehydrogenase 1	P28845
4	Beta sitosterol	Peroxisome proliferator-activated receptor gamma	P37231
		Peroxisome proliferator-activated receptor alpha	Q07869
		Sterol regulatory element-binding protein 2	Q12772
		Androgen Receptor	P10275
		Acetyl-CoA carboxylase 2	O00763
		11-beta-hydroxysteroid dehydrogenase 1	P28845
		Acyl-CoA desaturase	O00767
		Cytochrome P450 19A1	P11511
5	Borapetol A	Insulin receptor	P06213
		Peroxisome proliferator-activated receptor gamma	P37231
		Peroxisome proliferator-activated receptor alpha	Q07869
		Nitric-oxide synthase, endothelial	P29474
		Superoxide dismutase [Mn], mitochondrial	P04179
		Epidermal growth factor receptor erbB1	P00533
		Androgen Receptor	P10275
		Steryl-sulfatase	P08842
		11-beta-hydroxysteroid dehydrogenase 1	P28845
		Caspase-1	P29466
		Serine/threonine-protein kinase AKT2	P31751
		Glucokinase	P35557
6	Borapetol B	Epidermal growth factor receptor erbB1	P00533
7	Borapetoside A	Peroxisome proliferator-activated receptor gamma	P37231
		Epidermal growth factor receptor erbB1	P00533
		Steryl-sulfatase	P08842

No	Compound	Target	UniProt ID
		Caspase-1	P29466
8	Borapetoside B	Cannabinoid receptor 1 (by homology)	P21554
		TNF-alpha	P01376
		Epidermal growth factor receptor erbB1	P00533
		Caspase-1	P29466
9	Borapetoside C	Epidermal growth factor receptor erbB1	P00533
		Steryl-sulfatase	P08842
		Caspase-1	P29466
10	Borapetoside D	Peroxisome proliferator-activated receptor gamma	P37231
		Peroxisome proliferator-activated receptor alpha	Q07869
		Epidermal growth factor receptor erbB1	P00533
		Caspase-1	P29466
11	Borapetoside E	Peroxisome proliferator-activated receptor gamma	P37231
		Epidermal growth factor receptor erbB1	P00533
		Caspase-1	P29466
12	Borapetoside F	Epidermal growth factor receptor erbB1	P00533
		Caspase-1	P29466
13	Cycloeucalenone	Acetyl-CoA carboxylase 4	O00763
		11-beta-hydroxysteroid dehydrogenase 1	P28845
		Cytochrome P450 19A1	P11511
14	Dihydrodiscretamin	PI3-kinase p110-alpha/p85-alpha	P27986
		Insulin receptor	P06213
		Androgen Receptor	P10275
		1-acylglycerol-3-phosphate O-acyltransferase beta	O15120
15	Luteolin 4'-methyl ether 7-glucoside	TNF-alpha	P01379
		Epidermal growth factor receptor erbB1	P00533
		Caspase-1	P29466
16	Makisterone C	Peroxisome proliferator-activated receptor gamma	P37231
		TNF-alpha	P01377
		Epidermal growth factor receptor erbB1	P00533
		Serine/threonine-protein kinase AKT2	P31751
		Androgen Receptor	P10275
		Acetyl-CoA carboxylase 3	O00763
		11-beta-hydroxysteroid dehydrogenase 1	P28845
		Cytochrome P450 19A1	P11511
17	N-acetylanonaine	Epidermal growth factor receptor erbB1	P00533
		Nitric-oxide synthase, endothelial	P29474
		Cytochrome P450 19A1	P11511

No	Compound	Target	UniProt ID
18	N-Acetylnornuciferine	Epidermal growth factor receptor erbB1	P00533
		Cannabinoid receptor 1 (by homology)	P21554
		Androgen Receptor	P10275
		Cytochrome P450 19A1	P11511
19	N-cis-Feruloyltyramine	Insulin receptor	P06213
		TNF-alpha	P01375
		Epidermal growth factor receptor erbB1	P00533
20	N-Formylanonaine	Cannabinoid receptor 1 (by homology)	P21554
		Nitric-oxide synthase, endothelial	P29474
		Epidermal growth factor receptor erbB1	P00533
		Cytochrome P450 19A1	P11511
21	N-formylasimilobine 2-O-Beta-D-glucopyranoside	Peroxisome proliferator-activated receptor gamma	P37231
		Peroxisome proliferator-activated receptor alpha	Q07869
22	N-trans-caffeoyltyramine	Insulin receptor	P06213
		Epidermal growth factor receptor erbB1	P00533
		Androgen Receptor	P10275
		Cytochrome P450 19A1	P11511
23	N-trans-Feruloyltyramine	Insulin receptor	P06213
		TNF-alpha	P01378
		Epidermal growth factor receptor erbB1	P00533
		Serine/threonine-protein kinase AKT2	P31751
24	Paprazine	Insulin receptor	P06213
		Peroxisome proliferator-activated receptor gamma	P37231
		Epidermal growth factor receptor erbB1	P00533
		Androgen Receptor	P10275
25	Rumphioside A	Epidermal growth factor receptor erbB1	P00533
		Caspase-1	P29466
26	Rumphioside B	Epidermal growth factor receptor erbB1	P00533
		Caspase-1	P29466
27	Tembetarine	Androgen Receptor	P10275
		Adrenergic receptor beta	P07550
28	Tinocrispol A	Epidermal growth factor receptor erbB1	P00533
		11-beta-hydroxysteroid dehydrogenase 1	P28845
29	Tinoscorside A	Insulin receptor	P06213
		Epidermal growth factor receptor erbB1	P00533
30	Tyramine	Epidermal growth factor receptor erbB1	P00533
		Androgen Receptor	P10275
		Adrenergic receptor beta	P07550
		Cytochrome P450 19A1	P11511

437 **S3**

438 **Table S3. Molecular docking result of *T. crista* phytoconstituents to 7 selected**

439 **target proteins using AutoDock**

Protein target	TC Compound	Lowest binding energy	Hydrogen bond	Hydrophobic bond
PI3K	Tinoscorside A	-11.64	Asp96, Lys833	Ala 805, Thr 887, Met 804, Pro 810, Asp 950, Ile 953, Ile 879, Tyr 867, Met 953, Ile 831, phe 961, Glu 880, Ile 881, Val 882, Trp 812, Lys 890
PI3K	Borapetoside A	-9.88		Asn 951, Ile 963, Lys 833, Lys 807, Asp 964, Ile 831, Val 882, Tyr 867, Glu 880, Ile 879, Ile 881, Met 804, Ser 806, Pro 810
PI3K	<i>N-formylasimilobine 2-O-Beta-D-glucopyranoside</i>	-9.65	Val 883, Lys 890	Phe 961, Glu 880, Ile 881, Ala 885, Met 953, Ile 879, Tyr 867, Ile 831, Ile 963, Asn 951, Asp 950, Thr 887, Trp 812
PTPN1	Beta sitosterol	-8.65		Gly 720, Asp 691, Arg 721, Cys 715, Ala 717, Tyr 546, Phe 482, Asp 548, Ser 716, Gln 762, Ile 719, Gly 759, Val 549.
PTPN1	Jatrorrhizine	-7.79	Arg 721	Asp 681, Lys 620, Tyr 546, Ser 716, Asp 548, Val 549, Ile 719, Gln 762, Ala 717, Phe 682.
PTPN1	Magnoflorine	-7.71	Gly 720, Cys 715, Ala 717, Ser 716, Tyr 546, Lys 620.	Arg 721, Ile 719, Phe 682, Asp 548, Gln 762, Asp 681.
PPARG	Borapetoside A	-9.94		Arg 280, Ile 281, Met 348, Cys 285, Leu 353, Leu 330, Ser 342, Leu 333, Ile 341, Glu 343, Leu 340, Gly 284, Arg 288,
PPARG	Stigmasterol	-9.71	Arg 280	Leu 255, Leu 330, Leu 333, Ile 341, Val 339, Met 348, Leu 340, Ser 342, Arg 288, Cys 285, Gly 284, Glu 343, Phe 287, Ile 281.
PPARG	Beta sitosterol	-9.65		Lys 263, Ser 342, Phe 287, Gly 284, Arg 288, Cys 285, Ile 281, Leu 353, Met 364, Met 348, Ile 341, Glu 259, Ile 262, Gly 258.
INSR	Tinoscorside A	-11.43	Asp 1083, Ser 1086, Met 1079.	His 1081, Gly 1003, Ala 1028, Leu 1078, Val 1010, Leu 1002, Met 1139, Glu 1012, Arg 1000, Arg 1026, Gly 1082, Ala 1080.

INSR	N-trans-Feruloyltyramine	-8.85	Ser 1086, Asp 1083, Met 1079.	Ser 1090, His 1081, Leu 1002, Met 1139, Leu 1078, Gly 1082, Tyr 1087.
INSR	N-cis-feruloyltyramine	-8.52	Met 1079, Asp 1083.	Leu 1078, Ala 1080, Arg 1000, Leu 1002, Ser 1086, Tyr 1087, Ser 1090, His 1081, Met 1139, Gly 1082.
EGFR	Tinoscorside A	-9.65	Asp 855, Thr 845, Cys 797.	Leu 792, Pro 794, Leu 844, Met 793, Ala 743, Thr 790, Met 766, Lys 745, Val 726, Asp 800, Leu 718, Gly 796.
EGFR	Borapetoside B	-9.48		Pro 794, Leu 718, Met 793, Leu 844, Thr 790, Ala 743, Thr 854, Asp 855, Asn 842, Arg 841, Cys 797, Leu 792, Gly 796.
EGFR	Borapetosida A	-9.21	Thr 854	Leu 718, Leu 844, Val 726, Met 793, Ala 743, Thr 790, Leu 792, Ile 789, Leu 777, Leu 788, Met 766, Lys 745, Lys 755, Gly 796.
TNF	Borapetoside B	-7.9		Ile 155, Leu 57, Gly 121, Leu 120, Tyr 119, Tyr 151, Tyr 59.
TNF	Cycloeucalenol	-6.95	Gln 149	Tyr 119, Gln 61, Leu 120, Gly 122, Gly 121, Val 123, Leu 57, Tyr 59, Tyr 151.
TNF	Borapetoside H	-5.84		Tyr 151, Tyr 59, Tyr 119, Leu 120, Gly 121, Ser 60.
AKT2	Makisterone C	-11.3	Tyr 327, Cys 311, Lys 298, Arg 274, His 196.	-
AKT2	Higenamine	-8.63	Tyr 327, Cys 311, Lys 298, Arg 274, His 196.	-
AKT2	Borapetol A	-8.17	Tyr 327, Cys 311, Lys 298, Arg 274, His 196.	-

440

441

442

443

444

445

446 **S4**

447 **Fig S4. Docking sketch map of best ligand-binding position of T.crispa**

448 **phytoconstituents and 7 selected target proteins in insulin resistance.**

Fig 1. General workflow of present work using network pharmacology and molecular docking analysis.

Fig 4. Structure of active constituents prediction of *T. crista* as insulin sensitizer.
 (A) Tinoscorside A (B) Makisterone C (C) Borapetoside A (D) β Sitosterol (E) Borapetoside B.
 All structures were drawn using MarvinSketch based on the previous studies⁶.

Fig 5. Systematic prediction of 7 significant protein targets and therapeutic pathways of *T. crispa* constituents on insulin resistant.

The highlighted nodes represent the significant targets of *T. crispa* and other nodes represent their related target on insulin resistant. All the presented pathways were summarized by KEGG pathway database²¹.

Fig 6. (A) Intersection Venn diagram of *T. crispata* constituents and target protein related insulin resistance. There were 30 constituents of *T. crispata* that were predicted to be targeted to insulin resistance target protein. (B) Intersection Venn diagram between predicted target protein of *T. crispata* constituents, target proteins of insulin resistance pathogenesis, and high degree of connection value of target proteins of insulin resistance. There were 7 target proteins that meet the three intersections. In addition there were 22 proteins targeted by *T. crispata* constituents that were shown visually in figure 5C. (C) Compound-target network of *T. crispata* constituents targeted to insulin resistance pathogenesis. The blue dots represent *T. crispata* constituents, the yellow dots represent protein targets, and the connecting lines represent compound-target interaction.

Fig1

Fig2

Fig3A

PI3K Tinoscorside A

Fig3B

Fig4

Fig5

Fig6A

Fig6B

PI3K Tinoscorside A

bioRxiv preprint doi: <https://doi.org/10.1101/2021.05.05.442728>; this version posted May 5, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

INSR Tinoscorside A

PPARG Borapetoside_A

bioRxiv preprint doi: <https://doi.org/10.1101/2021.05.05.442728>; this version posted May 5, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder, who has granted bioRxiv a license to display the preprint in perpetuity. It is made available under aCC-BY 4.0 International license.

PTPN1_beta_sitosterol

TNF Borapetosida_B