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Triterpene saponins from *Barringtonia acutangula* (L.) Gaertn as a potent inhibitor of 11β-HSD1 for type 2 diabetes mellitus, obesity, and metabolic syndrome

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Abstract

Background: *Barringtonia acutangula* (L.) Gaertn, *Garcinia indica* (Thouars) Choisy, *and Feronia limonia* (L.) Swingle is widely utilized in traditional folk medicine against diabetes, obesity, and metabolic syndrome but lacks the evidence of compound-protein interaction for the treatment.

Methods: Phytocompounds were retrieved from herbs databases and public repositories. Probable protein targets were predicted using BindingDB ($p \geq 0.7$). The pathways modulated by compounds were analyzed using the STRING and KEGG pathways. The compound-protein-pathway network was constructed using Cytoscape v3.6.1. Druglikeness was predicted by Molsoft. Docking was performed by AutoDock vina by PyRx 0.8v.

Results: Among three plants, eleven triterpene saponins from *B. acutangula* showed druggable characteristics and identified to inhibit the 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1/HSD11B1) as a key protein target and also inhibit/modulate other 27 protein molecules involved in the 3 major pathways i.e. Metabolic syndrome, cGMP-PKG signaling, and insulin resistance pathways and also these compounds showed interactions with the active site amino acid residues of 11β-HSD1. Among eleven compounds Barringtogenol B scored the highest binding affinity by forming a hydrogen bond with Ile218 active site residue of 11β-HSD1.

Conclusion: Triterpene saponins contained in *B. acutangula* bark and seed inhibits 11B-HSD1 and this multi-compound contained enriched fraction could be the potent treatment regimen for T2DM, obesity, and MetS.

Keywords: 11-β hydroxysteroid dehydrogenase type 1, *Barringtonia acutangula* (L.) Gaertn, Diabetes mellitus, Docking, Metabolic syndrome, Network pharmacology, Obesity

Introduction

Metabolic syndrome (MetS) is a group of metabolic abnormalities involves both genetic and acquired factors and gained considerable attention worldwide due to exponentially increased risk of cardiovascular disease, hypertension, obesity and type 2 diabetes mellitus (T2DM) that includes dyslipidemia, insulin resistance (IR), hypertension and visceral obesity [1, 2]. Currently, conventional drugs such as insulin sensitizers, PPAR-γ agonists, statins, etc. are utilized for the management of T2DM, obesity, and MetS. However, these agents target a single protein molecule that could regulate the mechanism of other protein, alters the homeostatic proteins/pathways, and causes numerous side effects such as ketoacidosis, pancreatitis, genital mycosis, neuropathy risk, nausea, and vomiting and have various limitations [3, 4]. Limiting the use of single drug molecules at high doses and utilizing the multiple drug candidates belongs

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to the same drug class targeting multiple proteins at a low dose could be the potential treatment strategy for complex diseases like T2DM, obesity, and MetS [5].

Herbal plants play a key role in the treatment of complex diseases in humans/animals due to their complex mixture of secondary metabolites on the modulation of corresponding molecular protein targets [6]. Currently, the network pharmacology concept of multi-drug, multi-target, and multi-pathway interactions opened up new systematic insights into the holistic understanding of the effects of herbal compounds at a molecular level and can open up opportunities for the identification potent drug candidates against complex diseases like T2DM, obesity, and MetS [4, 7, 8].

Barringtonia acutangula (L.) Gaertn, *Garcinia indica* (Thouars) Choisy, *and Feronia limonia* (L.) Swingle are traditionally utilized herbs for the management of diabetes, obesity, hypertension, and cardiovascular diseases and used in various polyherbal formulations. Researchers demonstrated their anti-obesity, anti-diabetic potency, the anti-hypertensive effect under various animal models [9–13]. We aimed the current study to identify the specific group of phytocompounds from these plants responsible for the regulation of protein targets and modulation of disease-associated pathways in the management of T2DM, obesity, and MetS. Interestingly, we identified the triterpene saponins group contained in aqueous extract of *B. acutangula* bark, seed, fruit, and leaves [14–16] to inhibit 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1/HSD11B1) as a key target. Several studies have been shown its pathogenic role and as a therapeutic target in the treatment of T2DM, obesity, and MetS associated hypertension, cardiovascular disease [17–19].

Materials and methods

Mining of phytocompounds and target identification

Phytocompounds were retrieved from Dr. Duke's DB (https://phytochem.nal.usda.gov), Phytochemical Interaction DB (https://www.genome.jp/db/pcidb/), ChEBI (https://www.ebi.ac.uk/chebi/) and public repositories (Supplementary Table 1). Probable targets of each compound were predicted by BindingDB at a probable score of ≥0.7 with corresponding to the standard small molecules targeting the specific protein of interest [20]. Gene ID of each protein was retrieved from UniProt. Further, the phytocompounds targeting therapeutic targets involved in the pathogenesis of DM and obesity were retrieved from the Therapeutic Target Database (http://db.idrblab.net/ttd/). The probable targets modulated by the phytocompounds were shown in Supplementary Table 2.

Gene ontology, enrichment analysis, and network construction

Search Tool for the Retrieval of Interacting Genes/Proteins v11.0 (STRING; https://string–db.org) and its annotated tool KEGG pathway (https://www.genome.jp/kegg/pathway.html) was utilized to identify the phytocompounds modulating enriched pathways associated with the pathogenesis of DM and obesity. Further, the compound-protein-pathway network was constructed using Cytoscape v3.6.1 software. During the network construction, the edge count parameter was applied to identify the potentially modulated target by the phytocompounds. A degree sorted circular layout was applied to design the network.

Retrieval and preparation of ligand and protein

The phytocompounds were retrieved from the PubChem in 2D and 3D .sdf format. To eliminate the clashes within ligand atoms and to produce the reasonable staring pose, the mmff94 force field was applied for each compound for energy minimization using MarvinSketch. Further, pose having the lowest energy conformation was chosen and saved in protein data bank format (.pdb). The .pdb molecule then converted into AutoDock molecule (.pdbqt). The three dimensional (3D) x-ray crystallographic structure of 11β -HSD1 (PDB ID: 1XU7 and 1.8 A^0) was retrieved from the RCSB PDB [21]. The backbone dihedral angles ϕ and ψ were analyzed by Ramachandran plot obtained through PROCHECK (https://servicesn.mbi.ucla.edu/PROCHECK/) and ERRAT server was used to check the overall protein quality (https://servicesn.mbi.ucla.edu/ERRAT/).

Ligand- protein docking

The binding affinity of the compound with protein target was checked by AutoDock vina by PyRx 0.8v. The grid box for ligand and protein was set to maximum and other parameters were kept default. Discovery Studio Visualizer 2019v was used to visualize the non-covalent interactions of the ligand with active amino acid residues of the protein target.

Results and discussion

Twenty-nine isolated compounds from three plant viz., *B. acutangula* (12), *G. indica* (9), and *F. limonia* (8) were retrieved from the herbs databases and public repositories. Twelve compounds from *B. acutangula* bark, seed, and fruit and leaves were identified as triterpene saponins and predicted to modulate 28 protein targets involved in the T2DM, obesity, and MetS i.e. HSD11B1, HSD11B2, HMGCR, AKR1B10, AR, ALOX15, CYP19A1, CYP17A1, LSS, NR1H3, PTPN1, PTGS1, PTAFR, PRKCA, PRKCE, F2RL1, ATP5B, HSP90AA1, ATP2A2, SQLE, FDFT1, XDH, ATP1A1, ATP1A2, PPP2R5D, PPP2CA, PPP1CC, STAT3. Whereas, among 9 compounds from *G. indica,* 3 compounds were predicted to modulate 16 protein targets i.e. AKR1B1, ADRA2A, CA2, PDE5A, CHRM5, CYP3A4, IL2RA, ALPI, AMY2A,

PTPN1, RPS6KA3, TNF, ALOX12, PTGS1, KDR, and MAOA. Among 8 compounds from *F. limonia*, 6 compounds predicted to modulate 14 protein targets i.e. CA2, CBR1, CYP19A1, CYP2A6, CYP2C9, GPR35, HSD17B3, HSP90AA1, MAOB, PDE5A, PGR, PTGS1, PTPN1, and TYR associated with T2DM, obesity, and MetS (Supplementary Table 3). Further, the probable protein targets were queried into STRING to understand the protein-protein interaction network and to obtain disease pathways modulated by the phytocompounds. The result revealed that 79 pathways were highly enriched within the network. The peer interpretation of the pathways revealed that among 79 pathways, 10 pathways were associated with T2DM, obesity, and MetS. Among 10 pathways, metabolic pathway followed by insulin resistance and cGMP-PKG signaling pathway were identified as highly enriched pathways (Supplementary Table 4). Further, we constructed the network interaction between compounds with their probable targets and enriched pathways. Among all the compounds, a group of triterpene saponins from *B. acutangula* were highly enriched to interact with 11β -HSD1 (Fig. 1). The enzyme 11β-HSD1 is an ER-localized membrane protein catalyzes the interconversion of cortisone and cortisol. Excessive production of cortisol in adipose tissue by 11β -HSD1 progresses the pathogenesis of T2DM and obesity [21]. Based on the mode of action, 83 inhibitors and 2 modulators of 11β-HSD1 were developed and none of them progressed beyond Phase III [22]. Numerous researchers suggested a group of natural compounds could be suitable inhibitors against 11β-HSD1 for satisfactory pharmacological treatment and also utilization of triterpenes as a potent inhibitor of 11β-HSD1 [18, 23–25].

In obese and diabetic rodents and humans, there is an increased expression of the 11β-HSD1 in adipose tissue. Increased expression of 11β-HSD1 results in MetS with visceral obesity, dyslipidemia, T2DM, and hypertension [26]. The in vitro and in vivo studies reflects the potency of *B. acutangula* leaves, bark, seed, and fruits to play an important role in the treatment of DM, obesity, and MetS [27–30]. Babre et al. reported, due to the presence of saponins in the hydroalcoholic extract of *B. acutangula* root delayed the intestinal absorption of dietary fat via pancreatic lipase activity inhibition [27]. Further, 500 mg/kg dose significantly reduced blood glucose levels from day 7 in STZ-induced diabetic rats [28]. Aqueous extract of *B. acutangula* fruit showed hypoglycaemic activity in the STZ-induced hyperglycaemic rat [29]. Grogery et al. study results reflected *B. acutangula* leaf aqueous and ethanolic extracts (500 mg/kg) and glibenclamide (10 mg/kg) to exhibit equal anti-diabetic efficacy in STZ-induced diabetic rats [30]. This suggests triterpene saponins from *B. acutangula* interact with 28 protein molecules and 11β-HSD1 as a potential protein target and modulate the pathways associated with T2DM, obesity, and MetS.

The affinity and hydrogen bond interactions of eleven triterpenes from *B. acutangula* bark and seed with 11B-HSD1 active amino acid residue were analyzed by docking study. The active site residues were identified using the PDB ID 1XU7 and the protein quality was found to be 92.7%. The characteristics of 11β-HSD1 are shown in

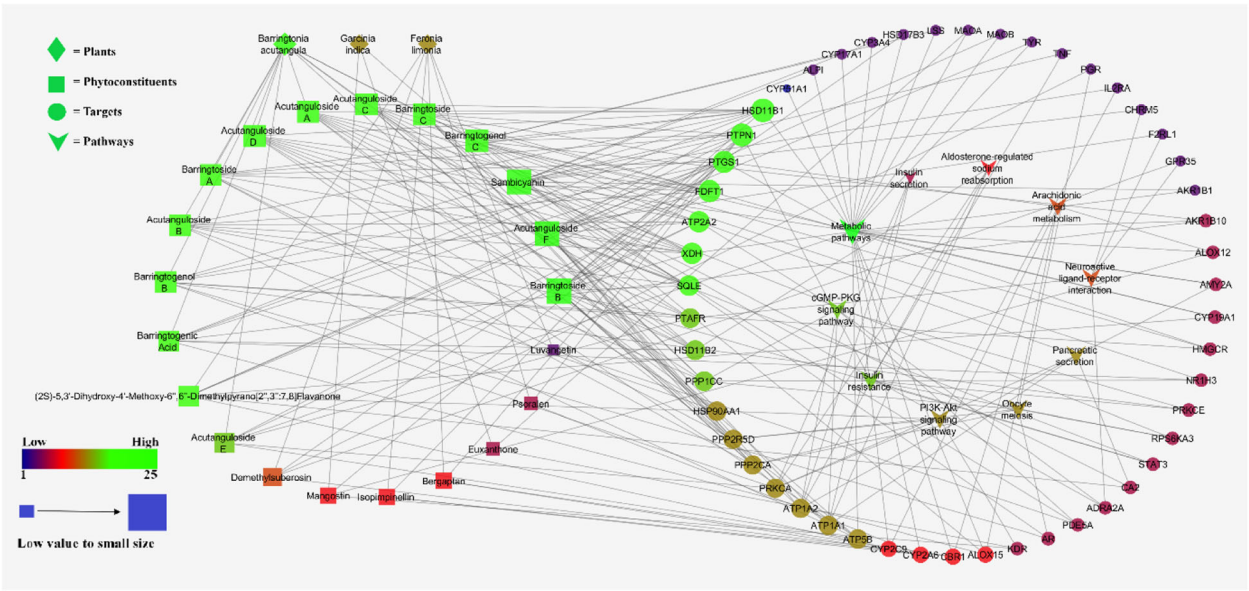

Fig. 1 Network representation of compound-target-pathway interaction

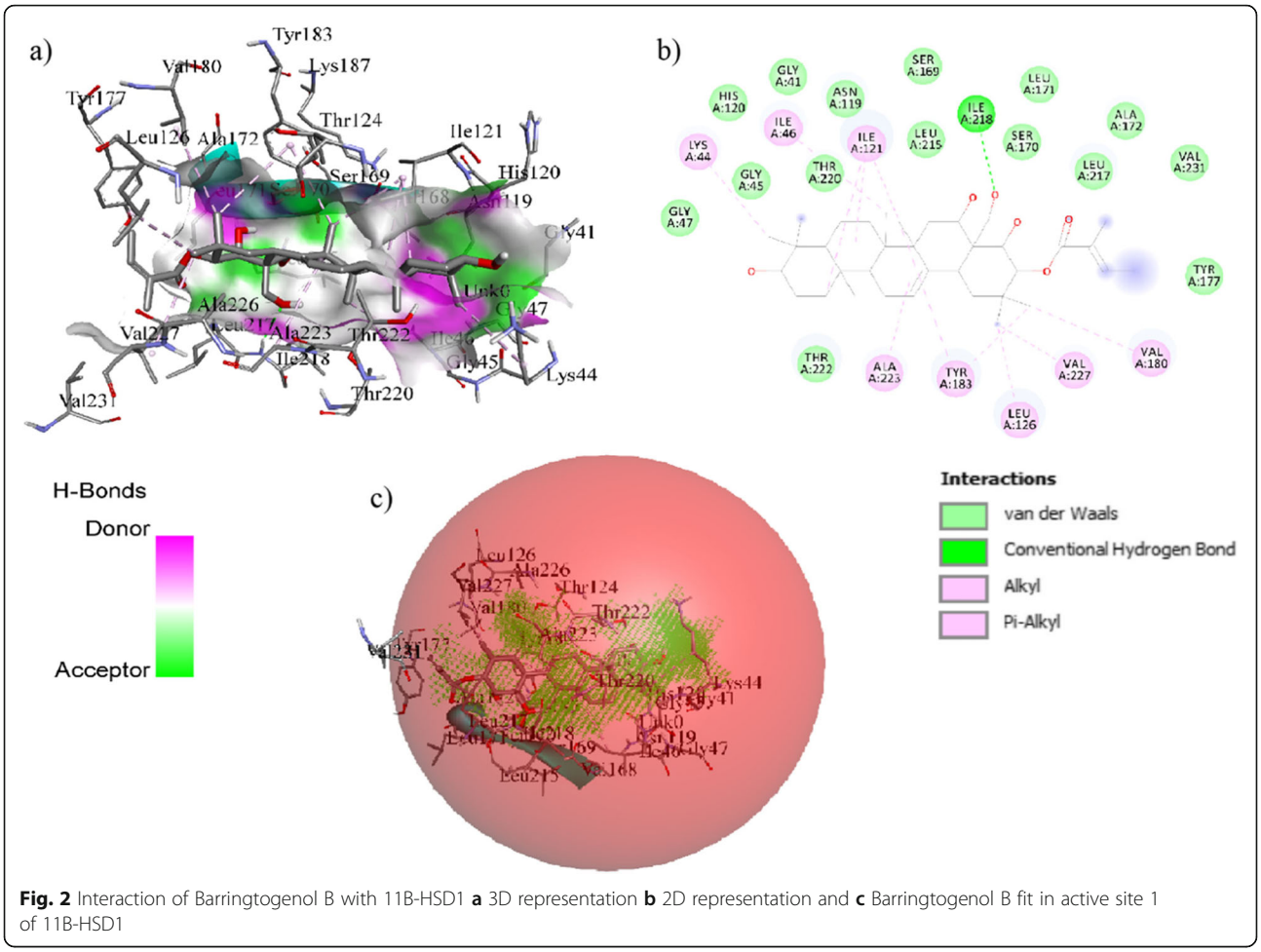

Fig. 2 Interaction of Barringtogenol B with 11B-HSD1 **a** 3D representation **b** 2D representation and **c** Barringtogenol B fit in active site 1 of 11B-HSD1

Supplementary Table 5. All eleven compounds from *B. acutangula* showed druggable characteristics (Supplementary Table 6), among them, Barringtogenol B scored the highest binding affinity (– 11.3 kcal/mol) by forming one hydrogen bond i.e. Ile218 and eight Alkyl, Pi-alkyl interaction i.e. Lys44, Ile46, Ile121, Val180, Val227, Leu126, Tyr183, and Ala223 with the active site residue of 11β-HSD1 (Fig. 2). Interestingly, all the compounds showed hydrogen bond, Alkyl, Pi-alkyl, Pi-sigma interaction with active site residue which suggests having the best fit with the target (Supplementary Table 7). It is important to note that, the triterpenes from *B. acutangula* not only predicted to interact with the 11β-HSD1 but also with other 27 protein targets involved in the T2DM, obesity, and Mets.

Conclusion

In conclusion, eleven triterpene saponins contained in aqueous extract of *B. acutangula* bark, seed, fruit, and leaves were identified to have druggable characteristics and these compounds targeted 28 protein molecules and 11β-HSD1 as a potential therapeutic target and identified to interact with the active site amino acid residues. The compound-gene set enrichment, network pharmacology, and docking analysis identified triterpene saponins from *B. acutangula* modulated 10 enriched disease pathways of T2DM, obesity, and MetS which suggest a potent therapy. However, the current study findings are based on the prediction using experimental based available database and computer simulations, so that subsequent wet-lab studies can be designed accordingly to test/verify 11β-HSD1 inhibitory activity using enriched fraction or isolated compound.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s40816-020-00210-y.

Additional file 1.

Abbreviations

DM: Diabetes Mellitus; T2DM: Type 2 Diabetes Mellitus; MetS: Metabolic Syndrome; 11β-HSD1 or HSD11B1: 11β-hydroxysteroid dehydrogenase type 1; BindingDB: Binding database; STRING: Search Tool for the Retrieval of

Interacting Genes/Proteins; KEGG1: Kyoto Encyclopedia of Genes and Genomes; PDB: Protein Data Bank; BE: Binding Energy

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Not applicable.

Declarations
VSP and NAK have approved this manuscript. The content of this manuscript or any portion thereof has not been published or submitted for publication elsewhere.

Authors' contributions
VSP and NAK equally participated in the study design, literature search, data analysis, preparing, and revision of the manuscript. The authors read and approved the final draft of the manuscript.

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Availability of data and materials
All data generated or analyzed during this study are included in this published article [and its supplementary information files].

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Not applicable.

Consent for publication
Not applicable.

Competing interests
All authors declared that they have no competing interests.

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