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Unveiling the molecular mechanisms and clinical implications of maslinic acid in diabetes mellitus: Insights from network pharmacology

Sarvesh Sabarathinam [a,1,*], Sanjana Satheesh [b,1]

[a] Center for Global Health Research (CGHR), Saveetha Medical College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai, 602105, Tamil Nadu, India
[b] Department of Biotechnology, Birla Institute of Technology and Science, Dubai Campus, Dubai International Academic City, Dubai, P.O. Box 345055, United Arab Emirates

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ABSTRACT

Maslinic acid(MA), a natural pentacyclic triterpene, has potent anti-tumor activity and exerts effects by various mechanisms, including apoptosis, cell cycle arrest, autophagy regulation, and angiogenesis alteration. We investigated the Network pharmacology and Molecular docking analysis of Maslinic Acid The network pharmacology report shows that 23 overlapping targets were identified with Maslinic Acid. Followed by the binding scores were found to be similar to the reference standard Rosiglitazone & Pioglitazone. Maslinic Acid exerts its effect on insulin resistance via inhibition of peroxisome proliferator-activated receptor, α-amylase, and α-glucosidase inhibition, glycogen phosphorylase inhibition, reduction in ghrelin concentration, downregulation of SGLT1 and GLUT2 genes, NF-κB suppression, Nrf2 activation, and AMPK/SIRT 1 pathway activation. The Network analysis and docking score confirm the diabetic activity of Maslinic Acid. This study aims to study various targets of Maslinic Acid in correlation to Diabetes mellitus and analyze their mechanism in detail. Our investigation of MA as a potential treatment target for insulin resistance or diabetes mellitus using network pharmacology revealed that it has significant roles in producing glucose-lowering activity by regulating glucose homeostasis via several insulin signaling pathways discussed above.

1. Introduction

Bringing a novel pharmacologically active compound into the market in a cost-effective manner is one of the challenging responsibilities for many researchers. Growth in computational biology and bioinformatics has increased the influence of pharmaceuticals at the early stages of drug development. Approaches based on network biology and polypharmacology have recently gained popularity and are being used extensively. An overabundance of repeated drugs on the same targeted proteins results in higher chances of drug resistance. Systems biology progress has made it clear that targeting individual proteins is ineffective for treating complicated disorders (Ashok Vardhan et al., 2018; Sharma et al., 2023). This forced drug researchers to accept polypharmacology, which they had previously viewed as an undesirable trait that needed to be eliminated or diminished to create pure medications that act on single targets. According to network biology, changing phenotypes requires simultaneous manipulation of several targets. Developing tools to support polypharmacology can increase effectiveness and forecast unintended side effects. Network pharmacology (NP), a combined approach, focuses on the multi-targeted profiles of all populations by examining how drugs affect both the interactome and the diseasome levels. It exploits computational power to technically categorize the molecular interactions of a drug molecule in a living cell (Kim et al., 2024).

Since a single plant contains numerous bioactive compounds, the traditional medicine system, and its mediated syndromes are directly related to the molecular network in human physiology. Unaware of the single plant's medicinal properties leads to synergistic and antagonist effects. NP is considered a new-generation research model since it plays a vital role in detecting multiple pharmacological effects of a drug on the targeted genes/phenotype. NP emerged as a crucial tool for comprehending the intricate connections between plant remedies in managing

* Corresponding author. Center for Global Health Research, Saveetha Medical College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai, 602105, Tamil Nadu, India.
E-mail address: sarveshtvg@gmail.com (S. Sabarathinam).
[1] Authors contributed to the manuscript equally.

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chronic disorders and their impact on the whole body. The Pathways of NP work through various informatics tools like Cheminformatics, ADMET parameters estimation, Molecular docking, chemical libraries, compound targets, compound networks, KEGG pathway, disease-gene interaction, Ontology mapping, and validation. The applications of NP include estimating novel targets, scientific evidence for the use of traditional medicines, developing new leads, and reducing cost and time through in silico evaluations (Hopkins, 2008; Noor et al., 2022; Paul et al., 2021; Zhang et al., 2019). Hence the present study objectives are to evaluate the potential of Maslinic Acid (MA) as a therapeutic agent for diabetes mellitus and to elucidate the molecular mechanisms through which MA exerts its anti-diabetic effects. Fig. 1: Overview & Application of NP (Samy et al., 2024; Ramya et al., 2024; Rithanyaa and Radhakrishnan, 2024).

2. Overview of maslinic acid (MA)

Maslinic Acid is a naturally occurring pentacyclic triterpene found in various plants, including edible fruits and vegetables, medicinal herbs, and the skin of olives in particular. Maslinic Acid makes up 0.8% of table olives' weight when extracted from solid leftovers. On the other hand, its concentration in olive oil ranges from 38 mg/kg in extra virgin olive oil to 721 mg/kg in crude pomace olive oil (Lozano-Mena et al., 2012). MA (2-α,3-β-dihydroxyolean-12-en-28-oic Acid), also referred to as crategolic Acid, pentacyclic triterpene acid derived from Olea europaea L. (Olive tree) and Crataegus pinnatifida Bunge (shanzha) is known for its broad pharmacological activity. Pentacyclic triterpenes are an essential class of secondary plant metabolites and play a significant role in exerting potent anti-tumor activity with a lesser chance of toxicity (Yu et al., 2021). MA exhibits its anti-neoplastic activity by inducing cell apoptosis and is involved in cell-cycle arrest, regulation of autophagy, and also alters the process of angiogenesis. MA activates both extrinsic and intrinsic apoptotic pathways via the activation of capase-8 and capase-3, which reduces Bcl-2 expression and up-regulates Bid cleavage. MA also prevents cell proliferation and growth in cancer by declining the activity of the IL-6/JAK/STAT3 signaling cascade, where IL-6 regulates immune and inflammatory responses, which leads to tumor development and metastasis. Oxidative stress, heme-oxygenase-1 (HO-1), contributes to most inflammatory diseases. Pre-existing studies suggest that MA induces or enhances the activity of HO-1, which alters the synthesis of tumor necrosi56s factor-α (TNF-α), INTERLEUKIN-1, and INTERLEUKIN-6. Regulation of hO-1 by MA is made by nuclear factor erythrocyte 2-related factor 2 (Nrf2), which promotes the antioxidant enzyme genes. The pathway of Nrf2-Antioxidant Response Elements is crucial for managing inflammatory diseases. MA also inhibits LPS-induced COX2, and PGE2 regulates iNOS/NO levels and NF-κB and the production of pro-inflammatory mediators. This shows that MA can be a potential target for airway inflammatory conditions (Lee et al., 2020). MA improves cognitive function in animal models by activating brain-derived neurotrophic factor (BDNF) signaling, giving a cholinergic blockade effect and enhancing LTP formation in the hippocampus. And also, the activation of ERK-CREB or PI3K-Akt pathways significantly improves cognitive deficits, suggesting that MA can be used in treating cholinergic-induced cognitive dysfunction like dementia, etc.(Bae et al., 2020; Li et al., 2021),.

3. Pharmacokinetic profile of MA

Maslinic Acid is a pentacyclic triterpenoid that is olean-12-ene substituted by hydroxy groups at positions 2 and 3 and a carboxy group at position 28 (the 2alpha,3beta stereoisomer). The basic triterpenoid skeleton of MA acts as anti-inflammatory activity. MA is a pentacyclic-triterinoid whose molecular weight is 472.2 g/mol ($C_{30}H_{48}O_4$), LogP is 6.2044, 01-rotatable bonds, 03 acceptors, 03 donors, and 206.148 total polar surface area. The Lipinski rule profile of MA is accepted; there is no violation. The synthetic accessibility score is 4.785(green alert). It confirms the ease of synthesis of molecules based on the combination of

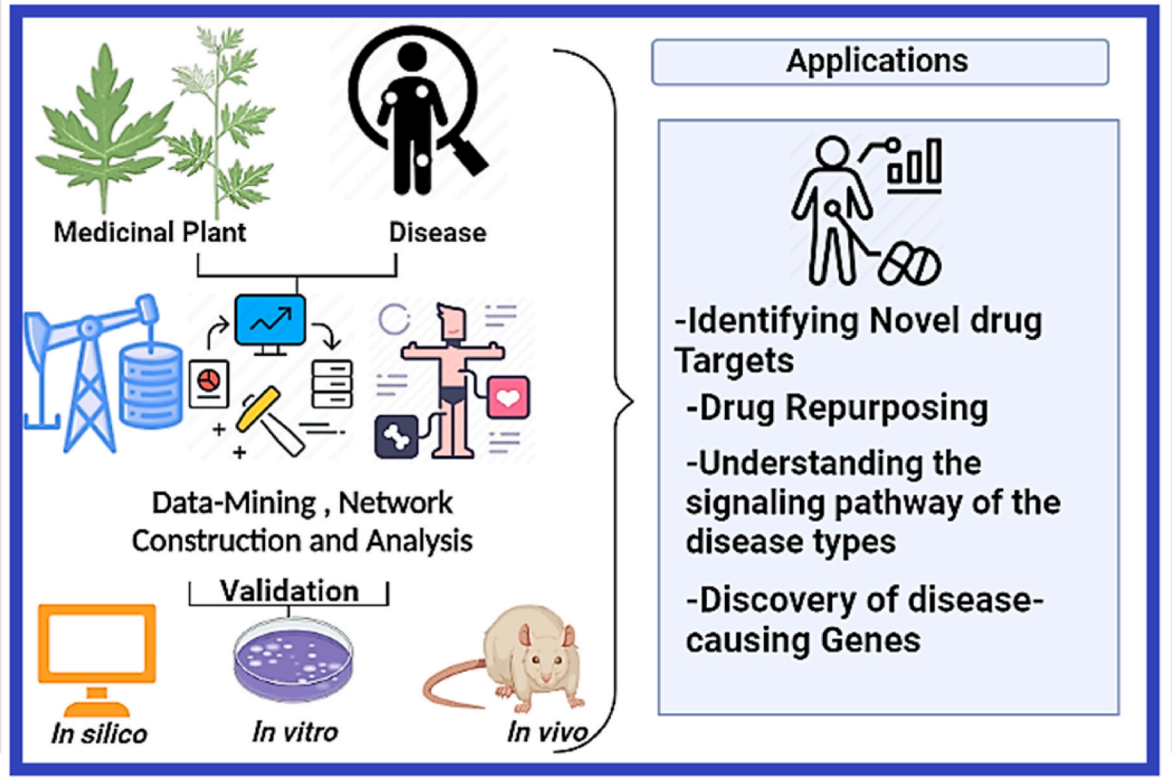

Fig. 1. Overview and applications of Network Pharmacology.

fragment contributions and a complexity penalty. It fails if the score is six or above. The drug-likeness value of MA is 0.397(Red). While coming to bioactivity scores, G-Protein coupled receptor 0.24, Ion channel modulator-0.15, Kinase inhibitor-0.41, Nuclear receptor ligand 0.81, Protease inhibitor 0.20, Enzyme inhibitor 0.62 MA has various pharmacological activities. The water solubility of MA is −3.042log mol/L, Caco2 permeability 0.629 log Papp in 10–6 cm/s, MA has 1000% intestinal absorption, and MA is known as CYP3A4 Substrate (Banerjee et al., 2018; Daina et al., 2017; Pires et al., 2015; Xiong et al., 2021).The chemical structure, Radar, and Toxicity Profile of MA are depicted in Figs. 2 and 5. The pharmacokinetic profile of the MA was collected from the following databases. PKCSM server [https://biosig.lab.uq.edu.au/pkcsm/] SwissADME [http://www.swissadme.ch/] Protox-II [https://tox-new.charite.de/protox_II/index.php?site=home] (Sabarathinam, 2024b; Sabarathinam et al., 2024). The pharmacokinetic profile of MA is given in Table 1. & The Physicochemical and Toxicity profile of MA is given in Table 2.

4. Target related to MA

Targets associated with MA (accepted by Lipinski's rule) were obtained via SwissTargetPrediction(Daina et al., 2019). The following disease targets Diabetes; CUI: C0011847(D), Hypercholesterolemia; CUI: C0020443(HC) targets were identified by DisGeNET (https://www.disgenet.org/).(Piñero et al., 2021) The overlapping targets between D, HC & MAs-targets were visualized on the Venn diagram by interactive (http://www.interactivenn.net/). (Heberle et al., 2015) The PPI network is constructed on STRING analysis (https://string-db.org/). Overlapping targets and STRING network V 11.5 (Szklarczyk et al., 2021) is illustrated in Fig. 3. Maslinic Acid is a pentacyclic triterpene found in a variety of natural sources. MA has various targets like enzymes, phosphatases, surface antigens, nuclear receptors, membrane receptors, and phosphodiesterase. The targets for diabetes and hypercholesteremia were recruited from DisGeNET, a database of gene-disease associations (https://www.disgenet.org). The target genes of the corresponding components were subjected to STRING v_11 to visualize and construct the PPI network for the same. The higher confidence protein interaction is depicted in Fig. 3. The PPI network consists of 23 nodes and 42 edges; average node degree: 3.65; average local clustering coefficient: 0.422; expected number of edges: 10, PPI enrichment p-value: 3.15e-14. The network was found to be significant.

Table 1
Pharmacokinetic parameters of MA.

Model Name	Predicted Value	Unit
Water solubility	−3.042	(log mol/L)
Caco2 permeability	0.629	(log Papp in 10–6 cm/s)
Intestinal absorption (human)	100	(% Absorbed)
Skin Permeability	−2.735	(log Kp)
P-glycoprotein substrate	No	(Yes/No)
P-glycoprotein I & II inhibitor	No	(Yes/No)
VDC (human)	−1.28	(log L/kg)
Fraction unbound (human)	0.033	(Fu)
BBB permeability	−0.493	(log BB)
CNS permeability	−1.477	(log PS)
CYP3A4 Substrate	Yes	(Yes/No)
CYP1A2/2C19/2C9/2D6/3A4 inhibitor	No	(Yes/No)
Total Clearance	−0.071	(log ml/min/kg)
Renal OCT2 substrate	No	(Yes/No)
AMES toxicity	No	(Yes/No)
Max. Tolerated dose (human)	0.127	(log mg/kg/day)
hERG I/II inhibitor	No	(Yes/No)
Oral Rat Acute Toxicity (LD50)	2.516	(mol/kg)
Oral Rat Chronic Toxicity (LOAEL)	1.889	(log mg/kg_bw/day)
Hepatotoxicity	Yes	(Yes/No)
T.Pyriformis toxicity	0.285	(log ug/L)
Minnow toxicity	0.24	(log mM)

Table 2
Physicochemical and toxicity profile of MA.

Parameter	Value
Molecular weight	472.7
Number of hydrogen bond acceptors	52
Number of hydrogen bond donors	3
Number of atoms	82
Number of bonds	86
Number of rotatable bonds	1
Molecular refractivity	137.82
Topological Polar Surface Area	77.76
Lipinski	Yes; 1 violation: MLOGP>4.15

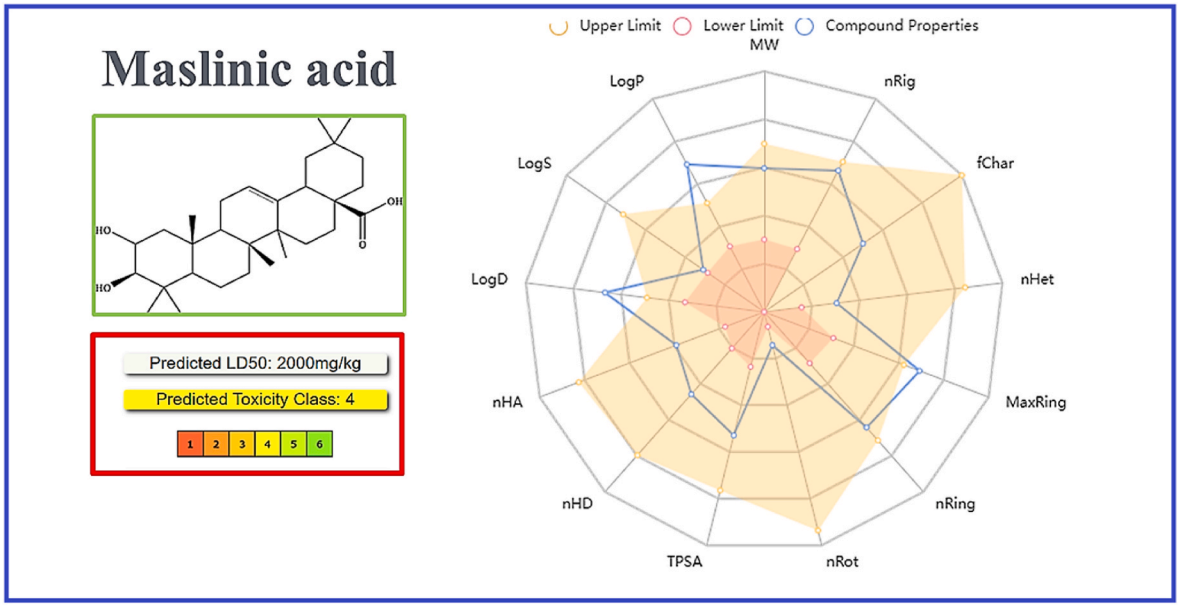

Fig. 2. Chemical structure, Lipinski rule radar and toxicity profile of Maslinic Acid.

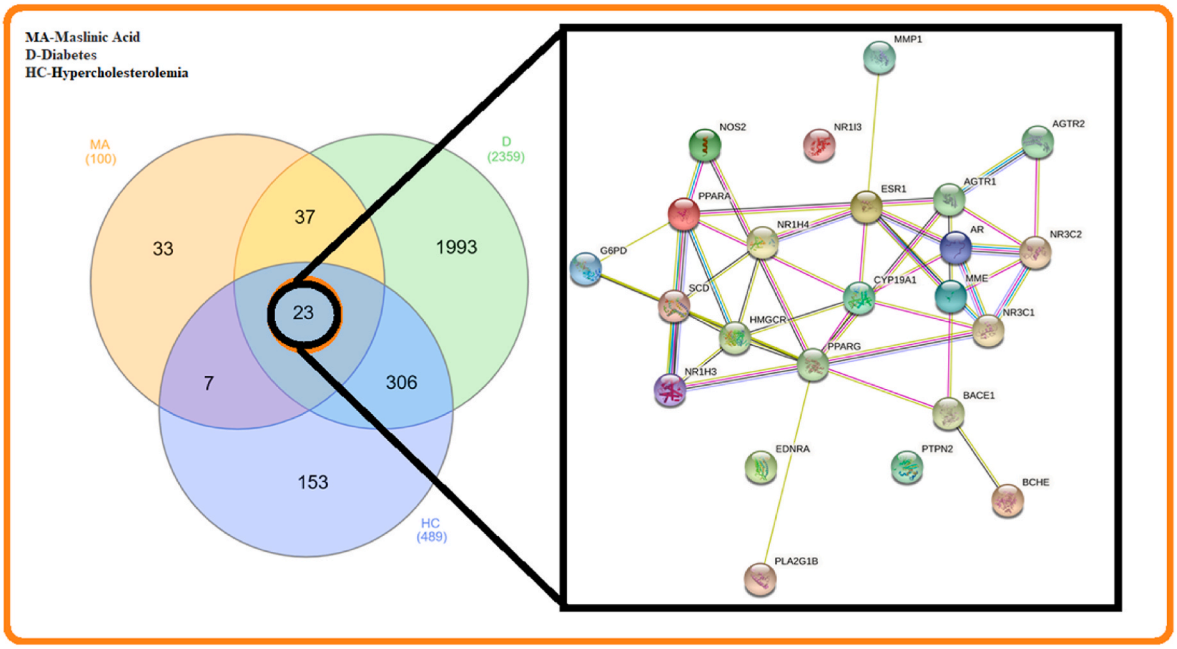

Fig. 3. Filtration of overlapping targets and network analysis.

MA can also be used for the treatment of other conditions, such as cancer and inflammation. MA was selected to examine the effects of diabetes and hypercholesterolemia, but via KEGG analysis (Table 3), it was found to be responsible for many cancers, renin-angiotensin pathway, AMPK signalling pathway, and vascular smooth muscle contractions. Due to the facts and visualization, MA may be a novel drug for treating various diseases and disorders. Based on the KEGG pathway profile, it is much easier to analyze the important genes related to the diseases through structure-based design of the components and their ability to predict the binding-conformation of small molecule ligands to the appropriate target binding site. The main reason behind selecting the proteins is that they played a major role in protein-protein interaction in KEGG analysis, and that these proteins are found to play a vital role in the mechanism of diabetes and cholesterol metabolism.

5. Molecular docking analysis of MA

The experimental technique used for the in silico molecular docking study was that described by Dhivya L. S. et al. With a minor alteration (Dhivya et al., 2022b). The interaction of the produced chemicals with the proteins (PDB ID:2ZNN, PDB ID:4EMA, and PDB ID:3K8S) was investigated using AutoDock tools 1.5.6. The compound's structure was retrieved from Pubchem with the appropriate 2D orientation. Utilizing the Avogadro tool, the molecule's energy was reduced, and the results were input into AutoDock for the docking simulation. The protein data bank was used to download the crystal structures of the human PPAR gamma in complex with rosiglitazone (PDB ID: 4EMA), the human PPAR gamma in complex with T2384 (PDB ID: 3K8S), and the human PPAR alpha ligand binding domain in connection with a synthetic agonist TIPP703 (PDB ID: 2ZNN). The target protein file was generated by leaving the related residue with protein using Auto Preparation of target protein file AutoDock 4.2.5.6. The protein preparation was carried out using the described standard technique by removing the co-crystallized ligand, water molecules, and cofactors (MGLTools 1.5.6). The grid box for the docking simulations was set using the graphical user interface application. The macromolecule's region of interest was put up in the grid so that it is encircled by it. The optimal docked conformation between the ligand and protein was looked for using the docking method included with AutoDock 1.5.6. For each ligand, a maximum of nine conformers were considered throughout the docking procedure. Discovery Studio Visualizer and PyMOL chose the conformations with the most beneficial (least favorable) free binding energy to study the interactions between the target receptor and ligands. The interacting residues and H-bonds are shown using a ball and stick model depiction, while the ligands are offered in various colors (Fekadu et al., 2022; Sabarathinam, 2024a; Vaithiyalingam et al., 2024).

6. Discussion

Our study intended to study the anti-diabetic effect of MA, which is known for its anti-neoplastic activity. The study method used to investigate the selected compound against insulin resistance in various pathways was achieved by in silico, network pharmacology, and molecular docking. A total of 23 target proteins were targeted by MA out of 100 targets. KEGG analysis renders several signalling pathways related to insulin resistance: insulin signaling, Alpha-amylase and alpha-glucosidase, NF-κBand Nrf2, ghrelin, and SGLT1 signaling show us that MA as a potential insulin sensitizer leading to balancing of glucose homeostasis. The selected compound amino acid's interactions with all three protein receptors were studied using molecular docking. Docking is a promising strategy for doing in silico screening on a huge library of compounds and putting forth structural ideas for how the ligands inhibit the target receptors. Lead optimization benefits significantly from this process (Dhivya et al., 2022a). While PPAR-γ activity increases glucose metabolism and makes the body more sensitive to insulin, PPAR- β/δ activation increases the metabolism of fatty acids. As a result, the nuclear receptors of the PPAR family regulate energy homeostasis and metabolic activity significantly. The current study critically examines the beneficial and harmful effects of PPAR agonists on obesity, cancer, dyslipidemia, diabetes, adipocyte differentiation, inflammation, lung illness, cancer, lung disorders, neurodegenerative disorders, pain, and inflammation(Tyagi et al., 2011b). To compare it to that of Rosiglitazone and Pioglitazone, a widely used therapeutic medication. Table .3 lists the selected compound binding affinities and their interactions with hydrogen bonds and amino acids. In the present work, the studied compounds had good binding relationships between 12.89 (2ZNN), −11.37 (3K8S), and-11.08 (4EMA) kcal/mol. The standard medications, rosiglitazone, and pioglitazone, had a less binding affinity to the studied

Table 3
KEGG pathways.

Term description	Observed gene count	Background gene count	Strength	False discovery rate	Matching proteins in present network
PPAR signaling pathway	5	75	1.75	1.07E-05	PPARG, MMP1, SCD,PPARA,NR1H3
Pathways in cancer	7	517	1.06	0.00027	PPARG, EDNRA,MMP1, NOS2,AR,ESR1,AGTR1
Renin-angiotensin system	3	23	2.05	0.0004	AGTR2,MME,AGTR1
AMPK signaling pathway	3	120	1.33	0.0328	PPARG, HMGCR,SCD
Neuroactive ligand-receptor interaction	4	330	1.01	0.0352	NR3C1,EDNRA, AGTR2,AGTR1
Vascular smooth muscle contraction	3	133	1.28	0.0352	PLA2G1B,EDNRA, AGTR1

compound, which displayed a critical relationship with a minimum energy of −7.2 and −6.9 kcal/mol. In comparison to standard drugs, the studied compound demonstrated similar residual interactions with the amino acid residues Gly-194, Phe-146, Met-153, Asp-64, and Leu 63, as well as the H-bonds Ile-95, Ile-122, and Phe-41 for the human PPAR alpha ligand binding domain in connection with a synthetic agonist TIPP703 (PDB ID: 2ZNN). The studied compound forms hydrogen bonds such as Ile-95, Phe-41, and Val-65 with the amino acid residue for the human PPAR gamma in complex with T2384 (PDB ID: 3K8S). Additional hydrogen bonding interactions with amino acid residues were seen in the studied compounds for the human PPAR gamma complex with rosiglitazone (PDB ID: 4EMA), such as Ile-122 and Val-65. Additionally, the studied compound displayed a higher binding affinity with Rosiglitazone and Pioglitazone against all three selected receptors. The 3D visualization and docking scores of MA are illustrated in Fig. 4 & Table 4.

7. Clinical correlation of MA with diabetes

Maslinic Acid is a pentacyclic triterpene derivative which is the most common class of oxidosqualene cyclase products are pentacyclic compounds. They are formed through various cyclization mechanisms, as evidenced by their diverse structural composition. Maslinic Acid is generated by folding and cycling squalene to produce oxidosqualene, which is then converted into the dammarenyl ring system. The compound goes through ring expansion and further cyclization to generate the skeletons of lupeol, β-amyrin, and β-amyrin from dammarenyl. β-amyrin is further oxidised to produce erythrodiol, oleanolic Acid, and then maslinic Acid. Numerous studies have found that pentacyclic triterpenoids significantly reduce the peroxisome proliferator-activated receptor. Alpha-amylase and alpha-glucosidase are crucial enzymes in the breakdown of carbohydrates. Long chain carbohydrates are broken down by alpha amylase, while starch and disaccharides are converted to glucose by alpha-glucosidase. Maslinic Acid, lupeol, ursolic Acid, and oleanolic Acid are examples of pentacyclic triterpenoids having well-recognized biological activity. Lupane, ursane, and oleanane types of compounds are also present. According to a study on the link between structure and action, the primary carbon skeleton has no bearing on the anti-diabetic properties of pentacyclic triterpenoids. Alcoholic groups at C-28 and carboxylic groups at C-28 or C-30 have been reported to have moderate inhibitory activities on α-glucosidase, respectively. Several investigations show triterpenic acids' functional group at position C-28 can serve as a hydrogen bond donor(Ding et al., 2018). On the other hand, it has been demonstrated that the oleanane skeleton (β-amyrin) has a higher inhibitory effect on α-glucosidase conformation than the ursane skeleton (β-amyrin). According to several studies, maslinic Acid has more hydroxyl groups at the C-2 position than oleanolic Acid, which confers antioxidant capabilities and inhibits peroxisome

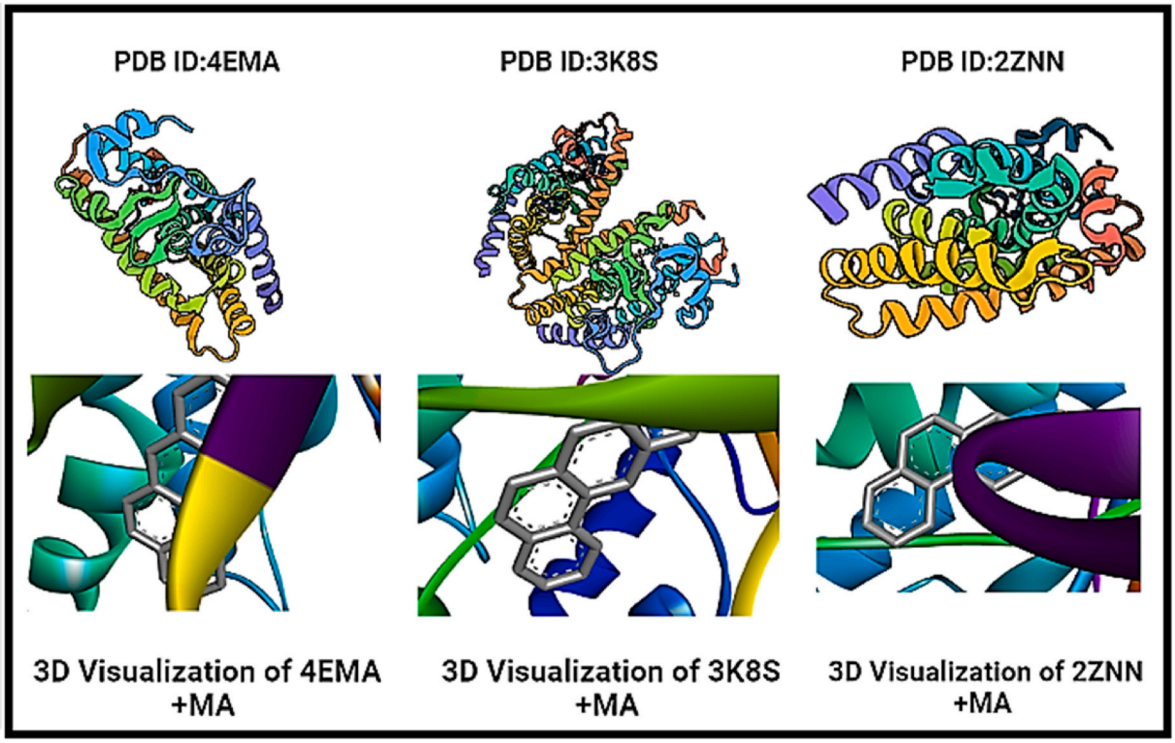

Fig. 4. 3D visualization of 4EMA, 3K8S & 2ZNN with MA

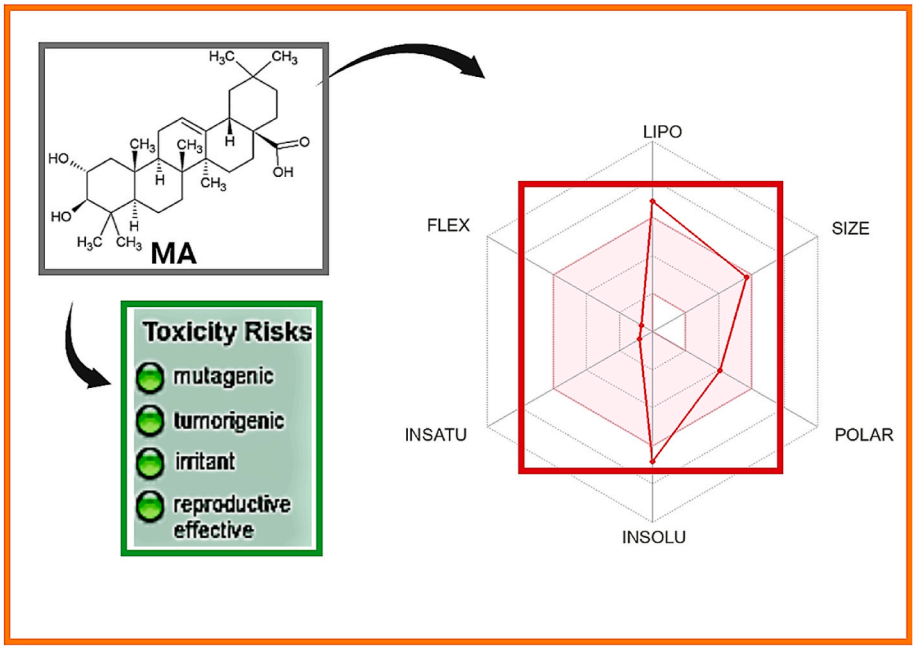

Fig. 5. Toxicity profile & Pharmacokinetic radar of MA.

Table 4
Binding Affinity and Amino acid interactions of Maslinic Acid.

S. No.	PDB ID and Standard Reference	Binding Affinity (-Kcal/mol)	Hydrogen Bond Interactions
01.	2ZNN	−12.89	Ile-95, Ile-122, Phe-41
02.	3K8S	−11.37	Ile-95, Phe-41,Val-65
03.	4EMA	−11.08	Ile-122, Val-65
	Rosiglitazone	−7.2	Ile- 95, Phe-41, Val 65
	Pioglitazone	−6.9	Ile −95, Val- 95

proliferator-activated receptors. The method by which maslinic Acid mediates suppression of peroxisome proliferator-activated receptors, however, is hypothesized to be independent of their antioxidant action (Tyagi et al., 2011a).

7.1. Effect of maslinic acid on α-glucosidase and α-amylase

Postprandially, carbohydrate hydrolysis in the GIT is predominantly performed by the enzymes α -glucosidase and α –amylase, which eventually results in the blood glucose levels rising. α-amylase initiates the carbohydrate metabolism by the hydrolysis of 1, 4-glycosidic linkages of polysaccharides to disaccharides and which are then hydrolyzed to monosaccharides such as glucose by the enzyme α-glucosidase. The glucose is then absorbed into the blood through the small intestines (Mwakalukwa et al., 2020) (Taslimi et al., 2018). Hence, the inhibitory effects of drugs on these hydrolyzing enzymes will have a better therapeutic effect on postprandial hyperglycemia. Several synthetic drugs available in practice are shown to produce some unwanted side effects, such as abdominal discomfort, bloating, headache, diarrhea(Alqahtani et al., 2020). Due to the increased side effects exerted by synthetic inhibitors, there is an extensive need for natural alternatives. The structural diversity exhibited by the natural products aids in effectively inhibiting the hydrolyzing enzymes without side effects. There are studies stating that maslinic Acid C-2 is methylene where there is a hydroxyl group. The triterpene acids C-2 might be suggested as an active group which enhances α-glucosidase inhibition activity (Hou et al., 2009). About the essential mechanism of action, comparatively little is known, even though several studies state the inhibitory effects of triterpenoids and diabetes (Lozano-Mena et al., 2014a). They conducted an invitro assay by taking the solvents such DMSO, water, and 100 μL of the enzyme (α-glucosidase, 5 U/mL in 0.15 M HEPES buffer) (Mwakalukwa et al., 2020) to the substarte 0.1 M sucrose solution and the mixture was homogenized and incubated at 37 °C. After the incubation, the formation of glucose was determined using the glucose oxidase method using a BF–5S Biosensor (Oji Scientific Instruments, Hyogo, Japan). The total α-amylase inhibitory effect was assayed by having potato starch solution and phosphate buffer followed by incubation at 37 °C for 30 min, then the reaction was stopped with 0.4 M HCl and 5 mM KI. The absorbance was read at 660 nm (Corona Electric Co, Japan). Boiled maize starch with 0.5 M Tris-HCl buffer (pH 6.9) containing 0.01 M CaCl2. After cooling, the reaction mixture was prepared with various concentrations (4.37–21.90 μmol/L) maslinic Acid. To which αamylase (porcine pancreas, 2.60 mmol/L) was added to initiate the reaction, incubated, and terminated the reaction with addition of acetic Acid. The inhibitory effects of MA followed a similar method above, except that 0.1 M K phosphate buffer was utilized. The reaction mixture was prepared with α-glucosidase and MA at various concentrations (4.37–21.90 μmol/L). The mixture was incubated, and the reaction stopped by adding 1.5 mL of 2 M Tris-HCl buffer (pH 6.9). The results suggest that MA dose dependently inhibited α-amylase and α-glucosidase activities. (Andile Khathi et al. 2013).(Khathi et al., 2013; Lozano-Mena et al., 2014b)

In an invivo study conducted by Jun Liu et al., 2007), they have investigated the antidiabetic effect of maslinic Acid in KK-Ay mice (male 6 weeks old, measuring 37–41g), which is an animal model of genetic type-2 diabetes with a blood glucose level greater than 300 mg/dl were included in the study. The animals were given MA orally at 3, 10, 30 mg/kg body wt dissolved in 0.5% CMC-Na solution. The control was subjected to an equal volume 0.5% CMC-Na solution (Jun Liu et al., 2007). For glucose determination, blood samples were collected at 2, 4, and 7hr. Non-fasting condition samples were included in the study. Glucose oxidase method was used to determine the blood glucose level in both treated and control samples. Blood glucose levels were also measured for repeated administration of MA (10 mg/kg) once a day for 2 weeks. Blood glucose levels were measured using the glucose oxidase method by taking blood samples, and the experiment was performed under nonfasting conditions. The study results suggested that MA showed a significant reduction in blood glucose levels in KK-Ay mice at 10 mg/kg and 30 mg/kg upon administration (p-value 0.05) on a single

oral dose at the 4th hour. MA 30 mg/kg concentration treated mice had reduced plasma glucose levels at the 4th and 7th hour with p value 0.01 in the repeated administration treatment modality (Liu et al., 2007).

7.2. Effect of maslinic acid on inhibition of glycogen phosphorylase

In glucose metabolism, glycogen phosphorylases play an important role in the glycogenolytic pathway. Several structural classes of GP inhibitors have been reported, whose binding sites identified in GP include the catalytic site, the purine inhibitory site (also known as I-site), the allosteric site, the glycogen storage site, and a novel allosteric inhibitor site. Attention attracted in this area remains high, as evidenced by the frequent appearance of related patents and publications (Wen et al. 2005).(Wen et al., 2005)

In a study conducted by (Wen et al., 2008) (Wen et al., 2008), they studied the Glycogen phosphorylase – Maslinic acid complexes to determine the inhibitory mechanism of maslinic Acid. They have learned the inhibition mechanism by studying the crystal structures of Glycogen phosphorylase-Maslinic acid complexes using the X-ray analysis method. The X-ray analysis depicts that the inhibitors bind at the site where the physiological activator AMP binds, which is the allosteric activator site. The x-ray analyses of the Glycogen phosphorylases inhibitory complexes show that GP exhibits a conformational change in the binding event, thus inactivating T state quaternary conformation In the enzyme. Therefore they exhibit nonpolar interactions with side chains of Tyr75, Phe196, and Val45' and form direct hydrogen bonds with Gln72, Arg310, and Asp42'. This stabilizes the inactive T state and inhibits the enzyme (Wen et al., 2008). Future detailed molecular mechanism studies can elucidate the anti-diabetic effects of malisinic Acid. In a study conducted by Liu et al., 2014); Liu et al. (2014))they have studied and investigated the anti-diabetic properties of maslinic Acid and their molecular mechanisms. In this study, various concentrations of Masilinic Acid were stimulated to HepG2 cells. The effects of MA on glycogen phosphorylase a (GPa) activity and the cellular glycogen content were measured in the study. HepG2 cells treated with PBS and Tris HCl were harvested. On centrifugation, the supernatant was used to measure the Gpa activity without AMP. The GPa activity was assayed by monitoring NADPH increase and measured at 340 nm. The results denote MA inhibits the activity of GPa and increases glycogen accumulation in HepG2 cells. They clearly suggest that MA has an anti-diabetic effect by inhibiting glycogen phosphorylase. In a study by Cheng in 2008 (Cheng et al., 2008),they have determined the inhibitory effect of Maslinic Acid against rabbit muscle GPa. The release of glucose – 1 – phosphate during glycogen synthesis was evaluated for studying the inhibitory activity of rabbit muscle GPa. The bioassay results were read using multiple readers, and they observed a significant inhibition against RMGPa, which was evident from the dihydroxy function in the pentacyclic triterpenes like maslinic Acid.

7.3. Maslinic acid downregulating the expression on SGLT1, SGLT2, and GLUT2 with inhibitory effect on alpha–glucosidase

A study was conducted by Andile Khathi in the year 2013 (Khathi et al., 2013)on STZ-induced diabetic rats; using the Western blot technique, they demonstrated that Maslinic Acid downregulated the gene expression of SGLT1 and GLUT2 genes and exhibited an inhibitory effect on carbohydrate hydrolyzing enzymes such as α-amylase and α-glucosidase in the small intestine which further indicates maslinic Acid as a potential drug for the treatment of postprandial hyperglycemia.

7.4. Maslinic acid suppresses NFkb and activates Nrf2

Diabetic retinopathy associated with type I diabetes mellitus is an essential factor to be considered in the treatment modality. In a recent study by Nasser A. Alsabaani (Alsabaani et al., 2022)in Feb 2022, they investigated the antioxidant potential of Maslinic Acid on T1DM rats. The treated rats showed a significant increase in Nrf2, indicating their antioxidant protective potential, and a decrease in protein levels of NF-κB, suggesting an improved retinal structure.

7.5. Activation of renal SIRT1/AMPK pathway in diabetic nephropathy

As previously discussed on Diabetic retinopathy, diabetic nephropathy is also a severe complication accompanying diabetes condition. In a recent invivo study conducted by Gao in the year 2022 (Gao and Wu, 2022),they have studied and investigated the effect of MA on STZ-induced diabetic mouse model. On gene expression analysis of renal tissues, they have observed activation of renal AMPK/SIRT1 pathway and potential protective effect of MA on renal structure and function, indicating its therapeutic advantage with reduced side-effects which many other diabetic drugs lack (Al-Abdan et al., 2021; Saxena et al., 2020).Thus, from a clinical point of view, it confirms that MA has a more robust correlation with the management of diabetes. Finally, it has been determined that the network pharmacology of MA includes static, dynamic, and narrow configurations. Our review will aid future researchers in their highly effective pursuit of network pharmacodynamics with chromatographic fingerprints.

8. Conclusion

Due to targeted efforts on the most severe chronic disorders, natural products/plant-based medicinal products now account for an increasing portion of the pharmaceutical sector. Regarding drug development, high throughput approaches, including in vivo, in vitro, and in silico, have made a solid case for assessing the pharmacological efficacy of herbal remedies. Before conducting comprehensive lab research, one original approach to understanding how active compounds work at the receptor level is essential. Drug discovery is quickly exploring the idea of creating multi-target medications against conditions considered challenging to treat, including cancer and cardio-metabolic disorder. In this regard, network pharmacology identifies disease mechanisms as networks best targeted by a combination of medications that work in concert. Network pharmacology has lately gained popularity as a tool for deciphering the mechanism of action of herbal medicines. Our investigation of MA as a potential treatment target for insulin resistance or diabetes mellitus using network pharmacology revealed that it has a significant role in producing glucose-lowering activity by regulating glucose homeostasis via several insulin signaling pathways discussed above. Further study on MA as an insulin sensitizer in vitro, in vivo, and clinical examination is essential to verify the *in-silico* outcomes.

9. Future perspective

The study suggests that Maslinic Acid (MA) could be a promising treatment for diabetes. Future research should include in vivo studies, clinical trials, and further investigation into MA's mechanism of action. Additionally, developing suitable formulations, exploring combination therapies, and obtaining patent protection are crucial steps for drug development. Finally, studying the synergistic effects of MA with other natural compounds could enhance its therapeutic potential.

CRediT authorship contribution statement

Sarvesh Sabarathinam: Conceptualization. **Sanjana Satheesh:** Nila Ganamurali, Data curation.

Consent to participate

Not applicable.

Consent to publish

Not applicable.

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Ethics approval

Not applicable.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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