Unraveling the Antiobesity Potential of *Balanites aegyptiaca Leaves* through Computational techniques

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Abstract
Obesity is a major health problem worldwide caused by the excessive accumulation of body fat which is linked to cause various diseases such as diabetes, heart disease, cancer and others. In this study, we used computational techniques (network pharmacology and molecular docking) for Balanites aegyptiaca leaves (BAL) to treat obesity. Three active compounds were identified by using network pharmacology which are: Quercetin, Acacetin, Apigenin while obesity related targets genes were obtained from GeneCards database. These genes are involved in fat metabolism, energy balance and inflammation. Molecular docking showed strong binding affinity of these phytochemicals.

AKT1 is the most significant target for obesity. The 3Dimentional structure of AKT1 protein (PDB ID: 3O96) was retrieved from protein data bank which is optimized and analyzed for its interactions. These findings suggest that Balanites aegyptiaca leaves may help in obesity management by targeting key biological pathways. However, further laboratory and clinical studies are needed to confirm effectiveness of Balanites aegyptiaca leaves in the treatment of obesity.

Keywords: Obesity, *Balanites aegyptiaca,* Network Pharmacology, AKT1, Molecular Docking.

Introduction

Obesity is a persistent and recurring condition defined as excessive buildup of body fat which negatively affects person overall health.[24] It is a major global health challenge and a rapidly growing epidemic that affects people of all ages from infants to adults.[34] Overweight can cause many chronic diseases such as cardiovascular disease, cancer, diabetes and metabolic syndrome. The World Health Organization (WHO) defines that obesity is imbalance between calories consumed and calories expended in order to categorize humans into one of three groups underweight, overweight and obese.

The basic formula of measurement of obesity is "Body Mass Index" (BMI) which is calculated as [(weight in kg)/ (height in m^2)].[32] There are many reasons for obesity making it a complex health problem. In addition to larger cultural impacts like bad eating practices and restricted availability to nutrient-dense food in some places, there is a hereditary component to obesity. Most studies concur that lifestyle choices such as overeating and inactivity have an important role in the development of the obesity. These actions have a big impact on how the illness progresses.[34]

In market, so many allopathic treatments are available to treat obesity but having certain adverse effects also. That is the reason why herbal medicines stand out among the various potential substituents as adjuvant therapy for obesity.

A various range of phytocompounds including alkaloid, flavonoid, polyphenols, saponin, terpenoids, tannin, terpenes, steroid are present in plants[35,41]. Flavonoid, phytosterol, phenolic amide and saponin shows excellent effect of modulating obesity.[13,16,33] This is the reason for investigating the naturally occurring phytochemical which may provide good effect as an alternative complementary remedy against the obesity.[1,26,33,37]

In this regards, after literature survey, *Balanites aegyptiaca* was found as indigineous plant giving antiobesity effect. *Balanites aegyptiaca* also known as 'Desert date' in English and in Marathi called as Hinganbeat, is member of the Zygophyllaceae family.[4,5,22] This is a commonly occurring yet largely unnoticed wild plant species in the drylands area of Asia. *Balanites aegyptiaca* has reported various pharmacological activities including the antioxidant, antimicrobial, hepatoprotective, anticancer, anti-inflammatory properties.[26] This plant contains flavonoids mainly quercetin and another apigenin, acacetin which are responsible to inhibition of adipogenesis, enhancement of lipolysis, suppression of appetite and food intake.[33]

Our study aims to investigate the anti-obesity potential of *Balanites aegyptiaca* leaves (BAL) through computational studies such as network pharmacology for identifying target genes. In addition, various *in silico* analysis techniques combined with the network pharmacology can improve the understanding of various health issues and use of natural product research.[21] Molecular docking is a computer-based technique that predicts how a drug or other molecule binds to a protein. It is a key tool in drug discovery and pharmaceutical research. Molecular docking study is for estimating the binding affinity of protein and ligand. [25]

Material and Methods

Material: Leaves of *Balanites aegyptiaca* were collected from at the place Mhaswad, Tal-Man, District Satara, Maharashtra and authenticated by Botinical Survey of India, Pune with authentication number: BSI/WRC/lden. Cer./2025/220 1250022474 dated on 24/01/2024.

Extraction of plant Compound: A total of 100 g of powdered (BAL) was weighed and soaked in 350 ml of methanol in a conical flask. The flask was shaken, sealed and left to stand at room temperature for 48 hours. After the extraction period, the mixture was filtered and the obtained extract was concentrated by evaporating to dryness in an evaporating dish.[42]

Screening of Phytochemical test in *Balanites aegyptica* leaves: Phytochemical identification screening was performed by using phytochemical tests for alkaloid, glycoside, tannin, flavonoid, terpenes, steroids, phenol, saponins, terpenoids, etc.[36] After preliminary phytochemical screening, IMPPAT Databases and Dr. Dukes phytochemicals databases were used for further analysis of phytochemicals.[10,11,25,40]

Identification of targets and construction of biological networks: Molsoft and SwissADME tools were used to check bioavailability, drug likeness score and molecular weight of BAL phytochemicals.[5,23] Later, PROTOX tool was used to predict toxicity of BAL[2]. These phytochemicals were entered in to SwissTargetPrediction to predict the potential target gene and diseases related gene was identified in the GeneCard database using keywords "Obesity".[8,39] The data obtained from SwissTargetPrediction and GeneCard is processed in Venny 2.1 to generate protein overlap results.[28] As a result, venn diagram was visualized on Vinny 2.1. The STRING database was used for PPI (Protein Protein Interaction) analysis in Cytoscape 3.9.0 version.[29]

Cytohubba plugin analysis is an essential hub analysis on the constructed protein network. The evaluation of the network hubs occurred by using closeness, betweenness, scoring and ranking methods. The top ranking hub of gene is analyzing for each scoring and ranking function, selected for computational molecular docking study. ShinyGO Database is used for annotation, visualization of gene ontology (GO) enrichment analysis and pathway analysis of Kyoto Encyclopedia of Genes and Genomes (KEGG).[14]

Preparation of Target Protein: The protein structure is selected based on findings from scientific literature of obesity and metabolic syndrome. Protein was selected for the docking study and downloaded from the RCSB Protein Data Bank (PDB ID: 3O96).[3,15,17] Protein structure was prepared using Chimera software.[12,30]

Preparation of ligand: The structures of orlistat, quercetin, apigenin, acacetin were generated with Chem Draw Ultra 8.0 (Cambridge Soft).[4] The 2D compound structures were transformed to 3D structures using Chem 3D Ultra 8.0. The optimization process and ligand geometry minimization occurred through density functional theory (DFT) method which produced .pdb format files for AutoDock vina program usage.[12]

Receptor Ligand Molecular docking: Molecular docking serves as a significant computational method in both structural molecular biology research along with drug design applications. The primary goal of molecular docking involves predicting dominant binding positions when ligands interact with target proteins whose three-dimensional structures are already known. High-dimensional space exploration success is achieved by docking methods together with suitable scoring criteria which properly evaluate studied molecules.

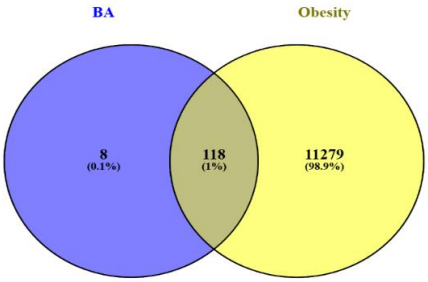
The virtual screening process using docking enables researchers to analyze vast molecular libraries and to generate structural hypotheses about ligand-target interactions for lead optimization. Active site of protein 3O96 was determined by using AutoDock tool (Grid dimensions X: 9.657; Y: -7.831; Z:10.604).[17] Docking of phytochemicals was carried out in PyRx tool and visualization of protein ligand interaction was done by using Biovia Discovery Studio.[12,18]

Results and Discussion

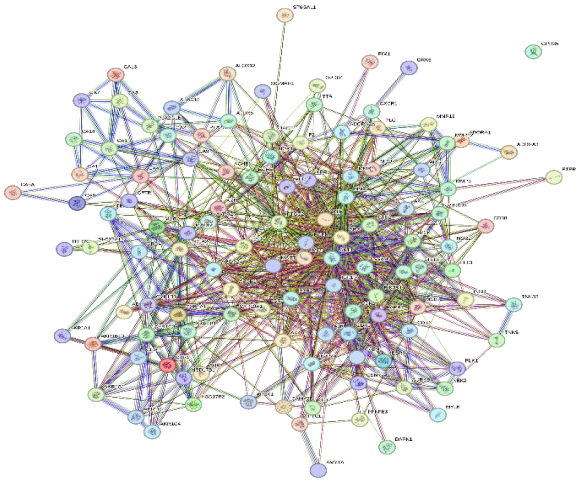
Phytochemical test of *Balanites aegyptiaca* leaves extract: Phytochemical compounds are present in *Balanites aegyptiaca* such as flavonoid, tannin, terpenes, saponin, terpenoids, cardiac glycoside, alkaloid, resin. Phytochemicals flavonoid, saponin, terpenoids, terpenes and alkaloid show the anti-obesity effect.

Table 1
Test compound identification from BAL for network pharmacology by using different tools[7]

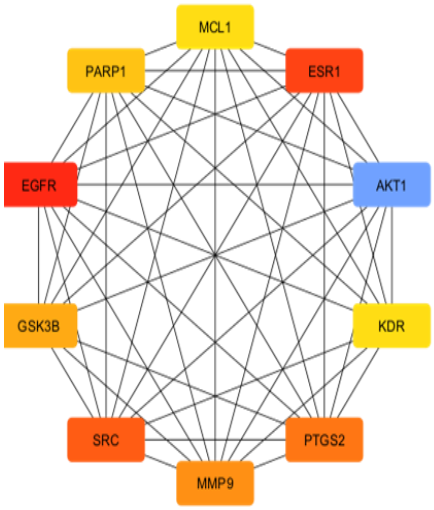
S.N.	Compound Name	PubChem CID	Oral bioavailability	Druglikeness Score	Molecular Weight	Carcinogenicity	Immunotoxicity	Mutagenicity	Cytotoxicity
1	Acacetin	5280442	0.55	0.2	284.07	No	No	No	No
2	Quercetin	5280343	0.55	0.52	302.04	No	No	No	No
3	Apigenin	5280443	0.55	0.39	270.05	No	No	No	No



(a)

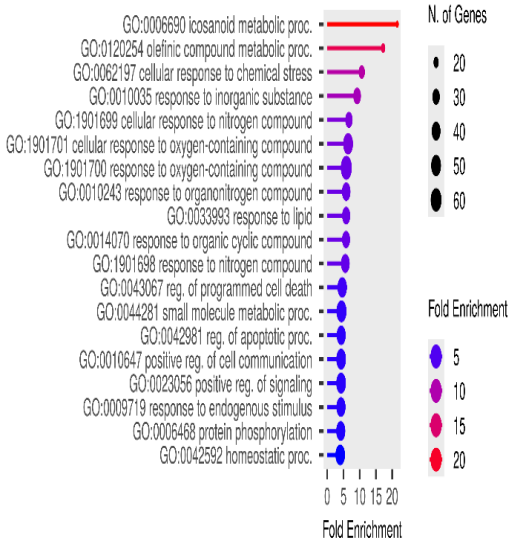


(b)

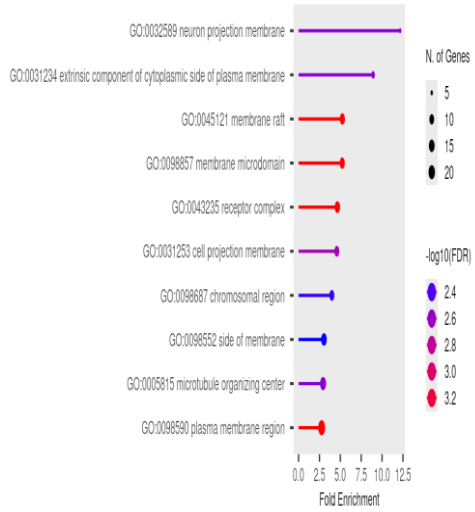


(c)

Name of Gene	Score
ATK1	62
EGFR	53
ESR1	52
SRC	51
PTGS2	48
MMP9	40
GSK3B	38
PARP1	35
MCL1	30
KDR	30



(d)



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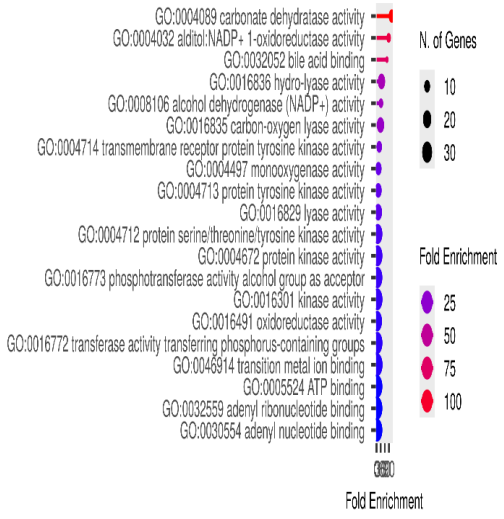

(f)

Figure 1: (A) Vennplot diagram of the common target found as potential target and these genes included in Pathogenesis of obesity, (B) String database network of all top 274 genes, (C) Network construction of top 10 genes, (D) Fold Enrichment analysis of common proteins by GO Biological Process, (E) Fold Enrichment analysis of common proteins by GO Cellular Component and (F) Fold Enrichment analysis of common proteins by GO Molecular function

Active Phytochemical compounds of *Balanites aegyptiaca* leaves: Phytochemical constituents present in the BAL show the anti-obesity potential detected by using the network pharmacology analysis, drug likeness score, bioavailability score, molecular weight and toxicity prediction.

Screening of Target Gene from bioactive compounds of *Balanites aegypyica*: SwissTargetPrediction identified approximately 126 potential targets of BAL phytochemical, while the GeneCard database shows over 11397 gene includes in the development of obesity. Only 118 target gene were common in tool Vinny 2.0 of both the target and gene shown in figure 1(A). These finding indicate that these genes could be essential in therapeutic action of BAL against obesity listed in figure 1(B) The detection of proteins is involved in the obesity response within the standard protein set. Therefore, targeting the anti-obesity pathway may serve as a promising strategy for obesity treatment.

Network pharmacology uses the concepts of closeness and betweenness of nodes. Nodes with high closeness are usually more central in the network and can serve as key hubs for information flow. In this network, ATK1 was identified first and is central gene of network. ATK1 ranked top highest amongst all genes in the network based on betweenness centrality in cytoscape which is shown in figure 1(C). Study reported that the inhibition of ATK1 has favorable outcome on achieving a better prognosis in obesity.

Thus, results suggest that the ATK1 is promising target for the management of obesity. On the other hand, gene ontology GO in figure 1(D) shows fold enrichment analysis of common proteins by GO biological process. Figure 1(E) Fold Enrichment analysis of common proteins by GO cellular component. Figure 1(F) shows fold enrichment analysis of common proteins by GO molecular function.

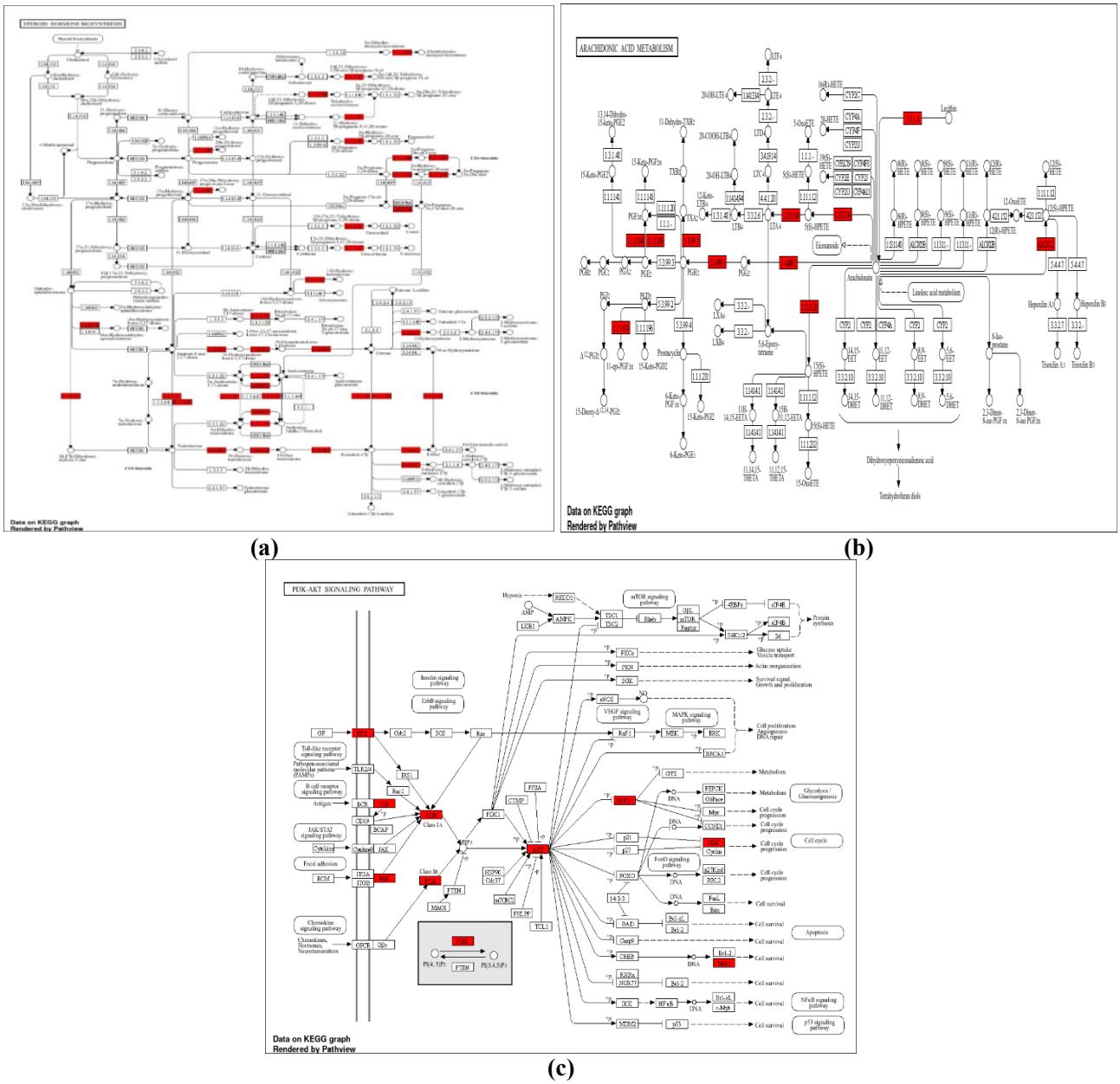

Figure 2: (A) Steroid hormone biosynthesis, (B) Arachidonic acid metabolism, (C) PI3K-Akt signaling pathway

KEGG enrichment analysis: In KEGG, finding top ten possible targets of BAL assisted obesity treatment were selected from twenty enriched pathways. From these ten pathways first pathway is steroid hormone biosynthesis (Fig. 2A), arachidonic acid metabolism (Fig. 2B) and PI3K-Akt signaling pathway (Fig. 2C). These three routes most likely have a key role in how *Balanites aegyptiaca* treats obesity.

Molecular docking of phytochemical of *Balanites Aegyptiaca*: Molecular docking is computational tool for drug design and to understand the interaction between the target protein and ligands. This method is helpful for designing and investigating the new drug with specific pharmacological action by the mechanism of drug receptor interaction. This is computer aided drug design help to identify the molecules by its orientation and scoring with the active site of the specific target protein. Phytochemicals of BAL show more binding affinity as compared to standard (Orlistat) is shown in table 2.

3O96 proteins have been extensively studied in adipogenic process. The phytochemicals of BAL show the antiobesity property and making effective candidate for downregulating the AKT1 which may ultimately prevent the adipogenesis by reducing the cholesterol, triglyceride, low density lipoprotein accumulation in the body as well as to enhance energy expenditure.[19,27,31,38] Significantly AKT1 acts to reduce the body mass, so BAL will be novel strategy for obesity management.

Table 2
Binding affinity with number of hydrogen bonds and amino acid involved in interactions.

Phytochemical Name	Binding Affinity kcal/mol	No. of Hydrogen Bonds	Common Amino Acid Residue
IQO (ligand)	-13.6	(LYS A:268), (SER A:205)	(TYR A:272), (THR A:82), (VAL A:271), (ASN A:53), (SER A:205), (LYS A:268), (TRP A:80), (LEU A:264), (VAL A:270), (LEU A:210), (THR A:291), (ILE A:290), (ILE A:84), (ARG A:273), (ASN A:54), (GLN A:79).
Orlistat (Inhibitor/Standard)	-7.5	(TRP A:80), (GLY A:294)	(GLN A:79),(LYS A:179),(LEU A:264),(VAL A:270), (SER A:205),(LYS A:268),(TYR A:263),(ASN A:53), (TRP A:80),(ILE A:290),(TYR A:272),(LEU A:210), (THR A:291), (VAL A:271),(ASN A:54),(ILE A:84), (ARG A:273),(GLY A:294),(THR A:82).
Acacetin	-9.5	(SER A:205), (LYS A:268), (ASN A:54)	(TYR A:272), (ILE A:84), (GLN A:79), (VAL A:271), (ASN A:54), (LEU A:264), (VAL A:270), (TRP A:80), (LYS A:268), (SER A:205)
Apigenin	-9.6	(THR A:211)	(GLN A:79),(LEU A:264),(LYS A:268),(SER A:205), (TYR A:263), (ILE A:290),(LEU A:210),(TRP A:80), (TYR A:272),(VAL A:270),(VAL A:271),(ASN A:54)
Quercetin	-9.7	(VAL A:271), (THR A:211), (SER A:205)	(SER A:205),(ILE A:290),(LEU A:210),(LEU A:264), (TRP A:80),(VAL A:270),(GLN A:79),(VAL A:271), (ASN A:54),(TYR A:272),(LYS A:268)

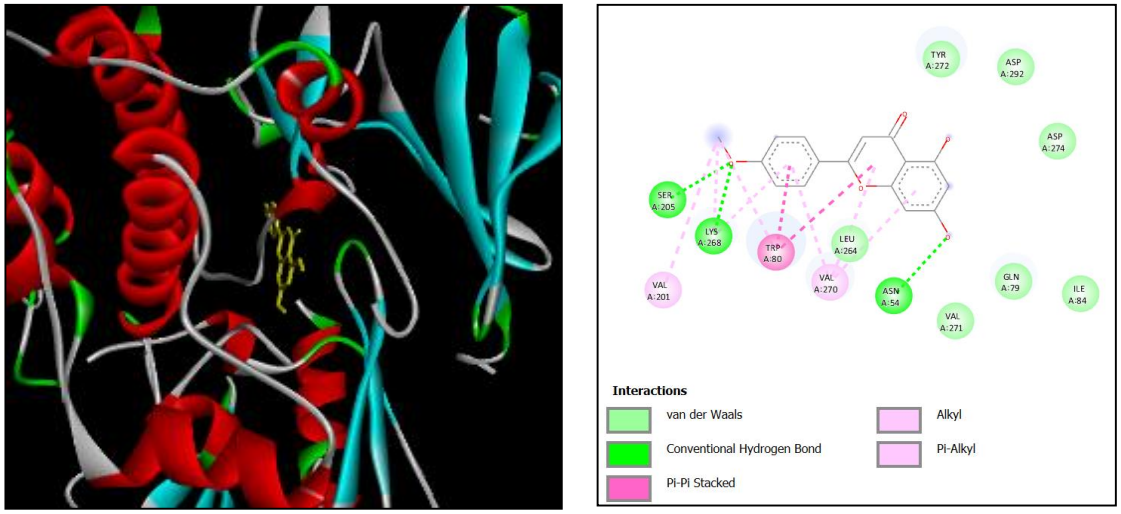

Figure 3: 3D and 2D Structure of Acacetin with 3O96

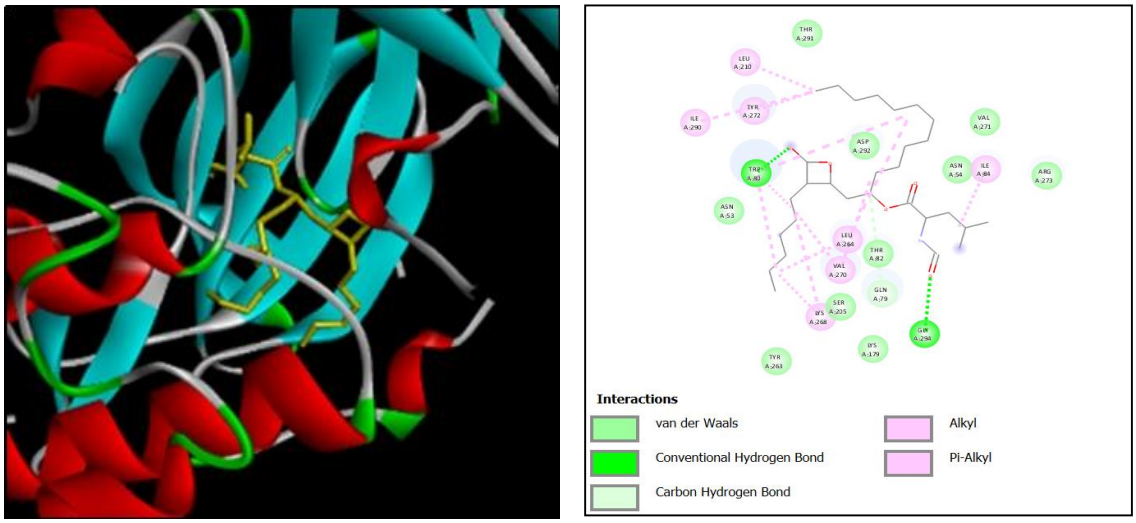

Figure 4: 3D and 2D Structure of Orlistat with 3O96

Conclusion

This study utilizes bioinformatics approaches including network pharmacology and molecular docking to explore the molecular mechanisms of BAL in obesity management. The effects of key phytochemicals such as acacetin, quercetin, apigenin on obesity were investigated. BAL exerts its anti-obesity effects through multiple mechanisms such as inhibiting adipogenesis, regulating lipid metabolism, promoting lipolysis as well as increase energy expenditure identified through network pharmacology analysis.

Acacetin is a dihydroxy and a monomethoxyflavone which shows the higher docking score as compared to orlistat. Therefore, this phytochemical could be considered as potent anti-obesity agent. These findings highlight BAL as a promising candidate for obesity management by modulating multiple biological pathways and targets. Further pre-clinical investigations are needed to confirm pharmacological effects of these phytochemicals.

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(Received 20th March 2025, accepted 25th May 2025)