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3D MOLECULAR DOCKING STUDIES OF BIOACTIVE COMPONENTS OF HYDROALCOHOLIC EXTRACT OF *Sauropus androgynus* LEAVES ON ILE5, 7MYJ AND 8GUR.

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ABSTRACT:

Sauropus androgynus (L.) Merr., known as Star gooseberry, is an essential plant in traditional medicine, particularly in South and Southeast Asia, praised for its high nutritional value and medicinal properties. This study explores the phytochemical profile and molecular docking potential of the hydroalcoholic extract of *S. androgynus* leaves. The leaves were shade dried, macerated in 70% ethanol, and subjected to phytochemical screening, revealing the presence of alkaloids, amino acids, carbohydrates, flavonoids, saponin glycosides, tannins, terpenoids, phenols, resins, phlobatannins, vitamins B2 and C. The hydroalcoholic extract showed a yield of 24.05%. For In silico studies, a network pharmacology approach identified 125 phytoconstituents, with 33 showing positive drug-likeness scores. Key compounds, including ascorbic acid, riboflavin, thiamine, chlorogenic acid, and rutin, were identified as potential bioactive agents. Molecular docking was conducted on selected proteins (NFKB1, CNR2, ACACA) with these compounds, revealing significant binding energies. Notably, 9-ethoxy-10-oxatricyclo [7.2.1.0] dodecan-11-one exhibited strong interactions with NFKB1 and ACACA, with binding energies of -3.9 and -6.6 kcal/mol, respectively. Riboflavin and thiamine also demonstrated substantial binding with NFKB1, with energies of -5.9 and -5.3 kcal/mol, respectively. These findings underscore the potential of *S. androgynus* as a source of nutraceuticals and pharmaceuticals, highlighting its broad-spectrum therapeutic properties and its relevance in developing new, effective treatments.

Keywords: *Sauropus androgynus,* hydroalcoholic extract, bioactive compounds, phytochemical screening, molecular docking.

INTRODUCTION:

Sauropus androgynus (L.) Merr., commonly known as Star gooseberry, is an important plant in traditional medicine and nutrition, especially in South Asia and Southeast Asia. This herb, belonging to the Euphorbiaceae family, is revered for its high nutritional value and diverse medicinal properties. Known locally as Thavasikkeerai in Tamil, it is often cultivated in vegetable gardens and used for ornamental purposes due to its manageable growth, which ranges from 50 cm to 3 meters in height. Historically, *Sauropus androgynus* has been employed to enhance lactation in breastfeeding mothers. A study by Saroni et al. (2004) demonstrated that the consumption of *Sauropus androgynus* leaves could increase milk production by 50.7% compared to mothers who did not consume the leaf extract. This has established the plant as a valuable resource for new mothers. Nutritionally, *Sauropus androgynus* is termed the "multivitamin plant" due to its rich content of vitamins and proteins. It is particularly noted for its high levels of vitamin C, which acts as a potent antioxidant. According to Zuhra et al. (2008), the vitamin C content ranges from 85.65% to 92.43 mg per 100g. This antioxidant property is crucial as it helps neutralize free radicals, thereby preventing infections and degenerative diseases. Furthermore, *Sauropus androgynus* is rich in flavonoids, which also serve as natural antioxidants. Selvi et al. (2011) reported that the flavonoid content in the plant is exceptionally high, measuring 831.7 mg per 100g. These compounds, along with other secondary metabolites such as alkaloids, phenols, and glycosides, contribute to its potential antimicrobial and antifungal properties. Andini (2014) highlighted these properties, indicating its use as a natural remedy for various ailments. The versatility of *Sauropus androgynus* extends beyond its medicinal uses. It is utilized in traditional communities for various purposes, including as food for humans and livestock, as a natural dye for cakes, and even as a food coloring agent. Research by Hayati (2016) and others has documented its application in treating fever and cough, improving egg quality in chickens (Santoso and Fenita, 2016; Bidura et al., 2017), and serving as an antidiabetic agent, among other benefits. The comprehensive study of *Sauropus androgynus* underscores its significance as a multi-functional plant with extensive applications in both traditional and modern medicine. Its high nutritional content and medicinal properties make it a valuable asset in the development of new drugs and dietary supplements, providing a natural alternative to synthetic chemicals. The integration of traditional knowledge with modern scientific research can potentially lead to the discovery of new, effective treatments derived from this versatile plant [1-6].

MATERIALS AND METHODS:

EXTRACTION:

The fresh leaves of Sauropus androgynus were collected, washed and shade dried. The shade dried plant was crushed by hand to coarse powder. 200 g coarse powder was subjected to maceration in 70% ethanol for 72 hrs. After the extraction the macerate was evaporated to dryness by a rotary evaporator. Further, any remaining solvent was removed through Lyophilisation. The dried extract was then weighed to calculate the extractive value and stored in a tightly sealed container [7,8].

PHYTOCHEMICAL SCREENING: Phytochemical screening involves identifying various classes of naturally occurring chemicals in different parts of plants. These phytochemicals include phenols, flavonoids, alkaloids, terpenoids, and saponins, among others. Rich in these compounds, botanical nutraceuticals not only promote health and wellness but also mitigate health risks. Their popularity is increasing due to their effectiveness in combating various physiological challenges. Phytochemical screening helps to uncover the constituents of plant extracts, identifying the predominant ones, and is crucial for discovering bioactive agents that can be used as dietary supplements.

1. **Test for Alkaloids**
    - **Dragendorff's Test**: Reddish brown precipitate with Dragendorff's reagent (potassium bismuth iodide solution).
    - **Mayer's Test**: Cream color precipitate with Mayer's reagent (potassium mercuric iodide solution).
    - **Hager's Test**: Yellow precipitate with Hager's reagent (saturated picric acid solution).
2. **Test for Amino Acids**
    - **Million's Test**: White precipitate with Million's reagent.
    - **Ninhydrine Test**: Violet color upon boiling with ninhydrine solution.
3. **Test for Carbohydrates**
    - **Molisch's Test**: Purple to violet ring at the junction with α-naphthol and concentrated sulfuric acid.
    - **Test for Reducing Sugars**: Red precipitate of cuprous oxide with Fehling's solution A and B.

4. **Test for Flavonoids**
    - **Shinoda Test**: Pink scarlet, crimson red, or green to blue color with magnesium turnings and HCl.
    - **Alkaline Hydrochloride Test**: Intense yellow color turning colorless with NaOH and dilute acid.
5. **Test for Glycosides**
    - **Anthraquinone Glycosides (Borntrager's Test)**: Rose pink to red color in the ammonical layer.
    - **Cardiac Glycosides (Keller-Killiani Test)**: Blue color at the acetic acid layer.
    - **Saponin Glycosides (Froth Formation Test)**: Stable froth formation.
6. **Test for Tannins**
    - **$FeCl_3$ Test**: Intense blue or black color with 1% aqueous iron chloride.
7. **Test for Terpenoids (Salkowski Test)**
    - Reddish brown layer at the interface with chloroform and concentrated H_2SO_4.
8. **Test for Phenols**
    - Blue or green color with 10% aqueous ferric chloride.
9. **Test for Resins**
    - Heavy resinous precipitate with boiling ethanol and 1% aqueous HCl.
10. **Test for Steroids**
    - Stable persistent froth with distilled water; emulsion formation with olive oil.
11. **Test for Phlobatannins**
    - Red precipitate with 1% HCl upon heating.
12. **Test for Vitamin B2**
    - Pale yellow-green color by transmitted light and intense yellow-green fluorescence by reflected light; disappears with mineral acids or alkali.
13. **Test for Vitamin C**
    - Yellow color turning blue with sodium nitroprusside, NaOH, and HCl [9-11].

INSILICO STUDIES:

NETWORK PHARMACOLOGY:

Phytoconstituents of *S. androgynus* were identified from available literature, scientific journals and traditional medicine books. The database was constructed from the phytoconstituents, their types, SMILES (Simplified Molecular Input Line Entry System) and PubChem CID (Compound Identification Number). During the construction of the database, duplication of phytoconstituents was eliminated. The canonical SMILES and PubChem CID for each phytoconstituent were then retrieved from the PubChem database. Canonical SMILES were used to predict the target in BindingDB with a 70% similarity to known ligand molecules. Proteins were identified based on known targets reported in the Therapeutic Target Database (TTD), and the gene ID for each protein was retrieved from UniProt.

Drug likeness prediction: Phytoconstituents were evaluated for drug likeness using MolSoft based on Lipinski's rule of five.

Prediction of side effects: The SMILES of each phytoconstituent were analyzed using ADVERpred to predict potential side effects. Side effects were considered if the probable activity (Pa) was higher than the probable inactivity (Pi) and if the Pa value exceeded 0.7. Any side effects identified during the prediction were removed.

Pathway and network analysis: Proteins associated with obesity were analyzed using STRING for gene enrichment, identifying pathways modulated by the phytoconstituents. KEGG pathway analysis was conducted, and Cytoscape 3.6.1 was used to construct a network linking phytoconstituents, protein molecules, and identified pathways. The network's interpretation used color and node size scales based on the number of edges, with the node having the highest edge count indicated by a colossal node12-14. Molecular docking: The 3D structures of compounds/ligands were retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) and then transformed into the appropriate format.[12-14].

MOLECULAR DOCKING:

We retrieved the 3D structures of compounds/ligands from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) and then transformed them into. Pdb format using the

Discovery Studio Visualizer (https://discover.3ds.com/discovery-studio-visualizer-download). The selection of proteins responsible for the desired activity was accomplished through the RCSB PDB website (https://www.rcsb.org/). Subsequently, we identified the active sites within the selected proteins using the prank web software (https://prankweb.cz/). Following the preparation of both the ligands and the proteins, we conducted protein-ligand interaction studies by employing PyRx software, enabling the docking of molecules to examine their interactions[15-17].

RESULTS AND DISCUSSION:

Extraction:

The yield of hydroalcoholic extract were 24.05% (crystals with brown color).

Phytochemical screening:

To identifying the various naturally occurring chemicals in plants, such as phenols, flavonoids, alkaloids, terpenoids, and saponins. These phytochemicals are essential components of botanical nutraceuticals, which are known for promoting health and mitigating health risks. The increasing popularity of these nutraceuticals can be attributed to their effectiveness in addressing various physiological challenges. Alkaloids can be identified through tests such as Dragendorff's, Mayer's, and Hager's, each producing distinct precipitates. Amino acids are detected using Million's and Ninhydrine tests, which reveal white and violet colors, respectively. Carbohydrates are screened through Molisch's test and Fehling's test, indicating the presence of reducing sugars. Flavonoids can be identified via the Shinoda test and Alkaline Hydrochloride test, which produce color changes. Various glycosides, including anthraquinone, cardiac, and saponin glycosides, are detected through Borntrager's, Keller-Killiani, and Froth Formation tests, respectively. Additionally, tannins, terpenoids, phenols, resins, steroids, phlobatannins, and vitamins B2 and C each have specific tests that indicate their presence through distinct color changes or precipitate formation. These methods collectively ensure a comprehensive analysis of plant extracts, facilitating the identification and utilization of bioactive compounds in nutraceuticals.

Table 1: phytochemical screening results

Sr.No.	Chemical test	Present (+) or absent (-)
1.	Test for Alkaloids	
a)	*Dragendorff's test*	+
b)	*Mayer's test*	+
c)	*Hager's test*	-
2.	Test for amino acids	
a)	*Million's test*	+
b)	*Ninhydrine test*	+
3.	Test for carbohydrates	
a)	*Molisch's test*	+
b)	*Test for reducing sugars*	+
4.	Test for flavonoids	
a)	*Shinoda test*	+
b)	*Alkaline hydrochloride test*	+
5.	Test for glycosides	
I.	Anthraquinone glycosides	
a)	*Borntrager's test*	-
II.	Cardiac glycosides	
a)	*Keller-killiani test*	-
b)	*Baljet's test*	-
III.	Saponin glycoside	
a)	*Froth formation test*	+
6.	Test for tannins	+
7.	Test for terpenoids (Salkowski test)	+
8.	Test for phenols	+
9.	Test for resins	+
10.	Test for steroids	-
11.	Test for phlobatannins	+
12.	Test for Vitamin B2	+
13.	Test for Vitamin C	+

Insilico study:

1) Network Pharmacology: Drug likeliness property of phytocompounds and target prediction: Total 125 compounds were obtained from Sauropus androgynus from databases such as PubMed, Google Scholar, Scihub and other literature. Among 125 compounds, 33 compounds showed positive DLS. Targets were obtained from TTD and KEGG pathways. Those are Ascorbic acid, Riboflavin, thiamine, chlorogenic acid, rutin, l-(+)-Ascorbic acid 2,6-Dihexadecanoate, gamma-tocopherol, β-sitosterol, 9,12,15-Octadecatrienoic acid, (Z,Z,Z)-Benzofuran, 2, 3-dihydro-, 2-Acetylpyrrolidine, N-Ethyl-2-carbomethoxyazetidine, 9-Ethoxy-10-oxatricyclo [7.2.1.0 (1,6) dodecan-11-one, Heptaethylene glycol monododecyl ether, 2-Methoxy-4-vinylphenol, Morpholine, N-Chloroacetyl-d-phenylalanine, Cyclopentasiloxane, decamethyl Pyrene, ethylchrysanthemumate, cyclopropanecarboxylic acid, 3-buten-2-one, 4-(4-hydroxy-2,2,6-trimethyl-7-oxabicyclo[4.1.0]hept-1-yl)-,2,5-pyrrolidinedione, ferulic acid, eugenol, syringic acid, lysine, methionine, threonine, phenylalanine, valine, leucine & isoleucine.

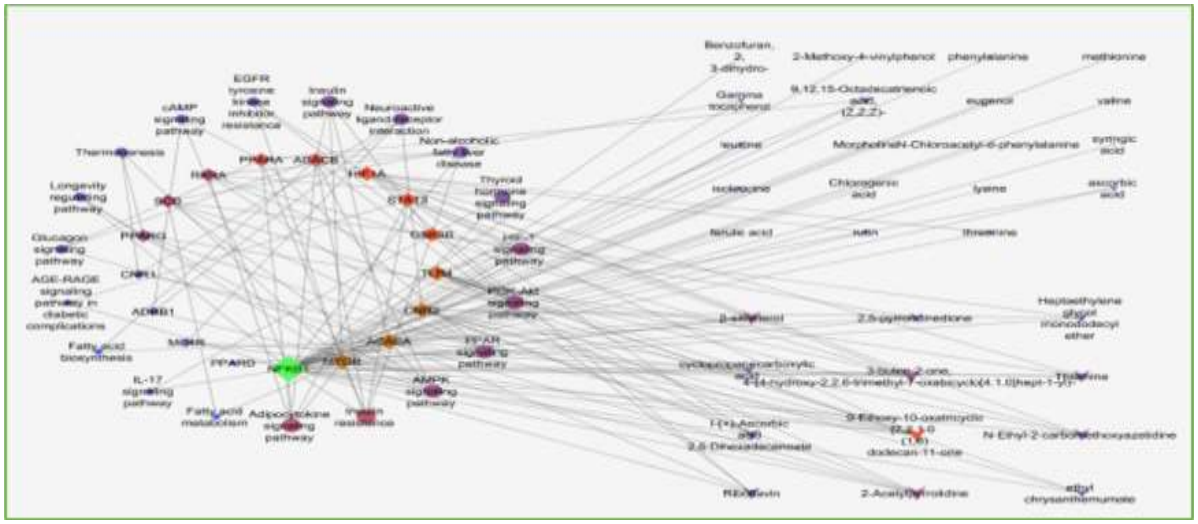


Fig 1: Network of *Sauropus androgynus* phytocompounds.

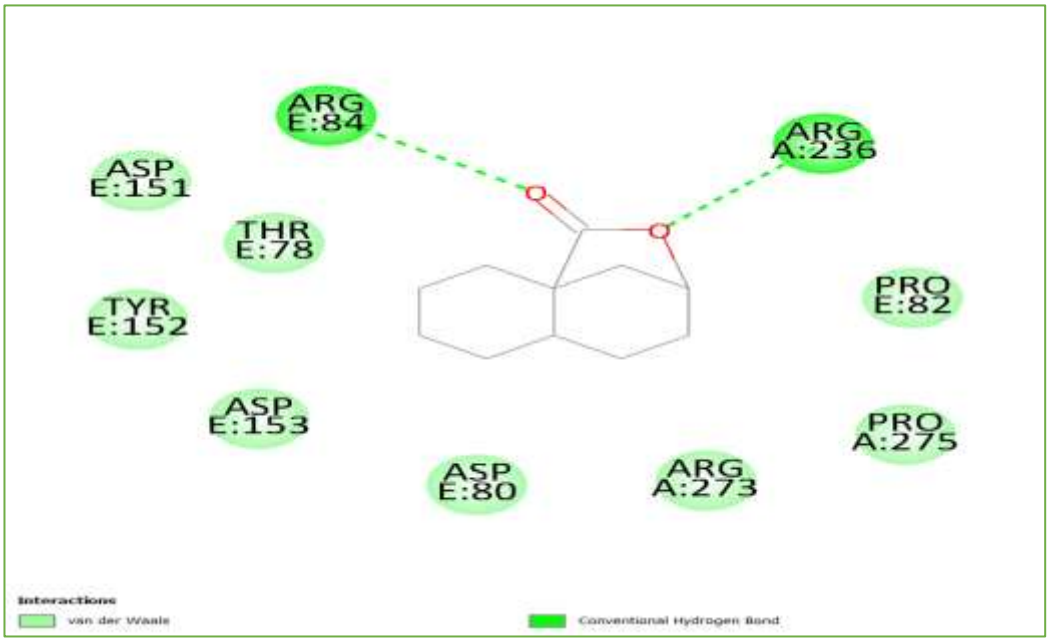
2) Molecular Docking:

Based on the literature review and the edge count, 3 proteins 1LE5, 7MYJ and 8GUR were selected for docking with selected phytocompounds.

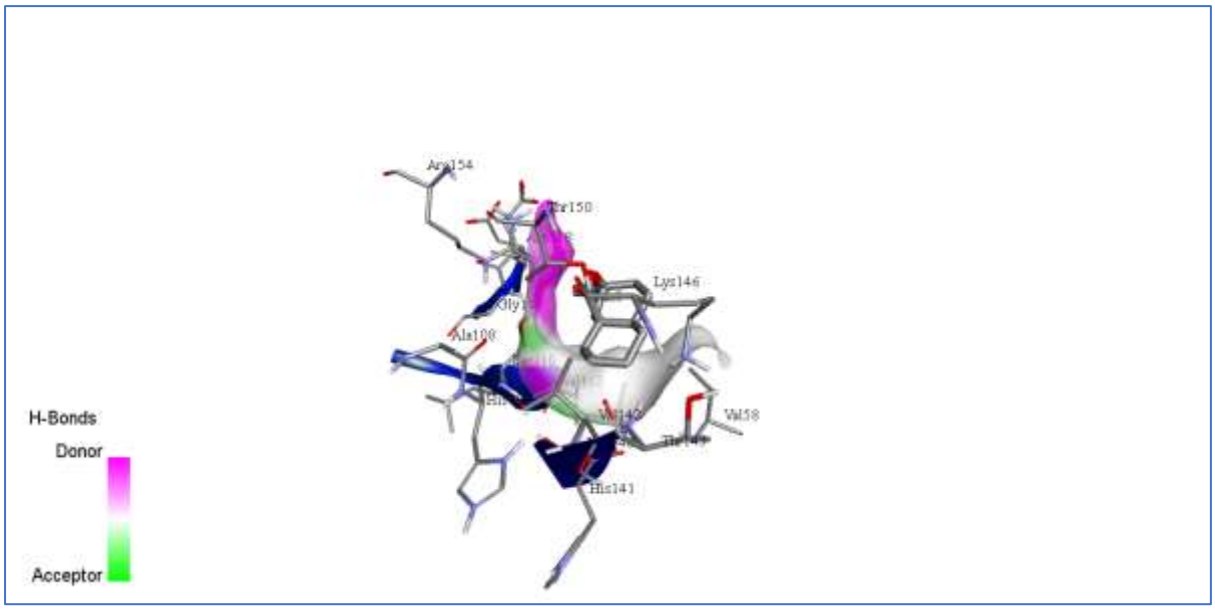
Table 2: binding energies

Target	Compound	Binding energy (Kcal/mol)
NFKB1	9-Ethoxy-10-oxatricyclo [7.2.1.0 (1,6) dodecan-11-one	-3.9
NFKB1	2,5-pyrrolidinedione	-3.5
NFKB1	Riboflavin	-5.9
NFKB1	thiamine	-5.3
CNR2	2-Acetylpyrrolidine	-4.5
ACACA	9-Ethoxy-10-oxatricyclo [7.2.1.0 (1,6) dodecan-11-one	-6.6
ACACA	2-Acetylpyrrolidine	-4.8

1) Interactions of 9-Ethoxy-10-oxatricyclo [7.2.1.0 (1,6) dodecan-11-one with 1LE5.

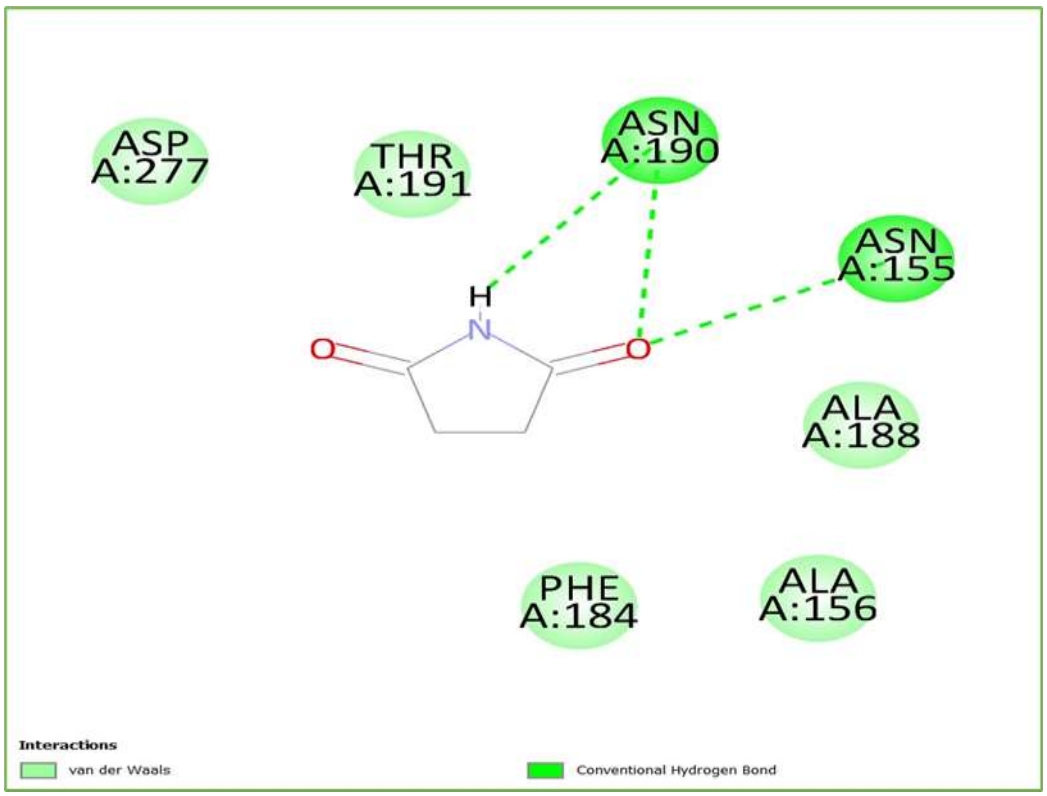


(a)2D interaction

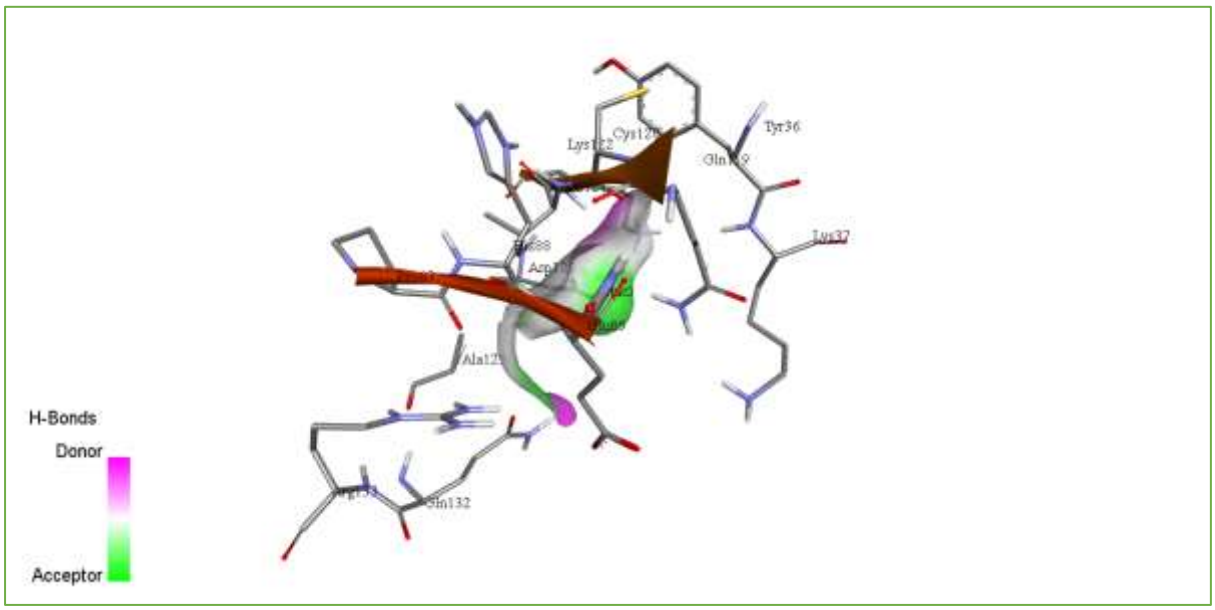


(b) 3D interaction

2) Interactions of 2,5-pyrrolidinedione with 1LE5.

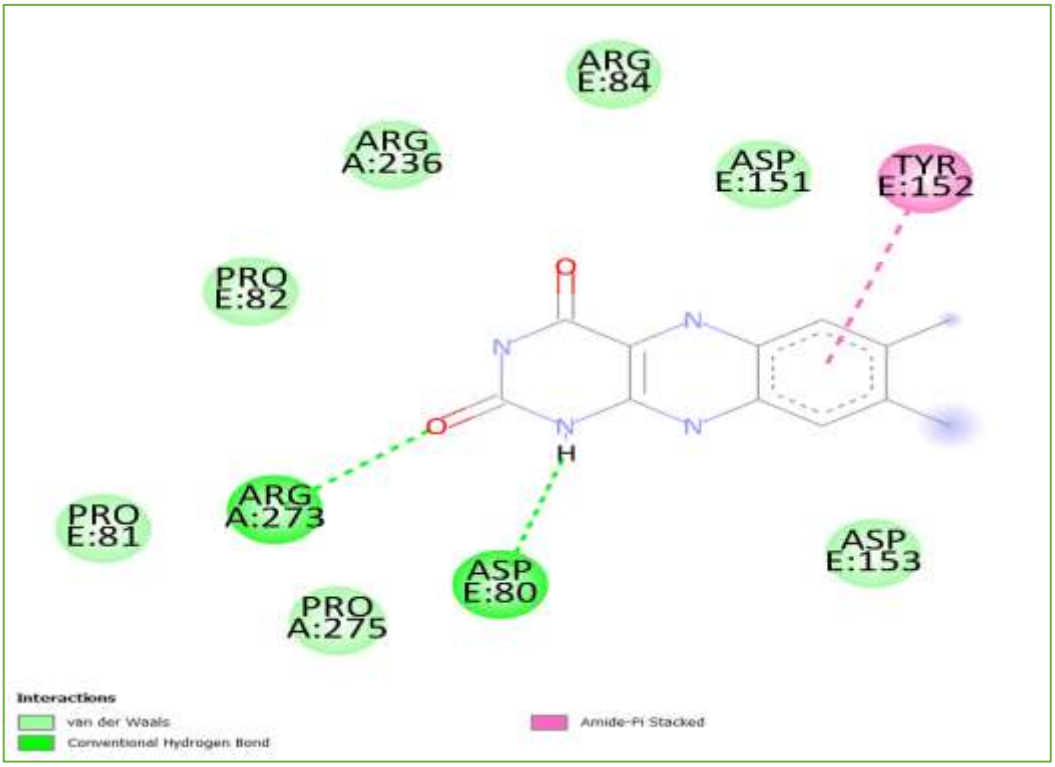


(a)2D interaction

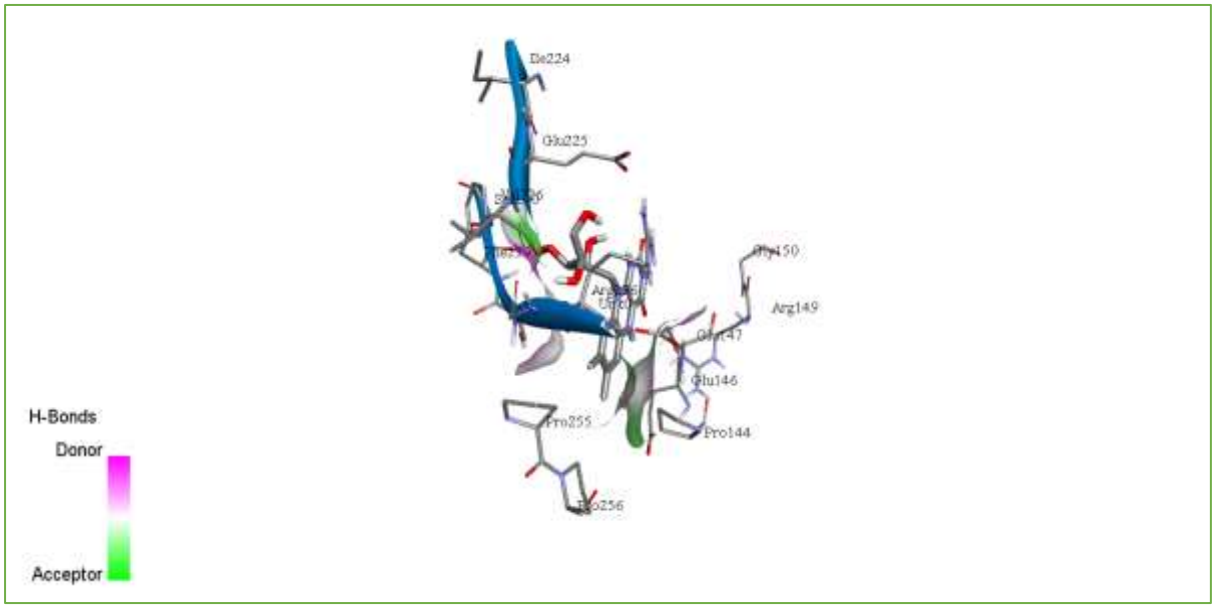


(b) 3D interaction

3) Interaction of Riboflavin with 1LE5.

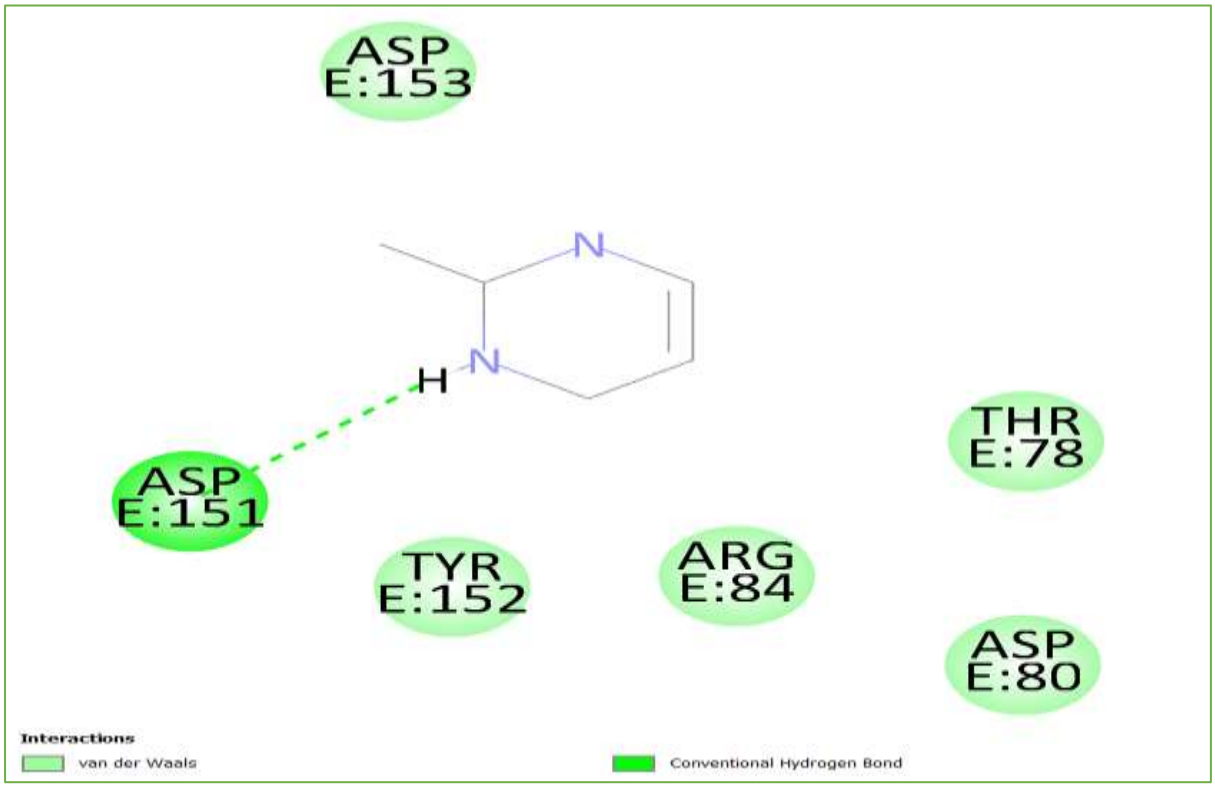


(a)2D interaction

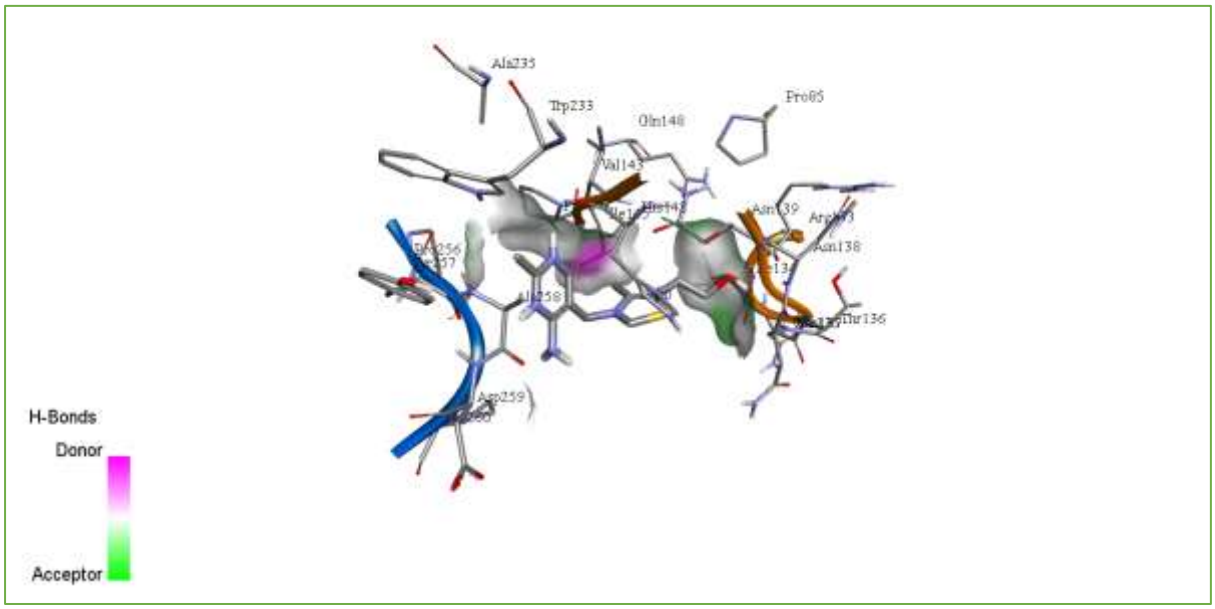


(b) 3D interaction

4) Interactions of Thiamine with 1LE5.

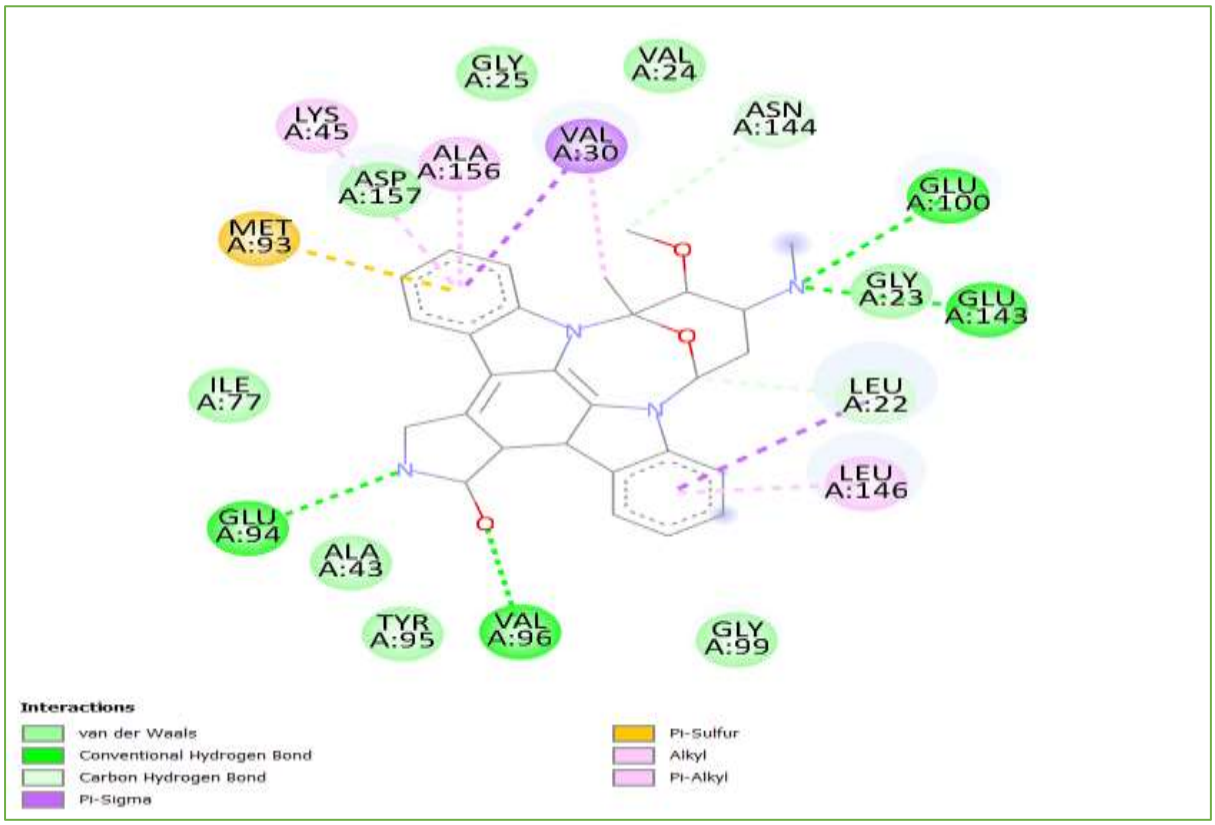


(a)2D interaction

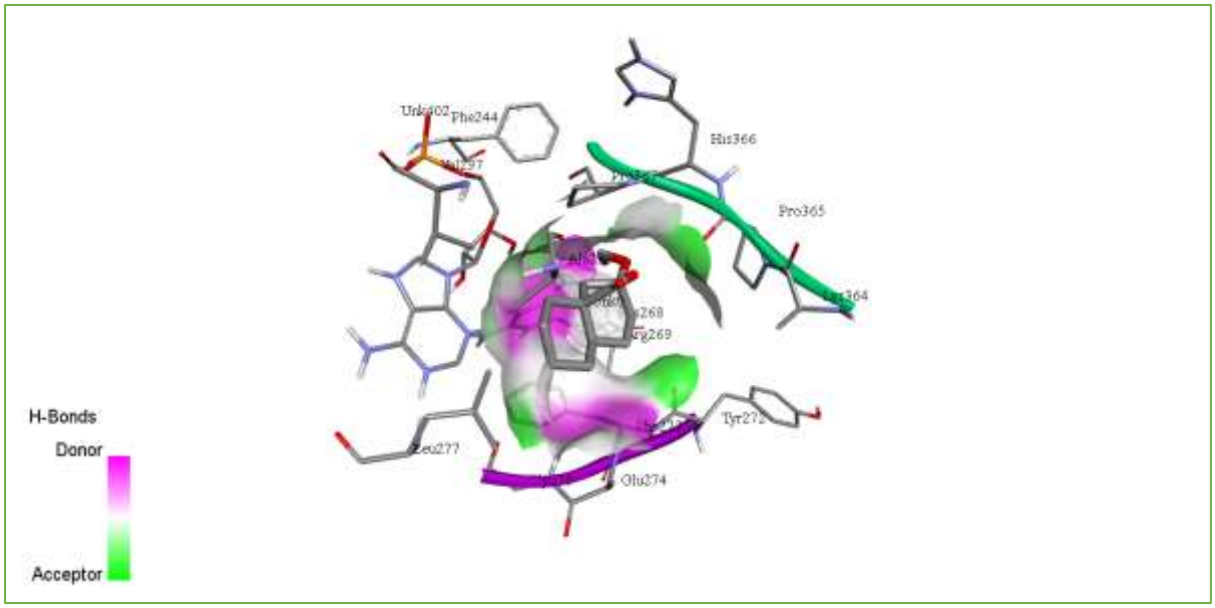


(b) 3D interaction

5) Interactions of 9-Ethoxy-10-oxatricyclo [7.2.1.0 (1,6) dodecan-11-one with 7MYJ.

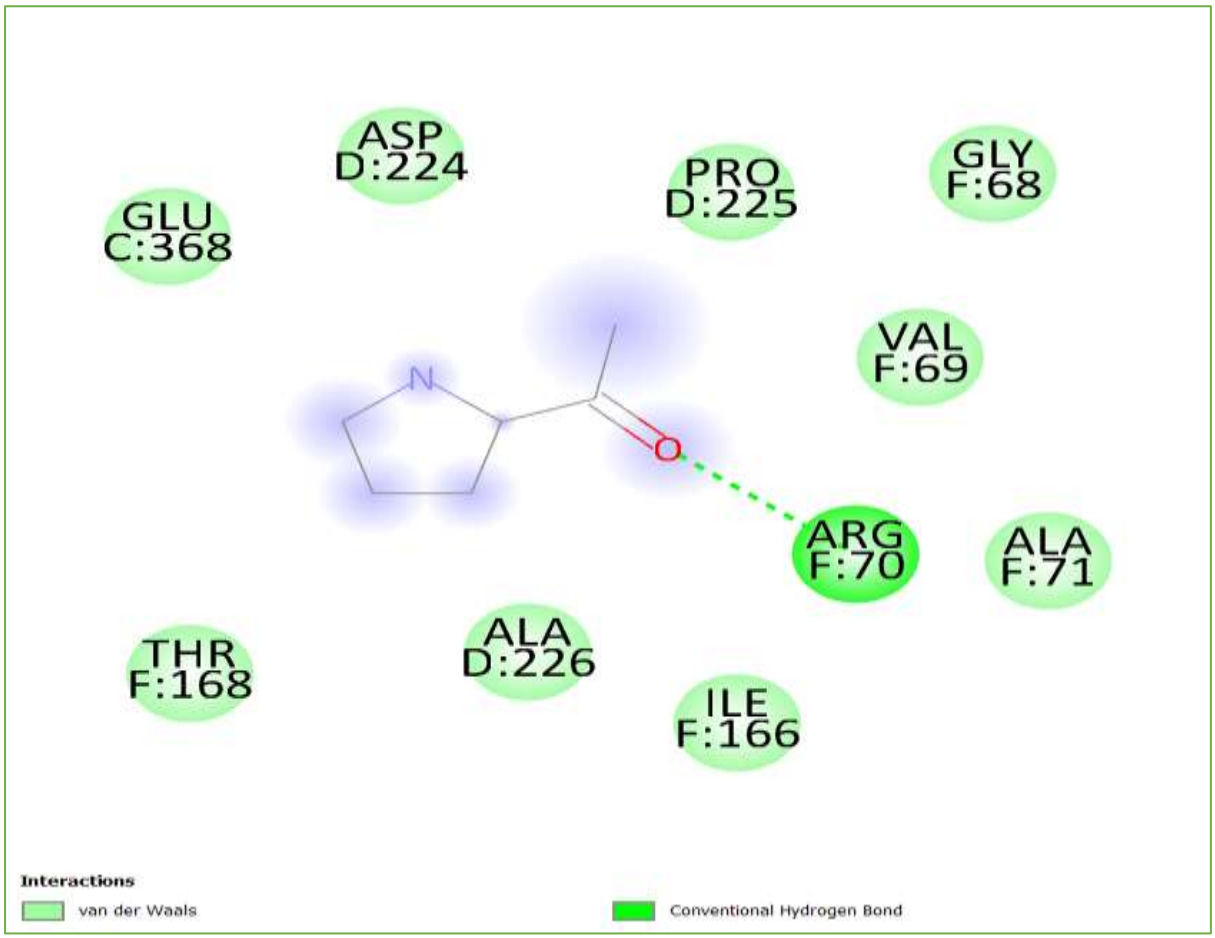


(a)2D interaction

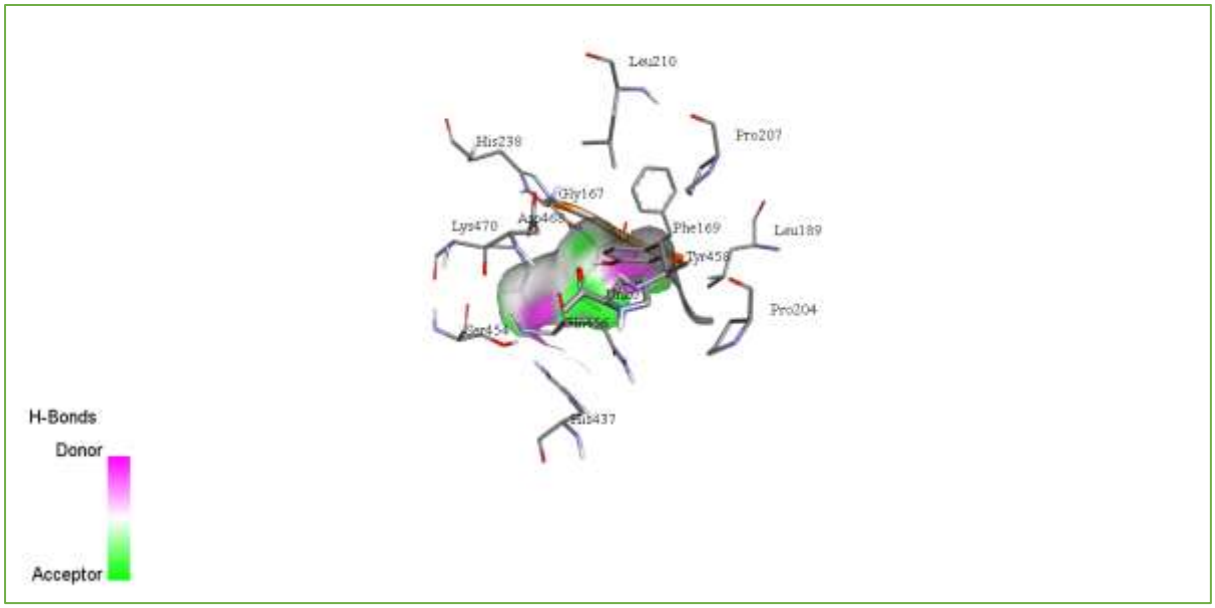


(b) 3D interaction

6) Interactions of 2-Acetylpyrrolidine with 7MYJ.

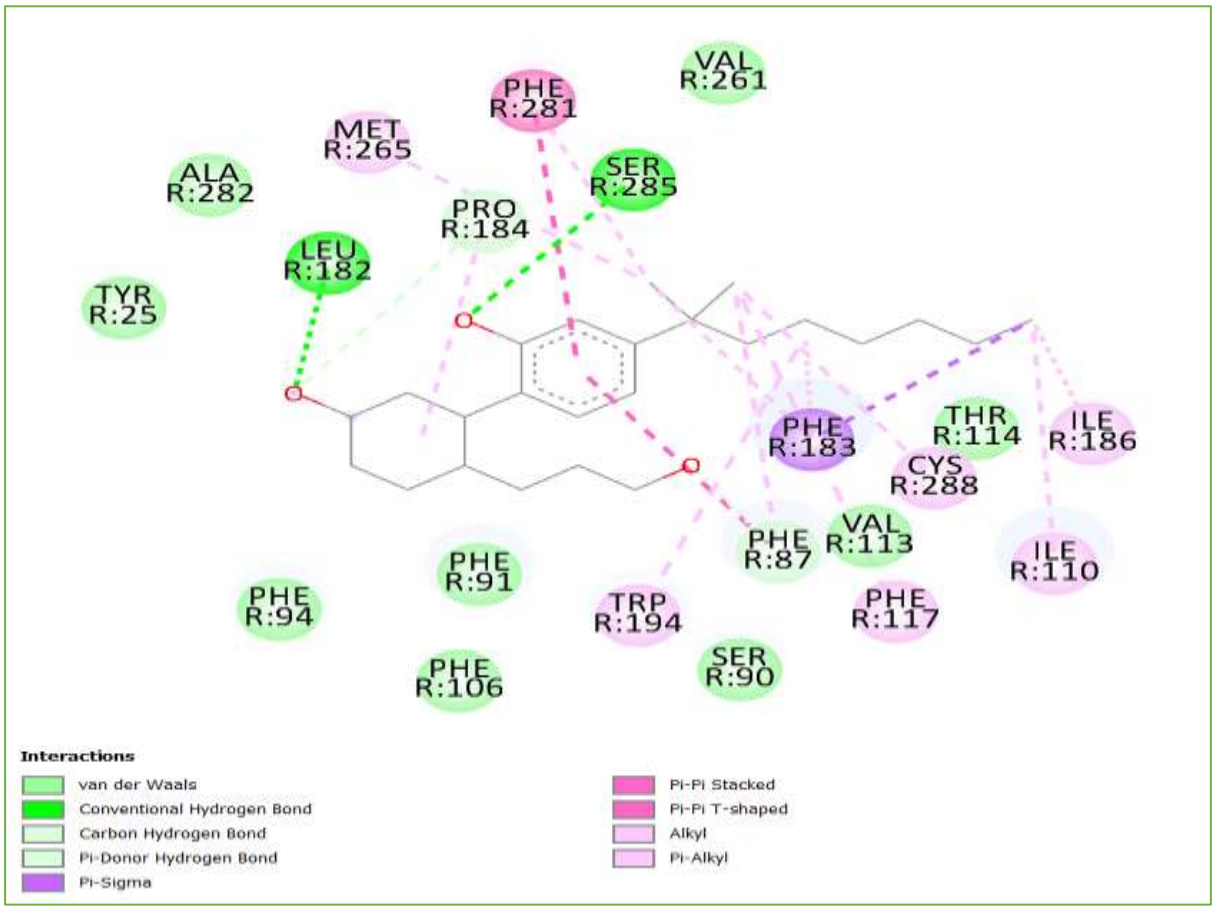


(a)2D interaction

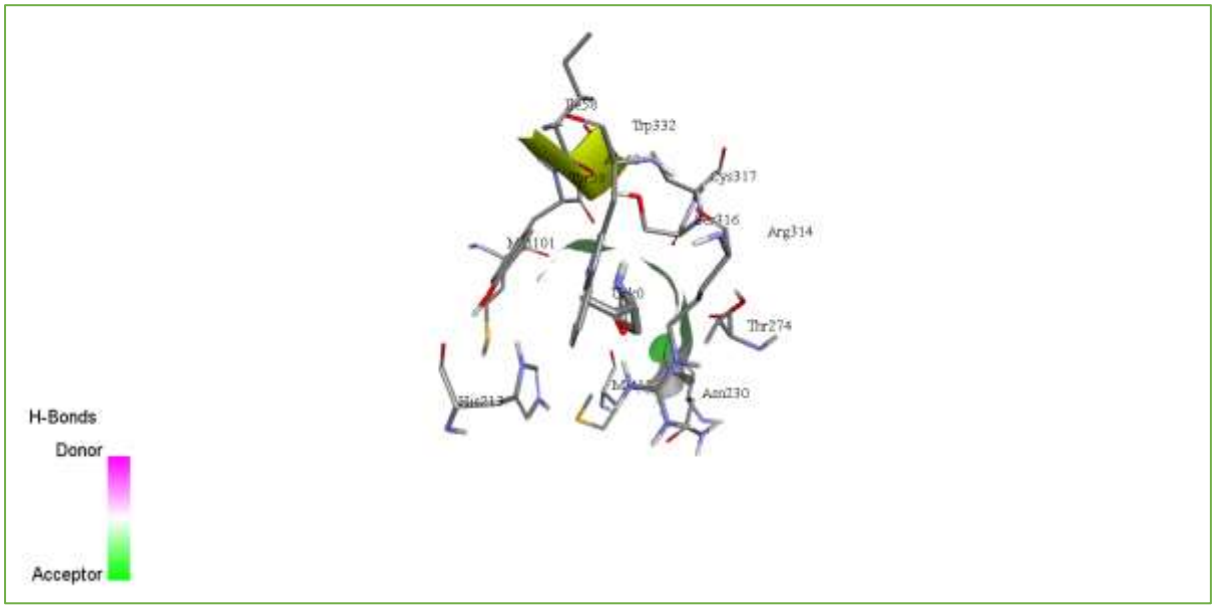


(b) 3D interaction

7) Interaction of 2-Acetylpyrrolidine with 8GUR.



(a)2D interaction



(b) 3D interaction

CONCLUSION:

The study on Sauropus androgynus leaves highlights its rich phytochemical composition and potential therapeutic applications through both experimental and computational approaches. Phytochemical screening revealed the presence of alkaloids, amino acids, carbohydrates, flavonoids, saponin glycosides, tannins, terpenoids, phenols, resins, phlobatannins, and vitamins B2 and C in the hydroalcoholic extract, affirming its bioactive potential. In silico analysis using network pharmacology identified 125 phytoconstituents, with 33 exhibiting favourable drug-likeness scores. Key bioactive compounds such as ascorbic acid, riboflavin, thiamine, chlorogenic acid, and rutin were highlighted for their potential therapeutic roles. Molecular docking studies further supported these findings, demonstrating significant binding energies of selected compounds with proteins NFKB1, CNR2, and ACACA implicated in various biological pathways. Notably, 9-ethoxy-10-oxatricyclo [7.2.1.0] dodecan-11-one showed strong interactions with NFKB1 and ACACA, indicating its potential as a bioactive agent. Riboflavin and thiamine also exhibited substantial binding with NFKB1. These results underscore Sauropus androgynus as a promising source of nutraceuticals and pharmaceuticals, bridging traditional knowledge with modern scientific validation. Its diverse phytochemical profile and molecular docking insights suggest avenues for developing new therapeutic agents and dietary supplements, leveraging its antioxidant, antimicrobial, and other health-promoting properties. Further research could focus on validating these findings through clinical studies, potentially translating them into practical applications for human health and wellness.

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