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The in-silico approaches; structural, functional proteins-association elucidation of Moringa oleifera phytochemicals against the tyrosine kinase receptor protein of Diabetes mellitus

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Abstract: The *Moringa oleifera* also called “Drumstick tree”, to its various pharmacological uses and nutritional adaptability worth is comprehensively all over the earth. The tree parts; stem, bark, gum, roots, and mostly leaves are great provenance of vitamins, minerals, and numerous clinically beneficial secondary-metabolites and also a significant role in diabetic-resistance. The virtual-study may exist significant in terms of expanding the number of successful antidotes derived through this herb and plan to obtain the potent-phytochemicals amalgam of miracle tree even an agent for the curative potential opponent the Diabetes-

Mellitus (DM) by computational screening. The structure of the top three selected phytochemicals was extracted from previous works of literature, Drug Bank database, PubChem-database, and screened with mutated protein from PDB structure (Crystal structure of insulin receptor kinase domain in complex with cis-(R)-7-(3-(azetidin-1-ylmethyl) cyclobutyl)-5-(3-((tetrahydro-2H-pyran-2-yl)methoxy)phenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine) through PyRx-docking tool. After, these three potent compounds: Anthraquinone, Serpentine, and Laurifolin were obtained, which showed successful binding within the targeted protein's active binding pocket; Anthraquinone-chain A: ASP-1177 aa (amino acid-complex) site, Laurifolin-Chain A: ASP-1110 aa, and Serpentine-Chain A: MET-1103 aa. The main features of the pharmacophore model based on ligands were revealed showed i.e. through molinspiration, swiss adme, admetSAR and exhibited acceptable drug-like properties; HBA (4,2,2), HBD (0,2,0), with the potent surface-binding active site: position A: 1159 by CASTp and structural visualized through Chimera Tool, and the protein functional network analysis of INSR Gene-associated with other via INSRR, IRS1, IRS2, SHC1, IGF1, PTPN1, INS, PTPN2, IGF1R, GRB14 of targeted plant Moringa oleifera against DM through STRING Database and gene-regulate expression were analyzed. Our finding proposes that docking potent these phytochemicals and gene functionality in M.oleifera may be utilized as a pharmaceutical candidate for diabetes and further investigate in future research.

Key words: Moringa oleifera, Screening analysis, phytochemicals, Pharmacokinetics, protein-association

Doi: 10.5281/zenodo.5516297

1.Introduction

Moringa oleifera Lam (suhanjna) the specie of Moringaceae family is a drought-resistant, rapidly-spreading tree of the Moringaceae lineage. M.oleifera is a most familiar and commonly

divided plant species. Around the world there are many different names of Moringa like Drumstick, Horseradish Sajna, Benzolive, Moonga, Marango, or Ben oil tree (Anwar & Bhanger, 2003; Mahmood, Mugal, & Haq, 2010). The extreme nutritional components, Industrial applications, medicative advantages are present in M.oleifera at a valuable socio-demographic interest. The Romanian, Greek, Egyptian physicians had M.oleifera as "phytotherapy" due to its distinct features as its parts are reported as highly effective bioactive compounds. M.oleifera is largely cultivated in various areas in the tropical (hot) regions and very popular as secondary metabolites because it is contributed remarkably to the therapeutic-usage of plant species in the primary-recovery process (Chhikara et al., 2020). The residents of Pakistan, Indian, Hawaii, Philippines, and Africa used M.oleifera's parts (roots, leaves, pods, gum, bark, and flowers) as edibles (Morton, 1991; Rani & Arumugam, 2017).

Due to its immense uses and suppleness *Moringa oleifera* is called a ''Miracle tree", because of its healthful and pharmaceutical values, it is widely planting globally. Many health beneficial secondary-metabolites enriched with vitamins, minerals are present in *M. oleifera* leaves, which are remarkably antibiotics having capabilities to fight against diseases. Thus, *computational* analysis significantly could expand productive antidiabetic drugs from this plant (Ogbe & Affiku, 2021).

DMT2 (Diabetes mellitus type-2) is a universally spreading health problem. People with DMT2 own a high threat in micro and macro-vascular tissues, (neuro-pathy, nephron-pathy, and retino-pathy) with cardio-vascular comorbidities respectively, causing higher blood glucose levels and regular-insulin abnormalities. During the zygote cell formation, the insulin whole-cell supplement receptor is raptured due to the DMTT2 inheritance. In accordance with our information still, no extensive in-silico investigation on several processes of metabolites of this targeted tree acts as an antihyperglycemic agent (Ben-Ami et al., 2017) (Swaminathan & Ng).

According to IDF (International Diabetes Federation) study, diabetes mellitus (DM) affects more than approximately 366 million people worldwide, with the forecast that this figure will grow to 552 million or more by 2030. DMT2 is an expanding worldwide health disorder with abnormal glucose tolerance, neuropathic, inadequate-insulin secretion, and microangiopathy. The deficiency of insulin does a key role in the disorders of metabolic-pathway linked with hyperglycaemia (higher blood sugar) and diabetic disorders (Cho et al., 2018).

These bioactive compounds like; carotenoids, flavonoids, glycosides, anthocyanin, steroids, alkaloids, tannins, saponins, terpenoids, and anthraquinone are found in almost all segments of *M.oleifera* having anti-cancer, anti-microbial, anti-oxidant, and anti-diabetic properties. These phytochemicals play a crucial role in the restriction of several clinical terms viz; diabetes, arthritis, cardiorespiratory diseases, and, tumors (Chandran, Meena, Barupal, & Sharma, 2020; S. Kumar, 2017).

There is no systematic screening analysis on various active-metabolic compounds of M. oleifera as an anti-diabetic product that we are aware of. Consequently, we examined some of the plant's main phytochemicals and compared them to the diabetes-mellitus mutated protein (MP). Our studies reveal further phytochemical research in this plant species by allowing as a means to integrate modification of plant-derived chemical substances (PDCS) as well as the afresh fusion of repeating unit of the targeted protein and network analysis of targeted gene.

2.Materials and Methods

2.1 Gene Finding

The RTKF (receptor tyrosine kinase protein family) coding gene INSR was retrieved through Human GeneDatabase-GeneCards (Safran et al., 2010). The causes of the several severe inherited insulin-resistance syndromes by mutation gene. Ligands and insulin bind with its

receptor by its pathway of signaling put-on, which manage the absorbing and production of glucose also storage and, synthesis of proteins, carbohydrates, and lipids.

2.2 Protein active sites prediction, and selection of protein

The protein was selected as following parameters R-Value Free: 0.198, R-Value Work: 0.171, R-Value Observed: 0.172, X-RAY DIFFRACTION with resolution 1.79 Å of the Crystal structure of insulin receptor kinase domain in complex with (Protein Data Bank) RCSB PDB ID: 5HHW was obtained. The IR (Insulin receptor-sense) is a type in which append trans-membrane receptors area, which encompasses to immense types of kinase of tyrosine (KT) receptor via shiftily in cancer and important in diabetes (metabolism) with IG_1, IG_2 (Kouranov et al., 2006). And the active site of 5HHW was predicted through CASTp 3.0 tool (Dundas et al., 2006).

2.3 Protein structure evaluation, Motif Prediction

The retrieved protein of insulin receptor-kinase was evaluation through SAVES v6.0 Tools (https://saves.mbi.ucla.edu/) with ERRAT and Verify-3D with RCSB-PDB: 5HHW, the targeted protein motif prediction by Motif Search (Kouranov et al., 2006) and the secondary structure prediction through online-tool SOPMA (Geourjon & Deleage, 1995).

2.4 Plant Selection

The health phytochemical plant is *Moringa oleifera* also called Drumstick tree is well recognized with health-beneficial characteristics. The *Guilandina moringa L* different sections of this plant such as; leaves, flowers, roots, and seeds are not well-useful for consuming, but own notable potential to treat different diseases. Many various bio-active medicinal compounds have been isolated and pick out from M. oleifera plant parts (Anwar, Latif, Ashraf, & Gilani, 2007).

2.5 Preparation of phytochemical structure of *Moringa oleifera*

The structural preparation with different phytochemicals of *Moringa oleifera* as; Serpentine, Laurifolin and Anthraquinone were analyzed, and extracted by using Drug bank, and Database of PubChem (Kim et al., 2016; Wishart et al., 2008).

2.6 Toxicity profiling

The molecular-based physiochemical properties and drug likeliness properties of best docking phytochemicals were analyzed using the tool Molinspiration server (Reena Roy, Kandagalla, & Krishnappa, 2020), which gives a prediction based results 'rule of five' (Ro5) based on molecular properties such as; H-bond-acceptors fewer as less than 10, less than 05 H-bond-donors, MloP value less than 05%, and a molecular weightless and equal 500 (Daltons) (Adhikari et al., 2020). Further, the quantitative analysis including ADMET profiling of top selected compounds was *in-silico* based observed through SwissAdme and admetSAR (Cheng et al., 2012; Daina, Michielin, & Zoete, 2017; Patel, Patel, Patel, Patel, & Kalasariya, 2020). and the selected compound's bio-activity was analyzed through Molinspiration (Wadapurkar, Shilpa, Katti, & Sulochana, 2018). And the potent three takeout the phytochemicals of *ruwag* were analyzed by DatabaseProTOX_2 (Drwal, Banerjee, Dunkel, Wettig, & Preissner, 2014).

2.7 Protein network analysis and physicochemical prediction

The Physicochemical properties of obtained protein targeted structure of *Moringa oleifera* were predicted through ProtParam (Garg et al., 2016), and insulin receptor kinase domain in complex domain-protein predicted through Pfam (Bateman et al., 2004). The INSR protein of M.oleifera protein network analysis by using STRING_database (Szklarczyk et al., 2016).

2.8 Prediction of membrane-spanning and Coiled-protein regions

The given targeted protein structure of insulin receptor kinase domain, PDB_ID: *5HHW*, the inside membrane of biomolecule spinning region were analyzed through ExPASY-TMpred Tool (Seth, Sun, Ea, & Chen, 2005), and Coiled-Coil Regions in Proteins were analyzed through ExPSY-COIL server (Gasteiger et al., 2003).

2.9 Protein potent ligand docking and structural visualization

We top three-potent phytochemicals of Moringa oleifera that effectuated the rule of Lipinski of Ro'05 and screened with mutated target protein of INSR of (DM) diabetes mellitus through PyRx docking tool (Dallakyan & Olson, 2015) and interaction ligand observed by using LigX-interaction MOE (Molecular Operating environment) (Vilar, Cozza, & Moro, 2008), visualized through Discovery studio Visualizer (Studio, 2008), also UCSF-Chimera tool (Pettersen et al., 2004), The solved/predicted structure of insulin receptor_kinase ensue the minimized through the UCSF-Chimera version 1.4.1 visualizing tool by selecting steepest steps (1000), conjugate gradient steps (1000) and adding the gasteiger and hydrogen charges to remove clashes and unnecessary atoms from protein structure, and also MOE version 2010.12 (Pettersen et al., 2004; Vilar et al., 2008).

3.Results and Discussion

The selected Mutated protein insulin receptor-kinase with chain-A, with structural weight: 35.45 kDa, Number of amino acids: 307, atoms adds: 2800, Residues: 306, chain of protein: 1/A of RCSB PDB ID_5HHW (Kouranov et al., 2006) with active site position A: 1159 by CASTp and visualize through Chimera Tool (Pettersen et al., 2004)are shown in figure no 01.

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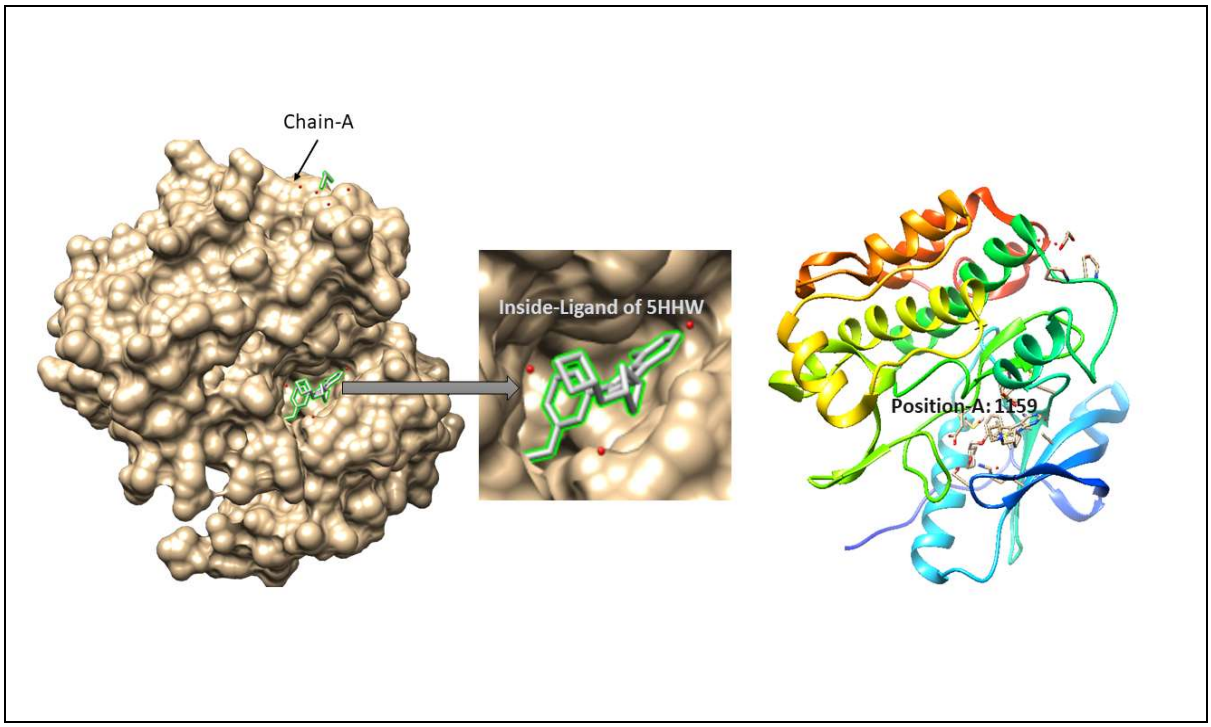

Figure 01: Cis-(R)-7-(3-(azetidin-1-ylmethyl) cyclobutyl)-5-(3-((tetrahydro-2H-pyran-2-yl)methoxy)phenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine (RCSB PDB ID: **5HHW**) through Chimera-Tool

After that the retrieved structural protein was evaluated by SAVES v6.0, the results of Verify 3D are pass with 94.44% of the residues-profiling and ERRAT_Tool are outcomes against the insulin receptor Kinase was Overall Quality Factor: 96.8421 (Pass) are shown in figure no 02.

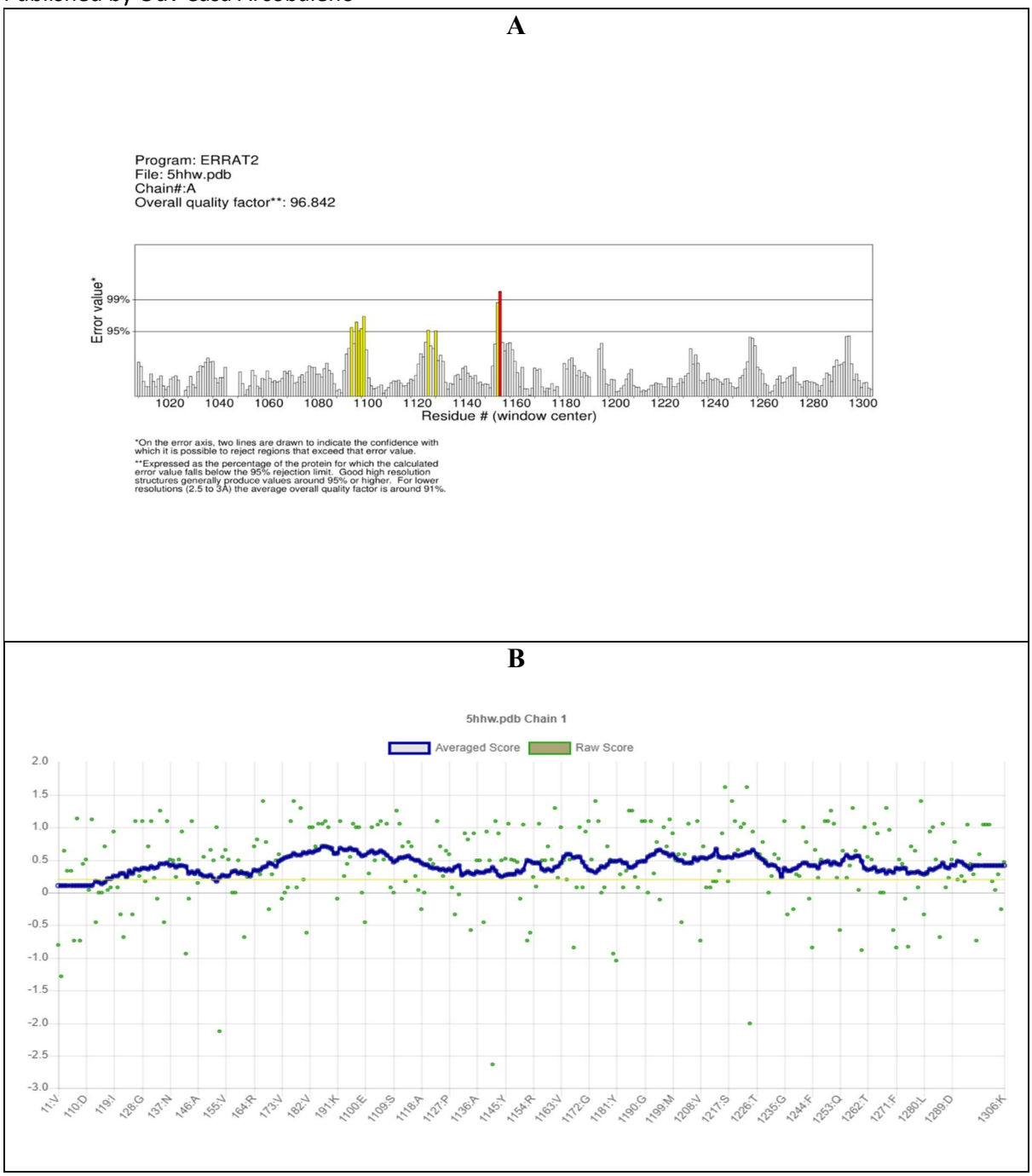

Figure 02: The targeted protein: RCSB PDB ID: 5HHW: evaluation results through SAVES v6.0; **A**: ERRAT (Overall Quality Factor 96.8421) and **B:** Verify3D Tools (94.44% of the residues have averaged 3D-1D score >= 0.2)

And the no motif was found in NCBI-CDD, no motif was found in Pfam of the targeted protein. The targeted protein (PDB ID: 5HHW) through CASTp results following as; nucleotide phosphate-binding region A: 1104-1110, active site A: 1159-Proton donor/acceptor, binding

site A: 1033, modified residue A: 1011-Phosphotyrosine: autocatalysis site, sequence variant-A: 1035G->V, DBSNP; rS_121913135, in typeA-IRAN with the interaction of GRB_7 via; amino acid sequence numbers is shown in figure no 03.

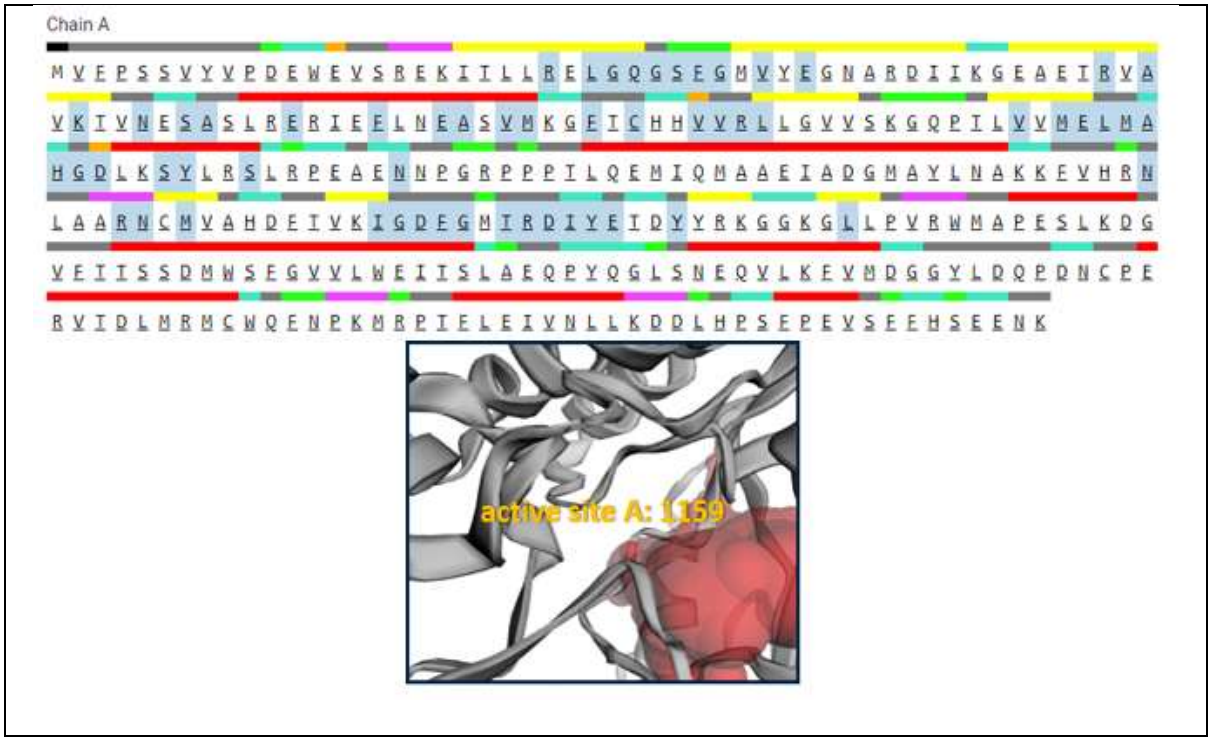

Figure 03: The Computed Atlas of Surface Topography of proteins results of target protein (5HHW) with active site no. of **A: 1159**

And some other medicinal uses of Moringa oleifera plant with different sections are shown in table no 01.

Table 01: Medicinal phytochemicals uses of certain parts of Moringa *oleifera* plant

Parts of plant	**Uses**	**References**
Leaf	Purgative, as poultice to sores, eye and ear infections used for piles, sore throat, bronchitis, fevers, scurvy and catarrh;	(Anwar et al., 2007; Kasolo,

	leaf juice is believed to reduces the swelling of glandular, control glucose levels.	Bimenya, Ojok, Ochieng, & Ogwal-Okeng, 2010)
Roots	Anti-inflammatory, Antilithic, circulatory tonic; used to treat rheumatism, articular pains, backache, kidney pain and constipation. cardiac, juices for toothache, earaches, and has anti-tubercular mechanisms	(Anwar et al., 2007)
Seed	In liver diseases	(P. S. Kumar, Mishra, Ghosh, & Panda, 2010)
Flower	Cure inflammations, tumors, spleen enlargement, hysteria and stimulant acts	(Anwar et al., 2007)
Bark of stem	Eye's diseases, tubular tumours, tuberculosis and ulcer	(Anwar et al., 2007; Kumbhare, Guleha, & Sivakumar, 2012; Satish,

		Kumar, Rakshith, Satish, & Ahmed, 2013)
Plants extract Gum	For Headaches, fevers, asthma, syphilis, rheumatism, astringent, rubefacients and intestinal problems	(Anwar et al., 2007; Liu, Wang, Wei, Gao, & Han, 2018)

The three potent phytochemicals of M. oleifera (Anwar et al., 2007) structural representation via; Serpentine, Laurifolin, and Anthraquinone through PubChem Database and Drug Bank database and visualized through PyMol tool (DeLano, 2002) is shown in figure no 04.

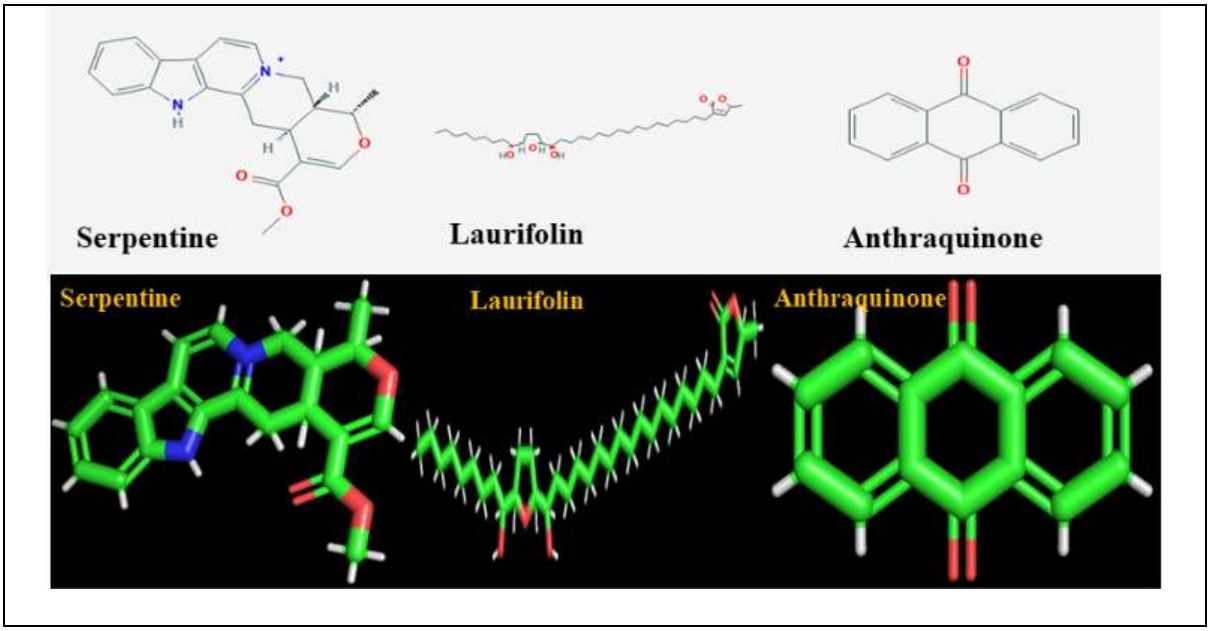

Figure 04: The three potent phytochemicals of M. oleifera of Serpentine, Laurifolin and Anthraquinone through PubChem Database and Drug Bank database (2D, 3D Structure) and visualized through PyMol

ADMET drug-likeness analysis of selected top three phytochemicals of *Moringa oleifera* was performed through molinspiration tool (Ubani et al., 2020) based on Lipinski rule of five (Ro5). The purposed top-phytochemicals displayed 0,1 violations to Lipinski's Ro5 (Lipinski, 2016) and exhibited acceptable drug-like properties like HBA (4,2,2), HBD (0,2,0), MloP values (0.64,0.64,6.00) are shown in Table no 02.

Table 02: Three potent phytochemical of *M. oleifera* Lipinski rule of five

Compounds	**Molecular weight (g/mol)**	**Number of HBA**	**Number of HBD**	**MLogP**
Lipinski rule of five	**<500**	**<10**	**<5**	**<5**
Serpentine	16.04	4	0	0.64
Laurifolin	16.04	2	2	0.64
Anthraquinone	371.52	2	0	6.00

Furthermore, pharmacokinetic properties were predicted through the Swissadme server for the validation of phytochemicals' drug-likeness (Saini, Sivanesan, & Keum, 2016) (GI-absorption of GI, permeant_BBB, P:gp of the substrate, CYPI_A2, CYP2_C19, CYP_3:A4-inhibitors, Kp of log, ghose, veber, egan, mugge, bioactivity scores, sub-cellular localization of the top-hits against the DM) are shown in Table no 03.

Table 03: ADMETSAR Profiling the prediction of top 03 phytochemicals of M. oleifera

Compounds	**Serpentine**	**Laurifolin**	**Anthraquinone**
GI absorption	High	Low	High
BBB permeant	Yes	No	Yes
P-gp substrate	Yes	No	No
CYP1A2 inhibitor	Yes	Yes	Yes
CYP2C19 inhibitor	No	No	No
CYP2C9 inhibitor	No	No	No
CYP2D6 inhibitor	No	No	No
CYP3A4 inhibitor	No	No	No
Log K_p (skin permeation)	-6.24	-1.34	-5.16
Ghose	Yes	No	Yes
Veber	No, TPSA>140	No	Yes
Egan	No,TPSA>5.88	No	Yes
Muegge	Yes	No	Yes
Bioavailability Score	0.85	0.17	0.55
Subcelluar localization	Mitochondria	Mitochondria	Mitochondria

The graphical-representation of dose-distribution of three potent phytochemicals of *M. oleifera* is shown in figure no 05.

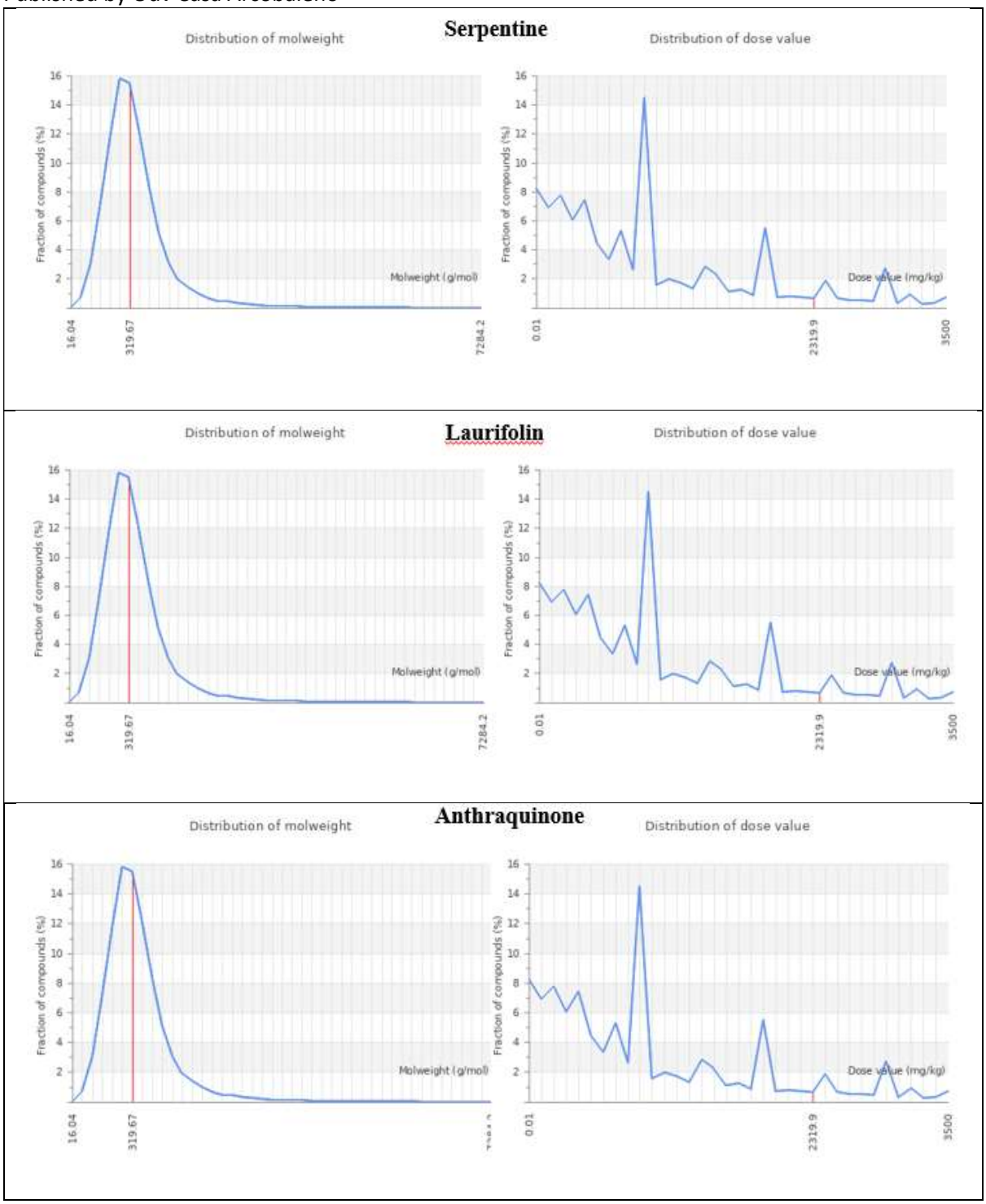

Figure 05: The graphical representation of dose distribution of three potent phytochemicals of M. oleifera (**black value** of input compound, **red value** of dataset)

Next, toxicity assessment of the top-ranked potential compounds obtained after the docking analysis with different toxicity-modules. These selected top compounds bioactivities result in PAINS: 0 alerts, Brenk: 1 alert: quaternary_nitrogen_3, Leadlikeness, yes, Synthetic

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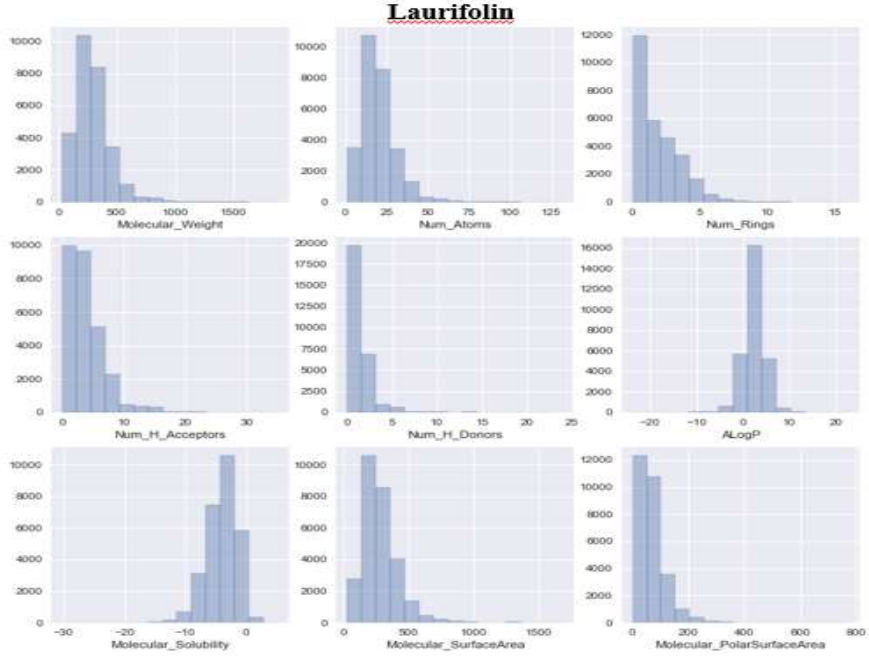
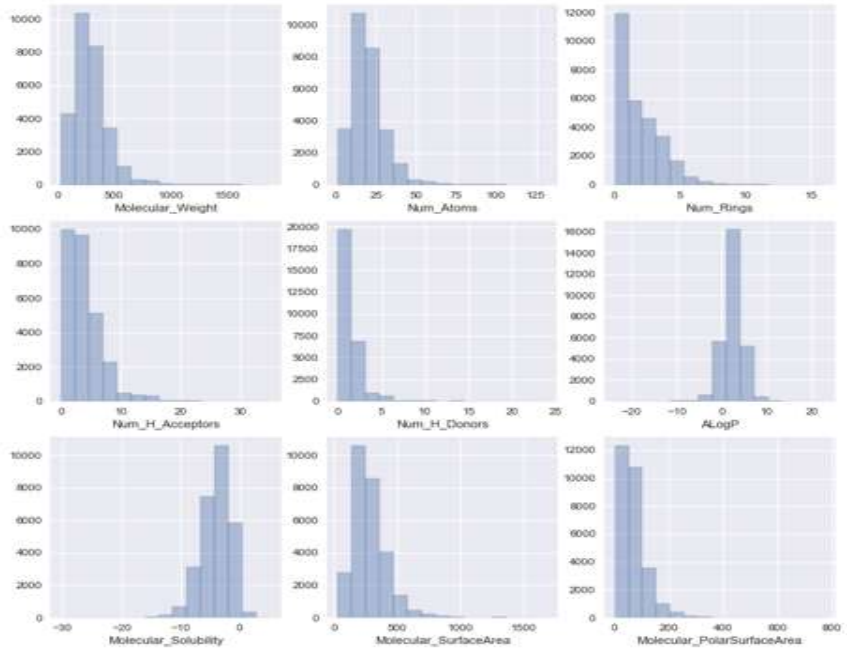
accessibility: 4.36, 6.94, 2.07 and, graphical representation admetSAR Applicability Domains

are shown in figure no 06.

Laurifolin



Anthraquinone



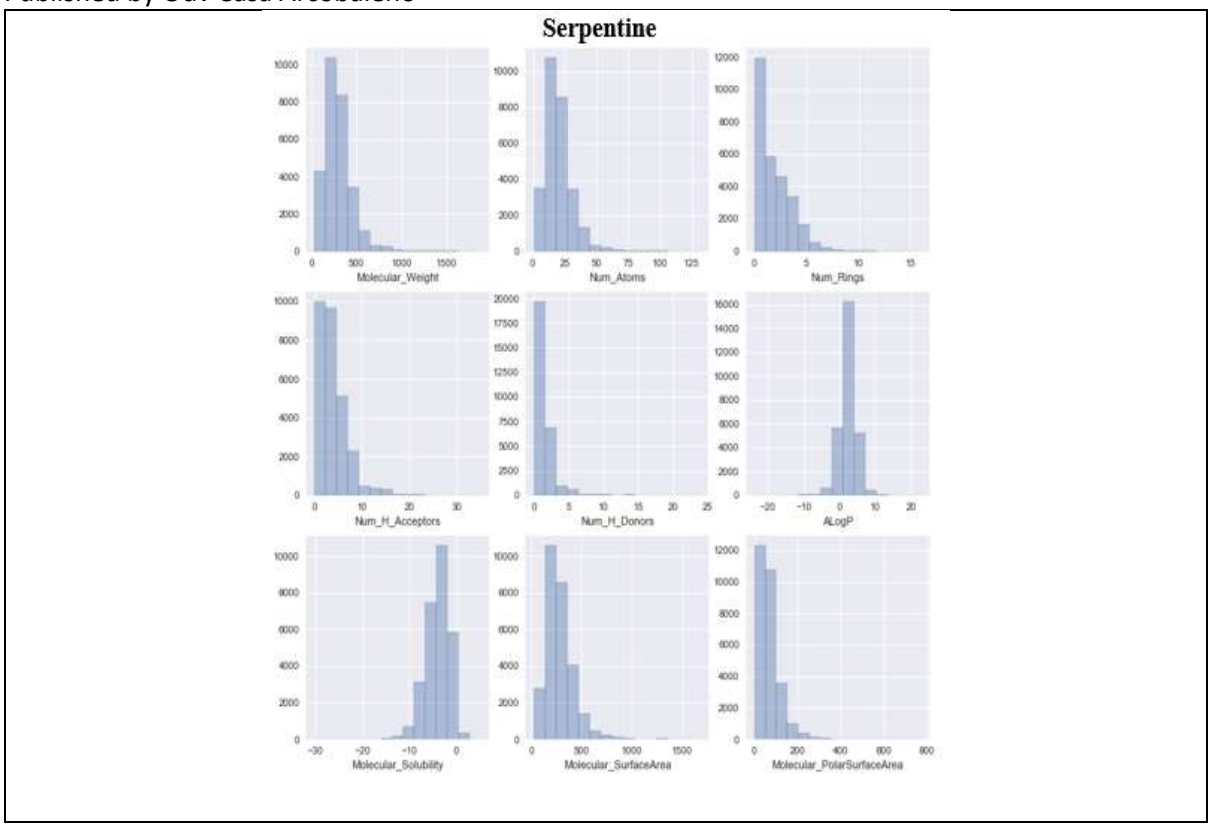


Figure 06: Toxicity profiling of three phytochemicals of graphical representation admetSAR Applicability **Domain variation-graph** in M. oleifera

The analysis revealed that physicochemical properties such as *M. oleifera*-protein length were 307, Theoretical pI: 5.49, amino acids having 34898.05 Dalton molecular weight. The GRAVY score and instability index was computed as (II) is computed to be 39.23, and Formula: C1554H2422N418O456S20, atoms of total: numbers: 4870, that classifies the protein as stable protein depicting those hydrophilic residues can establish hydrogen bonds through Protparam tool are shown in Table no 04.

Table 04: The Physicochemical properties of targeted protein structure in diabetes mellitus

Parameters	**M. oleifera**
Mol. Weight	34898.05
No. of amino acids	307
Theoretical *pI*	5.49

Instability index (II)	39.23
No. of Negatively Charged Residues (Asp+Glu)	42
No. of Positively Charged Residues (Arg+Lys)	34
Aliphatic Index	79.32
Grand average of Hydropathicity (GRAVY)	-0.289
Atomic Composition	Carbon **C** 1554 Hydrogen **H** 2422 Nitrogen **N** 418 Oxygen **O** 456 Sulfur **S** 20
Amino Acid Composition	Ala (A) 17 5.5%, Arg (R) 18 5.9%, Asn (N) 13 4.2%, Asp (D) 5.2%, Cys (C) 4 1.3%, Gln (Q) 9 2.9%, Glu (E) 26 8.5%, Gly (G) 21, 6.8%, His (H) 7 2.3%, Ile (I) 10 3.3%, Leu (L) 28 9.1%, Lys (K) 16 5.2%, Met (M) 16 5.2%, Phe (F) 15 4.9%, Pro (P) 17 5.5%, Ser (S) 19 6.2%, Thr (T) 14 4.6%, Trp (W) 5 1.6%, Tyr (Y) 9 2.9%, Val (V) 27, 8.8%, Pyl (O) 0 0.0%, Sec (U) 0 0.0% (B) 0, 0.0%, (Z) 0, 0.0%, (X) 0, 0.0%

And insulin_receptor_kinase_domain in complex with domain with Chain-A: 1023-1290, P06213: 1023-1290, Protein family: amino acid; PK_Tyr_Ser-Thr (PF07714) predicted through Pfam (Bateman et al., 2004) are shown in figure no 07.

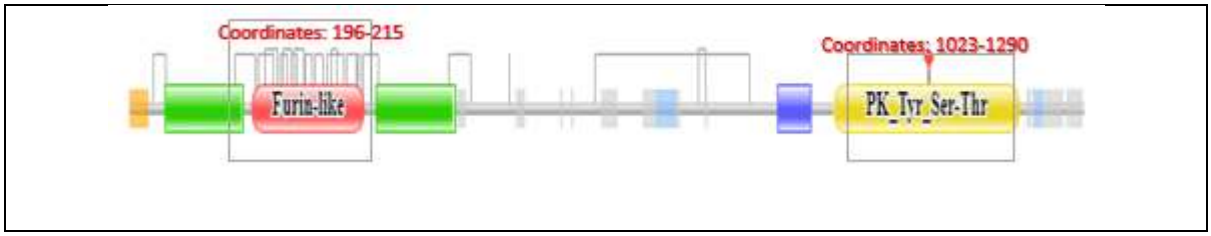


Figure 07: The structure of insulin receptor kinase domain in complex with cis-(R)-7-(3-(azetidin-1-ylmethyl)cyclobutyl)-5-(3-((tetrahydro-2H-pyran-2-yl)methoxy)phenyl)-7H-pyrrolo[2,3 d]pyrimidin-4-amine protein domain of Chain A (UniProt entry INSR_HUMAN): the domain of in **pink-reddish color; Furin-like, light-yellow color;** PK_Tyr_Ser-Thr.

The protein functional associated network analysis of targeted Protein of INSR was analyzed through STRING DATABASE (Mering et al., 2003), which results shows that; Insulin receptor; Src-homology-2 domains (SH2 domains) that identify unique phosphotyrosine residues, and the INSR Gene-associated with other via: INSRR, IRS1, IRS2, SHC1, IGF1, PTPN1, INS, PTPN2, IGF1R, GRB14. The network stat is as; the nodes: 11, edges: 48, average_degree of nodes: 8.73, avg. local clustering coefficient: 0.917, expected number of edges: 14, enrichment_ PPI p-v: 1.05e-12, and coExpression of score of the gene by RNA_expression analysis and regulation of Gene-INSR provided by Proteome_HD (Kustatscher et al., 2019) and Gene-COOCCURRENCE of INSR analysis network. (part: A, B, C) are shown in figure no 08.

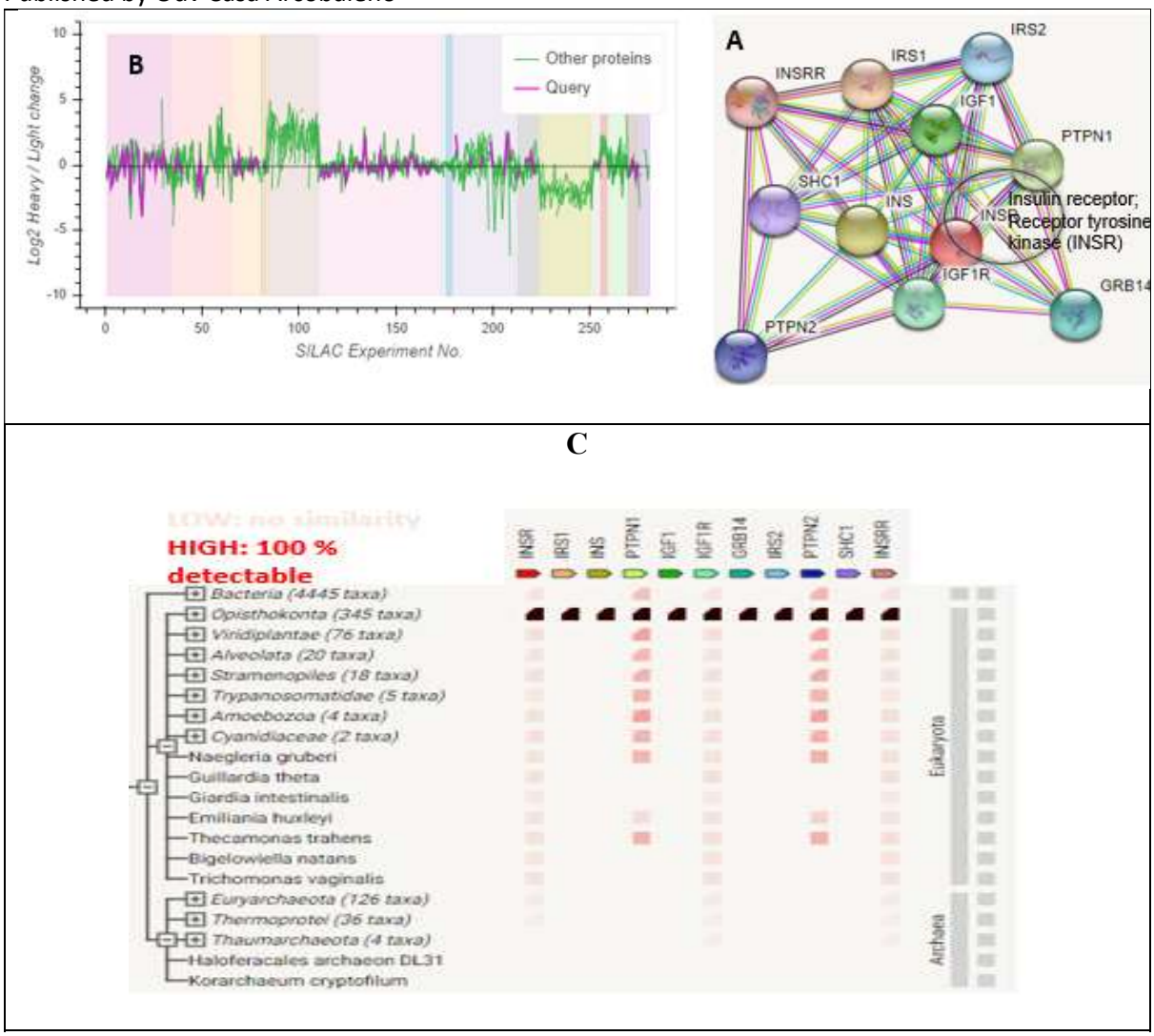


Figure 08: The functional network analysis of INSR by using String Database; **A**: INSR (Insulin receptor; Receptor tyrosine kinase), **B:** Co-expression analysis, Behavior of co-regulated proteins Heatmap in ProteomeHD, Groups of experiments are color-coded, **C:** GENE COOCCURRENCE of INSR analysis network.

The targeted structure of IRKD complex by using TMpred tool to predicts of membrane region of spanning and orientation, the results of IRKD; Sequence: MVF...ENK, length: 307, Prediction parameters: TM-helix length between 17 and 33, (209-212) 230 (230) 1030 220, and the outside region to 386-219, and protein coiled-coil regions graphs (Part: A, B) are shown in figure no 09.

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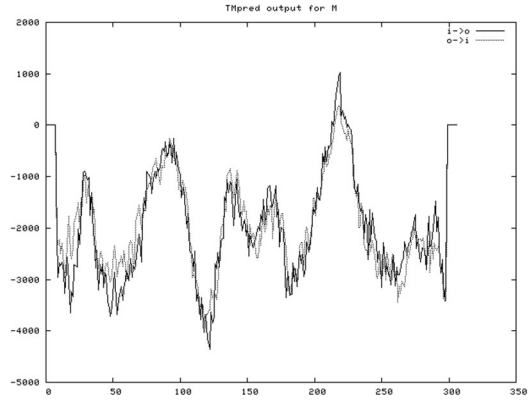
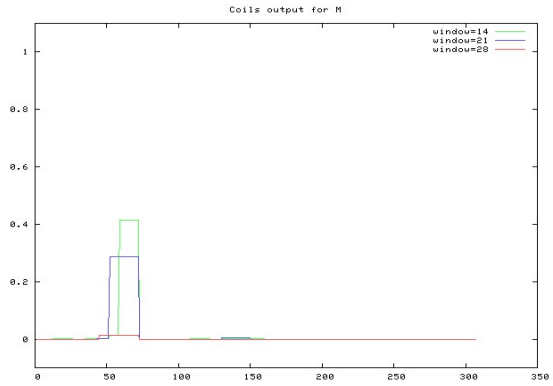
A: The structure of insulin receptor kinase domain in complex with cis-(R)-7-(3-(azetidin-1-ylmethyl)cyclobutyl)-5-(3-((tetrahydro-2H-pyran-2-yl)methoxy)phenyl)-7H-pyrrolo[2,3 d]pyrimidin-4-amine protein of membrane regions	**B:** The structure of insulin receptor kinase domain in complex with cis-(R)-7-(3-(azetidin-1-ylmethyl)cyclobutyl)-5-(3-((tetrahydro-2H-pyran-2-yl)methoxy)phenyl)-7H-pyrrolo[2,3 d]pyrimidin-4-amine protein of coiled-coil protein regions in graph plot color of red, blue, green
	

Figure 09: The result of membrane-spanning regions and their orientation of insulin receptor kinase domain in complex with cis-(R)-7-(3-(azetidin-1-ylmethyl)cyclobutyl)-5-(3-((tetrahydro-2H-pyran-2-yl)methoxy)phenyl)-7H-pyrrolo[2,3 d]pyrimidin-4-amine protein. **A:** In graph: helices shown in brackets are considered insignificant. A "+" symbol indicates a preference of this orientation. A "++" symbol indicates a strong preference of this orientation. The inside->outside | outside>inside membrane regions are: 209-230 (22) 1030 ++| (209- 231 (23) 386). **B:** In graph: coiled-coil protein region in targeted protein.

The secondary-structure of the coordinative protein model were through SOPMA_Tool are results shown in Table no 05.

Table 05: The secondary structure of targeted protein of receptor kinase data **(%Percentage)**

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Structural information	No.	Percentage %
Alpha helix-Hh	130	42.35
3_{10} helixGg	0	0
Pi helix-Ii	0	0
Beta bridge-Bb	0	0
Extended strand-Ee	42	13.68
Beta turn-Tt	20	6.51
Bend region-Ss	0	0
Random coil-Cc	115	37.46
Ambiguous states-?	0	0
Other states	0	0

And the figure no 10.

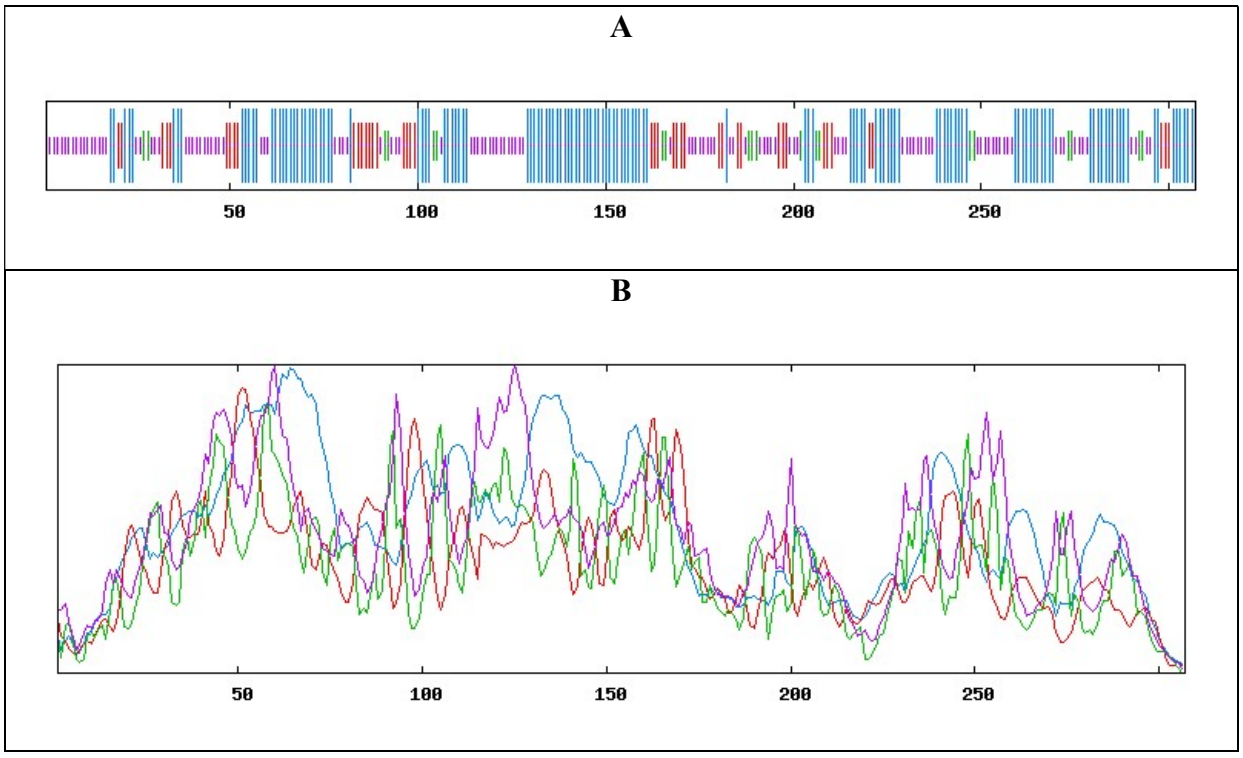

Figure 10: A: structural, no of amino acid with different color. **B:** highlighted with color like: alpha-helix, $3_{10,}$ pi-helix: dark-blue, beta bridge, extended strand: red, beta turn: black, bend region, random coil: orange, others: green

The 3D coordinate protein structure for the peripheral and rotation position of transmembrane proteins through OPM Server, as results shows that: hydrophobic thickness or depth are 2.2 ± 1.0 Å, Angle is: 52.± 8.°, Δ-Gtransfer: -3.6 kcal/mol, membrane residue embedded are: A-52-segments-1065 with heteroatoms included (Lomize, Pogozheva, Joo, Mosberg, & Lomize, 2012; Seth et al., 2005). The three top phytochemicals were docked through the PyRx tool, in which the protein-ligand docking can select the targeted compounds; M. oleifera of Serpentine, Laurifolin, and Anthraquinone through PubChem Database and Drug Bank database at a drug-design and discovery mechanism, these tops validate models were executed through PyRx tool (Dallakyan & Olson, 2015) to discover finally critical binding interaction with mutated protein with receptor and ligand complexes. The interaction of binding complexes through MOE-LigX interaction and potent site of phytochemical in protein were discovered from Discovery studio Visualizer tool by using ligand-receptor-interaction as; Anthraquinone-chain A: ASP-1177 aa (amino acid-complex) site, Laurifolin-Chain A: ASP-1110 aa, and Serpentine-Chain A: MET-1103. The screening successfully results were compared and analyzed through the UCSF-Chimera tool and Discovery studio Visualizer tool (Pettersen et al., 2004; Studio, 2008) and all binding pockets complexes are shown in figure no 11.

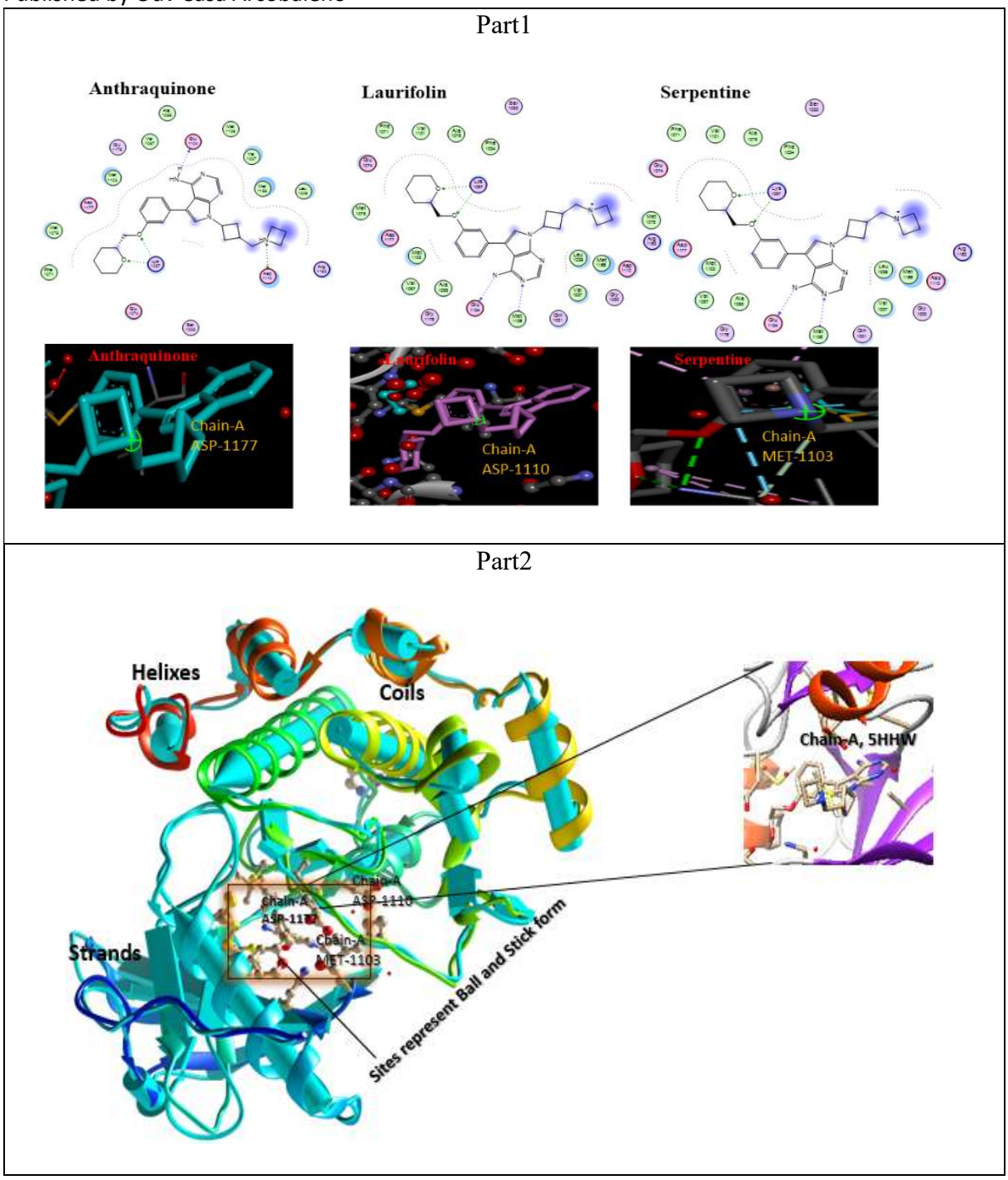

Figure 11: The potent three docking complexes of mutated target protein with binding sites; **Part1**: Anthraquinone-chain-A_ASP-1170, Laurifolin-chain-A_ASP-1110 and Serpentine-chain-A_MET-1103 AA (amino acid) also ligand interaction through LigX-MOE, docked with PyRx Tool and visualized from Discovery Studio Visualizer, **Part2**: the structural analysis through UCSF-Chimera Tool (the chain-A, binding interaction screening sites with

residual no's also color shows; helixes in red, coils in dark yellow orange, strands in light pink, blue and binding sites in ball, stick form: yellow, red, light white color) of *M. oleifera*

M.oleifera phytochemical analysis revealed the presence of biological-phytochemicals that facilitate gluconeogenesis in the tissue of perimetric, control the activity of carbohydrate enzymatic-metabolism, and support insulin secretion, probably affecting pleiotropic mechanisms to prevent diabetic complications. The anti_diabetic properties of phyto-based chemicals from M.oleifera were explored in this research, which was performed In-silico. Three hits' structures were derived from PubChem and Drug Bank databases, and their toxicity classes were determined. Docking analysis of potent phytochemicals with the modified-protein indicates that although it binds inside the active site there are undesirable bumps, suggesting insufficient interaction between amino acids and pharmaceutical elements. This analysis revealed that certain derivatives of phytochemicals become particularly protein-specific structure, they were designed to target Anthraquinone, Serpentine, and Laurifolin, respectively. The structural contour of a pharmacological-complex model, highlighting essential characteristics such as Aromatic ring, Hydrophobic, HBD, HBA, positive ionizable, protein-network analysis, functional analysis, membrane orientation, and many others necessary for receptor binding. These phytochemical pharmacophoric-properties will lead a role in the potential development of new anti-diabetic substances (Ehrman, Barlow, & Hylands, 2010; Nepolean, Anitha, & Emilin, 2009). Furthermore, computational elaboration showed that such studies play a crucial role in the formulation and procurement of drugs appropriate chemical compounds to treat a variety of diseases, including cardiovascular disease, pathogenic infections, cancer, viral diseases, neurodegenerative, diabetic disorders, and genetic disorders. Furthermore, the effectiveness of those phytochemicals that showed considerable potential was tested once more. Additionally, the potency of those phytochemicals that showed considerable potential was evaluated once more.

4.Conclusion

The pharmacokinetics, proteins-associate network, and screening analysis of *Moringa oleifera* have shown validated high potent bioactive-drug targets of this plant and obtained the three hits phytochemical-compounds Anthraquinone, Serpentine, and Laurifolin against the targeted DM's mutant enzyme (diabetes mellitus). These findings could pave the way for molecular modification of organic compounds as well as the newly synthesis of protein structural motifs and new phytochemical studies. The synthetic clusters were stable, and ligand sites appeared in the active binding pocket of the mutant protein. According to the results of the study, these filtered phytochemicals may be used as possible therapeutic active candidates to suppress diabetes mellitus in in-vivo and in-vitro research.

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