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The journal aims to address issues related to the third sector and community-based activities. The magazine therefore has the aim of spreading the culture of the third sector (voluntary organizations, social promotion associations, philanthropic bodies, social enterprises, mute aid companies, recognized and non-recognized associations, ecclesiastical bodies), the topics of interest are:

Social services

Health interventions and services

Education, health education and training

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Promotion of the culture of legality, peace between peoples, nonviolence and unarmed defence

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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

Muhammad Mazhar Fareed

Faculty of Life Sciences, Department of Bioinformatics and Biotechnology, Government College University, Faisalabad, Pakistan.

Maryam Qasmi

Faculty of Life Sciences, Department of Bioinformatics and Biotechnology, Government College University, Faisalabad, Pakistan.

Hira Zaheer

Department of Biochemistry, The Islamia University Of Bahawalpur, Pakistan

Hira

Faculty of Life Sciences, Department of Bioinformatics and Biotechnology, Government College University, Faisalabad, Pakistan.

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.

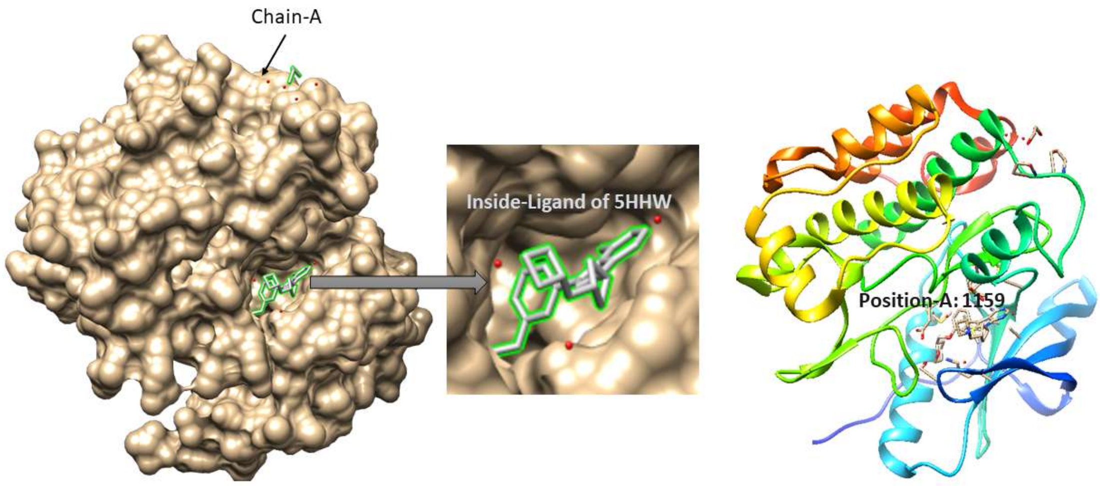
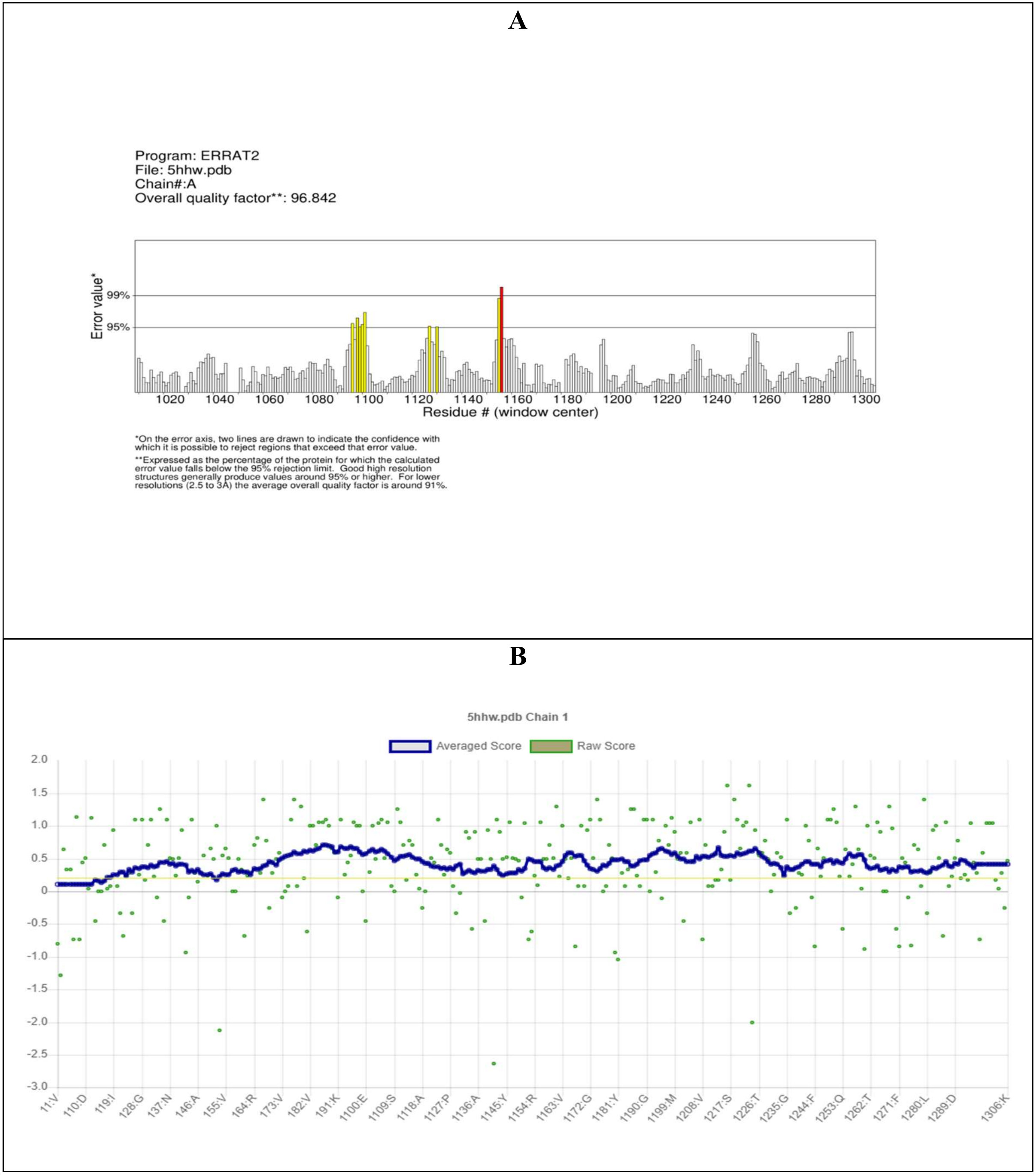


Figure 01: Cis-(R)-7-(3-(azetidin-1-ylmethyl) cyclobutyl)-5-(3-((tetrahydro-2H-pyran-2-yl)methoxy)phenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine (RCSB PDB ID: **5HHW**) through Chimera-Tool

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.



Program: ERRAT2
File: 5hhw.pdb
Chain#:A
Overall quality factor**: 96.842

*On the error axis, two lines are drawn to indicate the confidence with which it is possible to reject regions that exceed that error value.
**Expressed as the percentage of the protein for which the calculated error value falls below the 95% rejection limit. Good high resolution structures generally produce values around 95% or higher. For lower resolutions (2.5 to 3A) the average overall quality factor is around 91%.

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.

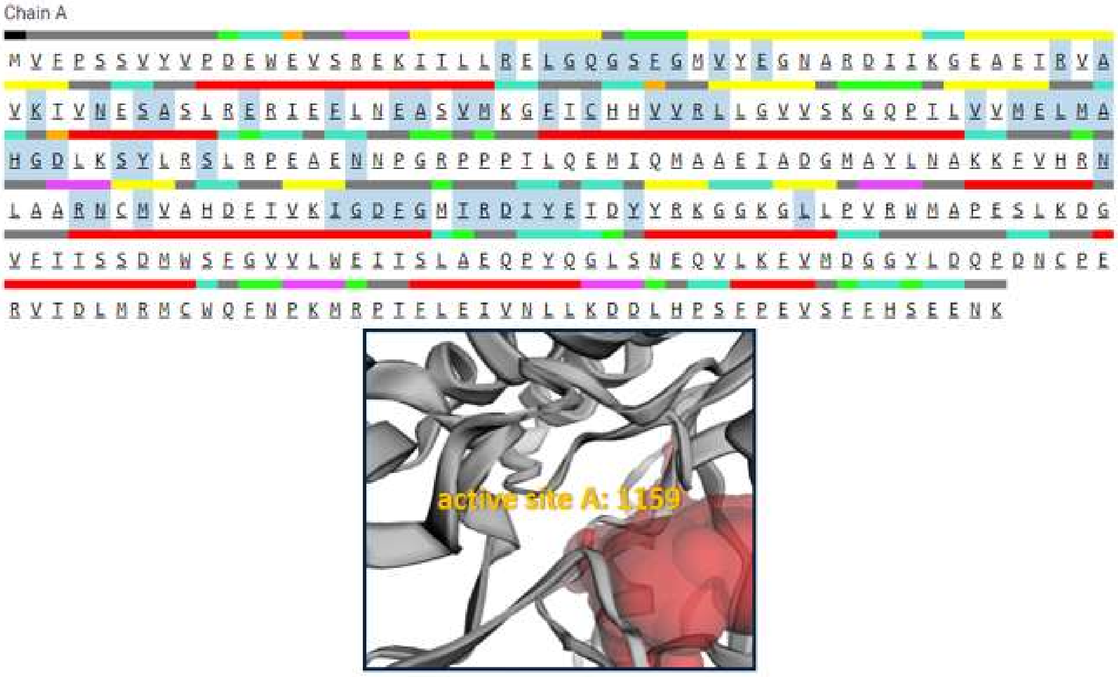


Figure 03: The Computed Atlas of Surface Topography of proteins results of target protein (5HHW) with active site no. of **A: 1159**

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.

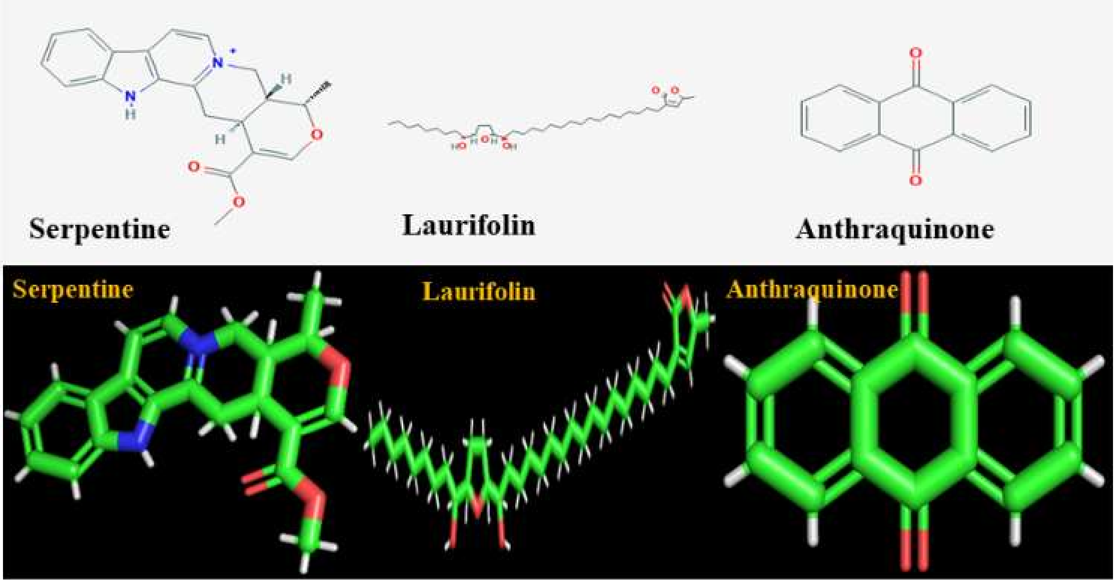


Figure 04: The three potent phytochemicals of M. oleifera of Serpentine, Laurifolin and Anthraquinone through PubChem Database and Drug Bank database (2D, 3D Structure) and visualized through PyMol

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.

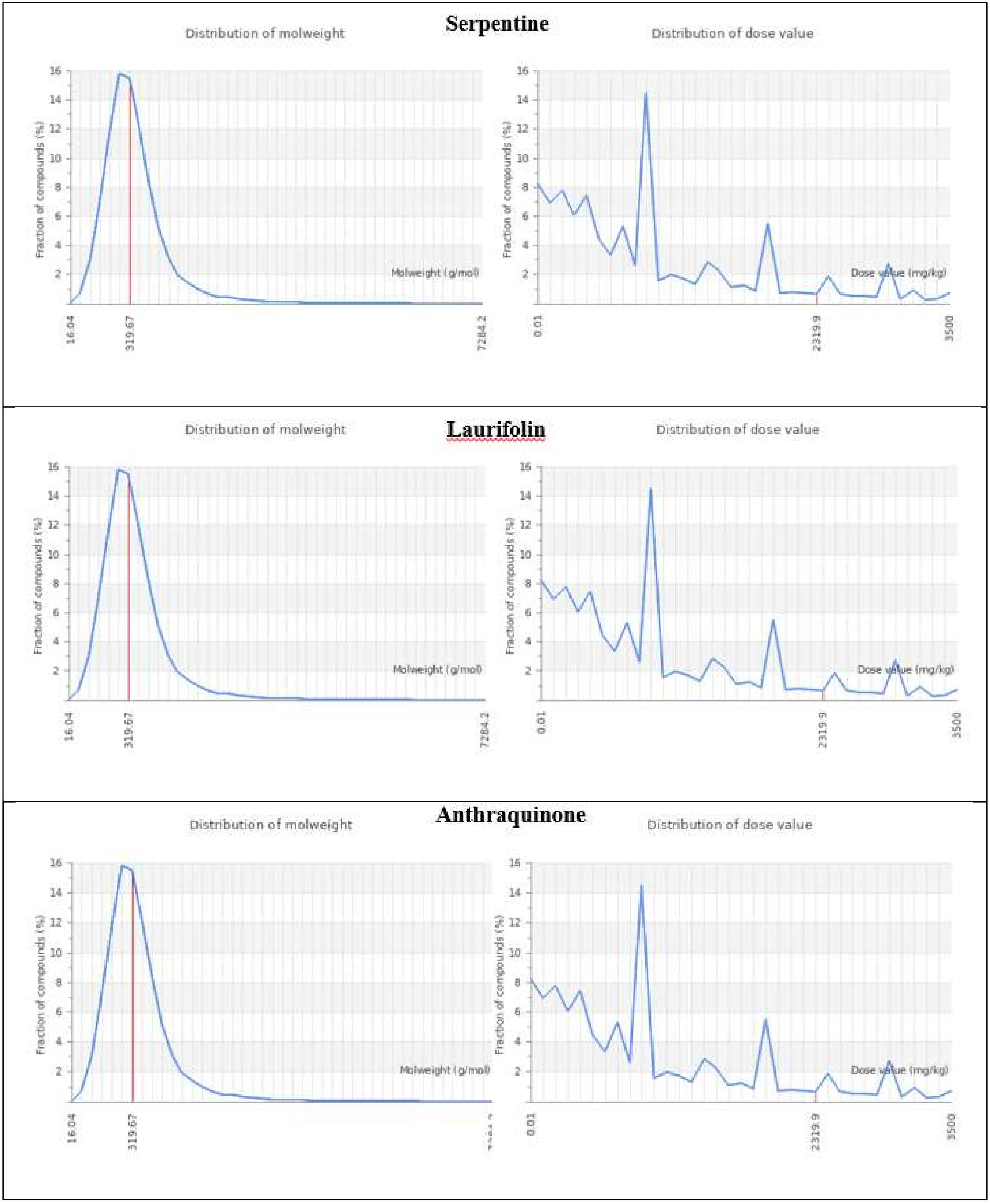
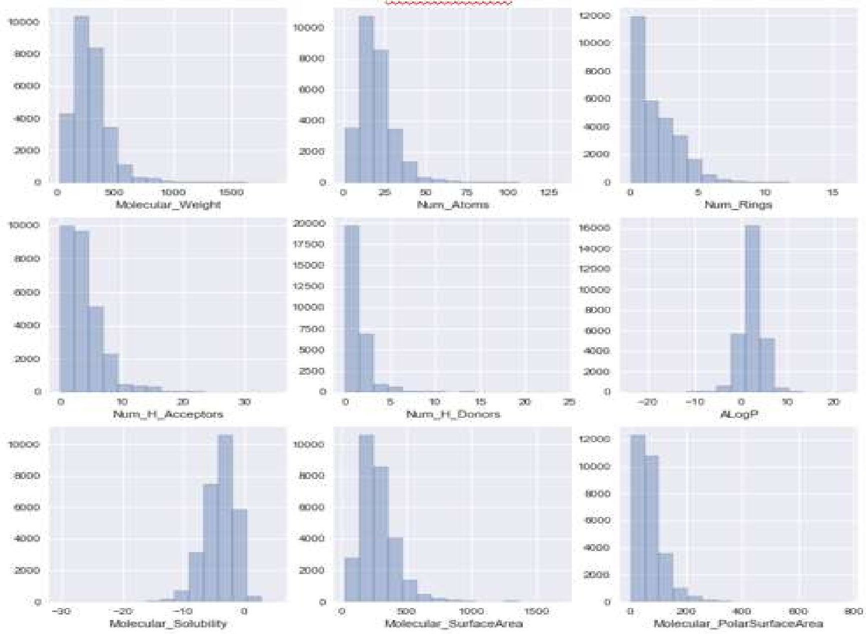
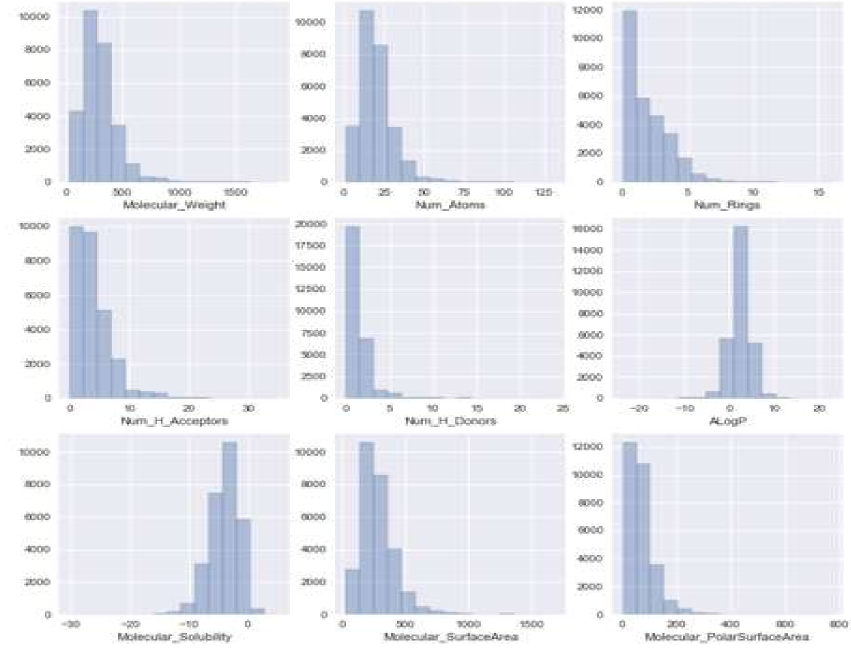


Figure 05: The graphical representation of dose distribution of three potent phytochemicals of M. oleifera (**black value** of input compound, **red value** of dataset)

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 accessibility: 4.36, 6.94, 2.07 and, graphical representation admetSAR Applicability Domains are shown in figure no 06.



Laurifolin



Anthraquinone

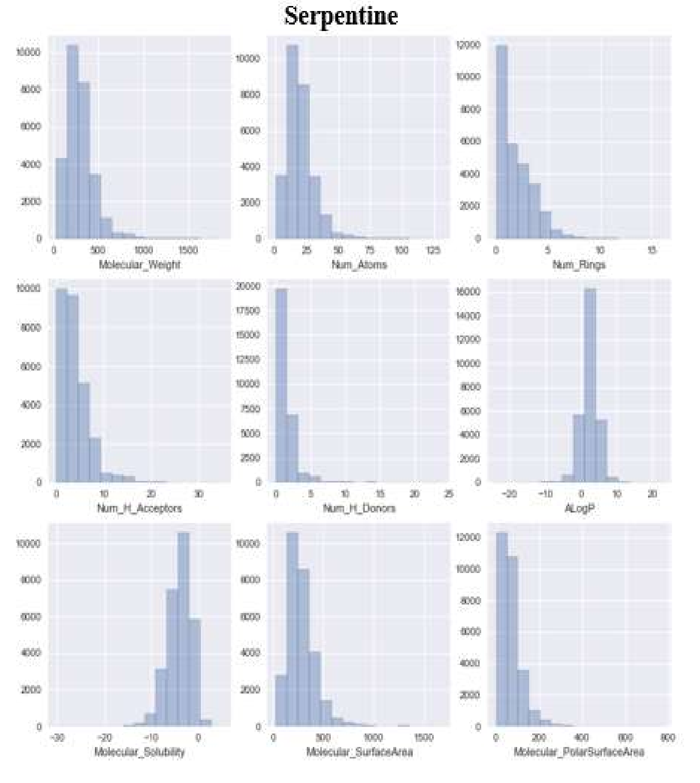


Figure 06: Toxicity profiling of three phytochemicals of graphical representation admetSAR Applicability **Domain variation-graph** in M. oleifera

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.

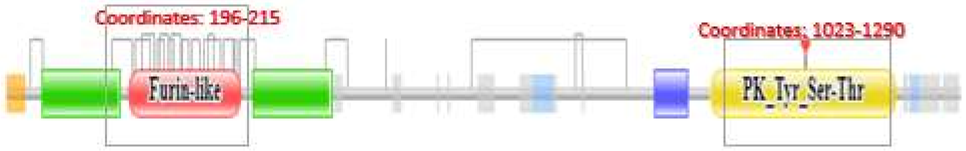


Figure 07: The structure of insulin receptor kinase domain in complex with cis-(R)-7-(3-(azetidin-1-ylmethyl)cyclobutyl)-5-(3-((tetrahydro-2H-pyran-2-yl)methoxy)phenyl)-7H-pyrrolo[2,3 d]pyrimidin-4-amine protein domain of Chain A (UniProt entry INSR_HUMAN): the domain of in **pink-reddish color; Furin-like,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

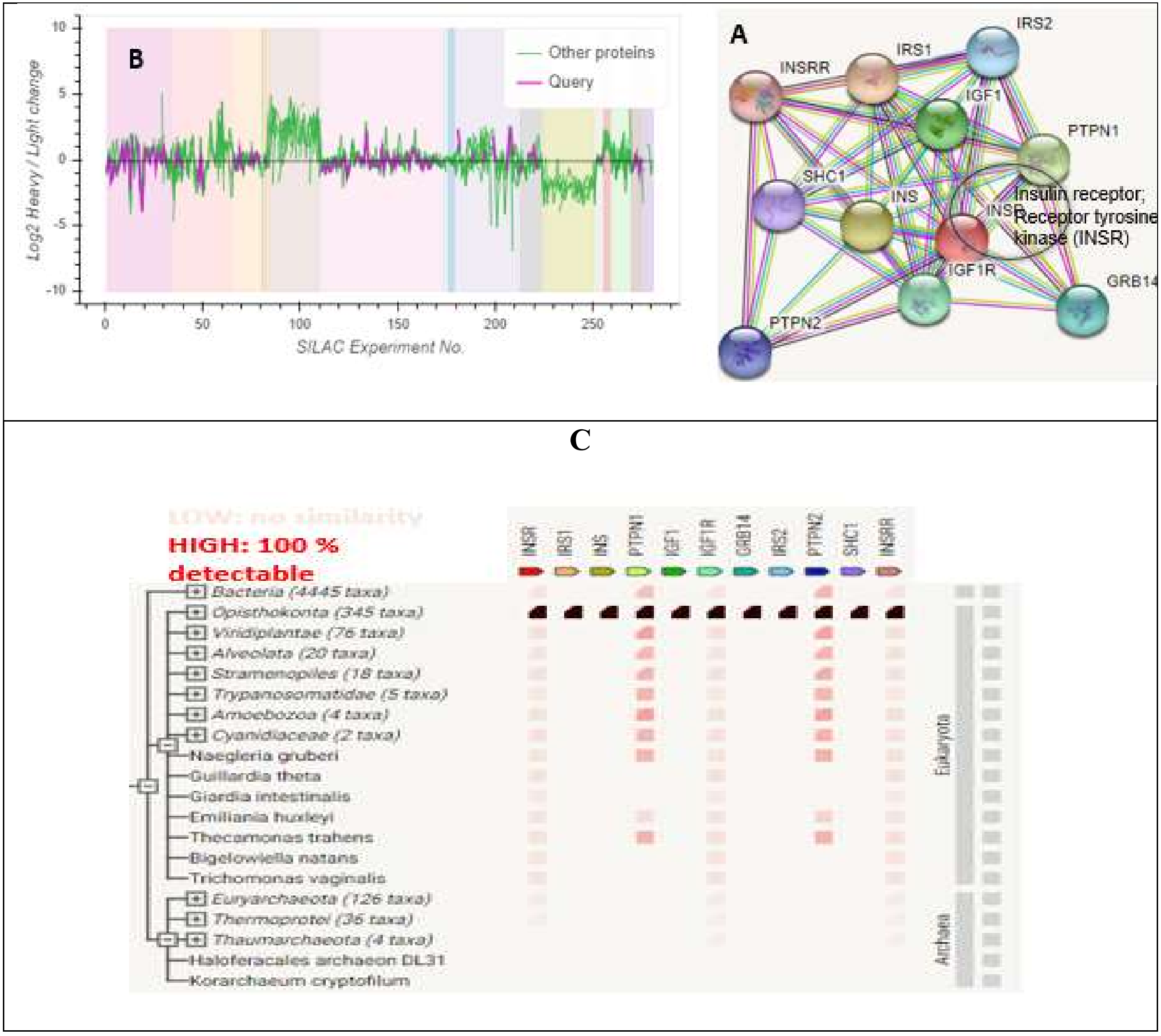
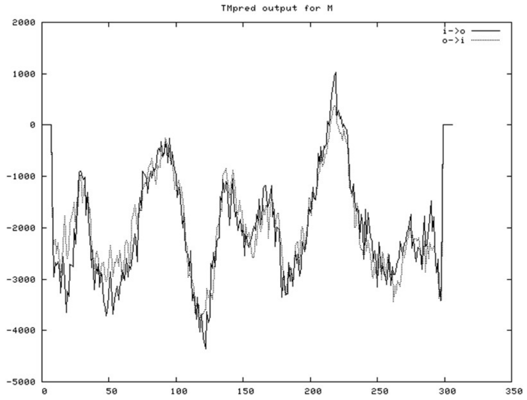


Figure 08: The functional network analysis of INSR by using String Database; **A**: INSR (Insulin receptor; Receptor tyrosine kinase), **B:** Co-expression analysis, Behavior of co-regulated proteins Heatmap in ProteomeHD, Groups of experiments are color-coded, **C:** GENE COOCCURRENCE of INSR analysis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

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

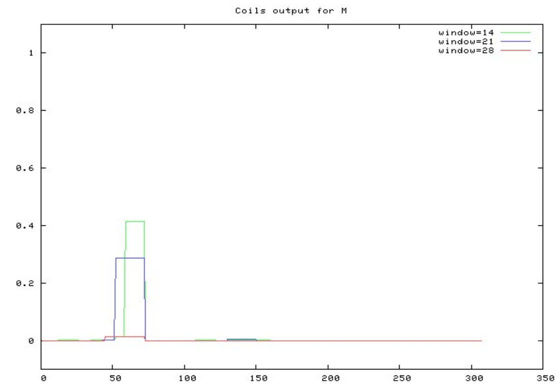


Figure 09: The result of membrane-spanning regions and their orientation of insulin receptor kinase domain in complex with cis-(R)-7-(3-(azetidin-1-ylmethyl)cyclobutyl)-5-(3-((tetrahydro-2H-pyran-2-yl)methoxy)phenyl)-7H-pyrrolo[2,3 d]pyrimidin-4-amine protein. **A:** In graph: helices shown in brackets 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)**

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.

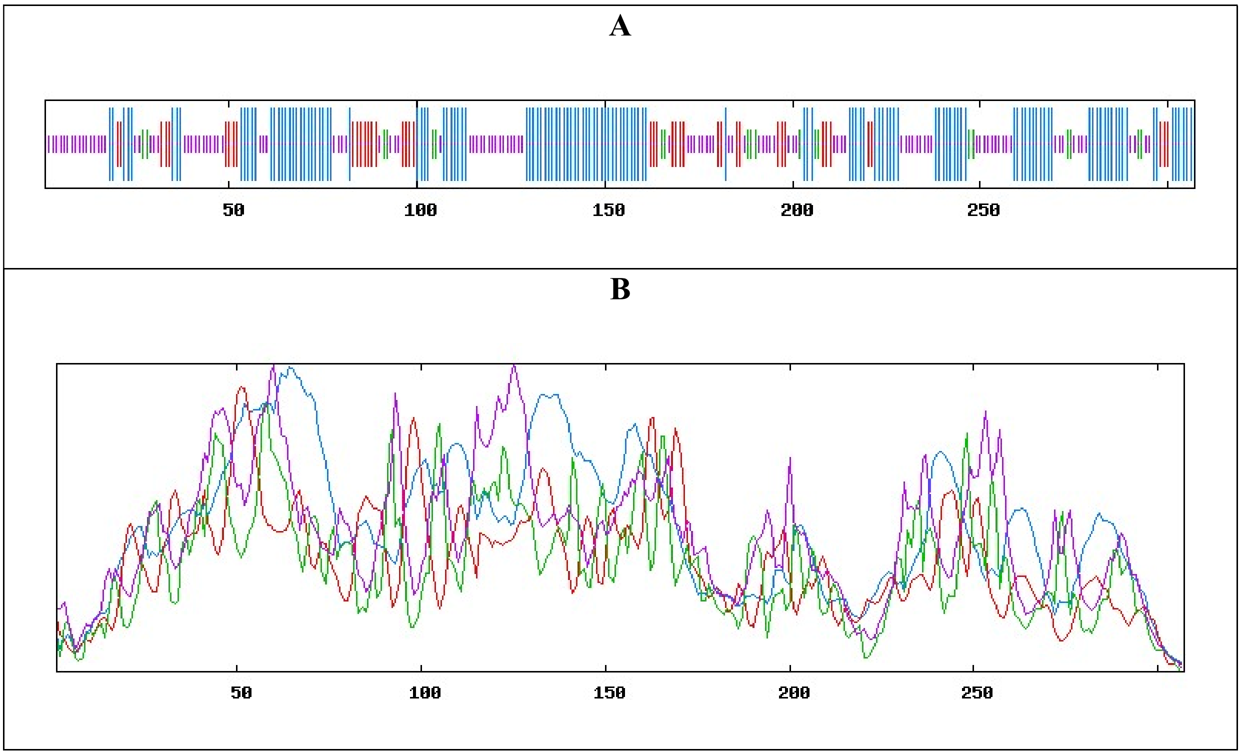


Figure 10: **A:** structural, no of amino acid with different color. **B:** highlighted with color like: alpha-helix, $3_{10,}$ pi-helix: dark-blue, beta bridge, extended strand: red, beta turn: black, bend region, random coil: orange, others: green

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.

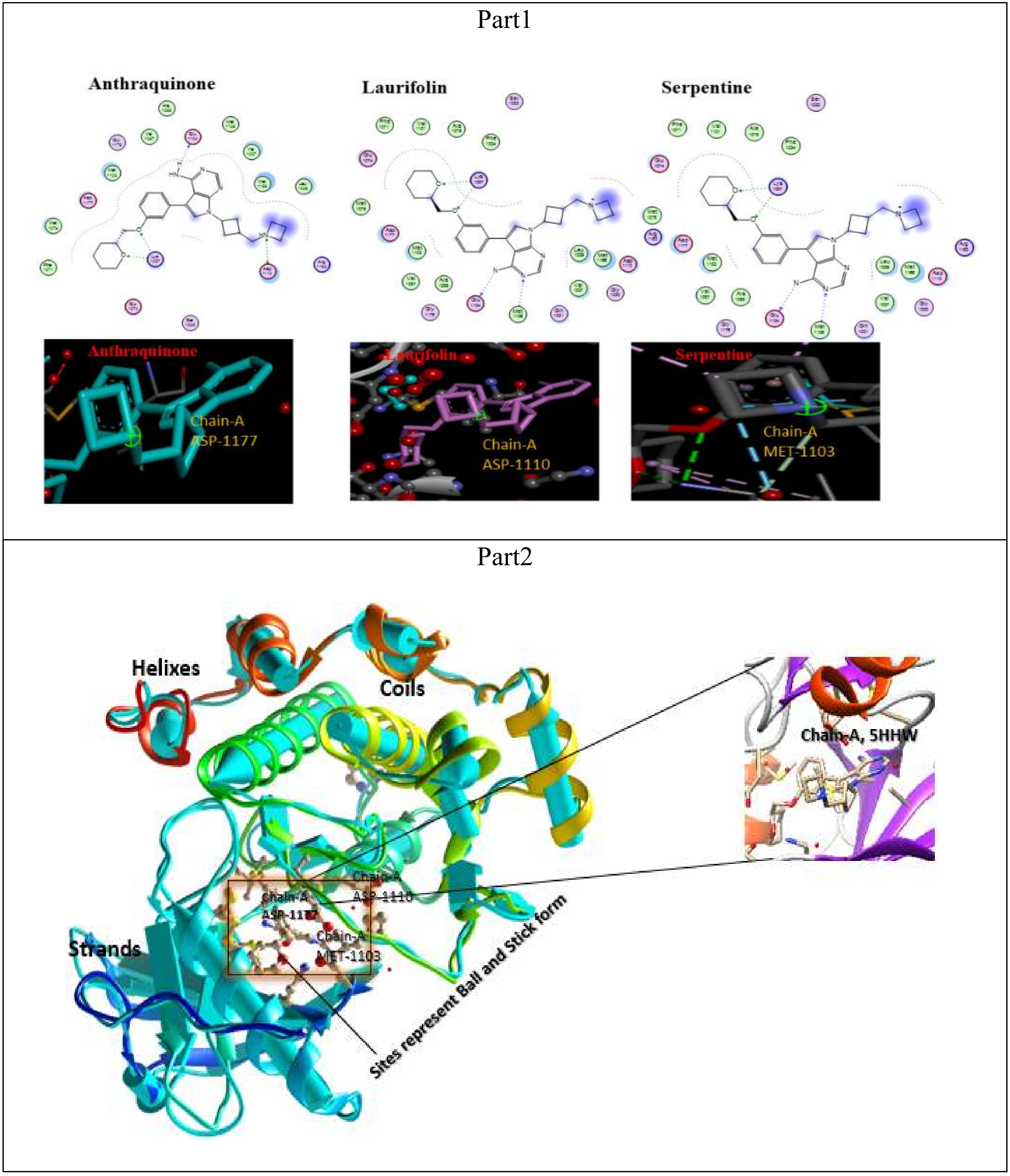


Figure 11: The potent three docking complexes of mutated target protein with binding sites; **Part1**: Anthraquinone-chain-A_ASP-1170, Laurifolin-chain-A_ASP-1110 and Serpentine-chain-A_MET-1103 AA (amino acid) also ligand interaction through LigX-MOE, docked with PyRx Tool and visualized from Discovery 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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Epidemology of down's syndrome among congential heart disease children in Faisalabad

Abdul Maajid Khokhar

Faculty of Medical Sciences, Department of Allied Health Sciences Radiography and Imaging Technology, Government College University Faisalabad, Pakistan

Muhammad Mohsin Ali

Faculty of Medical Sciences, Department of Physical therapy, Government College University Faisalabad, Pakistan

Sarwat Mahmood

Department of Physical therapy, Faisal Hospital Faisalabad, Pakistan

Muhammad Mazhar Fareed

Faculty of Life Sciences, Department of Bioinformatics and Biotechnology, Government College University Faisalabad, Pakistan

Abstract: Patients with Down's syndrome are prone to have congenital heart defects. This study was conducted to evaluate the frequency of various congenital heart defects in children with Down's syndrome. The data was collected from the Department of Cardiology in FIC (Faisalabad Institute of Cardiology) Hospital. Fifty-eight phenotypically Down syndrome children coming to the cardiology department for echocardiography, from birth to 13 years were included in this study. After detailed history and physical examination, all these patients were subjected to 2-dimentional echocardiography in addition to routine laboratory investigations. Congenital heart defects were found in 29 out of 58 patients (50%). Amongthe affected patients, 16 (55.2%) were males and 13 (44.8%) females with male to female ratio of 1.2:1. Acynotic lesions were more common (79.31%) than cyanotic lesion (20.69%). Among the isolated lesions ventricular septal defect, patent ductus arteriosus and complete atrioventricular defects were the commonest defects (20.69%) each, followed by pulmonary atresia (6.89%), atrial septal defect, tetralogy of Fallot, transposition of great arteries and double outlet right ventricle with ventricular septal defect (3.45%) each. Among the mixed lesions ventricular septal defect with atrial septal defect (VSD+ASD) was most common (6.89%), followed by patent ductus arteriosus with coarctation of aorta (PDA+CoA), univentricle with atrial septal defect (univentricle+ASD), and double outlet right ventricle with ventricular septal defect, patent ductus arteriosus and pulmonary atresia (DORV+VSD+PDA+pulmonary atresia) (3.45%) each. Congenital heart defects are found in 50% children with Down syndrome. The commonest are ventricular septal defect, patent ductus arteriosus and complete atrioventricular septal defect in our set-up. All children with Down syndrome should have a cardiac evaluation at birth. Down syndrome, Congenital heart disease, Transposition of great arteries, Pulmonary atresia, Tetralogy of Fallot, Ventricular septal defect.

Key words: Down syndrome, congenital heart disease, epidemiology, prevalence, echocardiography

Doi: 10.5281/zenodo.5516349

1.Introduction

Down syndrome or trisomy 21 is a congenital chromosomal disorder. It is most common genetic disorder caused by the addition of partial or extra copy of chromosome 21. This syndrome is a cause of retardation of mental and physical development of children associated with several physical dysmorphic features (Ahmed, Ghafoor, Samore, & Chattha, 2005). The common features are decreased muscle tone (hypotonia), short stature, delayed development, flat nasal bridge, flat occipital, microcephaly, short neck with excess skin on the back, low set ears with particular folded appearance, open mouth with protruding tongue, broad and short hands often with a single deep crease across the palm of the hand (simian crease), clinodactyly, upward slanting eyes with epicanthal folds, strabismus, nystagmus, white spots on the colored part of the eye (Brush field spots), congenital heart diseases, wide gap between first and second toe. All these anomalies can be detected at birth (Nussbaum, 2010).

In developed countries the incidence of Down syndrome varies from 1 in 732 live births (Sherman, Allen, Bean, & Freeman, 2007). Studies conducted in Australia by Collins and associates have shown the prevalence of Down syndrome as 10 per 10,000 live births (Collins, Muggli, Riley, Palma, & Halliday, 2008). In countries where abortion is illegal, the prevalence is higher such as United Arab Emirates and Ireland. Conversely in countries where pregnancy termination occurs in Down syndrome babies, the prevalence is low (Weijerman, 2011). The risk of Down syndrome with regular trisomy 21 increases with increasing maternal age however Robertsonian Translocation is not related to maternal age. There is a high recurrence risk in the carriers of Translocation (Nussbaum, 2010). A study conducted by a group of researchers in Korea showed that women older than 35 years old has increased risk of chromosomal aberrations in child (Kim, Lee, Kim, Shim, & Cha, 2013).

Congenital heart disease, in a definition proposed is “a gross structural abnormality of the heart or intra thoracic great vessels that is actually or potentially of functional significance” (Khajali et al., 2019). Congenital heart disease (CHD) includes major structural malformations of the heart and major vessels present at birth, or persisting abnormalities after birth. CHDs are a major cause of mortality and morbidity, especially in individuals where the heart defect is associated with additional organ malformations. Chromosomal aberrations are a frequent cause of CHDs, especially when they are associated with growth or developmental delay (Khajali et al., 2019; Thienpont et al., 2007).

Congenital malformation (CM) is emerging as one of the major childhood health disorder. CM can be divided into two main categories those with a single primary defect, and those with multiple malformation syndrome. Most of the single primary defects are of unknown etiology, and are based on multi-factorial inheritance. The etiology of disorders is divided into genetic, environmental factors and teratogenic agents, maternal condition, infections, mechanical problems, chemicals agents, drugs, radiation, hyperthermia, etc. and unknown. There are regional variations in the pattern of CM. Considerable variations in the frequency of CM in different populations have been reported, 4.3% in Taiwan, 7.92% in the UAE, 2.46% in Oman, 3.5% in Tehran (Iran), 1.04% in Arak (Iran) and 1.01% in Gorgan (Iran) (Gul, Nazir, Saidal, & Bahadur, 2020).

Echocardiography is the first line and indispensable investigation for the diagnosis of congenital heart defects. In fact, echocardiography has obviated the need of cardiac catheterization. Although most CHDs are diagnosed and dealt with during childhood, some of them are discovered for the first time on echo during adulthood in asymptomatic subjects (Sharma).

Congenital heart diseases are the main factor contributing in the course of downs syndrome. 4%-10% of all congenital heart diseases have association with down's syndrome and 40%-60% patients of down's syndrome have cardiac anomalies (Doná, Lawin, Maturana, & Felcar, 2015). Cardiac anomalies associated with down's syndrome include mainly patent ductus arteriosus (PDA), atrioventricular septal defect (AVSD), ventricular septal Defect (VSD), atrial septal defect (ASD), tetralogy of fallot (TOF), coarctation of aorta (CoA), transposition of great arteries (TGA), double outlet right ventricle (DORV), pulmonary valve atresia/stenosis and univentricle (Diogenes, Mourato, de Lima Filho, & da Silva Mattos, 2017).

Research has shown that neonatal mortality in DS is less dependent on CHDs and is caused by neonatal pathology such as asphyxia, low birth weight and prematurity, as in the general population (Weijerman, 2011). In these studies, we have shown the prevalence of congenital heart defect in patients of Down's syndrome 56.36% in Pakistan. It was 81% in Brazil, 45% in Libya, 43% in Netherlands ,58% in Mexico, 56% in Denmark, 56.9% in Korea, 41% in Bahrain, 56% in Italy, 38.7% in Spain, 44% in Atlanta, USA, 48% in Birmingham, UK (Al-Arrayed & Rajab, 1995; Diogenes et al., 2017; Doná et al., 2015; Elmagrpy, Rayani, Shah, Habas, & Aburawi, 2011; Freeman et al., 2008; JM, 1997; Khan & Muhammad, 2012; Paladini et al., 2000; Salih, 2011; Santoro & Steffensen, 2021; Weijerman, 2011).

2.Material method

2.1 Data collection procedure

A structured Performa was developed including all the variables of interest for use during the study. The Performa was pretested by before adopting a final version. All the data was collected by the investigator herself. After the investigator introduced herself, the echocardiography of phenotypically confirmed Down's syndrome patients was performed and findings of congenital heart defects were recorded on the Performa.

2.2 Trans thoracic Echocardiography

Trans thoracic 2D echo studies were done by a standard technique. Situs was analyzed in sub costal view. Pulmonary venous connections to LA were assessed in apical and suprasternal window and flow was assessed on color Doppler echo. Ventricular morphology was checked on apical four chamber and two chamber views. Accurate identification of great arteries was done in short axis views at the base of the heart. Pulmonary artery was further assed on para sternal long axis and short axis views while aortic arch on suprasternal short axis views. Right ventricle and its inflow tracts were visualized on apical and sub costal four chamber views para sternal long axis views and short axis views at the base. CoA was checked on supra sternal window. Doppler and short axis views were done for the detection of AS. 2D sub costal and apical dour chamber views were done to see ASD and VSD. Further analysis was done on M-mode and color Doppler imaging. Follow up was arranged according to the primary cardiac diagnosis.

2.3 Data analysis procedure

The data was managed and analyzed by using SPSS version 17. Data was described in terms of frequencies and percentages for categorical variables. Quantitative variables were expressed in the form of mean and standard deviation.

3. Results

Fifty-eight consecutive patients of Down's syndrome were studied. Echocardiography data was collected prospectively on a pre-designed Performa. All patients after clinical assessment were subjected to transthoracic echocardiography. Variables like patent ductus arteriosus (PDA), atrioventricular septal defect (AVSD), ventricular septal defect (VSD), atrial septal defect (ASD) tetralogy of Fallot's (TOF), coarctation of aorta (CoA), transposition of great arteries (TGA), double outlet right ventricle (DORV), pulmonary atresia/stenosis and univentricle were noted on the performa for each patient.

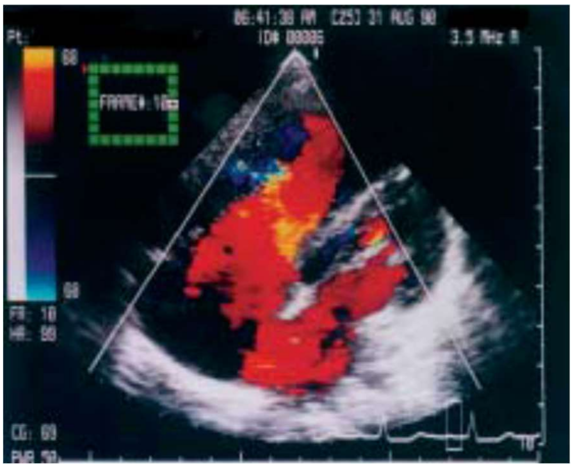

Fig 3.1: Four-chamber view is showing a large secundum type defect in the atrial septum. Blood flow crossing the ASD is apparent on **Doppler echocardiography.**

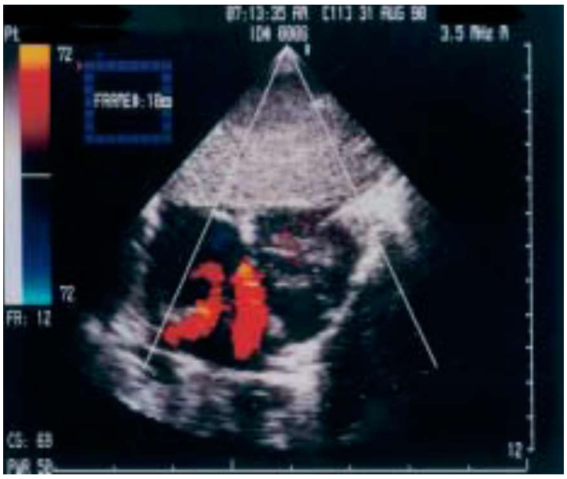

Fig 3.2: Sub costal four-chamber view with Doppler echocardiograph demonstrates blood flow crossing two defects, a primum ASD and a VSD of the inflow tract in a patient with an AVSD.

In 58 patients of Down syndrome, 63.793% (n=37) were males and 36.206% (n=21) females with male to female ratio of 1.7:1 and of these patients in which congenital heart defects were diagnosed were 55.171% (n=16) males and 44.827% (n=13) females with male to female ratio of 1.2:1 (Fig 4.1).

Figure 4.2 is a bar chart showing the percentages of isolated congenital heart defects along with their gender distribution in 58 cases of Down syndrome in the present study. In males patent ductus arteriosus (PDA) was most common (13.793%) followed by venticular septal defect (VSD) and complete atrioventricularseptal defect (CAVSD) (10.171%) each, least common were transposition of great arteries (TGA), double outlet right ventricle (DORV) and pulmonary atresia (3.448%) each. In females most common were venticular septal defect (VSD) and complete atrioventricular septal defect (CAVSD) (10.171%) each followed by patent ductus arteriosus (PDA) (6.8896%), atrial septal defect (ASD), tetrology of Fallot’s (TOF) and pulmonary atresia (3.448%) each. Figure 4.3 is also a bar chart showing the percentages of mixed congenital heart defects along with their gender distribution in 58 cases of Down syndrome in the present study. In males venticular septal defect with atrial septal defect (VSD+ASD), double outlet right ventricle+ ventricular septal defect+patent ductus arteriosus+pulmonary atresia (DORV+VSD+PDA+pulmonary atresia) and univentricle with atrial septal defect (ASD) were found (3.448%) each, while in females PDA+CoA and VSD+ASD were found (3.448%) each.

Patients were catagorized in four age groups; below 1 year , one to five years, six to ten years and more than ten years of age. Most of the congenital heart defects were found in 1-5 years age group about 51.724% (n=15) patients followed by below one year age group 41.379% (n=12), six to ten years of age 6.896% (n=2) and all patients above ten years of age group were without any cardiac defect (Table 4.1). Congenital heart defects were found in 50% (n=29) patients on echocardiography out of 58 paitients of down syndrome. Congenital heart defects were catagorized into cynotic and acynotic, isolated and mixed lesions. Among the 29 patients of congental heart defects acynotic lesion were more common in 79.310% (n=23) than cynotic lesions in 20.689% (n=6) patients. Among the acynotic lesions most common were isolated venticular septal defect (VSD) patent ductus arteriosus (PDA) and complete atrio venticular defect (CAVSD) each in 20.689% (n=6) followed by atrial septal defect with venticular septal defect (ASD+VSD) (mixed lesion) in 6.896% (n=2) and isolated atrial septal defect (ASD), double outlet right ventricle (DORV) and patent ductus arteriosus with coarctation of aorta (PDA+CoA) (mixed lesion) each in 3.448% (n=1) (Table 4.2).

Among the Cynotic lesions 20.689% (n=6) out of 29 patients, isolated pulmonary atresia was more common in 6.896% (n=2) followed by tetrology of fallots (TOF), transposition of great arteries (TGA) each in 3.448% (n=1) and mixed lesions of univentricle with atrial septal defect (univentricle+ASD) and double outlet rihht ventricle with ventricular septal defect, patent ductus arteriosus and pulmonary atresia (DORV+VSD+PDA+Pulmonary atresia) also each in 3.448% (n=1) (Table 4.3).

4 Statistical analysis

Figure 4.1: Pie chart showing distribution of patients with congenital heart defects according to gender in 58 cases.

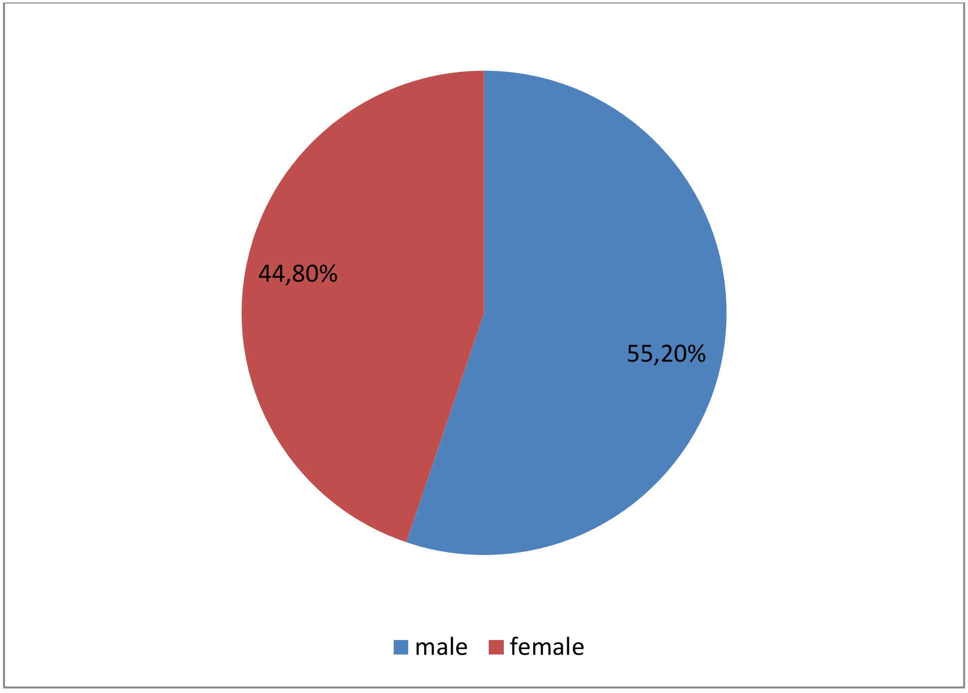

Figure 4.2: The percentages of isolated congenital heart defects along with their gender distribution in 58 cases.

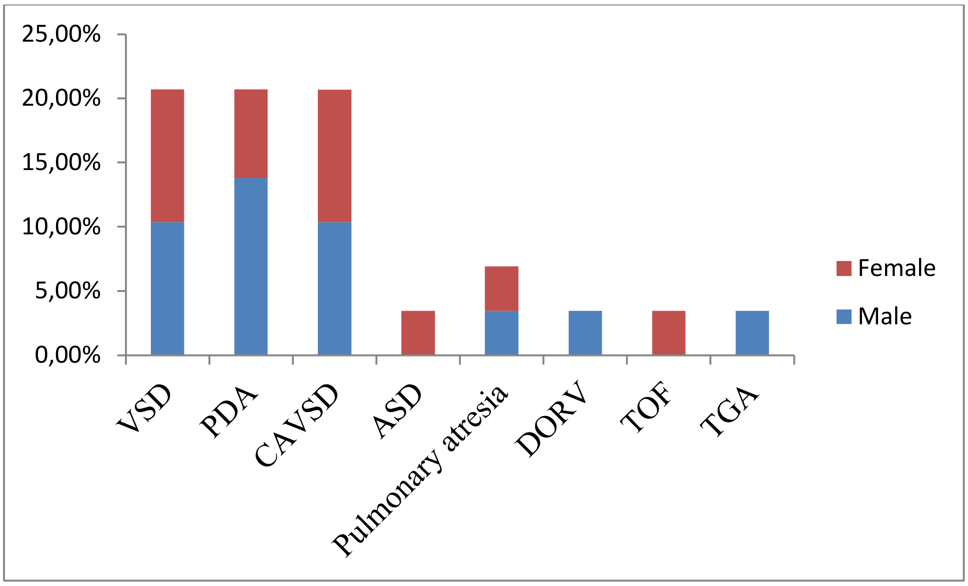

Figure 4.3: The percentages of mixed congenital heart defects along with their gender distribution in 58 cases.

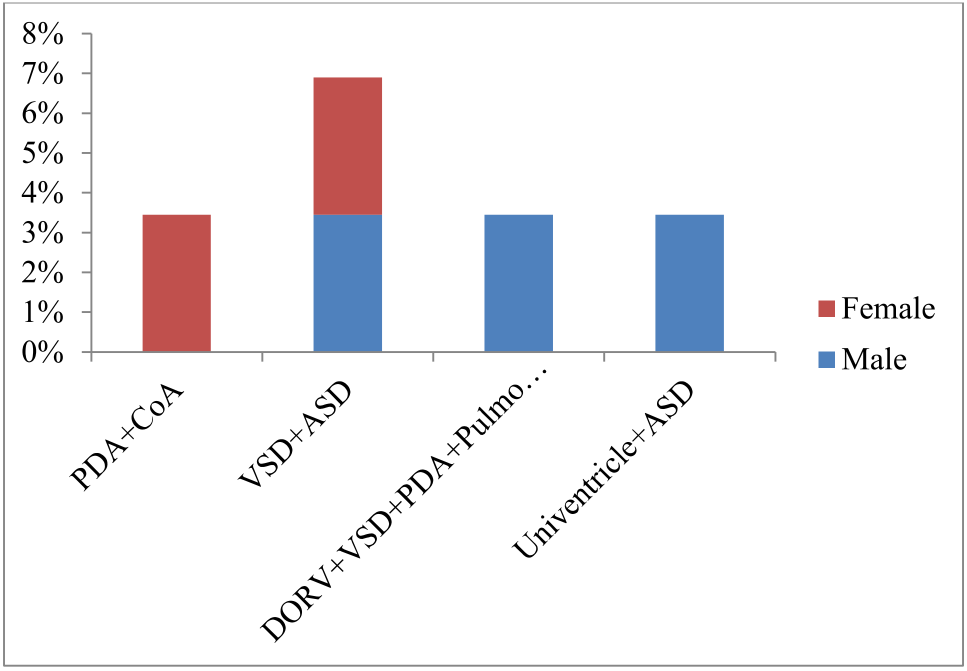

Table 4.1: Frequency distribution of different categories of age for 58 cases.

Age in years	Acyanotic lesions Number (%age)	Cyanotic lesions Number (%age)
Below 1 year	8 (27.586%)	4 (13.793%)
1 to 5 years	13 (44.827%)	2 (6.896%)
6 to 10 years	2 (6.896%)	0 (0%)
>10 years	0 (0%)	0 (0%)
Total	23 (79.310%)	6 (20.689%)

Table 4.2: Frequency distribution of different isolated cardiac lesions with major categories of acynotic and cyanotic lesions for 58 cases.

Isolated cardiac lesions (n=24)

Acyanotic lesions (n=20)	**Percentage**	**Cyanotic lesions (n=4)**	**Percentage**
VSD	20.989%	Pulmonary atresia	6.896%
PDA	20.989%	TGA	3.448%
CAVSD	20.989%	TOF	3.448%
ASD	3.448%		
DORV+ VSD	3.448%		

Table 4.3: Frequency distribution of different mixed cardiac lesions with major categories of acynotic and cyanotic lesions for 58 cases.

Mixed cardiac lesions (n=5)			
Acyanotic lesions (n=3)	**Percentage**	**Cyanotic lesions (n=2)**	**Percentage**
VSD+ ASD	6.896%	DORV+VSD+PDA+ Pulmonary atresia	3.448%
PDA+ CoA	3.448%	Univentricle +ASD	3.448%

5.Conclusion And Discussion

The high incidence of congenital heart disease in Down’s syndrome is well known, and many authors have published figures on the frequency with which congenital heart defects are found. These figures vary from 35 to 65 percent (Khan & Muhammad, 2012). The frequency of CHD in this study 50% is quite comparable to these studies. It is quite close to 56.9% in Korea and 56.36% in Khyber Pakhtunkhwa province in Pakistan (Khan & Muhammad, 2012). The frequency of congenital heart defects is quite higher 81% in Brazil and comparatively lower frequencies 45.10% in Libya and 43% in Netherlands (Elmagrpy et al., 2011; Mourato, Villachan, & Mattos, 2014; Weijerman, 2011). The various reasons for this difference may include the genetic make-up of each nation and the specific embryological mechanisms. In 58 patients with Down's syndrome, 37 (63.793%) were males and 21 (36.206%) females with male to female ratio of 1.7:1 and of these patients in which congenital heart defects were diagnosed were 16 (55.171%) males and 13 (44.827%) females with male to female ratio of 1.2:1. These results are very close to the study in which they found among the affected patients, 19 (61.3%) were males and 12 (38.7%) were females with male to female ratio of 1.5:1. These findings are quite different from study in Brazil, where females prevailed (56.1%) with male to female ratio of about 1:1.3 (Khan & Muhammad, 2012; Mourato et al., 2014). The most common type of congenital heart defects was isolated in 24 (82.758%) these values are very close to 90.3% in study (Khan & Muhammad, 2012). And are comparable to the Libyan population with 65% isolated lesion, 80% in Guatemala, 74% in Mexico and 78% in Turkey (Elmagrpy et al., 2011). This difference may be because of age at diagnosis where patients with more complex lesions die earlier before diagnosis. Among the acyanotic isolated and mixed lesions most common defects were ventricular Septal defect (VSD), patent Ductus Arteriosus (PDA) and complete atrioventricular septal defect (CAVSD) each in 6 (20.689%) followed by atrial septal defect with venticular septal defect (ASD+VSD) (mixed lesion) in 2 (6.896%) and isolated atrial septal defect (ASD), double outlet right ventricle (DORV) and patent ductus arteriosus with coarctationof aorta (PDA+CoA) (mixed lesion) each in 1 (3.448%). Among the Cynotic lesions isolated pulmonary atresia was more common in 2 (6.896%) followed by tetralogy of fallot (TOF), transposition of great arteries (TGA) each in 1 (3.448%) and mixed lesions of univenticle with atrial septal defect (univenticle+ASD) and double outlet right ventricle with ventricular septal defect,patent ductus artriosus and pulmonary atresia (VSD+DORV+PDA+Pulmonary atresia) also each in 1 (3.448%). These findings were quite different from the findings in Korean children in which atrial Septal defect (ASD) was most common about 30.5%. 2nd most common was ventricular Septal defect (VSD) with percentage of 19.3%, this was followed by patent Ductus Arteriosus (PDA) (17.5%) and AVSD (9.4%) (Diogenes et al., 2017). Also, in Brazil ASD secundum type was maximum (51.8%), followed by atrioventricular Septal defect (AVSD) (46.6%), ventricular Septal defect (VSD) (27.7%), tetralogy of Fallot (TOF) (6.3%) and other cardiac anomalies (12.5%) (Mourato et al., 2014). Elmagrpy also analyzed the association of CHD and DS in Libya. Among the isolated most common was ASD (23%) followed by AVSD (19%) and VSD (14%) (Elmagrpy et al., 2011). ASD was most common in (54%), followed by VSD (33.3%) and PDA (5.8%). PPHN was (5.2%) and it was higher than normal public incidence in Netherlands. In Sudan AVSD was most common in about (48%) followed by ASD in 23% and TOF in (6%) at the time of presentation (10%) had Eisenminger syndrome (Ali, 2009).

This study shows quite similar to the study in Khyber Pakhtunkhwa province where ventricular septal defect was the commonest defect (22.6%), followed by patent ductus arteriosus (PDA) (19.4%), atrioventricular septal defect (VSD) (19.4%), atrial septal defect (ASD) (16.1%) and tetralogy of fallot (Khan & Muhammad, 2012). The results were quite comparable to study in India where Atrioventricular Septal defect (AVSD) was most common in about 13 (37.142%) among 35 down's syndrome patients, while ventricular Septal (VSD) in 26 (68.421%) was most common among the 38 non syndromic patients (Tanghöj, Liuba, Sjöberg, & Naumburg, 2020).

From this epidemiological study it can be concluded that the congenital heart defects are common in children with down's syndrome. The commonest congenital heart diseases in down's syndrome are isolated acynotic lesions including ventricular septal defect (VSD), patent ductus arteriosus (PDA) and complete atrioventricular septal defect (CAVSD) in our set-up.

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The Evolutionary and Molecular impacts on Human Genetics; Review

Muhammad Mazhar Fareed

Faculty of Life Sciences, Department of Bioinformatics and Biotechnology, Government College University, Faisalabad, Pakistan.

Sabiqa Zulfiqar

Faculty of Life Sciences, Department of Bioinformatics and Biotechnology, Government College University, Faisalabad, Pakistan.

Abstract: The human genome holds a record of the evolutionary forces that have shaped our species. In human genome evolution, DNA sequences-genomics analysis, molecular-based analysis, population genetics, molecular genetics, functional genomics, genome function identification/profiling, and genomics modeling, and simulation have deepened our understanding of human genome evolutionary history, natural selection, and other studies. We study, some factors that influence the evolution of the human genome are the functional modification of DNA, post-translational modification (PTM), gene regulation, analysis of chromatin conservation, modification of histone, structural variations, mutational biases, single nucleotide polymorphism (SNPs) and gene-phenotypes interaction along the genome. By using the natural selection and evolutionary theory as study, these some phenomena will play a vital role in the understanding of the human genome and also breakthroughs in *in-vivo* and *in-silico* studies.

Keywords: Human Genome, Evolution, SNPs, DNA

Doi: 10.5281/zenodo.5516380

1.Introduction

The study in which genes change and change over time, molecular evolution. Is the study of DNA sequence knowledge an integral part of bioinformatics? The variation in cellular, molecular sequence composition like DNA, RNA, and protein. The field of molecular evolution is used in evolutionary biology theories and population genetics to illustrate the patterns of these changes. The human genome is a complete set of human genome sequences, encoded in DNA, into the cell nuclei or a small genome molecule located within each of 23 chromosomes pairs. The size of the human genome is 3,234 Mbp (Gibson, 2012). The human genomics is a basic goal to understand its evolution by decoding the biological program coded into the human genome accurately will reveal answers to the molecular human origin and biological specific characteristics issues. Studying the history of evolution and ancestry of our species also reveals how and why people of today get sick. Evolutionary forces have formed the genome of humans, that often no longer control the circumstances of most humans and disease can be caused by a mismatch between our genes and our environmental factors. The knowledge of human genomics has been provided by functional and comparative genomics, but we still know very little about their spatial mechanisms. What is the 3-dimensional genome structure? How is this system special and when? The structural organization of the genome has long been suspected of placing constraints on its growth. The dynamic physical environment of the human genome has been discovered recently through genome-wide data on chromatin structure, which gains unpredictable insights into chromosome composition, gene structure, mechanisms of mutation and selection (Lee, Abecasis, Boehnke, & Lin, 2014). The recent development and sequence by thousands of human genomes from various populations in the field of genetics, from the dynamics selection through populations and near related species to identify differences in the rate of mutations in the human population, revealed significant complexity in conventional topics, some Specific genetic factors and demographic factors are impacting the evolution of the human genomes (Gluckman, Beedle, Buklijas, Low, & Hanson, 2016; Henn, Botigué, Bustamante, Clark, & Gravel, 2015; Prado-Martinez et al., 2013).

2.The gene regulatory mechanism affects the production of the Human genome

There are some morphological variations in the human genome between closely associated species through gene regulatory changes like as cis-regulatory elements (CREs), such as promotor and enhancers in humans-diseases evolution with change of SNPs. The ability to map the gene, transcript factor sites histone modifications in several organisms and tissues in the genome. During gene expression, organisms like CREs are experiencing rapid turnover within similar tissue. Among other more than 20 mammalian species, liver promoters and enhancers found that 25% of the genes, enhancers, and approximately 10% of the promoters were highly conserved and unique among sequences, and different conserved findings have been observed in humans and mouse for similar organs (Lynch et al., 2015). The evolution and dynamics of the regulatory sequence across body tissues, organisms and groups of species by CREs. For example, transposable elements (TEs) have helped reprogram gene regulatory networks in tissues of mammals, humans and other evolution in different species. The rapid turnover of CREs and retained gene expression are typical features of the development of the mammalian genome with various development and tissue pressures. maintenance, alteration, and effect on genome variation of certain regulatory processes. Integrating genome-wide maps of CREs and expression in techniques for determining *in-vivo* chromatin conformation of DNA may provide a framework for modeling the influence of gene regulation on genome evolution. A recent study on chromatin-looping analysis of the function of genes with topological conserved domains in many human and mouse tissues through cells and species. Integrating genome structure and CREs data over several populations would possibly result in better modeling of the regulatory sequence evolution and acts with time and tissue on gene expression and regulation (Melé et al., 2015; Sakabe, Savic, & Nobrega, 2012; Siepel & Arbiza, 2014; Weirauch & Hughes, 2010).

3.Evolution of Human genetics with chemical modifications to DNA

The human body is made up of numerous different cell types, each containing the same genome, but play a different function in different locations of the human body by PTM. The roles of gene expression systems observed through cell types in diverse genomic organisms in which DNA and histone modifications as well as post-translational modifications (PTM) including methylation and acetylation (Brunet & Berger, 2014; Ptashne, 2013). These modifications can be influenced by environmental factors are inherited across generations. In extensive work, modifying all growth, disease, aging processes and influencing genetically modified sequence. For example, the chemical modification potentials impose constraints on the patterns of DNA sequence, the conservation degree of DNA, and histone changes among humans and closely related organisms. Paralogous regions are typical between closely related species by changing the status of orthologous for DNA acetylation and DNA methylation. There is a strong link between changes in acetylation-methylation promoters and changes in sequence. Understanding the evolution of these chemical modifications in future debates or other processes like transcription and many other types of post-translational modification (PTM) processes (Heard & Martienssen, 2014; Zhou, Goren, & Bernstein, 2011).

4.Mechanism of interaction between genes and phenotypes

The diversity of human clinical and developmental phenotypes is detected in the human genome by a variety of loci. Developing models that account for relationships between gene and phenotype and multiple genetic variations will be critical to fully dissecting the evolution and complex genetic human traits. Furthermore, histone modifications are a non-additive relationship between genetic variation, because a variety of technological and biological factors its effect on human traits was controversial. For these areas, new biostatistical methods are required to upgrade the models to allow full use of genotype and phenotype data complexity (Sivakumaran et al., 2011; Solovieff, Cotsapas, Lee, Purcell, & Smoller, 2013; Wood et al., 2014).

5.Mutational biases in Human genome

The most potential influential mutational biases are a recombination association process called GC-biased gene conversion (gBGC). The gBGC results from a slight preference for G-C alleles in the mismatch repair machinery that has the potential to promote the maintenance of deleterious alleles (alternative form, expression). The action of gBGC is widespread across humans and across diverse ecosystems (Lachance & Tishkoff, 2014; Wood et al., 2014). The gBGC's genome-wide modeling has shown variation in intensity around the heritage of humans and chimpanzees, and among humans. The origin and effect of gBGC are closely linked to the recombination dynamics, which differ greatly in the rate along the genome within the human population and between closely related species. The recombination patterns were affecting many genome evolution drivers including mutation rate, selection rate, and accumulation of de novo mutations (Coop & Przeworski, 2007; Torres, Szpiech, & Hernandez, 2018). We must develop high-resolution mutation rate maps for different populations, better models of how selection and recombination interact., and a deeper understanding of their impact on organismic performance in nature.

6.Functional impact of structural variation

The Indels and SVs make more variations in nucleotide between humans and chimpanzees than SNPs, and they have the genome restructured. Examples are the indels and SVs de novo levels of systemic changes observed in the human population including replication of variations and other rearrangements in genomics are more likely to have caused certain diseases. The calculated rate of de novo single nucleotide mutation was approximately 95 % per generation. SNPs are the ability to appears in different forms like as (AA**G**C-AA**T**C) difference as one nucleotide. These SNPs are in the form harmful, harmless and latent occur in the human genome. The effect of SNPs (silent) in the form directly and indirectly (Chuzhanova, Anassis, Ball, Krawczak, & Cooper, 2003; Montgomery et al., 2013; O'bleness, Searles, Varki, Gagneux, & Sikela, 2012; Stankiewicz & Lupski, 2010). For example, are the SNPs in cancer, in which DNA repairs, drug metabolize-enzymes are responsible for the metabolism/detoxification of carcinogenic acts as a cancer particular gene are involved? The potential significance in the genetic modeling and research relationship with SNPs of indels and SVs and new genes of phenotypically distinctive human and closely related species. Enough accurate maps of developmental events are possible in humans, and these data findings will help to establish new modeling approaches.

7.Conclusion

In human genome evolution, we understand the processes of evolutionary events occurs in human species and developmental advance programs in the human genome are encoded. The history of evolution is directly involved in our ability to treat the human genome fluctuation in basic and clinical science. In this study different research domains have highlighted, that have acts as a potential role in human genome evolution. We are also seeing the working of recent technical and research advancement in analysis of genome evolution, human genome, diagnostic disease profiling, analysis of gene sequencing, functional genomics, single-cell evolution analysis, experimental omics analysis will alter our understanding of the human evolutionary process and study design. Ultimately, the human genome analysis in an evolutionary framework for the origins of human-specific biological systems.

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The impact of the COVID-19 on policies and investments of the European Green Deal.

Paolo Biancone

Department of Management, University of Turin, Italy

paolo.biancone@unito.it

Valerio Brescia

Department of Management, University of Turin and DISEI Department, Department of Economics and Business Studies, University of Piemonte Orientale "Amedeo Avogadro", Italy

valerio.brescia@unito.it

Vahid Jafari-Sadeghi
Aston Business School, Aston University, United Kingdom
v.jafari-sadeghi@aston.ac.uk

Abstract: The paradigm generated on world systems and models by COVID-19 has led to the search for new solutions. The European Commission has generated and strengthened the previously defined sustainability and Green Deal policies. The NextGenerationEU fund could incentivize the application and adoption of the actions. The analysis intends to analyze how COVID-19 changed policies undertaken before the pandemic and how these were redefined. A content analysis (CA) technique, through LEXIMANCER software, is adopted to answer the research questions considering articles collected by the Business Source Ultimate platform from February 2020 to June 2021 in Europe. The analysis identified 35 key concepts that describe the path and approach that influenced and guided the new Green Deal policies. The analysis identifies the main areas on which an impact is expected and the confirmation or variation of the approach.

Keywords: Green Deal, NextGenerationEU, COVID-19, content analysis

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1. Introduction

On 14 July, the European Commission adopted a series of proposals to transform EU policies on climate, energy, transport, and taxation to reduce net greenhouse gas emissions by at least 55% by 2030 compared to 1990 levels. It is essential to reduce emissions over the next decade to make Europe the first climate-neutral continent by 2050 and translate the European Green Deal into a concrete reality (Montanarella & Panagos, 2021). The European Commission has defined a roadmap of key points that should be developed. Themes involve clean air, water and soil, and biodiversity, energy-efficient buildings, affordable healthy food, more public transport, clean energy and technological innovation for clean energy, longer-lasting products and recycling, adequate jobs and training, and competitive and resilient industry (European Commission, 2019; Sukumar et al., 2020). The European Green Deal is also a lifeline for us in terms of escaping the COVID-19 epidemic. The pandemic had already pushed technological development and the adoption of new approaches and immediate changes in production systems with greater efficiency, demonstrating the ability of companies to respond to the needs of the context (European Union, 2020). The European Green Deal will be funded with a third of the € 1.8 trillion investment in the Next Generation EU recovery plan and the EU's seven-year budget. The theme of environmental sustainability, the green deal and the impact of the policies adopted will have a consequent impact on the well-being of the population and on the impact of community behavior by launching new decision-making and cognitive processes and new public interest-oriented projects involving all sectors across the board (Brescia, 2020).

Automated content analysis of 178 articles related to magazines and journals was conducted to identify priorities and short-term responses. The use of LEXIMANCER on environmental issues such as the adoption of Green Deals policies is in fact more relevant than the scientific articles, applications and changes (Nunez - Mir et al., 2016). The analysis conducted focuses on the short-term effects of the pandemic crisis and the future change of economic and political models and paradigms in progress (Campra, Esposito, & Lombardi, 2020; Campra, Esposito & Brescia, 2020; Campra et al., 2021). The president of the European Commission Ursula von der Leyen highlighted how the pandemic influenced and pushed change during the Berlin Energy Transition Dialogue: "The European Green Deal is as important today as it was before Covid, indeed, it has become even more important ". Therefore, the study intends to answer two questions:

RQ1: Has COVID-19 affected the effective implementation of Green Deal policies?

RQ2: What priorities related to COVID-19 have been given in Europe with respect to Green-Deals policies?

The result intends to provide starting tools for understanding changes and priority elements of policies.

The next section analyzes the Green Deal policies and the relationship with the Next Generation EU Found. Subsequently, the methodological approach guarantees the replicability of the results and focuses attention on the choices made. The third section highlights the results of the automated content analysis which are explained by defining evidence in the fourth section. The last section answers the research questions by relating what was politically planned and what was adopted or given precedence. Research limitations and ideas for future analysis are explicit.

2. Green Deal Policy and Next Generation EU

The reduction of emissions envisaged by the European Commission defined on 23 and 24 October 2014 provided a reduction of at least 40% domestic reduction in economy-wide greenhouse gas emissions by 2030 compared to 1990 (EU, 2018). The start of the policy activities had already started in March 2016. The European Commission sent a document dubbed the European Green Deal (EGD) to the European Parliament and the Council on December 11th, 2019. The EGD was essential in the Von der Leyen Commission's approval and political agenda (Claeys, Tagliapietra, & Zachmann, 2019). During Von der Leyen's first 100 days in office, it was the most heavily lobbied topic in Brussels (Von der Leyen, 2019; Abels & Mushaben, 2020). The EGD is described as a "growth plan aimed at transforming the EU into a fair and affluent society with a modern, resource-efficient, and competitive economy" by 2050. (European Commission,2019, p. 2). The Green Deal proposes some pillars formed by a series of macro-actions containing strategies for all sectors of the economy, particularly transport, energy, agriculture, construction, and industrial sectors such as steel, cement, textiles, and chemicals. Furthermore, through specific funds, the reduction of the consumption of fossil fuels and the transition to less-polluting technologies was sought in addition to the proposal of a 'law for the climate' to make irreversible the path towards a zero-emission Europe in 2050 and a plan to increase the EU emissions reduction targets from 40%

to 50-55% by 2030 (Birindelli & Chiappini, 2021; Secinaro et al., 2020a). The law is associated with the launch of the European Climate Pact, followed by the proposal of the eighth action program for the environment 2020. The European Commission also proposed a plan that provides resources to support the circular economy through neutral markets focused on textiles, construction, electronics, plastics, specific legislation on the reuse and recycling of batteries, and a vast reform on waste (Zhong et al., 2020; Whiteman et al, 2011; Halluite et al.; 2005). The policies conceived also embrace sustainable and smart mobility through proposals for sustainable alternative fuels, combined transport, better use of water and rail transport, more restrictive policies for road transport and related emissions (Secinaro et al., 2020; 2021). The "Farm to Fork" policy also encourages the direct production chain to reduce the use of chemical pesticides, fertilizers, and antibiotics. The last topics dealt with the protection of biodiversity and deforestation. Several academics (European Commission, 2019; Ossewaarde & Ossewaarde-Lowtoo, 2020; Leonard et al., 2021). Disagree with the EGD's goal of decoupling economic growth from resource inputs. The European Commission's commitment to a just, inclusive, and people-centred transition is also mentioned in the EGD (European Commission, 2019). Several studies show that transitions are deeply political, requiring consideration of power relations and vested interests within energy systems (Rosenow & Eyre, 2013; Pettifor, Wilson & Chryssochoidis, 2015), examining the impact of deploying renewables and gas infrastructure to grow industrial output and consumption and scrutinizing ownership of the means to producer (Ziabina & Pimonenko, 2020; Brahmana & Kontesa, 2021). Furthermore, social opposition to sustainability agendas emphasizes the importance of recognizing the socioeconomic impacts of green transitions, as well as the disparity between workers' concerns about meeting the "end of the month" versus the climate community's demand to avoid the "end of the world" (Martin & Islar, 2021). In this context, Green New Deals (GNDs) offer an alternative to climate change mitigation by focusing investments on vulnerable, marginalized, and frontline communities and promoting "egalitarian policies that prioritize public goals over corporate profits" (Pettifor, 2020). Mastini, Kallis, and Hickel (2021) found that little attention has been paid to the fact that the content and framing of GNDs has altered through time in their research of brief histories of GNDs. An ecological modernization paradigm based on investments in technical solutions is dominant in what they refer to as GND 1.0. This framework is described as a technocratic exercise aimed at reviving capitalist investments by allocating financial resources to research and development, light subsidies, and carbon pricing (Mastini et al., 2021). In this way, the ‘GND 1.0' narrative can be linked to a neoliberal approach to climate politics in the sense that it advocates market-friendly regulation, market stabilization, and resource mobilization and capital accumulation. Mastini et al. (2021) used the term "GND 2.0" in the aftermath of the G20's growth-friendly budget consolidation in 2010 when 16 of the G20's member nations failed to meet UNEP's 2009 proposal of spending only 1% of GDP on green initiatives. The ‘GND 2.0' “rejects the primacy of market-based environmental policy instruments” that view our current ecological meltdown as a market failure that can be fixed through pricing, rather than a social crisis that can only be addressed by redistributing economic and political power (Mastini et al., 2021). As a result, the ‘GND 2.0' adopts command-and-control environmental regulation while prioritizing decarbonization in terms of speed, scale, and breadth through leveraging public investment and coordination (Aronoff et al., 2019; Falk & Hagsten, 2020; Palea & Drogo, 2020). Across the Atlantic, H. Res. 109 declares the US government's responsibility to create a GND to achieve zero net greenhouse gas emissions through a fair and just transition for all communities and workers; create millions of good, high-wage jobs and ensure prosperity and economic security for all; and invest in infrastructure and industry to meet the challenges of the twenty-first century in a sustainable manner (Samper, Schockling, & Islar, 2021). The discrepancy between intentions and reality leads to researching what has actually been implemented especially in a period of pandemic crisis in which the influence and incidence of COVID-19 could have significantly influenced the policies adopted. Due to the flu caused by Covid-19, the European Commission, the European Parliament, and EU leaders have agreed on a recovery plan that will help the European Union repair the economic and social damage caused by the coronavirus health emergency and help lay the foundations to make the economies and societies of European countries more sustainable, resilient and prepared for the challenges and opportunities of the ecological and digital transition. On 18 December 2020, Parliament and the Council reached a final agreement on Next Generation EU, the 750 billion euro program for the relaunch of an EU economy overwhelmed by the pandemic crisis. Next Generation Eu (NGEU) is a tool for relaunching the EU economy from the crash of Covid-19, incorporated in a seven-year budget 2021-2027 worth approximately 1,800 billion euros (750 from Next Generation plus over 1,000 billion budget). The instrument is closely correlated with the EGD and GND policies as 30% of European funds will be reserved for it and is tied to the fight against climate change, the highest percentage ever for the EU budget.

3. Sample and Research Method

The Leximancer text analysis program was utilized in this study to find statistical algorithms and compare important themes in news articles. Leximancer is a software system that uses mathematical methods to find, define, and illustrate links between concepts (Leximancer Pty Ltd., Brisbane, Australia). Leximancer is a technique for "unsupervised translating lexical co-occurrence information from real language into semantic patterns" (Smith and Humphreys, 2006, p.262). The Leximancer system uses an automated content analysis (ACA) technique in which the thesaurus is utilized as a classifier and the resulting co-occurrence data is used to produce concept maps (Jafari-Sadeghi, 2021; Smith and Humphreys, 2006). Themes are represented as heat maps, with intense colors like red and orange indicating the most essential topics (Leximancer, 2018). Clusters of concepts are displayed, each of which is surrounded by a colored circle that represents a theme. The distance between two concepts reflects how frequently they arise in similar mental situations, while the linkages between concepts show the most likely relationship between concepts (Sotiriadou et al., 2014). Leximancer version 4.5 was used to complete the ACA since it included a number of unique features for analysis and visual display (Leximancer, 2018). It was also chosen above other software because of a body of work that supported and validated Leximancer's capabilities (e.g. Cretchley et al., 2010; Crofts and Bisman, 2010, Smith, 2003; Smith and Humphrey's 2006; Penn-Edwards, 2010). The stability and reproducibility of Leximancer make it trustworthy (Verreynne and Parker, 2011; Middleton et al., 2011). Additionally, the program's ability to reliably identify text in the same way across multiple analyses illustrates its consistency (Jafari-Sadeghi, et al., 2020; Smith and Humphreys, 2006). It also allowed researchers to quickly identify concepts with little manual intervention, which is a limitation of manual coding tools like NVivo or ATLAS (Wilk et al., 2019; Lemon and Hayes, 2020). There are two more advantages to adopting ACA versus manual review. First, given the timeframes and resources available, it allowed us to review a far bigger amount of entries than we could with a manual review. Second, completing an ACA helped to eliminate human subjectivity that might arise when determining whether a report is an accurate and full portrayal of the comments received during a public review. If the increased exploitation of gas was a resource already included in the EGP for the industrial sector (Ziabina & Pimonenko, 2020), it is flanked by some alternatives on which the significant incentives and plans have been launched.

3.1 Automated content analysis two-stages process

A two-stage approach was used to analyze the data. The two-step approach and the use of the LEXIMANCER program follow the approach adopted by Martin & Rice (2010), Stewart & Gapp (2014) Massaro et al., (2021). The data cleaning process was the initial step, which deleted any unneeded or irrelevant information. All sources that could represent the short-term effects on Green Deal decisions following COVID-19 were selected. The keywords used to search for sources are "green deal" and "green new deal". The sources were selected by the Business Source Ultimate platform from February 2020, start of the pandemic period in Europe (Campra, Esposito & Brescia, 2020), to June 2021. Database containing full text and bibliographic citations of articles taken from over 3,500 scientific, economic, and social science periodicals (2,000 peer-reviewed) and numerous other specialist sources. It also gives 40,000 other specialist sources, including e-books, Company Profiles, SWOT analyses, Industry Reports (including those published by Datamonitor), Case Studies, and Market Research Reports. Allows navigation by subject through a Thesaurus created by EBSCO specialists. It also provides detailed information on over 1 million public and private companies. From the first screen, it is also possible to select the Econlit with Full Text and Historical Abstracts with Full-Text databases to consult the databases simultaneously. The time frame represents the response and potential effect of the pandemic crisis on Green Deal choices. Initially, the platform found 822 sources, including national reports, conference proceedings, scientific articles in open access, magazines, and newspapers. The selection of sources from English-language magazines and newspapers reduced the sources to 478, of which only 396 in full text. The analysis of the abstracts excluded duplication and sources not strictly related to the European context. The final results are 173. The results were then imported into Leximancer, where concepts (i.e. a set of weighted terms) were automatically constructed without any changes, resulting in an impartial list of concept seeds (i.e. the most often occurring words in the text) (Smith and Humphreys, 2006; Secinaro et al., 2021a). This initial evaluation assisted in identifying seeds that were either irrelevant or did not provide value to the concept seeds created (e.g. report title, subheadings, inquiry location, references, etc.). The text classification for each concept seed was examined in the second stage. Even though the correctness of Leximancer's weighted single words has been proven (Smith and Humphrey, 2006; Penn-Edwards, 2010), an iterative manual validation method was used to identify any conceptual disparities between the results and submissions. First, all text segments inside the created concepts were retrieved. The text segments within each notion were carefully read to determine whether the document part was accurately categorised. Concepts that were identified as erroneous or inaccurate were highlighted and examined during this procedure.

4. Results

In the first stage of the analysis, Leximancer discovered 35 primary concepts with an absolute concept count range of a maximum of 273 "plan" (69%) and a minimum of 45 "oil" (11%). The data is net of the two key concepts identified relating to the EUROPEAN context (present in 100% of the sample) and COVID expressly present in 32% of the sample. As can be seen from the clusters (Figure 1), European policy is closely linked to energy management, considering both the carbon emission market and sustainable investments. The other clusters depend on single macro groups. The EU cluster is closely related to the response to COVID. The lockdown highlighted how in many areas of the world, reducing production has led to cleaner air. Therefore, Europe's intention is not to reduce production but to change the production process with a zero-pollution approach. The Green Deal promises to reduce EU greenhouse gas emissions to zero by the middle of the century, in a massive environmental clean-up that will impact everything from energy production to agriculture and city planning. The European cluster is significantly conditioned by food emission, production, and management policies and consequently to the economy of sustainable investments to prevent carbon emission. The cluster of energy, in turn, is associated with management in the pandemic and post-pandemic period and with European policies and is conditioned by debates related to the period of transition and accompaniment towards clean energy (wind, photovoltaic, and gas), the debates on the use of nuclear energy and the emission policies of companies.

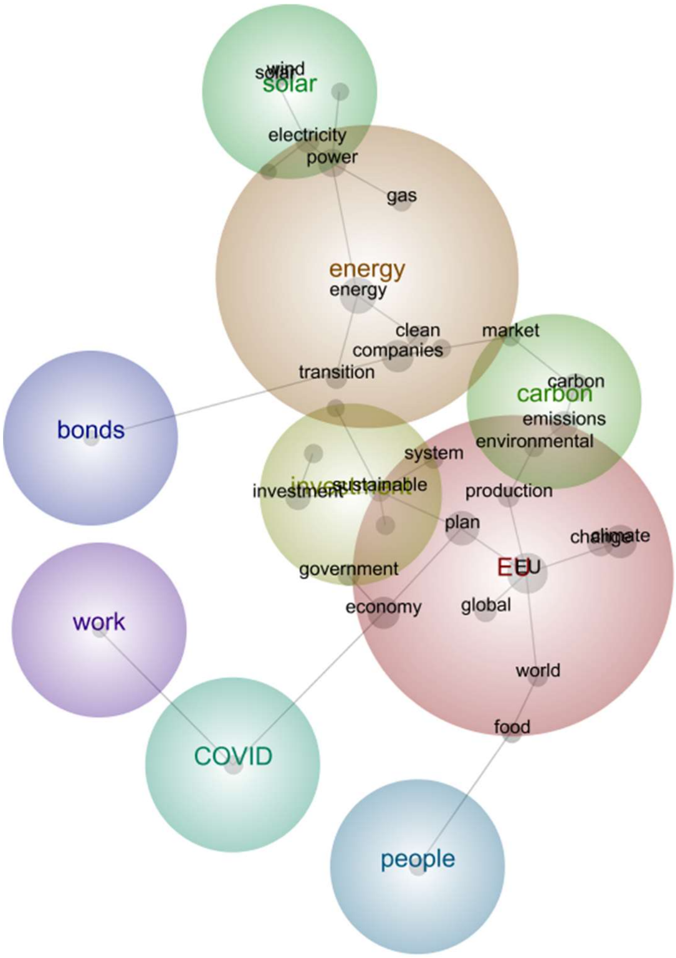

Figure 1 Themes, concepts and concept map for the Green Deal in Europe

5. Discussion

The section explores the content of the results obtained by LEXIMANCER. Through the relationships previously detected and the clusters, the second stage allows to determine the issues and policies on which short-term effects caused by the pandemic have been experienced. The priority for European policy associated with the Green Deal and recall from the Next Generation EU focus on relaunching sustainability. However, the frustration of various leaders unable actually to achieve the objectives aimed at avoiding climate change is evident from various sources. However, the analysis of the results reveals that we are not completely at the starting point concerning previous agreements on climate and environmental protection. For example, between 1990 and 2018, the EU reduced greenhouse gas emissions by 23%. The need to relaunch environmental and climate sustainability policies was, in fact, already restarted by Ursula von der Leyen during the UN climate conference in Madrid, promising that Europe would become the world's first carbon-neutral continent within the next 30 years. The relaunch of a sustainable policy occurred when President Donal Trump left the Paris Climate Accord, and at the same time, China, one of the leading producers of pollution, defined expansion plans. Therefore, European policies will be correlated with prospects at a global level and will affect the different world visions. However, the short-term period that began with the spread of the pandemic could have conditioned the debate and the policy choices undertaken.

The energies adopted are among the main priorities dealt with to bring about the transition of companies towards clean perspectives. One of the main topics of interest in the debate appears to be Hydrogen, which can store renewable energy and be a raw material in energy-intensive industries, such as the steel sector. The issue is particularly delicate because it affects all those companies that during the twentieth second have produced thanks to combustion plants that used coal, oil, gas, and fossils and that still several workers. Policies are therefore figuring out how to re-employ the many workers employed in the sector. One of the main themes is the real change in the energy consumption of European cities. Buildings account for 40% of energy use, and the goal is to renovate 3% of buildings annually in the EU over the next decade. The Energy Performance of Buildings Directive is a piece of EU legislation that sets out requirements for energy efficiency in buildings. Europe is taking an example from Canada which has already initiated this type of policy. Europe intends to invest as already done in Australia and Canada in hydroelectricity and the exploitation of natural gas. Parallel to the identification and investments in renewable energies, debates on the harmfulness of nuclear energy have begun in some states, including Germany.

The different European nations have initiated various actions aimed at governing the complicated economic period. While the exit from the pandemic could mark the end of a crisis not only social but also economic, it is true that it has deprived the various states of strength to evade the commitments made with the Green Deal. In such a difficult moment, European nations have the opportunity for economic revitalization to consider the priorities of the Green Deal as part of the solution. The European Commission and the international debate highlight how the recession and the possibility of a cohesive policy of European reinvestments can guarantee a decisive change. It mainly involves Germany, France, Italy, Austria, and Spain, overcoming each nation's previously detected blocks. Unfortunately, in reality, many states and industries, instead of respecting and orienting choices towards the Green Deal due to lost revenues during the pandemic, have cancelled what was planned. The founder of the Solar Impulse Foundation highlights how these choices go against the prospect of reviving the economy and new job positions. Globalization has led to changes that have somewhat damaged the sustainable policies implemented. It is highlighted that the states with the most advanced technologies, China, Russia, the United States, India, Japan, are instead boycotting the financial power of London capable of imposing the Green Deals desired instead by Europe and North America.

The Green Deal is based on sustainable energy policies; during the period, numerous newspapers question which energies to invest more in based on the power generated. Energy efficiency refers to the efficiency with which an energy source is converted into electricity, as well as the power plant's online (non-intermittent) reliability. The size of the various sites is being determined by the amount of power required in each area. Sun and wind are the two renewable energy sources most endorsed in the debate. For example, a wind farm of 1,000 MW rated capacity takes 7-10 years to build, and its “median performance”—that is, actual electricity generated — is half or less than half of a 1,000 MW coal-fired plant takes 2-3 years to build. Photovoltaics for the same investment conditions on average generate much more energy than wind power, so the debate often points the choice in this direction. Nonetheless, it is highlighted those renewable technologies take three or four times to produce the same energy previously produced through coal.

The zero-carbon or low carbon objective to reduce CO2 emissions does not currently find incentive policies for carbon reduction by non-European producers. It was also stated that a Carbon Border Adjustment system would boost European steelmakers' efforts to cut carbon emissions while simultaneously incentivizing non-European companies to do so. The proposed law on emissions has partly absorbed the recommendations of some critical stakeholders that will be affected by the new rules, and the logistics service representatives participated in the work providers, seaport and inland port authorities, shippers, combined transport operators, rail freight operators, port and terminal operators, marine equipment manufacturers, and shipyards. To date, particular attention has been paid to the production of low-emission steel and the parallel importation of only "green steel", however, leading to higher costs that affect the European market from 35% to 100% compared to the previous situation. In order to reduce emissions, the European Community is focused on recycling and the possible circular economy linked to batteries. On a global level, the economic push to develop green finance came precisely from the pandemic crisis. Investments in ESG Bond allowed for expansion of vaccination plans and more excellent prevention of industrial accidents. The major investments are destined for the production of steel, chemicals and cement which produce the highest emissions to date and are considered strategic sectors by Europe. Many European and extra European states have started issuing green bonds, identifying them as a possible short-term response to the crisis. However, Covid has changed the commitment of some dissident powers; China has committed to reducing carbon emissions to zero by 60. Some electricity producing companies have simultaneously launched initiatives aimed at respecting the Green Deals objectives, in particular, in Europe not having received EU grants, Enel has still started for 70% of the planned investments in renewable energy. Extra-EU the FTE Energy present in America has committed to be net-zero carbon by 2050 and the reduction of CO2 emissions 50 percent by 2030 and 80 percent by 2040.

Covid has led to a change in the work paradigm by accelerating smart working and forcing digital change. The challenge undertaken in the pandemic period concerns automation, digitization and the use of other technologies. Digital change has initiated a greater incidence in investments aimed at the digitization of cities (Jafari-Sadeghi et al., 2021; Garousi Mokhtarzadeh et al., 2020). At the same time, there has been an increase in the number of freelancers who have tried to respond to a period of crisis in the labor market for those who did not yet have a job. A crisis has reduced the possibility of finding an occupation only in the areas adjacent to those of residence, thus reducing the possibilities. On the other hand, many companies have invested in employees, recognizing in training a more remarkable ability to foster loyalty, customer satisfaction and positive brand perception. Among the sectors particularly affected by the pandemic, the one relating to the crisis linked to the supply-chain process, mainly affected by the pandemic and influenced by future investment policies, emerges (Riahi Dorcheh et al., 2021).

The policy proposed to food producers has been to produce more with less. Less soil but also fewer nitrates and other supporting chemicals. The policy started affects the lower use of pesticides, antibiotics, and fertilizers. A production that continued during the pandemic crisis despite the difficulties in importing and exporting what was produced. Furthermore, a lower taxation policy can increase the sale of organic fruit and vegetables. Some significant factors to date are absent in the proposed European policies, such as high yield seeds, adding more work, or cushioning the impact of reduced production reducing waste in the food chain. The possibility of personalized training has allowed us to understand what works and what are the limits of smart working. The process initiated a change in the redefinition and redesign of the work. The environment and the well-being of workers were debated issues in the pandemic period. Despite the intention not to affect jobs, the European Commission released its "Farm to Fork and Biodiversity Strategies "in May 2020. In practice, a smaller quantity of food will be produced by seeking a better quality. The choice was adopted following an analysis that finds that one-fifth of the food produced is wasted while 36 million citizens cannot afford a quality meal every second day. At the same time, it provides for 30% of the agricultural land across Europe to be returned to nature. The agriculture output drops 12% overall, and worldwide production drops 1%. The United States Department of Agriculture (USDA) has opened the debate on the political choice made by the European Commission with the “Farm to Fork Biodiversity Strategies” which, according to estimates, could affect the actual possibility of access to food. Insecurity found equal to 185 million people would be thrown into food insecurity (lack of reliable services and/or enough food) over ten years. The current European export policy outside the EU intends to consider the world as the only region with considerable repercussions also economically.

6. Conclusion

The Green Deal policy influences the entire globe but has had a specific influence in the short term as the analysis carried out shows a relapse between theoretical objectives and priority themes also developed due to the pandemic crisis. While on the one hand, it has reduced attention to some priority sectors and some issues such as sustainable transport, textiles, general waste management, electronics, plastic management and the circular economy has, on the other hand, focused and given priority to some issues that affect the well-being of the community rather than the profit of corporations (Pettifor, 2020). The investment plan envisaged by the Next Generation Found EU can relaunch the objectives of the Green Deal, the analysis highlights the political priorities and the most debated sectors, and precisely because of COVID-19, they have been more developed than others. The analysis of clusters and relationships highlighted how the short period had disruptive effects. Compared to what was highlighted by the EGD (Brindelli & Chiampini, 2021), the debate and international application have mainly focused on the steel production sector, identifying the exploitation of hydrogen as an element in which to invest. Another element recalled concerning the provisions of the EGD concerns only the recycling and management of electric batteries in which Europe is investing (Zhong et al., 2020). The particularly relevant issue for the EGD concerns farm management, the "Farm to Fork" policy (European Commission, 2019) intervenes on the exploitation of inputs and the volume of food produced, but in fact, opens a rift between the intentions of the European Commission to waste less food with better territorial quality and fewer imports and the possible increase in the impoverishment of subjects who already had difficulty in accessing quality meals. The de facto policy also aims to increase local biological diversification, self-sufficiency and reducing deforestation, elements driven by the pandemic crisis, and the difficulty of the supply-chain sector (Sadraei et al., 2018). According to the analysis, the change in spaces and smart working driven by COVID-19 and the lockdown has increased the attention to decarbonization, and the change that cities must have, investments aimed at prioritizing lower energy consumption are already underway buildings. The theme confirms the attention already paid by GND 2.0 (Aronoff et al., 2019; Palea & Drogo, 2020). Change and energy consumption not only affect public regulation but also private regulation, confirming carbon prices policies (Mastini et al., 2021). The pandemic has sought resources through the London financial market with green bonds to immediately deal with the pandemic crisis and still finds interest today on the part of the European market (Mastini, Kallis, and Hickel, 2021; Fatica & Panzica; 2020), however, opposed by other non-European productive powers. Investments aimed at exploiting natural resources such as natural gas and water for electricity production have been defined as priorities. However, a debate is open regarding adequate power and the best renewable source among those already widespread (wind and photovoltaic) with possible effects on spaces required according to the required volume required. Nuclear is not considered a clean resource to invest in Europe. The involvement and relaunch of work concerning the sectors on which the Green Deal policies will have the most significant impact have not been addressed and is the most critical in a period of recession and social difficulty caused by the pandemic; the issue significantly deviates from the objectives that the European Commission had set itself (Samper, Schockling, & Islar; 2021). In the short term, the Green Deal finds a push and a relaunch caused in part by the pandemic by the investments of the European Commission. However, it has been conditioned and opposed by the previous United States government and by China which is still trying to define the future policy between expansion and environmental effects detected following the lockdown. The recharge results have evidence and impact in several industrial sectors and on the community, effectively conditioning both the production processes, the materials and energy used, and the consumption and policies that impact the community and services of public interest. Future research could analyze the actual investments and projects carried out thanks to the Next Generation Found EU, and the incidence of the priorities pushed up to now by COVID-19 that influence on the one hand the well-being of citizens on the other the European production sector and worldwide export and import policies.

6.1 Limitations

As with any research, there are limitations. The analysis considers only issues developed by the media and which have been given complete transparency. Some information and decisions of the European Commission may not have been fully publicized. The small sample represents significant economic and political information but does not consider other socio-cultural information that could be collected through the aid of other information databases. Additionally, many studies (qualitative and quantitative) could also be done to compare the situation of European Green Deal investment applications.

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