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In Silico Analysis of SNPs and Gene Disease Association Analysis, Homology Modelling and Molecular Docking of Human GST Gene using Bioinformatics tools

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Abstract---GST gene codes for metabolic isozymes catalyze the conjugation reaction where glutathione is reduced to xenobiotic substrate and mutation in GST gene can cause cancer. During this study, mutation found in GST and efficacy of current treatment is determined using computational and web-based tools. Diseases related to GST were obtained using DisGeNET. Primarily breast carcinoma and malignant tumor of prostate are selected for further analysis. Using Cytoscape network analysis is obtained. Highest scores were displayed in breast carcinoma and malignant tumor of prostate for GSTZ1 and GSTA1 respectively, which allows predicting role of GST in disorder. Disease specific mutations were collected from ClinVar, DisGeNET and UniProt databases. I-Tasser platform is employed to perform homology modelling and validate by Ramachandran plot. Molecular docking was performed by PyRx of generated model against drugs/ligands commonly used to control the disease. Drugs showed high binding affinity towards generated protein model of GST might be used as potential drug target for the disease.

Key Words--- GST- Glutatione S Transferase; Cytosolic family: SNP- Single Nucleotide Polymorphism; GST signaling pathway; MAPEGs -Microsomal GSTs also referred to as 'membrane associated proteins in eicosanoid and glutathione metabolism'

I. INTRODUCTION

Enzymes are the catalyst that enhances the biological reaction. These enzymes are coded by specific sequence of gene on DNA which is transcript and translated to protein that would be an enzyme. The change in these DNA sequences could end in formation of variants or commonly called SNPs of that gene which has the power to regulate the expression of gene and may also hinder gene regulation and their metabolic pathway. During this study particularly human GST gene and their variants are studied.

Glutathione-S-transferase (GST) family encodes for the genes that are captious for the life processes. GST depicts a crucial group of enzymes that detoxifies both endogenous compound and foreign compound [Daniel WB et.al. 2004]. Majorly GST is involved in the nucleophilic attack on electrophilic substrate (GSH). GSTs acts as ligands for signaling kinases namely ASK1, JNK, Akt, RyRs and EGFR [Nerino A, et al.,2018]. The GST family interacts with various MAPK in non stress condition [Julie Pajuad et al.,2012].

The protein:protein interaction is facilitated by GST and their isozymes, which increases their efficiency of removing toxic substance from cell [Nissar S, et al.,2017]. With bioinformatics tools the deleterious SNPs can be ppredicted before population study. This will make the study cost and time effective.

Human GSTs have categorical three main families: Cytosolic, Mitochondrial and Membrane bound microsomal families. The Cytosolic and mitochondrial families are soluble enzymes with 3-D fold structural similarities. Every family is coded with variable gene located on different chromosome. [Nissar et al.,2017].

Whereas, MAPEGs are structurally different but have similar function to carry the reaction of GSH to electrophilic substance. [Hayed JD., 2005].

A. Signaling Pathway of GST gene:

Cells square measure exposed to internal and external stress unceasingly, that triggers communication pathways associated degreed leads to an initiation of various cell programming including apoptosis. Regulation of those pathways involves upstream activation of three protein kinase family: MAP3K, MAP2K and MAPK. The GST family interacts with various MAPK in non stress condition. In stress condition, these interactions are disrupted and results in activation of signaling pathways. [Julie Pajaud et

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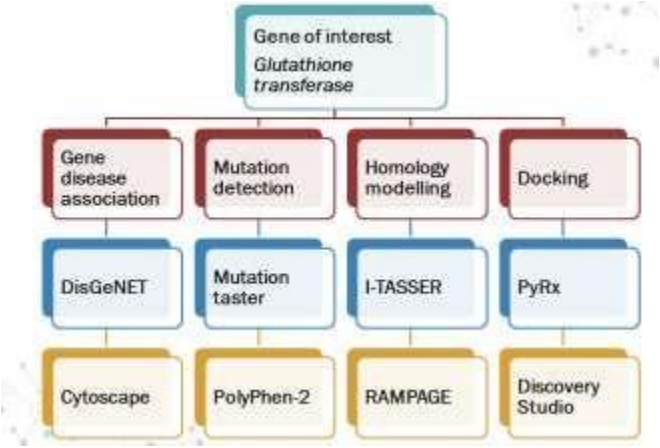

Figure 1: Diagrammatic representation of the work pipeline.

II. MATERIALS AND METHODS

The pipeline for the study is showed in the flowchart as depicted in Figure 1.

A. DisGeNET and Cytoscape:

To rule out the disease caused thanks to the mutation within the gene of interest (GST) directed to, use of bioinformatics tools like DisGeNET and Cytoscape. DisGeNET characterizes the extent of linkage of the gene to disease within the sort of scores and provides details of published literature during which mutations are mentioned. Cytoscape presents the gene-disease association network to ease the visualization and additionally gives the sort of association of gene with the actual disease.

B. Polyphen2 and Mutation Taster:

Mutation doesn't necessarily imply for the disease. a number of them are synonymous while some are non-synonymous which will be non disease causing. Web based tools like Polyphen2 and Mutation Taster gives functional effect or an involvement of the mutation leading to the disease thanks to alteration during amino acid(s) in a protein as scores in various ranges.

C. I-TASSER and RAMPAGE:

Genes encodes for proteins. Eventually these proteins perform various functions during a cell. Therefore, when a mutation results in alteration(s) of any type in coded proteins, their effects are often observed as anomaly in normal functions performed by cells of the organ or body and eventually as ailments. Thereupon, predicting the structural changes within the protein is significant. Hence, to work out an ingenious and an altered structure of protein encoded by gene of interest before and after mutation(s) respectively, I-TASSER developed by Zhang Lab University of Michigan are often used. I-TASSER refers to Iterative Threading Assembly Refinement. it's used for structure and performance prediction of protein whose sequence is understood but 3D structure is unknown.

The Ramchandran plot analysis of proclaimed protein model is often completed by using RAMPAGE software developed by Crystallography and Bioinformatics Group at University of Cambridge. it's wont to visualize an energetically favored region for psi angles against phi angles of an amino acid(s) in any protein structure.

D. PyRx and Discovery studio:

To rule out a binding affinity of protein translated from gene of interest with selected molecules like drugs and protein, PyRx are often used. PyRx is an open source software that vividly enhances the accuracy with time efficient limit of docking with a completely unique separating ability, optimization and multithreading (Chen and Ren 2014). to see the binding, softwares like Discovery studio and PyMol are often used.

III. RESULTS AND DISCUSSION

A. Identification of disorders associated with GST:

Analysis of diseases related to GST (Cytosolic family) was performed using DisGeNET database by entering GST (along with their family) as query term and afterward network was built using Cytoscape version 3.6.1.

In the case of GSTA1, total diseases associated were 74, ranged from Schizophrenia to neoplasms (some experimental) to Myocardial disorder. supported score, Schizophrenia and malignant tumor of prostate was the foremost closely related disorder with a score of 0.320. it had been followed by Acute renal failure, which had a score of 0.310. Likewise, disorders like myocardial disorder, psychotic disorder, drug toxicity were also mentioned with a score of 0.3. Similarly, the info from DisGeNET was obtained for other Cytosolic GST family gene that is: GSTM1,GSTZ1, GSTT1, GSTO1, GSTP1 and GSTS1.Using DisGeNET app installed in Cytoscape, a network is obtained which demonstrates 74 disorders previously mentioned in DisGeNET, each within the sort of a circle(node) linked to GSTA1 via edges, as shown in Figure 2.

As the network is just too large, to mapped out the disorders with which GSTA1 and GSTZ1are more closely linked, the source was changed to CURATED in cytoscape, as shown in Figure 2. After this, another network was obtained. Same networks were obtained for remainder of the member of

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Cytosolic family of GST gene.

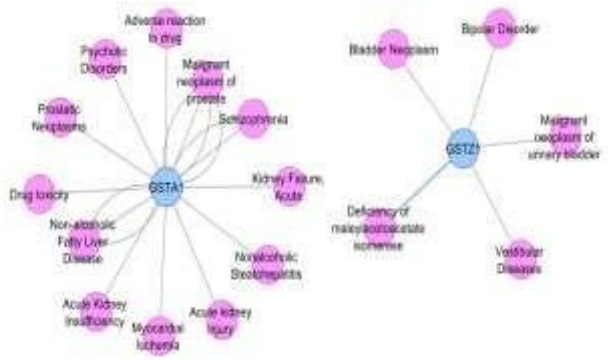

Figure 2: Curated merged network generated by Cytoscape for GSTA1 and GSTZ1

The diseases mentioned in the network and other diseases also showed their relation with GST Cytosolic family. In some of the in vivo studies performed showed that GSTP-/- mouse model, namely JNK activity is enhanced, in liver, lung and fibroblast. That shows JNK pathway is upregulated results in increase DNA binding and mRNA expression. [Elbsy R et.al., 2003]. It is also observed that for Parkinson's disease model, GSTP-/- models were hyersensitive than wild type to this stress condition [Castro-Caldas et al.2012]. Therefore, in the brain and in stratum, GSTP acts as regulator of JNK pathay. GSTA1 is involved with JNK in caco-2 cells. There results showed lower levels of GSTA1 in preconfluent cells. It shows JNK dependent apoptosis signal was increased in preconfluent cells [Romero et.al.,2006].

Exploring the details mentioned in tables below for the latter network, in all two types of associations were observed between GSTA1 and GSTZ1 with diseases. Except Deficiency of maleylactoacetate isomerase, Breast carcinoma, Bipolar disorder, Carcinoma of bladder, Schizophrenia, Malignant neoplasm of prostate in which GSTA1 and GSTZ1 is a genetic variant, in case of all other disorders GSTA1 and GSTZ1 are linked as a biomarker. The diseases were differentiated based on their type of association with GSTA1 and GSTZ1 as in Table 1.

Table 1: Type of relation of GSTA1 and GSTZ1 with disorders obtained from DisGeNET tool in Cytoscape.

Sr. no	Association type	Name of the disease
1	**Biomarker**	**Malignant neoplasm of urinary bladder, Neoplasm Metastasis Tuberculosis, Drug toxicity, Acute kidney failure, Non- alcoholic fatty liver**
2	**Genetic Variation**	**Deficiency of maleylactoacetate isomerase, Breast carcinoma Bipolar disorder, Carcinoma of bladder, Schizophrenia, Malignant neoplasm of prostate**

Biomarker association indicates that gene either is involved in an etiology of the disorder or is a biomarker for it. Genetic variation association on the other hand indicates that a sequence variation is associated with the disease phenotype, but there is still no evidence that the variation causes the disease. In the case of GSTA1, 7 out of 10 closely related disorders show biomarker type of association whereas 2 (Malignant neoplasm of prostate) show genetic variation type of association. In case of GSTZ1, 5 out of 10 closely related disorders show biomarker type of association whereas rest (Breast Carcinoma) show genetic variation type of association.

B. GST polymorphism:

Polymorphism of GSTs are associated with various disease such as neruodegerative diseases, Cancer disorders, Non-alcoholic fatty liver and other caused due to metabolic disorders. GSTs polymorphism is associated with Parkinson's and Alzhaimer neurodegenerative conditions [Board P et. al., 20016 and Kumar A et. al., 2017]. As GSTs are involved with JNK and MAPK pathway they are also responsible for down regulating apoptosis. The risk is significantly high for colorectal cancer with GSTM1 null genotype in Lebanese population. [Darazy et. al.,2011 and Hlavata et. al., 2010].

Polymorphisms in GSTP have a role in cancer drug treatments. As, SNPs are results of nucleotide change, for example, change from from Ile to Val and from Ala to Val, generates 4 GSTP1 alleles. [Osma et.al.,1997].The size and hydrophobicity of residue acts as determining factor for each of the GSTP1 isozymes [Hu et.al.,1998].

C. GST SNPs analysis:

The GSTs SNPs were submitted to SIFT to screen out the deleterious SNP. This was performed on the basis of the SIFT score, the SIFT score of SNP being 0 is the deleterious and the increase from 0 to 1 they prove to be less deleterious [Pauline C. Ng and Steven Henikoff.,2001]. This SNPs were mapped to native structure by I-Mutanat 2.0. It is neutral network-based tool used to study protein stability alteration caused by single- site mutation [Dhabi B et al., 2014].

SNP&GO depicted 2 SNPs with a damaging effect of a scoring accuracy of 80%. PROVEAN characterize functional amino acid change via evolutionary relationship classification in GSTA1 and GSTZ1 protein. By PROVEAN, 2 out of 10 SNPs are predicted deleterious,

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where variants equal or above the threshold of −2.5 are considered as deleterious. When results of all the tools were used to detect high-risk SNPs the functional SNPs at positions E33G and G32R showed a positive damaging effect in four servers. And they were used for further analysis [Joshi B et.al.,2015].

D. Mutation detection and Homology modeling:

From the data of all SNP for GST family, the significant SNPs were screen, and are mentioned in Table 2 which were further used in the study.

Table 2: Data of variants and their score obtained from various bioinformatics tools. (*-Deleterious GST variants, **-Novel GST variants, SIFT Score: <1= deleterious and >1=non-deleterious)

dbSNP_ID	Amino acid substitution	PolyPhen-2		Mutation Taster Score/effect	SIFT score
		Human Var	Human Div		
rs367590266*	E33G	0.971	0.994	Disease causing	**0.001**
rs4925	A140D	0.106	0.234	Polymorphism	**0.358**
rs156697	N142D	0.001	0	Polymorphism	**0.712**
rs34400162	V41I	0.328	0.820	Polymorphism	**0.034**
rs2266633	D8N	0.001	0.001	Polymorphism	**0.407**
rs7972*	G42R	0.996	1	Disease causing	**0.006**
rs1050851	A102A	0.001	0.100	Polymorphism	**0.87**
rs1799983	D298E	0.120	0.280	Polymorphism	**1**
rs227415	G102S	0.304	0.340	Polymorphism	**0.247**
rs4150795	K55R	0.100	0.128	Polymorphism	**1**
rs141645977**	P104L	0.996	1	Disease causing	**0.001**
rs150506133**	P79T	0.998	1	Disease causing	**0.002**
rs374294843**	P73S	0.998	1	Disease causing	**0**
rs11509436**	S86C	0.996	1	Disease causing	**0**
rs45529437**	C32Y	0.998	1	Disease causing	**0**
rs371083091**	G12V	1	1	Disease causing	**0.001**
rs376564748**	R18H	1	1	Disease causing	**0.001**
rs374361982**	R187G	0.958	0.997	Disease causing	**0.003**
rs199846502**	**R13W**	**0.996**	**1**	**Disease causing**	**0**

The homolog model of GSTA1 and GSTZ1 was built using I-TASSER online server. For non- mutated GSTA1 and GSTZ1, a single homolog model was obtained with C-score of 1.45, as shown in Table 3. For mutated GSTA1 and GSTZ1 Homolog models were also prepared for individual mutations, out of which the one with highest C-score was selected for further stability analysis using RAMPAGE.

Table 3: C-Score for individual variants obtained using I-TASSER

dbSNP	Model 1	Model 2	Model 3	Model 4	Model 5
rs367590266	1.45	1	1.3	1.0	**1.2**
rs7972	**1.45**	**1.2**	**1.0**	**1.25**	**1.0**

The homolog models of mutated and non-mutated proteins were downloaded in PDB format and further analysed for its stable conformation using Discovery Studio. The mutated protein showed 85.8% of residues in favoured region in GSTA1 and 85.9% in GSTZ1, their homolog models are shown in Figure 3.

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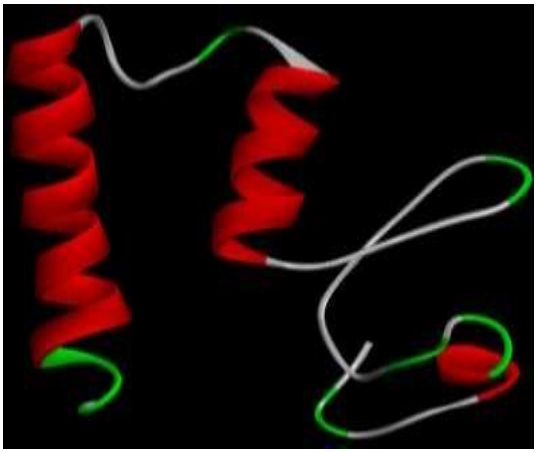

Figure 3: Model for GST variants of GSTA1 and GSTZ1 (respectively).

E. Molecular Docking:

Molecular docking of GSTA1 and GSTZ1 was carried out using PyRx. The exhaustiveness score was kept 8 and the dimensions of the grid box were set as default. Total 8 drugs were used for docking. [Nerino et. al.,2018]. The drugs used in this in silico study are mentioned in Table 4. EAA is the diuretics used to treat cardiovascular diseases. It also has antiproliferative property. [Mignani S et.al., 2016]. Binding Energy of each of these drugs is shown in table below. Binding energy of GSTA1 and GSTZ1 ranged from -7.2 to -5.4 kcal/mol. The drugs Spinesterol and ADH showed least binding energy value, which implies that they have the highest affinity for GSTA1 and GSTZ1. The conformations of drug-ligand complex with zero RMSD value obtained after docking are as shown below. Ligand binding interaction of these drugs was analyzed using Discovery Studio and PyMol.

Table 4: Total drugs used for docking.

Drugs	Properties
Ethyl aceto acetyl	It is a diuretic used to treat various cardiovascular diseases
Anti-diuretic hormone	Used in hormonal treatment for cancer disorders
Spinesterol	It is phytosterol found in spinach and has anti-microbial activity
Gamma elemene	Compound of turmeric with anti-biotic property
Piperene	It is a natural compound with anti-tumor activity
Calebine	Compound of turmeric with anti-biotic property
Metformin	Inhibitor for GST gene
Canfosamide	Inhibitor of GST gene

Multidrug resistance is a phenomenon that involves various mechanisms. GSTs shows its involvement in the process of enabling resistance towards anticancer multidrug used in treatment of various cancers. There is an observable increase in GSTs expression in cancer cells compared to regular cells. [Hayes et.al.,2005 and Gate et.al.,2001]. There are examples of drugs to which resistance is developed due to GST activity, they are: Cisplatin, Busulfan and Dicholoacetate[Allocati et.al.,2018]. GST inhibitors enhances sensitivity of cancer cells to antitumor drugs and that makes a use in therapeutic applications [Mahajan et.al.,2005]. Some of the examples of inhibitors of GSTs are: NBDHEX,Ethacraplatin and Ethacrynic acid and analogues[Allocati et.al.,2018]

Also the prodrugs that are defined as pharmacologically inactive compounds that are converted to active compounds in present of drug, is used to improve the bioavailability of active drugs by increasing their amount in target cells. Examples of prodrugs for GSTs: Canfosamide, Metformin analogues and Doxorubicin analogues. [Allocati et.al.,2018] Thus, by performing docking we can know which drug interacts with macromolecule(protein) with higher affinity and thus proves to be more effective, and their results are mentioned in Table 5. And the docked model of GST proteins with high affinity drugs are showed in Figure 4.

Table 5: Docking results of GSTA1 and GSTZ1 against drug agents

.(NS= Not Significant)

Drug name	Protein	Binding affinity (kcal/mol)	RMSD (Å)	RMSD LB (Å)
EAA	GSTA1, GSTZ1	-6.2, NS	0.0	0.0
ADH	GSTA1, GSTZ1	-7.2,-7.2	0.0	0.0
Spinesterol	GSTA1, GSTZ1	-7.0,-6.4	0.0	0.0
Gamma elemene	GSTA1, GSTZ1	-6.9, -7.2	0.0	0.0
Piperene	GSTA1, GSTZ1	-6.0, NS	0.0	0.0
Calebine	GSTA1, GSTZ1	-4.9, -6.4	0.0	0.0
Metformin	GSTA1, GSTZ1	-5.9, NS	0.0	0.0
Canfosamide	GSTA1, GSTZ1	NS, -6.0	0.0	0.0

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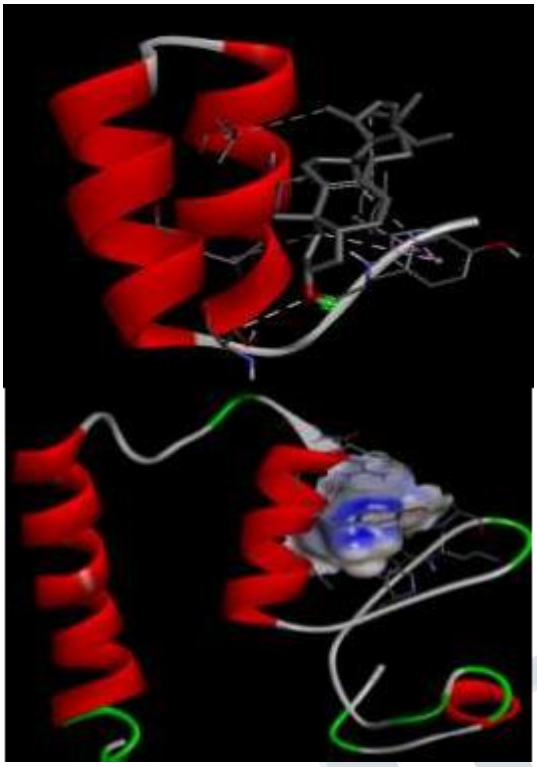
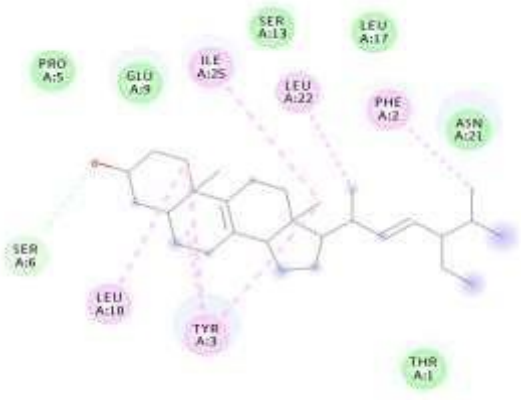


Figure 4:(a) and (b) Model for GST variants of GSTA1 and GSTZ1 docked with ligand spinesterol and gamma elemene (respectively).

These results are obtained by docking of respective GST protein with the ligands as named above. Here, the docking is performed in PyRx software and results are visualized in discovery studio. Binding affinity is a determing factor for drug being effective or not in the virtual space. Furthermore, 2-D interaction results are obtained for the study which amino acid interacts with particular site of GST protein, as shown in Figure 5.



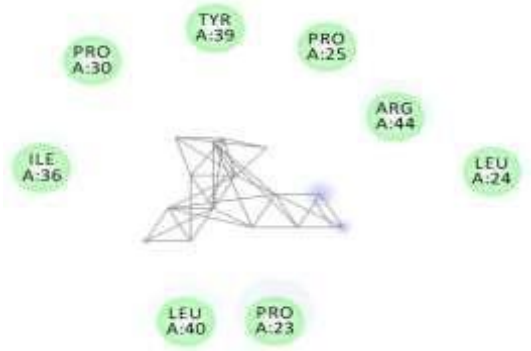


Figure 5: 2-dimensional interaction of GST variants of GSTA1 and GSTZ1 docked with ligand spinesterol and gamma elemene (respectively).

In this 2-dimensional interaction it shows the interaction of particular amino acid interacting with the targeted GST protein at a specific site.

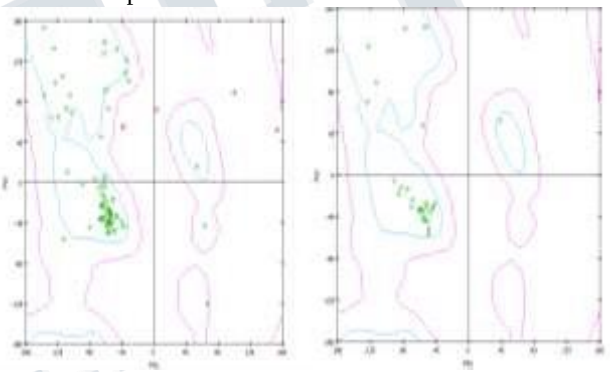


Figure 6: Ramachandran plot of GST variants of GSTA1 and GSTZ1 docked with ligand spinesterol and gamma element (respectively).

Ramachandran plot validated the protein model obtained from the SWISS-MODEL workspace, according to which 430 and 550 residues were obtained in the final GSTA1 and GSTZ1 model docked with their ligand respectively. Out of all the amino acids, 83% and 80% were in the most favored region (that is seen with green color) as shown in Figure 6. Thus, the overall structure of GSTA1 and GSTZ1 protein docked with their ligand structure obtained from the Ramachandran plot can be considered as the appropriate one. Stability is determined by the percentage of favored region that is, favored region percentage is directly related to stability.

IV. CONCLUSION

From this study it is concluded that variants of cytosolic GST family have a damaging impact which results in diseased conditions in humans. Variants rs 367590266 and

rs7972 are damaging and results in Malignant neoplasm of prostate and Breast carcinoma respectively, whereas SNPs rs141645977, rs150506133, rs37294843, rs11509436, rs455294437, rs3710830091, rs376561982, and rs199846502 are novel SNPs as it shows the damaging score but its disease is unknown. These SNPs can be studied further to know where and in which pathway they are involved in to recognize the disease they cause. After molecular docking Spinasterol, showed potential ligand affinity towards GSTA1. Gamma elemene and Beta elemene showed potential ligand affitnity towards GSTZ1. Hence, they can be used for therapeutic purpose.

V. FUTURE PROSPECTS

Very few studies have been carried out in India that highlight the importance of GST Cytosolic family specifically GSTA1 and GSTZ1 mutations in cancer disorders. This study can be considered as one such study that aims for advanced research in Gene-Disease association analysis. GSTA1 and GSTZ1 can be targeted as a therapeutic target for non-neoplasms and cancer disease. The mechanism of Malignant neoplasm of prostate and breast carcinoma can be studied by keeping normal GSTA1 and GSTZ1 (respectively) gene expression and changes in its expression under diseased condition as the focus. Anti-Diuretic hormone and Ethyl aceto acetate can be used as potential ligands in the drugs for the treatment of their respective disease. Also, natural compounds spinesterol and gamma elemene can be used in their treatment as they showed equal potential in binding affinity with mutated protein.

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