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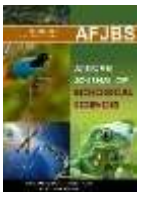

In Sillico Discovery And Evaluation Of *Momordicacharantia L* Phytocomponents Against Dinp Phthalates Induced Oxidative Stress Proteins Associated With Neurodegenerative Diseases

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ABSTRACT

The Momordicacharantia plant, also known as bitter gourd or bitter melon, is a crucial herbal remedy with a wide range of medicinal properties. It contains various essential phytocompounds, which contribute to its anti-inflammatory, antioxidant, and neuroprotective effects. DiNP phthalate is a common plasticizer used in polyvinyl chloride products, exposure is linked to increased oxidative stress and deregulations of genes linked to neurodegenerative diseases like Alzheimer's. In the present study, in silico molecular docking analysis of phytocomponents present in M. charantia fruit was studied against Oxidative Stressed genes (ACHE, MAPK9, SP1) associated with neurodegenerative disease pathways like Alzheimer's Diseases (A.D.). We docked the receptors are ACHE, MAPK 9, SP1 with the phytocompounds ofMomordicacharantiafruit. The result revealed that out of these 19 phytocompounds, Ajmalacine, Alkaloid AQC2, Alkaloid DF, Steroid U, and quinine were ranked the highest, with binding scores ranging from −11.0 kcal/mol to −9.1 kcal/mol compared with the standard, Armepavine, with a binding score of −6.6 kcal/mol. From the results obtained, it can be concluded that Ajmalacine, Alkaloid AQC2, Alkaloid DF, Steroid U, and quinine act/treat against Oxidative Stressed genes (ACHE), which are associated with neurodegenerative diseases like Alzheimer's Disease (A.D.).

Key Words: - Oxidative Stressed Genes, Ajmalacine, Alkaloid AQC2, Alkaloid DF, Steroid U, Quinine, Docking.

INTRODUCTION

Neurodegenerative diseases, including Alzheimer's, Parkinson's, and Huntington's diseases, represent a growing global health challenge due to their complex etiologies and lack of effective treatments(Blaikie et al.,2019). Oxidative stress plays a very important role in the pathway of these diseases through neuronal damage, resulting in corresponding losses of function in

cognitive abilities(zhao et al.,2013). DiNP phthalates, a course of plasticizers broadly applied to different industries, have been involved in the exacerbation of processes connected to oxidative stress and may therefore contribute to the acceleration of the progression of neurodegenerative conditions(Boberg et al.,2011). In light of such pervasive DiNP exposure and its adverse health impact, identification of novel therapeutic agents that could mitigate oxidative stress and protect neuronal integrity is of prime importance(Safiri et al.,2023).

Momordicacharantia L is a widely used medicinal plant, wherein different traditional cultures have been using the herb in various types of therapies owing to its diverse therapeutic properties (Basch et al.,2003). The bioactive phytocomponents present in this plant include flavonoids, alkaloids, and triterpenes. Literature available in these regards envisages that flavonoids, alkaloids, and terpenes are very potential antioxidants, thus exerting anti-inflammatory and neuroprotectiveeffects(Anilkumar et al.,2015). Gradually, the adjoining properties unfold the potential utility in counteracting the oxidative damage induced by environmental toxins like DiNPphthalates(Chen et al.,2015).

In silico approaches, such as computational simulations and molecular docking studies, have turned out to be very powerful tools in the process of discovering and evaluating potentially therapeutic agents. In essence, this offers a platform where large libraries of phytocomponents can be rapidly screened against specific protein targets involved in the disease pathways, offering insight into efficacy and mechanism of action. In this context, proteins related to neurodegenerative diseases, including amyloid-beta, tau, alpha-synuclein, and various antioxidant enzymes, are key players in the progression of oxidative stress-linked neuronal damage(Kim et al.,2004).

Therefore, the present study will employ computational approaches to identify and check on the efficacy of phytocomponents of *Momordicacharantia* against key oxidative-stress proteins implicated in neurodegenerative diseases, especially in the aspect of exacerbation upon DiNP phthalate exposure(Chen et al.,2014). We will further try to comprehend the association of these phytocomponents with target proteins through molecular docking and dynamic simulation studies and their possible therapeutic lead identification. It may pinpoint some novel plant-based intervention strategies for intervening in neuropathologies arising due to environmental pollutants and promoting neuroprotection.

A fusion of this traditional medicinal knowledge with such advanced computational methodologies evolved at present certainly has opened a very promising frontier against neurodegenerative diseases. This work, therefore, holds tremendous potential regarding the understanding of therapeutic potentialities of *Momordicacharantia* but finally, ends up underlining the importance of addressing environmental factors in the development of comprehensive treatment strategies against neurodegenerative conditions.

In the present study, a selected bunch number of gene pools are considered, and In-silico molecular docking analysis of phytoconstituents present in *M. charantia* fruits was studied against Oxidative Stressed genes (ACHE, MAPK9, SP1) which is associated with neurodegenerative disease like Alzheimer's Diseases (A.D.).

2. Material and Method

2.1 Selection of Phytocompounds

Through an extensive literature review, key phytocomponents have been identified for investigation in the present study.By focusing on these selected phytocomponents, our research aims to elucidate their mechanisms of action and explore their potential in drug development and disease management **(**Basch et al.,2015**)**.

2.2 Ligand Preparation and Filtration

Nineteen-phytocompounds were extracted from *Momordicacharantia* and used as ligands for the docking analysis. These chemicals' 3D structures were obtained from the PubChem database. After that, these ligands were cleaned up, their 3D coordinates were computed, and ligand conformations were produced using Discovery Studio 4.0's "prepare ligand protocol." In order to forecast the compounds' solubility and permeability in drug development, they were filtered after production according to their molecular characteristics. Lipinski's "rule of five," which emphasizes bioavailability, is the most well-known of the physical property filters. The rule states that the compounds have a molecular mass of less than 500 daltons, not more than 5 hydrogen bond donors, ten hydrogen bond acceptors, and an octanol-water partition coefficient log P not greater than 5 **(Pubchem database)**. The filtered compounds were then used for docking analysis.

2.3 Genes selection

The reported molecular targets responsible for oxidatively stressed proteins, which are associated with neurodegenerative disease pathways, in Humans **(8)** were retrieved from the Protein Data Bank (PDB). The retrieved PDB contains water molecules, heavy atoms, cofactors, and metal ions. Hence, the downloaded PDB structures were prepared using the 'prepare protein' protocol of Discovery Studio 4.0.

2.4 Molecular modeling of the protein expressed in fish and model validation.

Due to the unavailability of the protein structures of oxidative Stressed genes taken from zebra fish, modeling of the proteins was done by using the Roberta server **(**Roberta server 2.0**)** and was computationally validated by the Ramachandran plot by using the PROCHECK server **(Laskwsli 2006)**.

2.5 Prediction of binding sites of proteins selected for the study

CastP server wasused to predict the binding sites of the selected proteins **(Tian et al.,2015)**. The binding sites of the receptor proteins were predicted based on the 'receptor cavity method' using the CastP server. Using this protocol, active sites of the target receptor were identified based on the inhibitory property of the amino acid residues present in the binding sites.

2.6 Molecular docking

The anti-inflammatory activity of all the 19 phytochemicals reported from *Momordicacharantia*was assessed by docking these compounds against the respective active sites of the target proteins. Discovery Studio 4.0 was used in this study to find the interacting compounds of *Momordicacharantia* with the selected targets of Oxidative stressed genes. The strategies of Discovery Studio 4.0 are to exhaustively dock or score possible positions of each ligand in the binding site of the proteins. Using scoring functions, a docking study of the target proteins was done with natural compounds derived from *Momordicacharantia*to find the preferred orientation and binding affinity of the compounds with each target protein. After predicting the binding sites of the selected proteins taken for the study, molecular docking was performed using the auto dock vina tool **(Rentzsh et al.,2015),** and the molecular interaction was viewed using the Biovia Discovery tool **(kim et al.,2017)**. Ligands were docked to the proteins and scored for their relative interaction strength to identify drug development candidates. The final poses were then scored based on the total docking energy, composed of the intramolecular energy of the ligand and the ligand-protein interaction.

2.7 Drug Likeness

A qualitative notion called "drug-likeness" is applied in drug design to assess how a chemical behaves like a drug in terms of things like bioavailability. A molecule's long-term therapeutic effectiveness is acknowledged as one of the primary factors of its potential for successful drug development, along with its molecular characteristics that affect absorption, distribution, metabolism, excretion, and toxicity **(**Swiss–ADME Tool). According to **(Swiss ADME)**, these factors account for almost 60% of all medication failures throughout the clinical phases; hence, predicting ADMET qualities is essential for discovering novel treatments. Designing lead compounds that are quickly absorbed by the stomach, delivered to their intended site of action, and do not readily transform into harmful metabolic products has thus become essential. It is now essential to create lead compounds that are easily absorbed by the stomach and quickly delivered to their intended site of action, demanding to transform into harmful metabolic products and quickly excreted from the body before reaching large enough concentrations. For drug-like candidates, the compounds' ADMET characteristics were examined.

3. Results & Discussion

3.1 Phytocompounds selection:

Different literature studies were referred to select the compounds that can have a better effect on Oxidative Stressed Genes associated with Alzheimer's disease (A.D.) and progressive neurodegenerative disorder. Around nine compounds were selected for the study. Regarding the literature review, 19 phytocomponents from *Momordicacharantia* were taken as ligands for docking analysis. The ligand molecules with the least binding energy are compounds with the highest binding affinity. This binding affinity indicated a focused interaction between the above compounds and the targets compared to others. The parameters for finding the best inhibitors, such as AutoDockVina, interaction energy, and number of hydrogen bonds, were also evaluated. The result shows that out of 19 phytocomponents, eight phytocomponents show high binding accuracy against Oxidative Stressed Proteins (ACHE, MAPK9, SP1).

3.2 Protein selected for the study and structural modeling

In the current study, three human-based proteins that, are Acetylcholinesterase, Mitogen-activated protein kinase nine, and Transcription factor Sp1 **(Boberg et al.,2011)** are taken for the study. The Detailed information on the proteins obtained from the UniProt database **(Uniport 2015)** is given in Table 1. The protein data bank (PDB) database used to retrieve the structure of the Acetylcholinesterase, Mitogen-activated protein kinase nine, and Transcription factor Sp1 and the corresponding PDB IDs are 6O5V, 7N8T, and 1SP1, respectively., In silico modeling was performed using the Robetta server and validated through the Ramachandran plot of the PROCHECK server. Here, 93.1% of amino acid residues were found in most favored regions, and no residues were found in dis- allowed regions. Figure 1 shows the 3D structure of the protein and Ramachandran plot details. ACHE proteins found 88.3 % of amino acid residues in most favored regions and no residues in dis- allowed regions. Figure 2 shows the 3D structure of the protein and Ramachandran plot details.

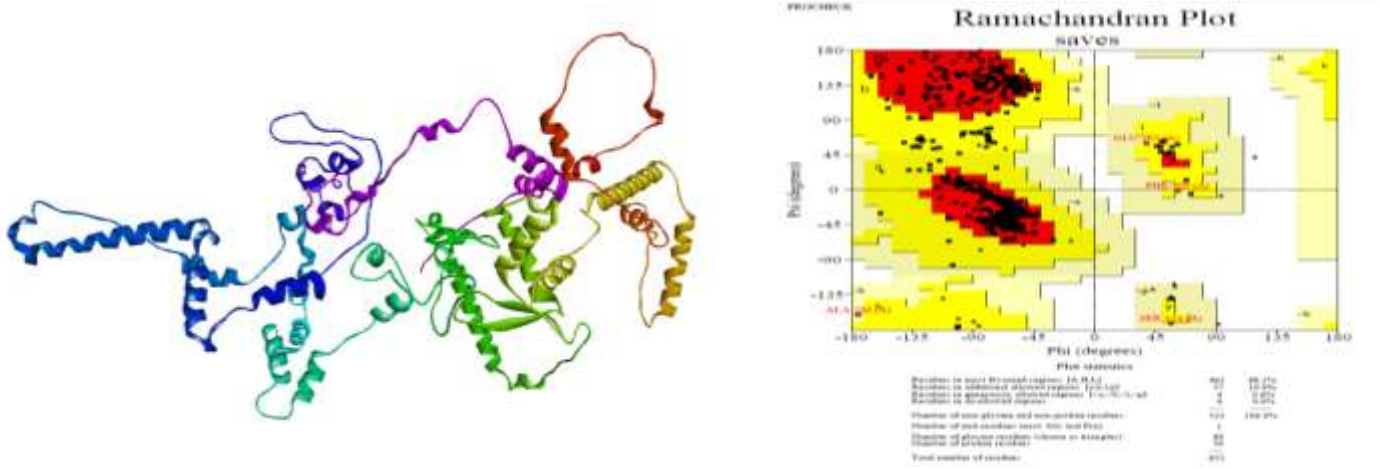
Table 1: Target proteins information of oxidative stress

Sl. No.	Uniport entry	Protein Name	Gene Name	Protein length	PDB entries
1.	P22303	Acetylcholinesterase (human)	ACHE (docked)	614	6O5V
2.	P45984	Mitogen-activated protein kinase 9	MAPK9	424	7N8T
3.	P08047	Transcription factor Sp1 (human)	SP1	785	Available 1SP1

Table 1 describes the ACHE, MAPK9 AND SP1 genes contains respective protein length with amino acid sequences , which is derived from uniport software and drawn by provert Software and validated in PROCHECKER Software

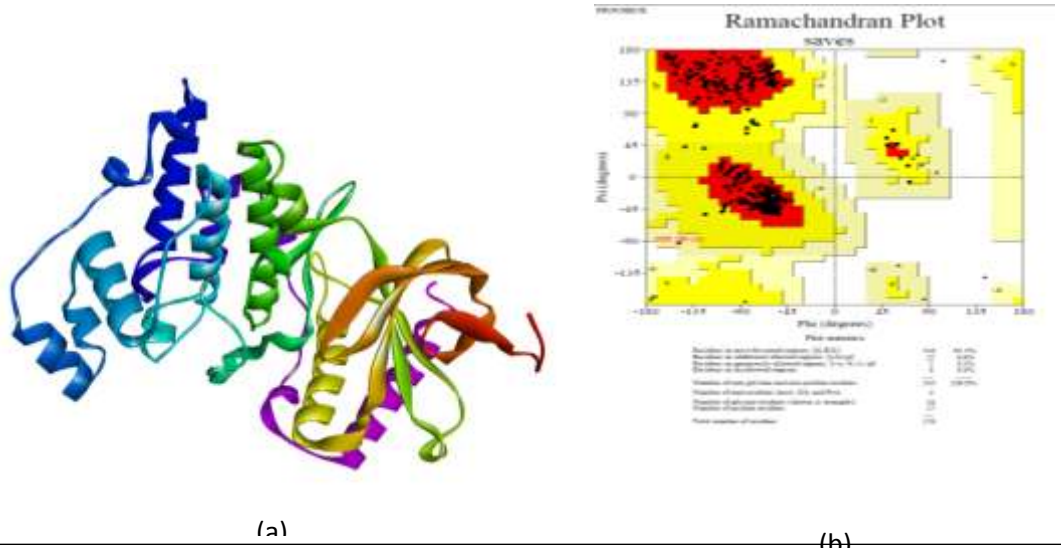
3.3 Ramachandran Plot for targeted Genes

The Ramachandran plot is one of the most essential ideas in structural biology, as presented in both articles and textbooks. As a graduate student in G.N. Ramachandran's research group, Sasisekharan explored using torsion angles to characterize the conformation of polypeptides and proteins. His research focused on the structure of collagen chains. This strategy's effectiveness was immediately apparent, and its application spread swiftly. In the last fifty years, this so-called Ramachandran plot, also known as the υ, ψ-plot, has remained primarily constant and is still an essential tool for protein structure research and education.



(a) (b)

Figure 1: ACHE(a) 3D-structure (b) Ramachandran plot



(a) (b)

Figure 2: MAPK9 (a) 3D-structure (b) Ramachandran plot

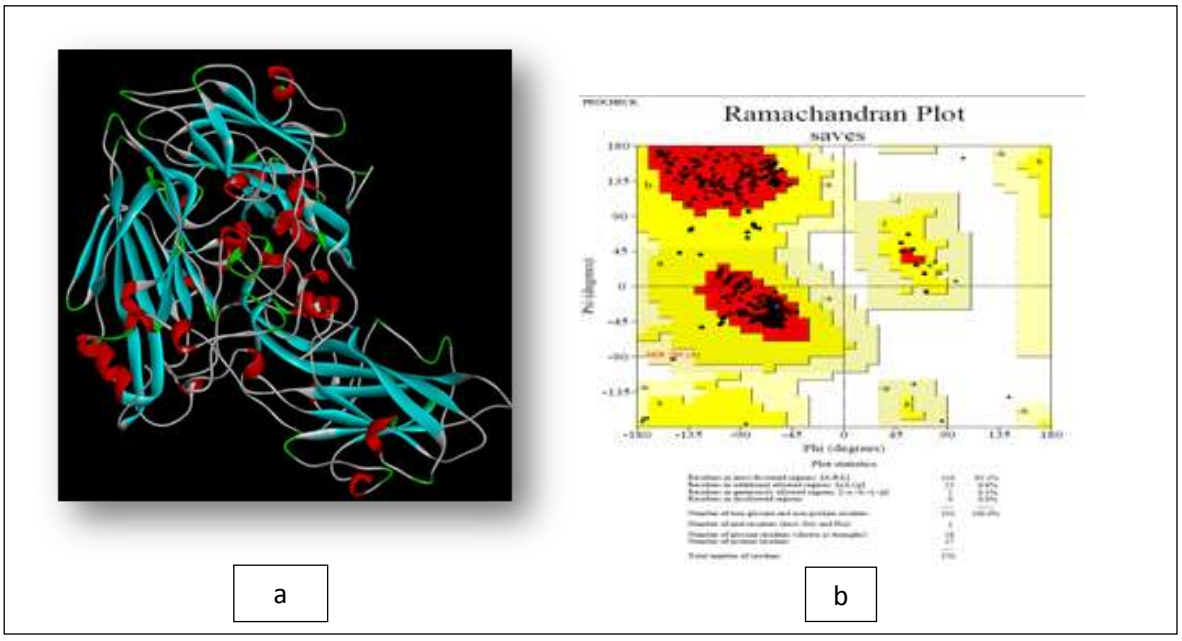


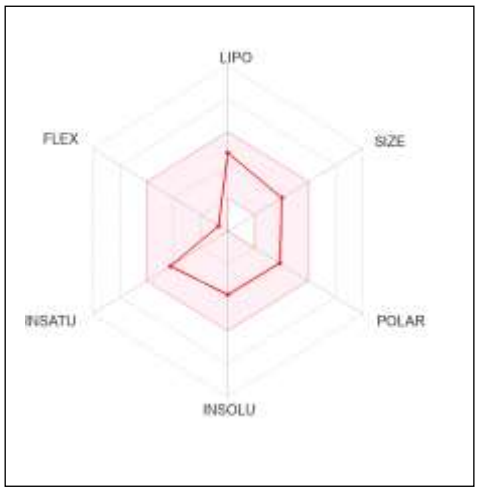
Figure 3: SP1(a) 3D-structure (b) Ramachandran plot

3.4Insillico ADMET Analysis:

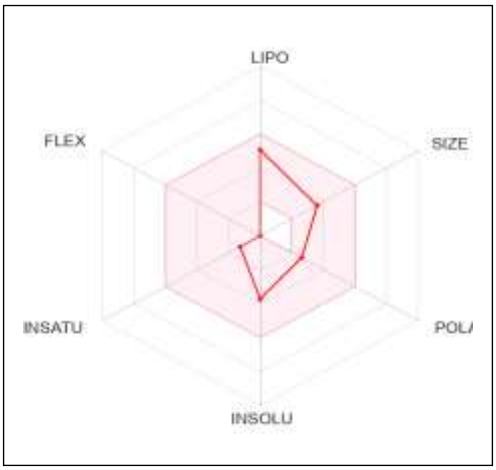
Physiochemical changes:

Considering the comparable AutoDockVina energy, interaction energy, and binding energy, three compounds were forwarded for ADMET analysis. These studies are based on the compounds' ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties. These properties provided insights into the pharmacokinetic properties of the compounds and were checked using SWISS-ADME built-in ADMET protocol. The parameters tested in this study were aqueous solubility, Blood blood-brain barrier (B.B.B.) level, Hepatotoxicity, and Absorption level. Pharmacokinetic properties of the best-fit phytochemicals showed that 19 of the compounds had passed all the pharmacokinetic parameters. These compounds were thus selected as the best compounds in this study as they had good interaction scores and ADMET properties.

a



b



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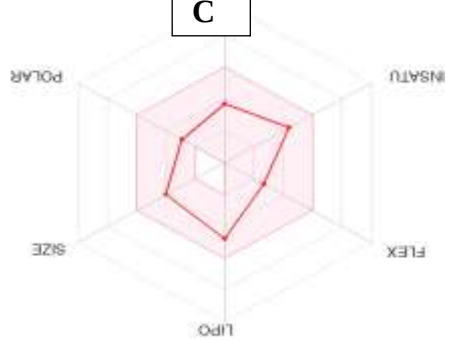

e

Figure 3 :- ADMET Analysis report of Best Bioavalibility lipophilic Phytocomponents : (a) Ajamalacine ADMET,(b) Alakaloid AQC2 ADMET, (C) Alkaloid DF ADMET, (d) Steriod U ADMET, (e) Quinine ADMET analysis done by SWISS ADME Tool.

3.5 Prediction of binding sites and molecular docking analysis.

3.5.1 The CastP server predicted the most active sites of the selected five proteins.

For the human proteins Acetylcholinesterase the active sites are Asn233, Glu313, Ile316, Pro410, Gln413, Try503, Gln508, Cys529, Asn533, and the grid box dimension value for this are ; X-36, Y-40 and Z-62. In the case of Mitogen-activated protein kinase nine, the active sites are Ile32, Ser34, Ala53, Lys55, Arg72, Leu106, Met111, Asp151, Gly171, Pro184, Arg192, Ser210, and grid dimensions areX-66, Y-80 and Z-82. In the case of Transcription factor Sp1, the active sites are Lys2, Phe3, Phe12, and Met13, and the grid dimensions are X-40, Y-40, and Z-40.

After setting the grid box value, a molecular docking study was conducted. Thenine-teen compounds selected from the literature review were docked against the selected genes using the Autodockvina tool. In the molecular docking study, the compound Steroid U scored the highest docking affinity of -11.0 kcal/mol against the Acetylcholinesterase and Mitogen-activated protein kinase nine proteins, followed by the other compounds. The compound Ajmalecine scored the highest docking affinity of -6.8 kcal/mol against the Transcription factor Sp1 protein, followed by the other compounds. Table 2 gives the docking results. Figure 3 shows the 3D interaction of the compounds, scoring the highest docking affinities against their respective protein structures

Reported compound	Acetylcholinesterase	Mitogen-activated protein kinase 9	Transcription factor Sp1 (Human)
2-Nitrohexane	-5.4	-4.8	-3.4
2-Palmitoylglycerol	-7.0	-5.0	-3.8
3-Epi-Sclhammericine	-7.7	-7.4	-5.5
Acrifoline	-8.3	-7.8	-5.5
Ajmalecine	-10.8	-9.6	-6.8
Alakloid AQc2	-10.6	-10.1	-6.5
Alakloid DF	-10.0	-8.3	-5.0
Alakloid SP-B	-8.5	-7.6	-5.4
Alakloid SP-K	-9.2	-7.8	-6.1
Alkaloid 27	-9.5	-7.7	-6.0
Alkaloid X	-7.5	-7.3	-4.6
Armepavine	-6.6	-6.4	-5.2
Conqunamine	-9.3	-8.2	-5.6
Flabelline	-9.7	-8.0	-6.1
GlycidylPalmitate	-6.8	-5.1	-3.4
Hexane, 1-(3-butenyloxy)-	-5.4	-4.7	-3.6
P.M alkaloid IV	-8.5	-9.3	-6.0
Quinine	-9.1	-7.6	-6.2
Steroid U	-11.0	-11.0	-6.7

Table 2: It shows the bindinding score of 19 phytocompounds against Oxidative stressed proteins (ACHE, MAPK9, SP1,)

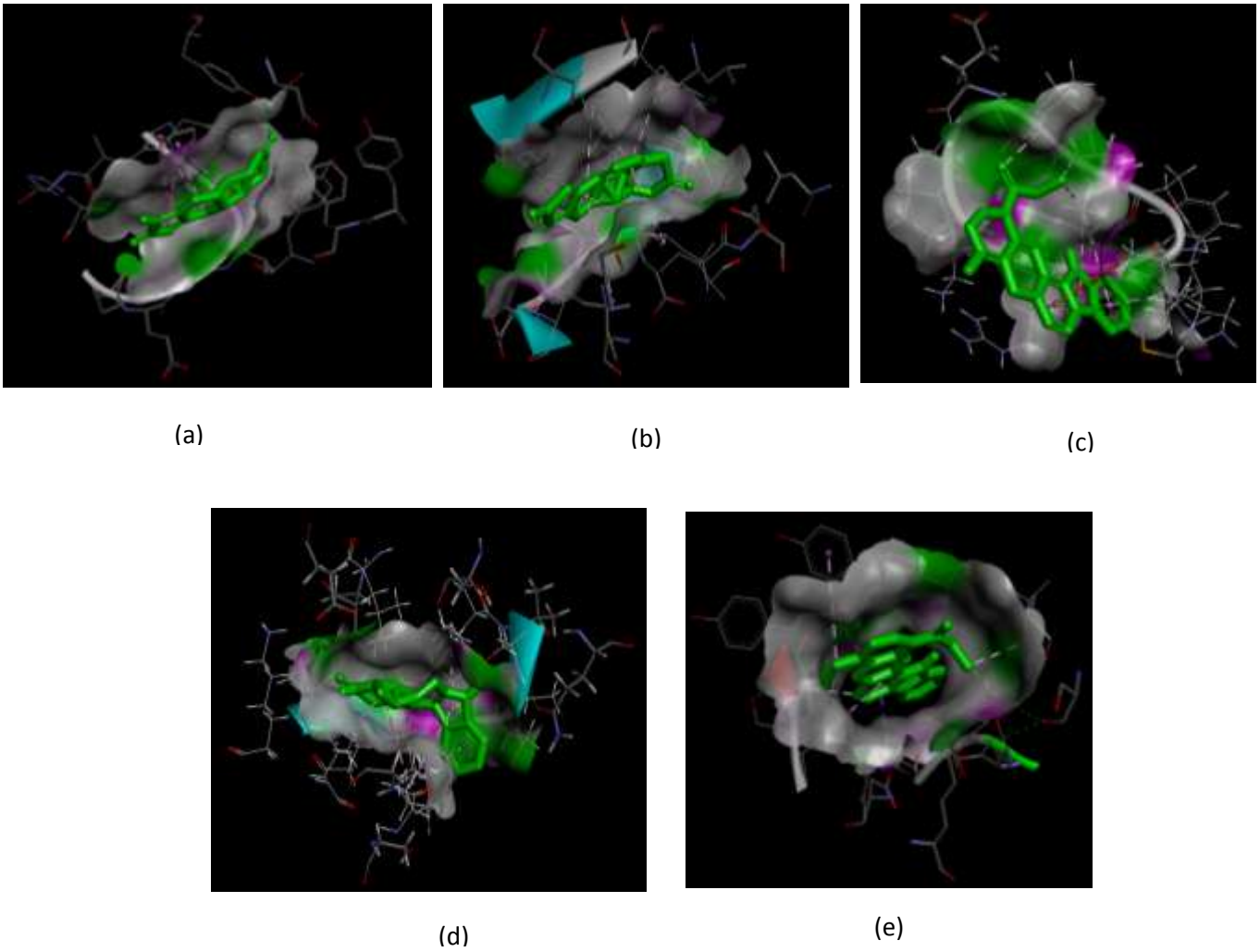

Figure 3. Molecular 3D-Interaction of a. Ajmalacine, b.Alkaloid AQC2, c.Alkaloid DF, d.Steroid U act highest binding affinity against ACHE

4. Discussion

In all these theories, the accumulation of ROS-induced damage is posited as a potential mechanism leading to age-related functional decline, failure of endogenous repair mechanisms, and neurodegenerative diseases, of which A.D. is the most common (30,31). Acetylcholinesterase (AChE) is an essential enzyme in the cholinergic nerve system, as this special issue reviews in great detail. Although there is a significant loss of forebrain cholinergic

neurons and a steady decrease in acetylcholine, numerous distinct types of neurons degenerate as A.D. progresses **(32).** Numerous nutritional and nutraceutical benefits can be derived from the *Momordicacharantia L* plant. The fruit extract revealed that Mc had significant amounts of ajmalacine, alkaloid AQC2, alkaloid D.F., steroid U, and quinine. Aligning sequences in the Short Protein Data Bank (PDB) database yielded the protein sequence. 6O5V, 7N8T, and 1SP1 are the transcription factors Sp1, Mitogen-Activated Protein Kinase 9, and PDB ID obtained. The Protein Data Bank (PDB) database obtained the Acetylcholinesterase structure, in silico modeling was carried out using the Robetta server and confirmed using the PROCHECK server. An *In silico* study was performed to determine the active biopeptide of these oxidatively stressed proteins. The result revealed, Out of these 19 phytocompounds, Ajmalacine, Alkaloid AQC2, Alkaloid DF, Steroid U and Quinine were ranked the highest with binding scores ranging against ACHE protein from −11.0 kcal/mol to −9.1 kcal/mol compared with the standard, From the results obtained, it can be concluded that Ajmalacine, Alkaloid AQC2, Alkaloid DF, Steroid U act highest binding affinity against ACHE, which are associated with Neurodegenerative Disease like Alzheimer's Diseases(A.D.),which are discussed in Table 2. In our future work, we will examine the ACHE activities in Adult Zebrafish (*Daniorerio*) and also try to solve the mechanism of Ache enzymes causing Alzheimer's Disease (A.D.) in Adult Zebrafish (*Daniorerio*).

5. Conclusion:-

Medicinal plants are a potential source of several bioactive compounds to treat various diseases. The receptor (Protein) and ligand are essential in structural-based drug design. In the present study, in silico molecular docking analysis of phytoconstituents present in *M. charantia* gourd phytocomponents collected from several Review of Literature was studied against Oxidative Stressed genes (ACHE, MAPK9, SP1) associated with neurodegenerative disease like Alzheimer's Diseases (A.D.) and Parkinson's Disease (P.D.). We docked the receptors ACHE, MAPK9, SP1 with the phytochemicals. From this, Ajmalacine, Alkaloid AQC2, Alkaloid DF, Steroid U, and Quinine from the plant gourd show promising lead target formation against ACHE based on molecular docking analysis (minimum hydrogen bond length and maximum docked score). Therefore, *in vivo*, and *in vitro* approaches are recommended to elucidate these compounds' molecular mechanism to act as potent drugs against the Oxidative Stressed genes associated with neurodegenerative diseases like Alzheimer's disease (A.D.).

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References

1. Blaikie, L., Kay, G., & Lin, P. K. T. (2019). Current and emerging therapeutic targets of alzheimer's disease for the design of multi-target directed ligands. *MedChemComm*, *10*(12), 2052-2072.
2. Zhao, Y., & Zhao, B. (2013). Oxidative stress and the pathogenesis of Alzheimer′ s disease. *Oxidative medicine and cellular longevity*, *2013*(1), 316523.
3. Boberg, J., Christiansen, S., Axelstad, M., Kledal, T. S., Vinggaard, A. M., Dalgaard, M., ...& Hass, U. (2011). Reproductive and behavioral effects of diisononyl phthalate (DINP) in perinatally exposed rats. *Reproductive Toxicology*, *31*(2), 200-209.
4. Safiri, S., Noori, M., Nejadghaderi, S. A., Mousavi, S. E., Sullman, M. J., Araj-Khodaei, M., ...&Gharagozli, K. (2023). The burden of Alzheimer's disease and other types of dementia in the Middle East and North Africa region, 1990–2019. *Age and ageing*, *52*(3), afad042.
5. Chen, X., & Pan, W. (2015). The treatment strategies for neurodegenerative diseases by integrative medicine. *Integrative Medicine International*, *1*(4), 223-225.

6. Basch E, Gabardi S, Ulbricht C. Bitter melon (Momordicacharantia): a review of efficacy and safety. American Journal of Health-System Pharmacy. 2003 Feb 15;60(4):356-9.
7. Anilakumar KR, Kumar GP, Ilaiyaraja N. (2015). Nutritional, pharmacological and medicinal properties of Momordicacharantia. International Journal of Nutrition and Food Sciences. Jan 1;4(1):75-83.
8. Kim, D. E., Chivian, D., & Baker, D. (2004). Protein structure prediction and analysis using the Robetta server. *Nucleic acids research*, *32*(suppl_2), W526-W531.
9. Laskowski, R. A., MacArthur, M. W., & Thornton, J. M. (2006). PROCHECK: validation of protein-structure coordinates.
10. Tian, W., Chen, C., Lei, X., Zhao, J., & Liang, J. (2018). CASTp 3.0: computed atlas of surface topography of proteins. Nucleic acids research, 46(W1), W363-W367.
11. Rentzsch, R., &Renard, B. Y. (2015). Docking small peptides remains a great challenge: an assessment using AutoDockVina. *Briefings in Bioinformatics*, *16*(6), 1045-1056.
12. Kemmish, H., Fasnacht, M., & Yan, L. (2017). Fully automated antibody structure prediction using B.I.O.V.I.A. tools: Validation study. PloS one, 12(5), e0177923.
13. UniProt Consortium. (2015). UniProt: a hub for protein information. *Nucleic acids research*, *43*(D1), D204-D212