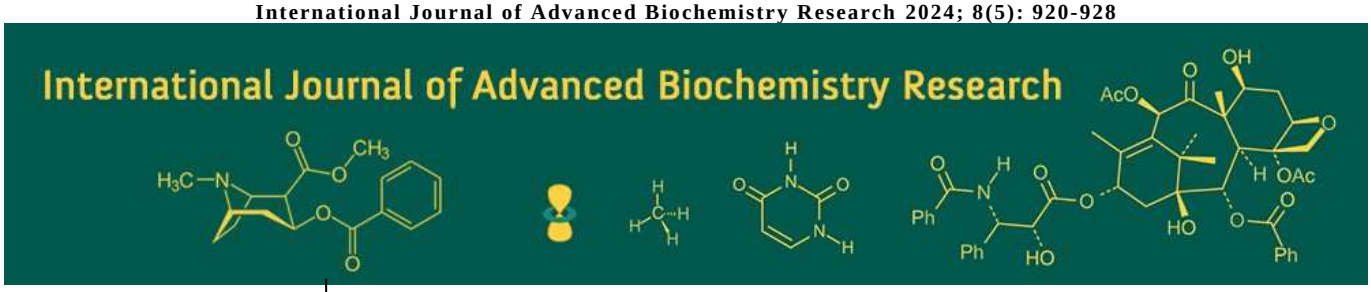

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A comparative evaluation of polyphenols from virgin coconut oil against EGR-1 for memory consolidation and reconsolidation in Alzheimer's disease

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Abstract
Virgin coconut oil (VCO) possesses antioxidant properties that may help to manage Alzheimer's disease (AD), the most prevalent kind of dementia and an incurable neurodegenerative disease marked by progressive synaptic dysfunction, neuronal death, and cognitive decline. When mitochondria are severely damaged, the antioxidant defense lowers, which increases the production of reactive oxygen species (ROS) that further damage mitochondria and increase the generation of free radicals. Additionally, altered mitochondrial function, dysregulated electron transport chain, and other sources elevate aggregated Aβ and neurofibrillary tangles, which further stimulate the production of ROS, reducing or depleting the antioxidant capacity. When a learning experience is retrieved, the memory becomes labile and needs time for protein synthesis to stabilize before being reconsolidated into a fixed state. The main signalling pathways that OS-induced AD involves, as well as the medications that are being developed to target these pathways in the hopes of advancing AD treatment. The *C. auratus* were trained with and without anisomycin (ANI), a brain protein synthesis inhibitor (PSI) for behaviour and spatial memory analysis. Polyphenols found in VCO were analysed *in silico* against EGR-1, an enzyme required for memory consolidation and reconsolidation showed significant binding affinity during molecular docking studies. In another independent investigation, oral VCO administration did not result in any appreciable harm, including behavioural abnormalities in rat models. Consequently, more investigations including clinical studies is required to ascertain the biological efficiency of the oils for the AD management by targeting EGR1 through the best candidate polyphenol identified (Myrystin 3-o glucoside) *in silico*.

Keywords: VCO, AD, learning memory, polyphenols, EGR-1

1. Introduction
Virgin Coconut oil (VCO) is attained more attention nowadays against the world's most common neurodegenerative disease Alzheimer's diseases (AD) [1] characterized with Aβ plaque. The unique polyphenol composition of VCO with antioxidant activity plays imperative role to prevent neuronal damage due to reactive oxygen species (ROS) attack [2]. In line with molecular docking investigations the two psychoactive phenolic compounds found in olive oil,, luteolin and oleuropein aglycone have shown the ability to hinder Acetylcholinesterase (AChE) enzyme, which renders them possible candidates in the treatment of AD [3]. A systematic study on how chemical brain processes that control brain processes and social behaviours in AD and natural remedies based on this will be a major breakthrough in AD management. Zinc finger TF EGR1 (also known as NGFI-A, KROX24, or ZIF268) has been demonstrated to express itself in the brain in a way that is responsive to social interactions and fosters pro-social behaviour across phylogeny [4]. Moreover, in a D-GAL/AlCl3-induced AD rat model, coconut oil has shown protective effects against oxidative injury, synaptic transfer, AD pathology markers, cognitive and non-cognitive impairment, and cholinergic function [5]. The objective of this study is to understand the molecular mechanism of spatial learning memory loss and behavioural changes and effect of natural polyphenols identified from VCO [6] to use as a natural remedy for memory loss associated with AD on the candidate gene EGR 1 [7] which is associated with memory consolidation and reconsolidation (8) using *in silico* and *in vivo* models.

2. Materials and Methods

The virgin coconut oil was prepared in a culinary method [9, 6]. Two independent studies were conducted to analyse the role of EGR 1 in AD management using major antioxidant polyphenols [9] present in vco.

2.1 Learning and behavioural analysis in *C. auratus* using a protein inhibitor anisomycin

An hour before the behavioural training, each of the three Aniso groups (n = 3) had an intracranial injection of 10µl Anisomycin (with DMSO as a carrier) into their telencephalon. At the same time, the Control group (n - 3) stayed in the same condition but weren't given any injections. The goldfishes (*Carassius auratus*) were maintained in a clean home tank throughout the exploration period of 7 days. The fishes were identified individually by their phenotype for behavioural observations and allowed to explore the experimental tank during the exploration. After exploration the fishes were transferred back to their home tank. After the seven days of exploration, training started.

On every day before one hour of the training 10µl of 100µg DMSO and protein inhibitor anisomycin was injected into the telencephalon of control and Aniso group respectively. After one hour of the injection the fishes were allowed to take the feed from the experimental tank with the maximum duration of 15 minutes (900 seconds). During each trial, a fish was introduced into the Starting chamber (SC), and the food tray was kept in the Target chamber (TC). In order to get their food, fish should reach TC by passing through a shaped passage from the SC. Scored the number of attempts (bumps against the gate) made to pass through gates, and time taken to cross the gate was recorded for each fish. Fishes were transferred back to their home tank after every trails (15 min, 1trail /day for 7 days) [10].

2.2 Molecular docking of major polyphenols from VCO against EGR 1 gene

To examine the docking of binding orientation of substrates with EGR 1, Autodock Vina in PyRx virtual screening tool PyRx 0.8 was chosen. For EGR 1 protein, crystal structures of EGR 1 (PDB ID: 4r2a) was retrieved from RCSB Protein Data Bank [6].

2.3 Preparation of the ligand structures

The 3D structures of the ligands [6] Myrystin 3 o glucoside, Vanillic acid hexoside, Ferullic acid, Kampherol 3 0 pentoside and Hydrosol were retrieved from PubChem [9].

2.4. Docking

The crystal structures of EGR 1was opened in PyRx virtual screening tool as a starting protein structure in pdbqt format. The ligand was chosen, and automatically converted to pdbqt format. After both of them were chosen, the grid box was automatically appearing and the centre of the target site was assign along with the dimensions. The centres of the box were assigned for 4r2a (X =35.8725, Y = 83.0836, Z = 30.8264) together with the exhaustiveness equalling to 8. The dimensions of the box were set to 25 × 25 × 25 Å. The docking was performed with autodock vina and re-docking ligand to observe the precision of the docking condition. In this study, one receptor was compared with five ligands. The best free energy of binding values would be obtained in PyRx virtual screening tool GUI and log files. The poses were chosen by compared the orientation with reference ligand x-ray structure of the certain enzyme. The DS Visualizer 4.0 was used to perform for all figures. The binding energy of the best pose was identified and compared with other [9]. The accuracy of a docking result is normally evaluated by the RMSD between the experimentally docking- observed and the x-ray ligand, which usually used RMSD cut- off value in a range of 2 - 3 Å [11, 12].

2.3 Phylogenetic tree construction of the EGR 1 gene

Fasta Sequences of Egr1 of *Homo sapiens* and *Carassius auratus* were retrieved from uniprot. Using, phylogenetic analysis tool Clustal w tree was created [13, 14].

3. Results and Discussions

3.1 Anisomycin, a protein synthesis inhibitor attenuates spatial learning in *C. auratus*

The Anisomycin received fishes showed reduced movements and took more time to cross the ‘ ‘passage and to take feed when compared to control group. The learning disability of anisomycin treated fishes is because of protein synthesis inhibition in the telencephalon of the animals, which received anisomycin during the training accurately 1 hour before the trails [15-17]. It is inferred that the proteins responsible for learning starts synthesizing during the learning task. As the protein synthesis inhibited during the learning phase, the aniso treated fishes showed reduced movements and took higher latency to take feed. While the control group easily crossed the passage and took feed by searching the feed chamber (FC).

Most of the studies revealed that the peak time of protein synthesis is during or immediately after training and the second time window for protein synthesis starts hours after training [15-17]. For any memory need to be deactivate should be in the active state (18). Memory in the active state vulnerable to the effects of protein inhibitors. Anisomycin is known to inhibit the synthesis of new protein it disrupted the synthesis of protein required to prevent the potential amnesia causing retrieving the memory [19]. The implication of this hypothesis is that some retrieved memories may have to be rebuilt. In addition to ability to block the synthesis of proteins needed to restabilize the memory, Anisomycin has side effects that can negatively impact on neural circuits supporting a recently activated memory. ANI did not affect pre-existing memories or the capability to memorize once the effect of the inhibition stopped. ANI impairs the consolidation of long-term memory in a dose-dependent manner without affecting short-term memory [10]. Here we showed that the anisomycin inhibited protein synthesis during learning spacial task *in C. aurus* which reduced movements including longer time to take feed compared to control groups.

3.2 Molecular docking studies on phenolic compounds of virgin coconut oil

Studies have shown that kaempferol administration can be useful both in preventive and post-insult therapeutic treatments [20-22]. Docking results shows ligand Myricetin 3 o glucoside has high affinity with EGR1 with a binding affinity of -5.6. Results are shown in fig 1-6 and table 1

In our previous study we have shown that the major polyphenol hydroxytysrosol acetate from VCO can act as an anticarcinogenic agent against *PTEN* of prostaste cancer (9). In the present study we have shown that the main active compounds like Myrystin 3 o glucoside, Vanillic acid

hexoside, Ferullic acid, Kampherol 3 0 pentoside and Hydrosol of VCO can regulate targets such as EGR 1 and may protect against neuronal injury by reducing the oxidative damage and activating antioxidant activity with Myricetin 3 o glucoside having maximum activity.

3.3 Phylogenetic tree of the EGR 1 gene

The phylogram of three species indicates EGR1 of human is homologous with *Carassius auratus*. The results are shown in fig 7. The phylogenetic tree represents behavioural investigations in gold fish can be extrapolated to vertebrate humans [20, 23, 24].

Molecular docking studies on phenolic compounds of virgin coconut oil

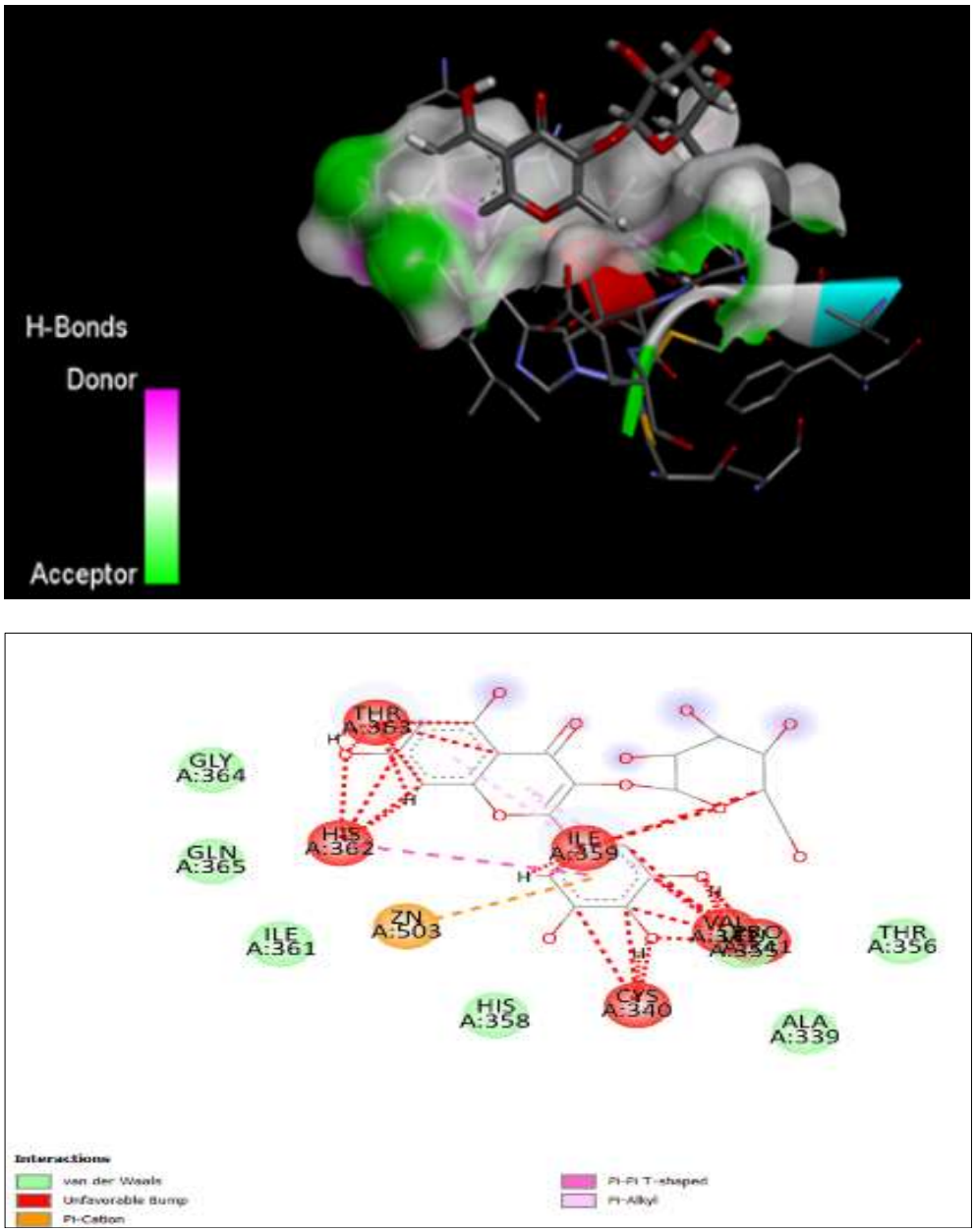


Fig 1: 3D & 2D interaction of EGR1 with Myrystin

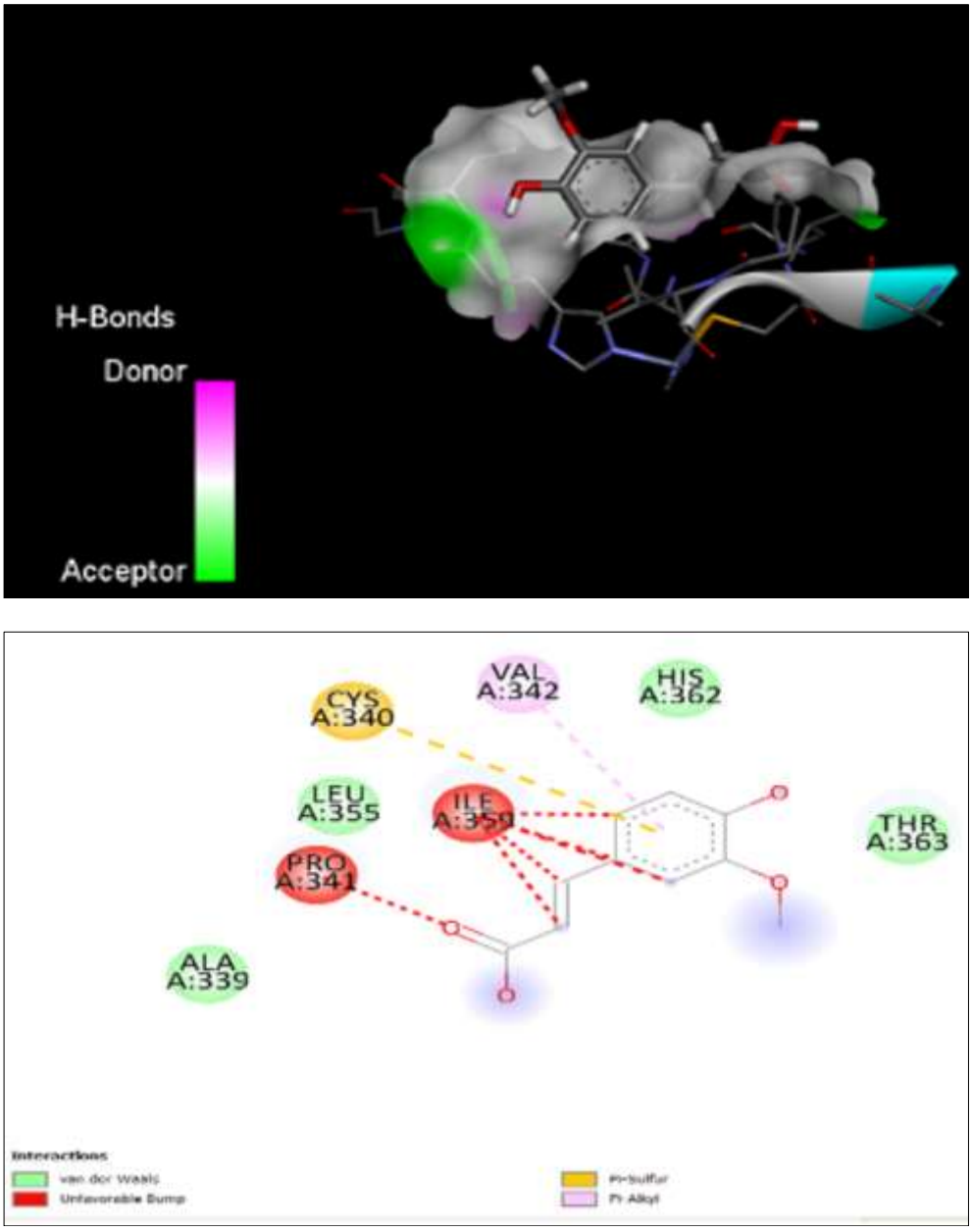
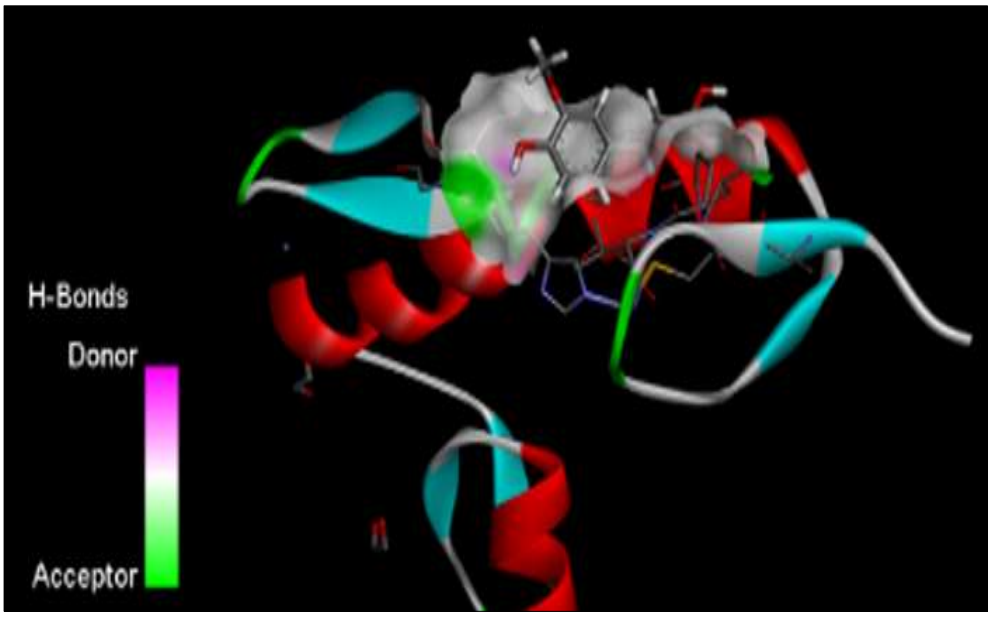


Fig 2: 3D & 2D interaction of EGR1 with Vanillic acid hexoside



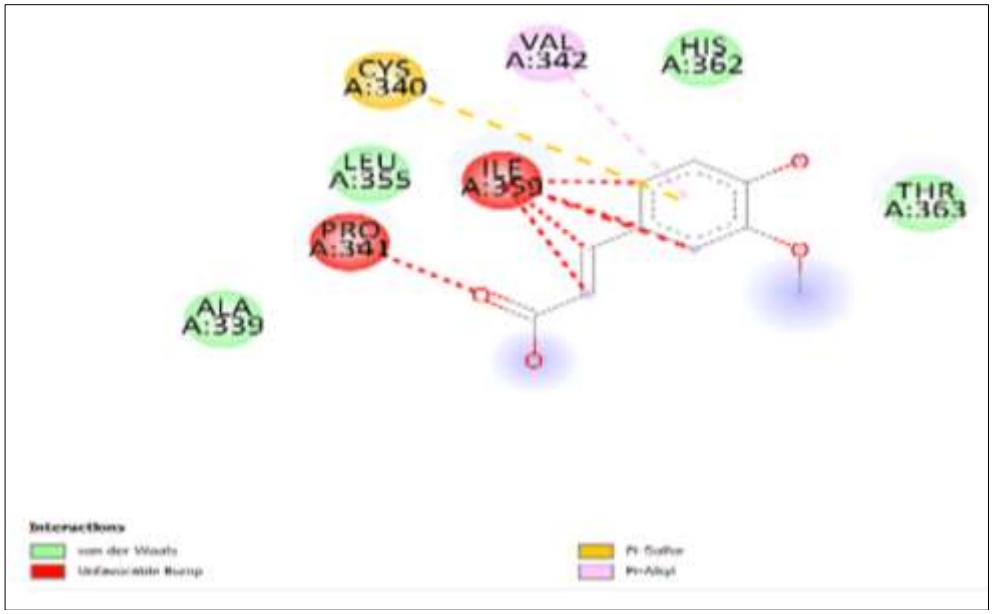
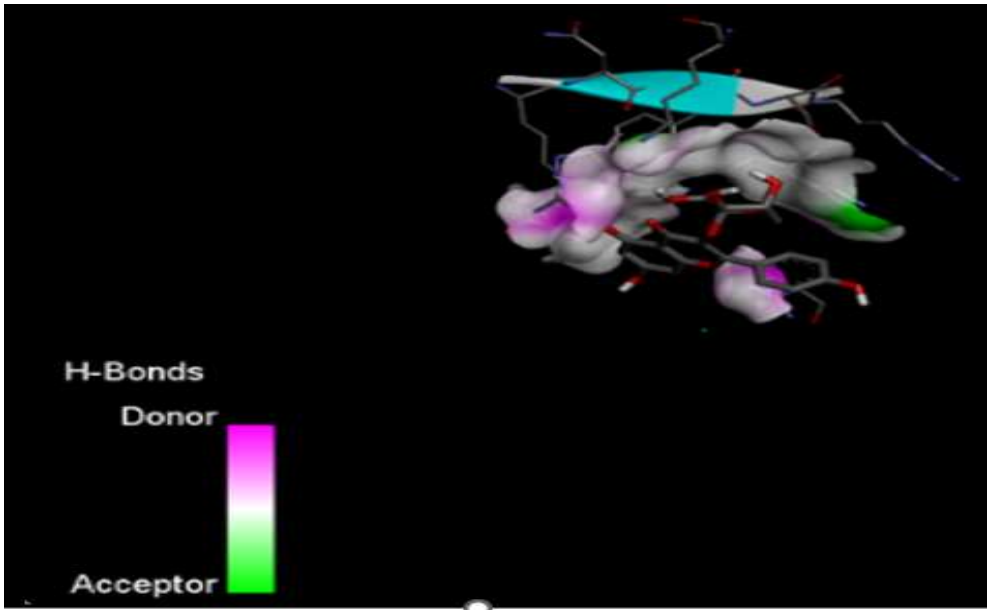


Fig 3: 3D & 2D interaction of EGR1 with Ferullic acid



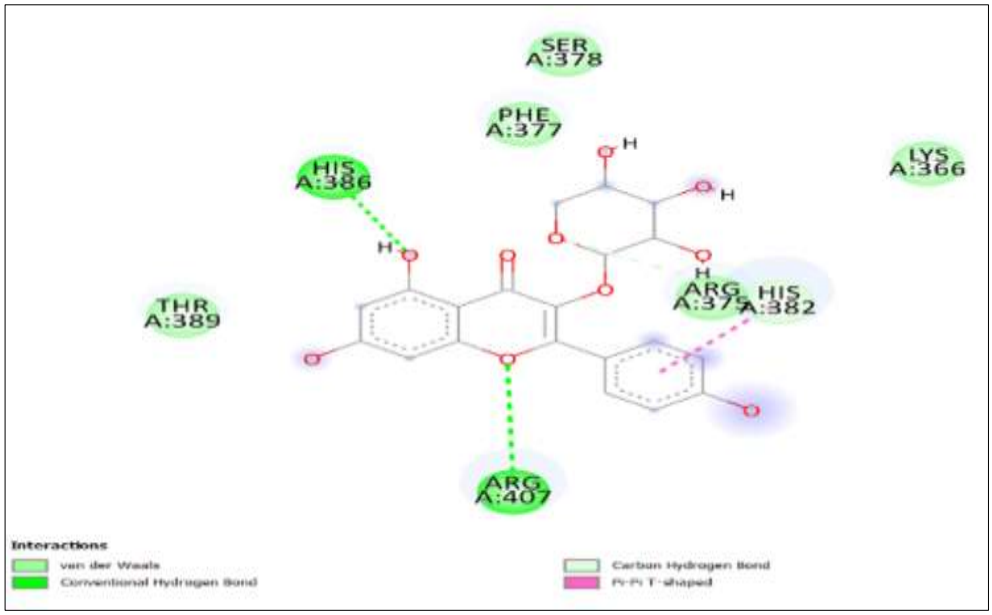
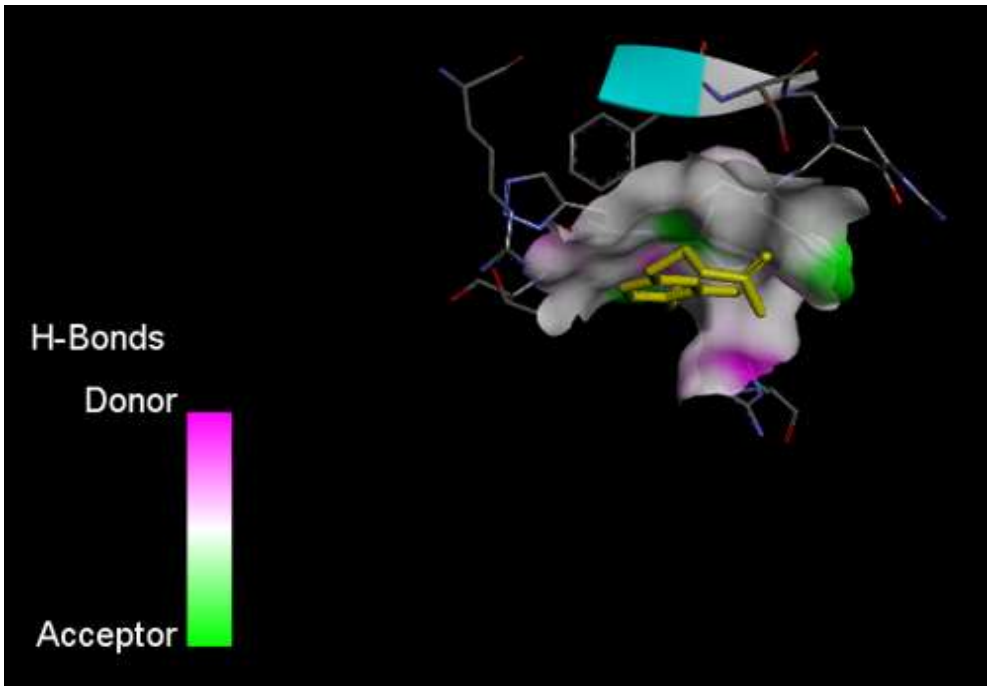


Fig 4: 3D & 2D interaction of EGR1 with Kampherol



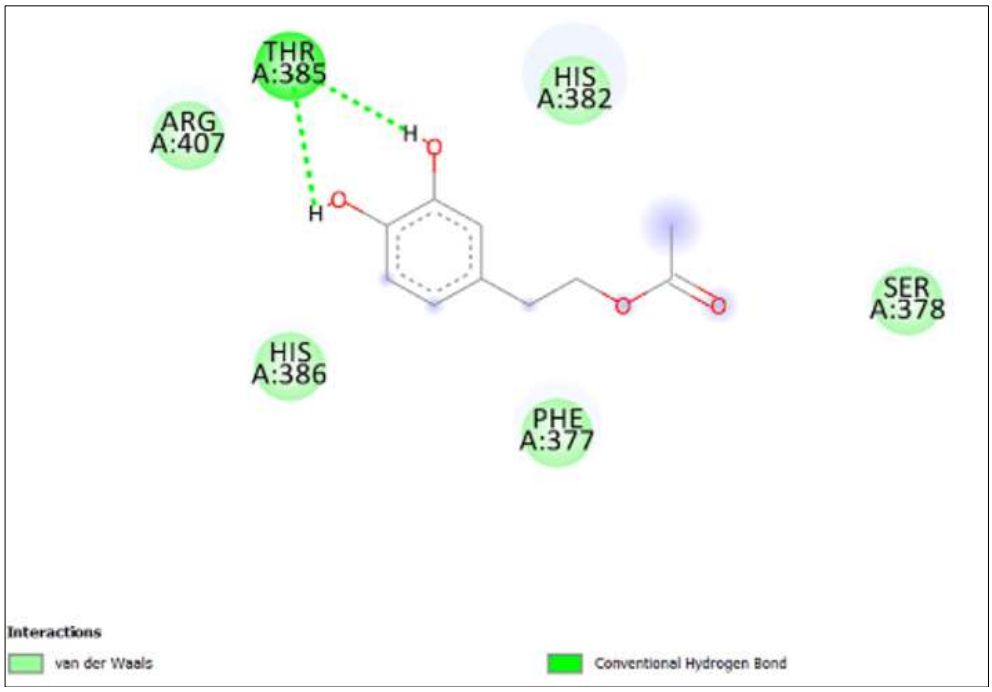


Fig 5: 3D & 2D interaction of EGR1 with Hydrosol

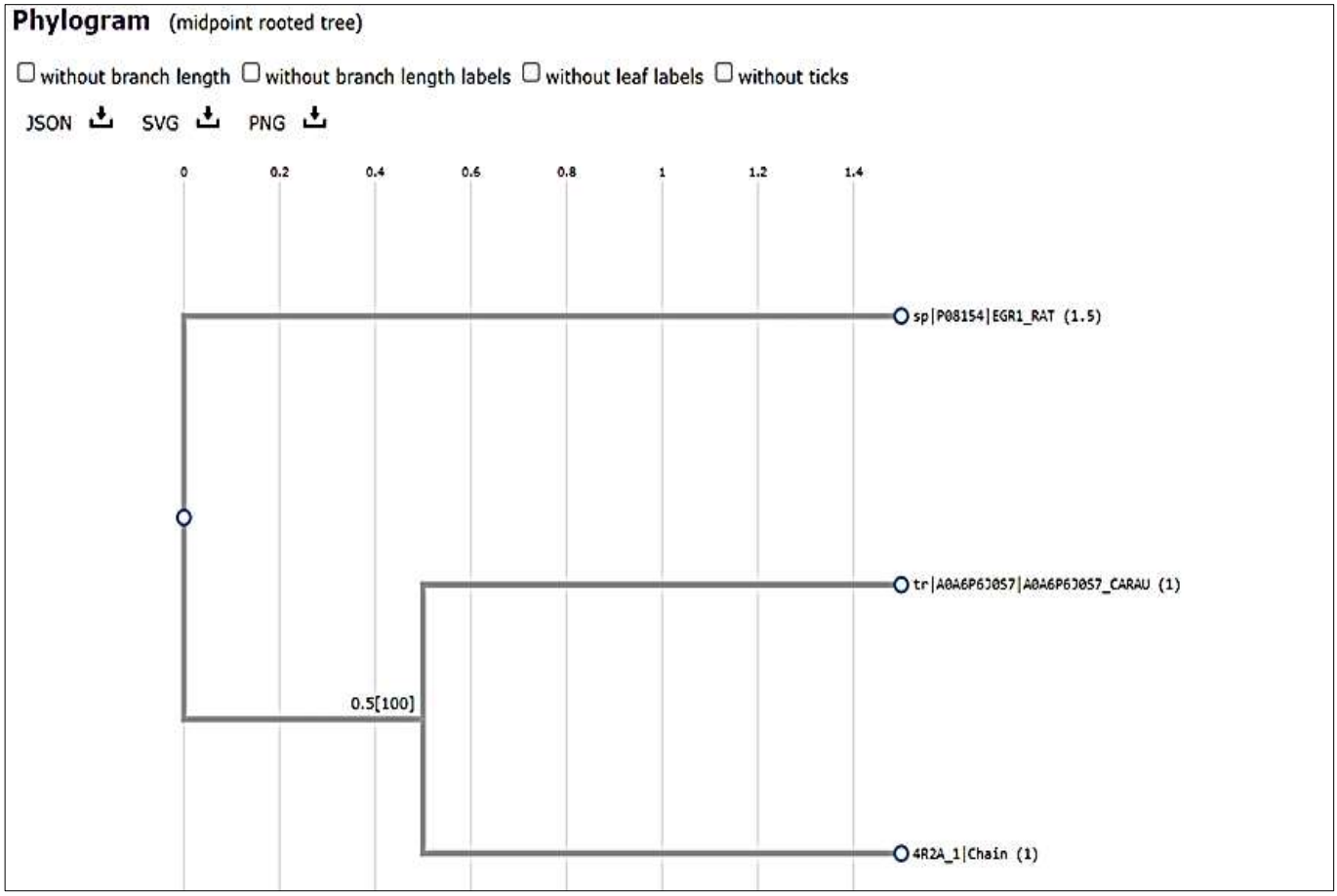


Fig 6: Phylogram

Table 1: The RMSD values and binding energy of docking result of 5 ligands with EGr1 were shown in Table 1

Ligand	Binding Affinity	RMSD/UB	RMSD/LB
4r2a_A_Hydroxytyrosol_acetate_uff_E=99.14	-4.9	0	0
4r2a_A_Hydroxytyrosol_acetate_uff_E=99.14	-4.9	5.506	2.952
4r2a_A_Vanillic_acid_hexoside_uff_E=171.00	-4.9	0	0
4r2a_A_Vanillic_acid_hexoside_uff_E=171.00	-4.7	6.223	2.518
4r2a_A_ferulic_acid_uff_E=171.00	-4.9	0	0
4r2a_A_ferulic_acid_uff_E=171.00	-4.6	5.996	2.535
4r2a_A_kaempferol_3_o_pentoside_uff_E=595.33	-5.3	0	0
4r2a_A_kaempferol_3_o_pentoside_uff_E=595.33	-5.3	5.937	3.269
4r2a_A_myricetin_3_o_glucoside_uff_E=572.54	-5.6	0	0
4r2a_A_myricetin_3_o_glucoside_uff_E=572.54	-5.6	7.01	3.308

4. Conclusion

As per procedures both the control and Aniso treated fishes were subjected to training and behaviour data were collected and analysed. The control group fishes initially showed more number of attempts and took more time to cross the gate 1 and to take feed. As the number of trails increases the fishes has learnt to cross gate 1. When compared to control group the Anisomycin treated group showed very less number of attempts and took uneven time to cross the gate I, but no fishes had taken the feed all over the training period. This reveals that the protein synthesis inhibitor Anisomycin blocks the learning process by inhibiting the protein synthesis (19). Phylogenetic analysis of the EGR1 of three species revealed that EGR1 of Human is homologous with *Carassius auratus*. Moreover, the docking studies also showed different secondary metabolites in the VCO (21) has a therapeutic effect against AD(20) . Docking results highly recommended myristine present in the VCO is to clinical trial along with further *in vitro* and *in vivo* analysis of the compound studied here.

The molecules tested here represents only a small fraction of interactions with candidate genes associated with AD (25-27). Therefore, it is necessary to conduct further studies in this concept including clinical trials. The management of this disease which do not have efficient treatment and which affects the quality of life style of patients and care givers may be benefited using this edible oil as part of diet. The raw use of this purest oil without further heating while cooking and nutrional support it can provide are added advantages considering severe nutritional loss due to severe brain damage in advanced stage of AD leads to death of patients. The antioxidants in polyphenols of vco can act as a ROS scavenger to prevent or slow down the further oxidation of biomolecules leading to neural damage in VCO consumers (28). To confirm their potential as psychoactive agents, additional study should be done, including the purification of the key components as well as an

investigation of their biological activities and mode of action.

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References

1. Lanctôt KL, Hahn-Pedersen JH, Eichinger CS, Freeman C, Clark A, Tarazona LRS, et al. Burden of Illness in People with Alzheimer's Disease: A Systematic Review of Epidemiology, Comorbidities and Mortality. Journal of Prevention of Alzheimer's Disease. 2023;1(11):97-107.
2. Zeb A. Chapter 61 - Neuroprotection induced by edible oils. In: Oliveira MR, editor. *Bioactive Nutraceuticals and Natural Products* (First Edition). Academic Press; 2024. p. 1487-505. Available from: https://www.sciencedirect.com/science/article/pii/B9780443237638000373
3. Younis M, Hashemi A, Maktoumi HA, Akhtar J, Khan SA. Phenolic constituents of three commercial olive oils: A comparative study. Pharmacological Research - Natural Products [Internet]. 2024;2(January):100012. Available from: https://doi.org/10.1016/j.prenap.2023.100012
4. Truby NL, Kim RK, Silva GM, Qu X, Picone JA, Alemu R, *et al*. A zinc finger transcription factor tunes social behaviors by controlling transposable elements and immune response in prefrontal cortex. bioRxiv Preprint Service for Biology. 2023;(November 2023).
5. Belviranlı M, Okudan N. Coconut oil ameliorates behavioral and biochemical alterations induced by D-GAL/AlCl3 in rats. Brain Research [Internet]. 2024;1823:148704. Available from: https://www.sciencedirect.com/science/article/pii/S0006899323004754
6. Illam SP, Narayanankutty A, Kandiyil SP, Raghavamenon AC. Variations in natural polyphenols determine the anti-inflammatory potential of virgin coconut oils. Journal of Food Science [Internet]. 2021 May 1;86(5):1620-8. Available from: https://doi.org/10.1111/1750-3841.15705
7. Ali M, Huarte OU, Heurtaux T, Garcia P, Rodriguez BP, Grzyb K, et al. Single-Cell Transcriptional Profiling and Gene Regulatory Network Modeling in Tg2576 Mice Reveal Gender-Dependent Molecular Features Preceding Alzheimer-Like Pathologies. Molecular Neurobiology [Internet]. 2024;61(2):541-66. Available from: https://doi.org/10.1007/s12035-022-02985-2
8. Wallace CH, Oliveros G, Xie L, Serrano P, Rockwell P, Figueiredo-Pereira M. Potential Alzheimer's early biomarkers in a transgenic rat model and benefits of diazoxide/dibenzoylmethane co-treatment on spatial memory and AD-pathology. Scientific Reports [Internet]. 2024;14(1):1-13. Available from: https://doi.org/10.1038/s41598-024-54156-z
9. Editors C, Editors A. Proceedings of National Seminar on Phytochemistry and Molecular Taxonomy of Flowering Plants.
10. Fontanini A, Czarnecki L. Neural Processing of Taste Information. In: Oxford Research Encyclopedia of Neuroscience; c2023.
11. Philothra BD, Alona I, Situmorang E, Limbardon P, Salsalina VG. Narra J. Cerebellum. 2023, 1-11.
12. Rahmi U, Goenawan H, Sylviana N, Setiawan I, Putri ST, Andriyani S, *et al*. Exercise induction at expression. 2024, 1-11.
13. Lu XJ, Zhu K, Shen HX, Nie L, Chen J. CXCR4s in Teleosts: Two Paralogous Chemokine Receptors and Their Roles in Hematopoietic Stem/Progenitor Cell Homeostasis. Journal of Immunology. 2020;204(5):1225-1241.
14. Lailatul Mufidah KT. No 主観的健康感を中心とした在宅高齢者における健康関連指標に関する共分散構造分析Title. 2021;7(3):6.
15. Abstract; c2024.
16. Wingrove JS, Wimmer J, Elda Saba Echezarreta V, Piazza A, Spencer GE. Retinoic acid reduces the formation of, and acutely modulates, invertebrate electrical synapses. Journal of Neurophysiology [Internet]. 2024 Apr 3; Available from: https://doi.org/10.1152/jn.00057.2024
17. Hermi F. Phylogenetic, molecular and functional characterization of prostaglandin receptors in teleost fish [Internet]. Proyecto de investigación: Available from: http://hdl.handle.net/10201/112103
18. Sepahvand T, Power KD, Qin T, Yuan Q. The Basolateral Amygdala: The Core of a Network for Threat Conditioning, Extinction, and Second-Order Threat Conditioning. Biology. 2023, 12.
19. Shi T, Feng S, Wei M, Zhou W. Role of the anterior agranular insular cortex in the modulation of fear and anxiety. Brain Research Bulletin [Internet]. 2020;155:174-83. Available from: https://www.sciencedirect.com/science/article/pii/S0361923019308032
20. Owoloye AJ, Olubode SO, Ogunleye A, Idowu ET, Oyebola KM. Computational identification of potential modulators of heme-regulated inhibitor (HRI) for pharmacological intervention against sickle cell disease. Journal of Biomolecular Structure and Dynamics [Internet]. :1-13. Available from: https://doi.org/10.1080/07391102.2024.2331097
21. López-Sánchez C, Lagoa R, Poejo J, García-López V, García-Martínez V, Gutierrez-Merino C. An Update of Kaempferol Protection against Brain Damage Induced by Ischemia-Reperfusion and by 3-Nitropropionic Acid. Molecules. 2024;29(4).
22. Hao B. Advances in Flavonoid Research: Sources, Biological Activities, and Developmental Prospectives. 2024, 2884-925.
23. Baratti G, Potrich D, Lee SA, Morandi-Raikova A, Sovrano VA. The Geometric World of Fishes: A Synthesis on Spatial Reorientation in Teleosts. Animals. 2022;12(7).
24. Yuan MH, Zhong WX, Wang YL, Liu YS, Song JW, Guo YR, *et al*. Therapeutic effects and molecular mechanisms of natural products in thrombosis. Phytotherapy Research [Internet]. 2024 Feb 23; Available from: https://doi.org/10.1002/ptr.8151
25. Pang C, Yang H, Hu B, Wang S, Chen M, Cohen DS, et al. Identification and Analysis of Alzheimer's

Candidate Genes by an Amplitude Deviation Algorithm. Journal of Alzheimer's Disease & Parkinsonism. 2019;09:1-32.
26. Baldassarre FF, Santovito S, Campo R, Dilorenzo G. Sustainable and healthy purchasing behaviors towards palm oil-based food in Italy. British Food Journal. 2024;126(1):156-72.
27. Golam RM, Tawfiq IM, Mohammed A. BCL2 a Hub Protein in Genes Expressed in Early Parkinsonism Patients. 1-7.
28. Zhen J, Villani TS, Guo Y, Qi Y, Chin K, Pan MH, *et al*. Phytochemistry, antioxidant capacity, total phenolic content and anti-inflammatory activity of *Hibiscus sabdariffa* leaves. Food Chemistry [Internet]. 2016;190:673-80. Available from: http://dx.doi.org/10.1016/j.foodchem.2015.06.006