Original Research Article

In silico studies of phytochemicals from Aframomum melegueta leaves against Acetylcholinesterase, Butyrylcholinesterase and Glycogen Synthase Kinase-3 beta as anti-Alzheimer's disease target.

ABSTRACT

Folklore medicine has been practiced for ages across the world using plants as a memory booster and in the treatment of neurodegenerative diseases such as dementia and amnesia. The tau, amyloid hypothesis and neuroinflammation hypothesis are major partakers in a combinational approach in the development of therapy against Alzheimer's disease (AD) and Acetyl and butyryl cholinesterases inhibitors have been investigated in clinical trials of AD for a very long time. This research involved an *in-silico* approach to study the interactions of phytochemicals in *Aframomum melegueta* leaves with some enzymes that have been reported in the literature which contribute to neuronal death and memory loss associated in AD. From the molecular docking result, curcumin and lilacin are shown to have a better binding poses than the co-crystallized ligands of either Acetylcholinesterase, Butyrylcholinesterase or GSK-3β, they also have higher docking scores than AChE inhibitor Rivastigmine. The ADMET/tox properties of our lead compounds (curcumin and lilacin) showed that they are good candidates for drug development, they also possess the highest docking scores against acetylcholinesterase, butyrylcholinesterase and GSK-3 beta suggesting potent compounds in Alzheimer's disease therapy. The pharmacokinetics studies also showed that curcumin and lilacin would pass through the blood brain barrier into the brain. This work is in line with recent multi-dimensional approach in drug development in that a single compound might possess many active groups which can activate/inhibit more than one protein without any toxic effect.

Keywords : AChE , BChE , GSK-3β , Alzheimer's disease, molecular docking, curcumin

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INTRODUCTION

Alzheimer's disease (AD) is the most commonly diagnosed dementia in aging individuals older than 65 years [12], as this disease advances, the symptoms can include problems with language**,** disorientation (including easily getting lost), mood swings, loss of motivation and behavioral issues. Folklore medicine has been practiced for ages across the world using plants as a memory booster and in the treatment of neurodegenerative diseases such as dementia and amnesia [25].

The tau, amyloid hypothesis and neuroinflammation hypothesis are major partakers in a combinational approach in the development of therapy against Alzheimer's disease [26], therefore research has been focusing on developing effective treatment for AD. *Aframomum melegueta* is a Zingiberaceae family plant spice used widely in Africa [31]. It's an herb whose seeds have traditional usage mostly as a pungent spice to season foods [41]. Acetyl and butyryl cholinesterases inhibitors have been investigated in clinical trials of AD for a very long time [17] while other approaches focus on the discovery and development of anti-inflammatory agents [18].

Acetylcholinesterase (AChE) is a significant therapeutic target for AD [17] which has been well documented in AD patients [46]. Butyrylcholinesterase (BChE) like AChE also regulates metabolism of the neurotransmitter acetylcholine in the brain of humans. BChE is majorly expressed in the glia and white matter in areas that are important in cognition. In AD, BChE is also linked to the pathologies observed around the cerebral cortex where it's not found predominantly in normal brains [8]. Glycogen Synthase Kinase-3 Beta (GSK3β) is expressed predominantly in the Central Nervous System, and it is the main kinase involved in the phosphorylation of tau protein whose activity increases in AD cases [20] leading to alterations in axons transport and neurodegeneration in the hippocampus [38].

Molecular docking has become an increasingly important tool for drug [1,4]. It is a scientific method that predicts the preferred orientation of a molecule bound to another forming a stable complex [34], the science of the more favorable orientation may then be used to

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make a prediction of the strength of the association between these two molecules using scoring functions, as molecular docking is one of the methods which is frequently used in the structure-based drug design.

This study involves an *in-silico* approach to study the interactions of some enzymes that have been reported in the literature to contribute to neuronal death and memory loss associated to AD and phytochemicals in *Aframomum melegueta* leaves. Computer-aided drug design is an important tool for understanding the binding interactions between a ligand and a target protein [33]. This has emerged as a reliable, cost-effective and timesaving technique for the discovery of lead therapeutic compounds.

MATERIALS AND METHODS

Schrodinger suites software (version 2017-1)) was used as the computational tool for this study.

Protein preparation

The 3D structures of acetylcholinesterase (PDB :4MOE), butyrylcholinesterase (PDB: 6EP4) and GSK-3 beta (PDB: 4ACC) were all retrieved from the online database (www.rcsb.org/pdb/home.do). These structures were chosen due to their high resolution. The proteins were imported to maestro interface for the preparation procedure, this was carried out with the protein preparation wizard [44] where missing side chains, loops were filled with prime, missing hydrogen and bond orders were added. The proteins were prepared at neutral pH of 7± 2.0 and Het states were generated for the co-crystalized ligands at the active sites of the proteins of interest during the pre-process. Unnecessary water molecules and interfering ligands were removed from the proteins using the review and modify tab followed by H-bond assignment using PROKA to optimize the crystal structure and Restrain minimization at RMSD 0.3 Å with OPLS3 force-field [14].

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Preparation of Ligands:

In this study, phytochemicals from *Aframomium melegueta* leaves were retrieved from literature [42]. Rivastigmine (a known AChE inhibitor) was adopted as the reference ligand and its molecular interaction vis a vis docking score pattern was compared to that of *Aframomum melegueta* leaves phytochemicals. The 2D structures of the phytochemicals were retrieved from PubChem database in sdf format and the ligands were prepared with the ligand preparation tool implemented in Schrodinger suite (LigPrep, Schrödinger, 2017-1). The ligands were prepared at a pH of 7± 2.0 using an OPLS3 force field [14]), desalt and generate were selected and the stereoisomer was allowed to keep the specific chirality so as to generate maximally thirty-two ligands.

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Grid Coordinate of the Receptor:

The interaction between ligands and proteins occurs at the active site, areas around this region define the binding pocket in x, y, z coordinates. In the maestro 11.5 is embedded a tool for receptor generation which was used in the mapping out of the coordinate of each target protein receptor complexed with its native co-crystallized ligand.

Table 1: Grid map around the active site of 4M0E, 3TPP and 4ACC proteins.

Protein Grid	Grid coordinates			Grid Range (Å)	Grid Box ligand range
Map	X	Y	Z	XYZ	XYZ
4M0E	-17.291	-42.307	25.96	30	10
3TPP	30.513	41.522	2.253	27.212	10
4ACC	17.471	18.86	10.742	27.152	10

Docking Experiment:

The docking procedure used in this experiment was carried out using Glide (Grid based ligand docking with energetics) tool v7.5 on Schrodinger maestro (version 2017-1) [10]. The process involves the interaction of the retrieved library of compounds with the active site of the prepared proteins. The co-crystallized ligands and library of compounds were docked with a scaling factor of 0.80 and partial charge cut off of 0.15 into the receptor grid using the standard precision algorithm (SP) leaving the ligand sampling at flexible. Extra precision algorithm (XP) was performed on the glide SP docking protocol with ligand sampling at none (refine only) for further optimization [10].

ADMET/Tox Screening:

The pharmacokinetics properties of all the retrieved compounds were estimated using qikprop module in maestro 11.5 (QikProp, Schrödinger, 2017-1).

Validation of Molecular docking Result:

The Accuracy of the docking experiment was validated by extracting the co-crystallized ligands from the proteins, these ligands were prepared using the ligprep tool and docked back into the active site of the protein. All docking protocols were able to replicate the

orientation of the ligand to the protein. The RMSD values for each protein to its active site was calculated and they were below 2.5Å.

RESULTS

Table 2: Docked results of showing lead compounds (curcumin and lilacin) of *Aframomum melegueta* leaves phytochemicals against of human acetylcholinesterase, butyrylcholinesterase and GSK-3 beta.

COMPOUND NAMES	GLIDE SCORE (Kcal/mol)		
	AchE	BchE	GSK-3β
Co-crystalized ligand	-10.142	-3.890	-6.746
Rivastigmine	-4.837	-8.102	-4.687
Curcumin	-11.203	-9.269	-8.381
Lilacin	-9.694	-9.044	-7.191

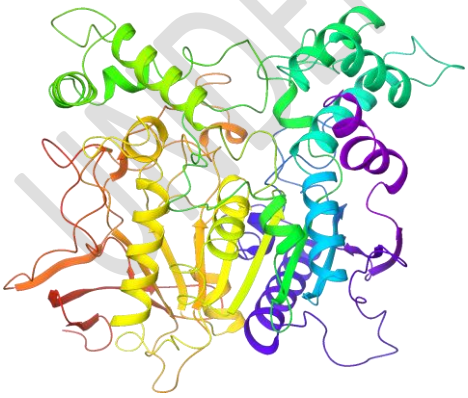

Figure 1: Crystal structure of human acetylcholinesterase (PDB: 4M0E) in ribbon representation

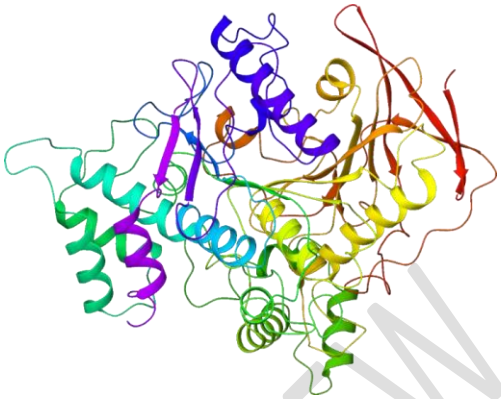

Figure 2: Crystal structure of human butyrylcholinesterase (PDB: 6EP4) in ribbon representation

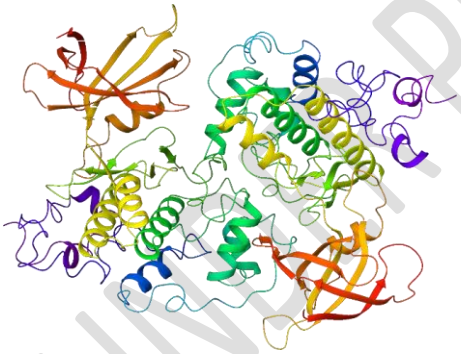

Figure 3: Crystal structure of human Glycogen Synthase Kinase 3β (PDB: 4ACC) in ribbon representation

ACHE BINDING POSES

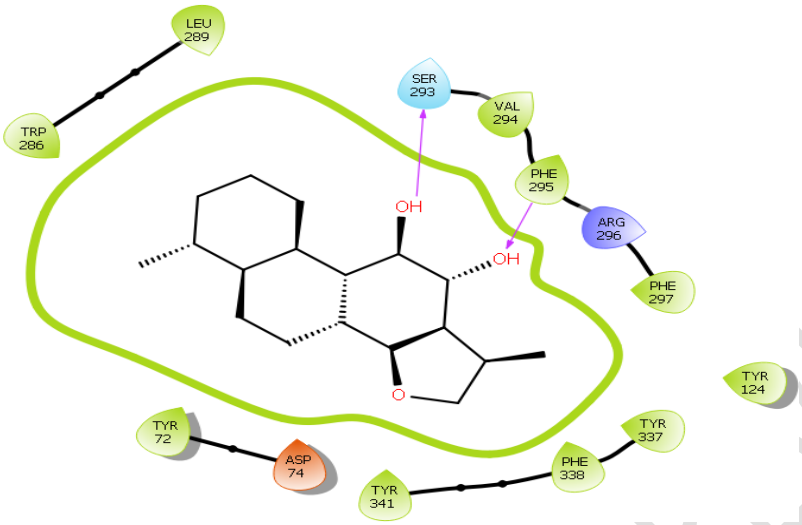

Fig 4: Binding pose of Co-Crystalized Ligand with human acetylcholinesterase

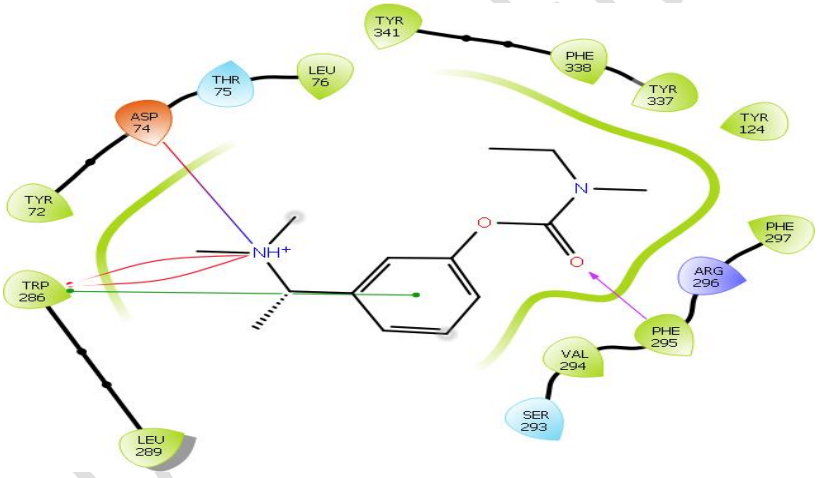

Fig 5: Binding pose of Rivastigmine with human acetylcholinesterase

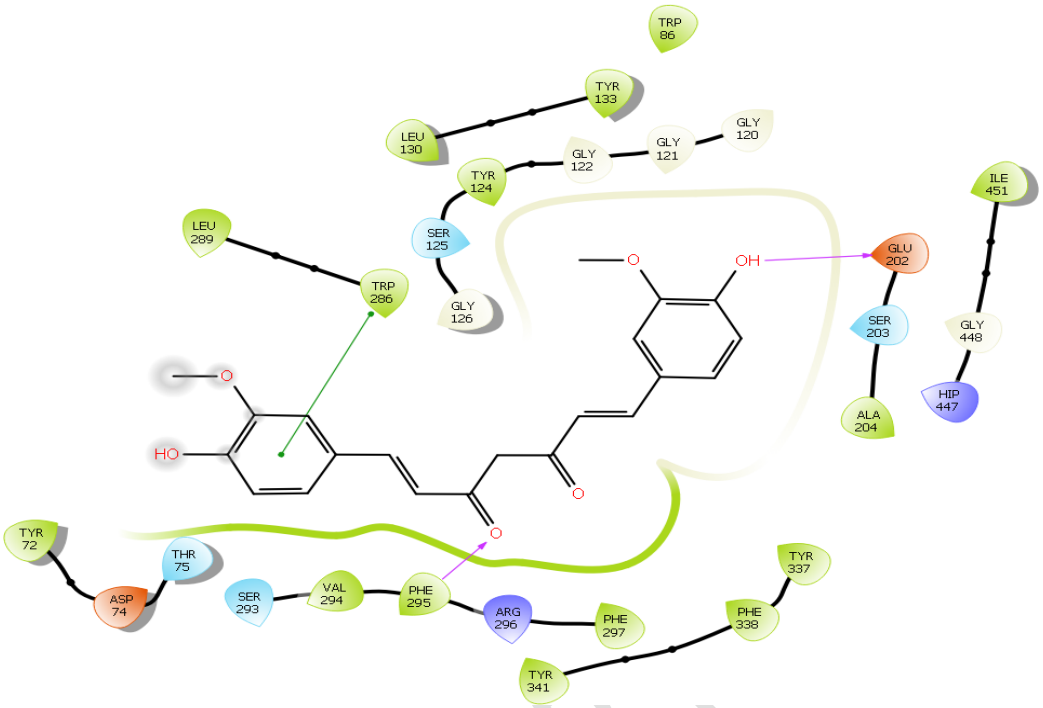

Fig 6: Binding pose of Curcumin with human acetylcholinesterase

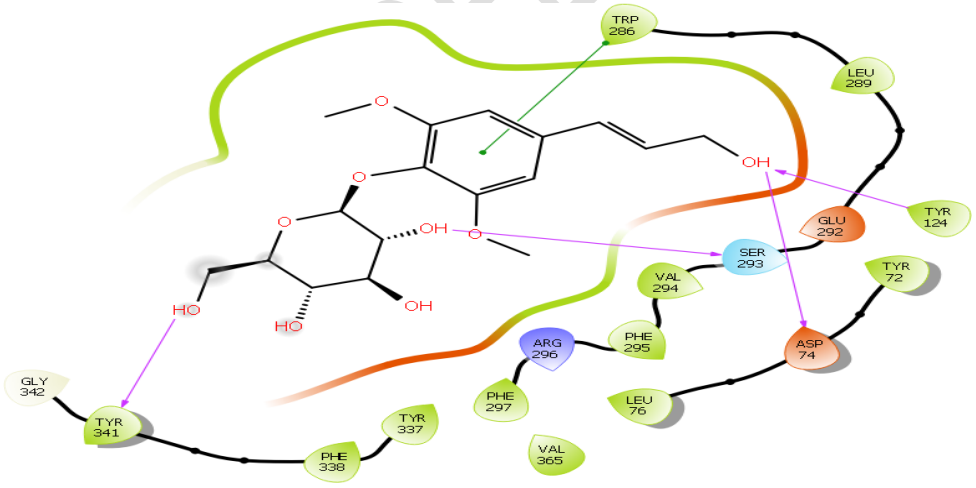

Fig 7: Binding pose of Lilacin with human acetylcholinesterase

BChE BINDING POSES

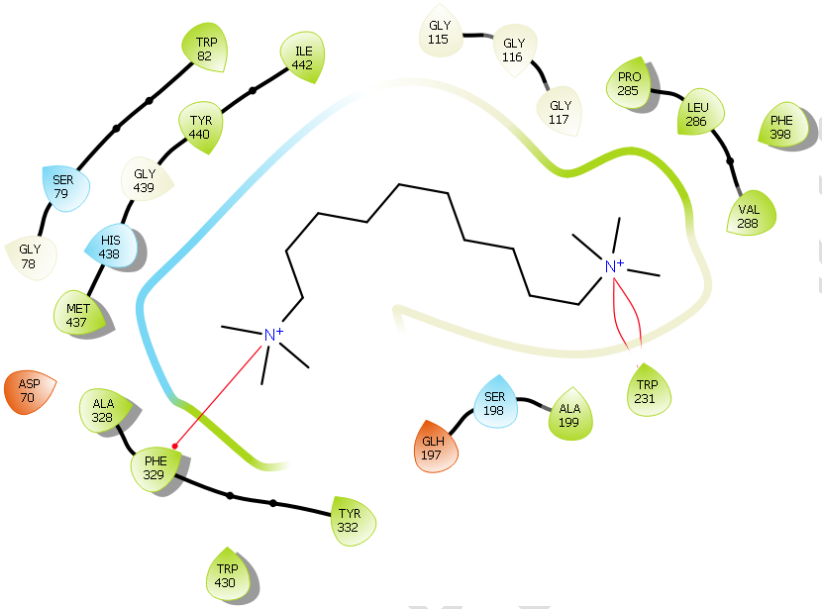

Fig 8: Binding pose of Co-Crystalized Ligand with Human butyrylcholinesterase

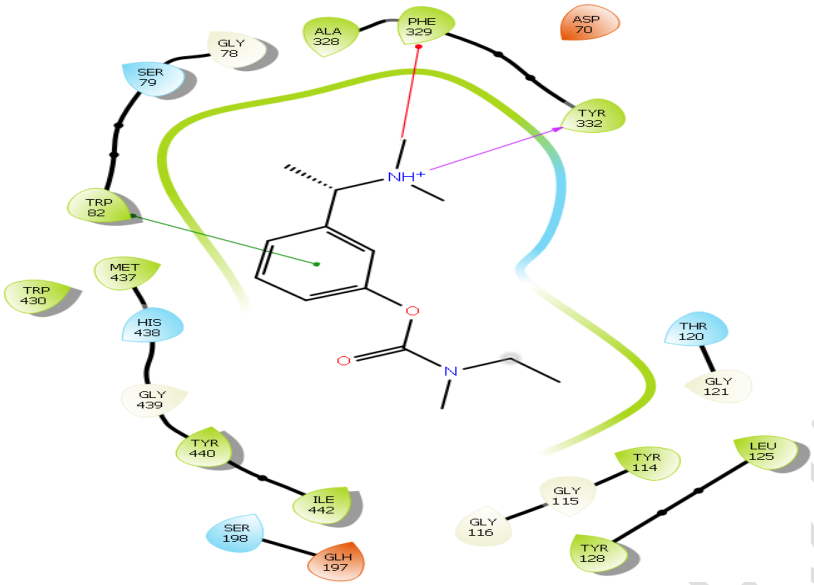

Fig 9: Binding pose of Rivastigmine with Human butyrylcholinesterase

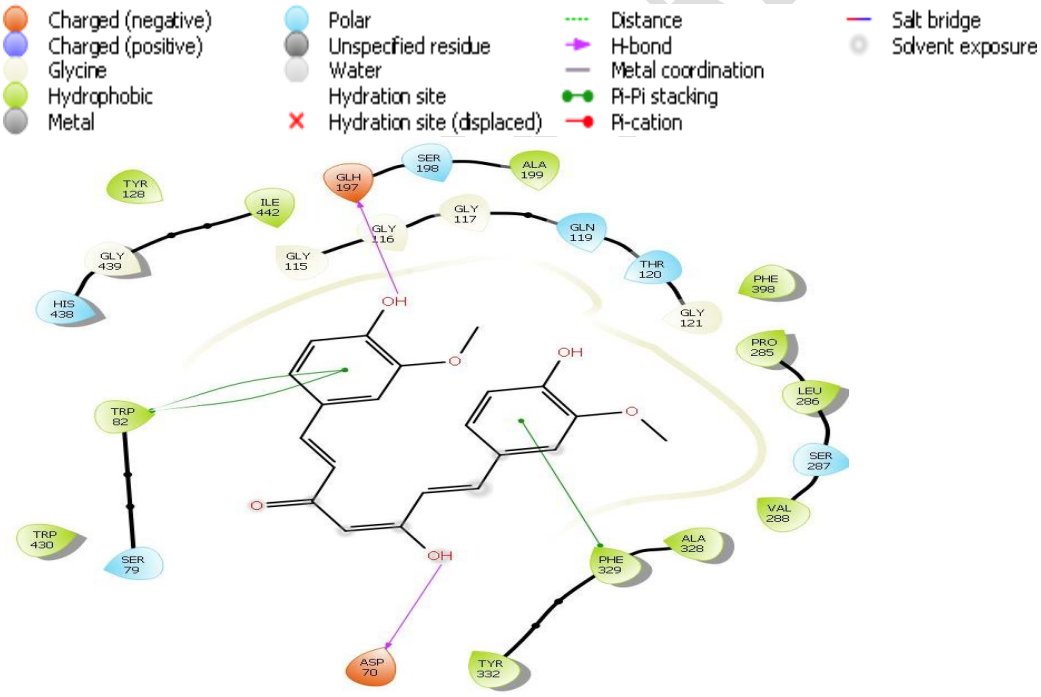

Fig 10: Binding pose of Curcumin with Human butyrylcholinesterase

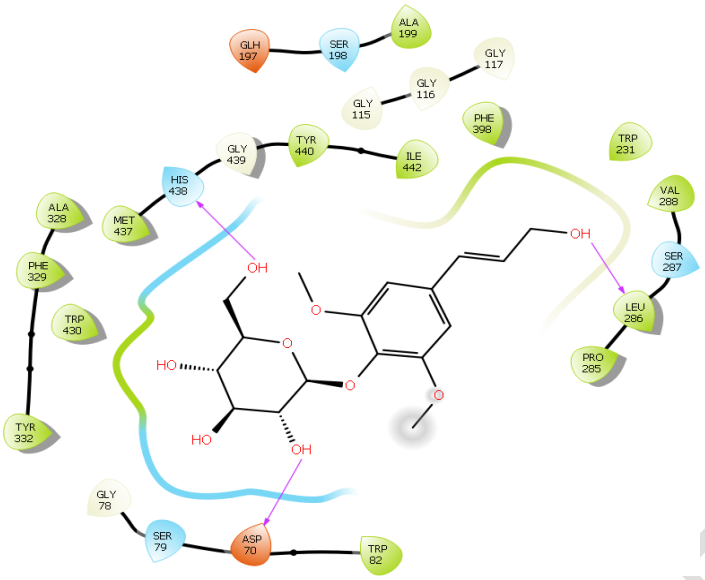

Fig 11: Binding pose of Lilacin with Human butyrylcholinesterase

GSK-3β BINDING POSES

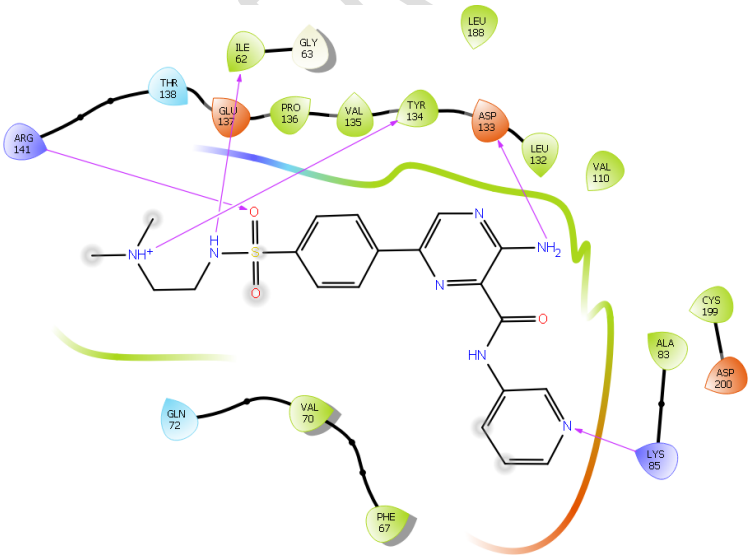

Fig 12: Binding pose of Co-Crystalized Ligand with human GSK3β

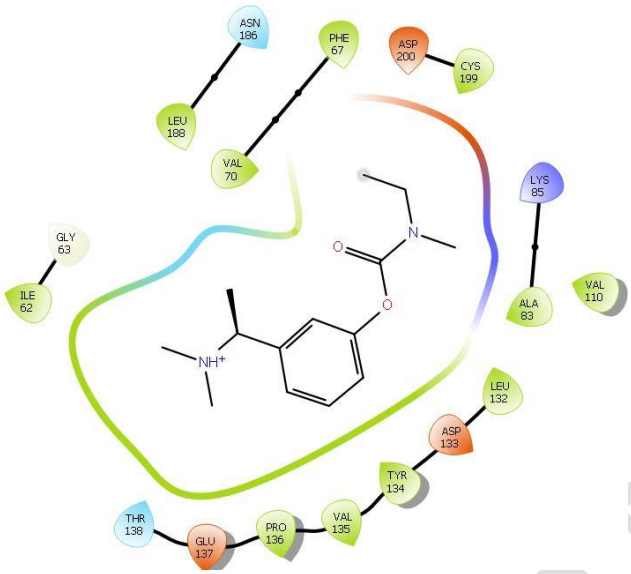

Fig 13: Binding pose of Rivastigmine with GSK3-beta

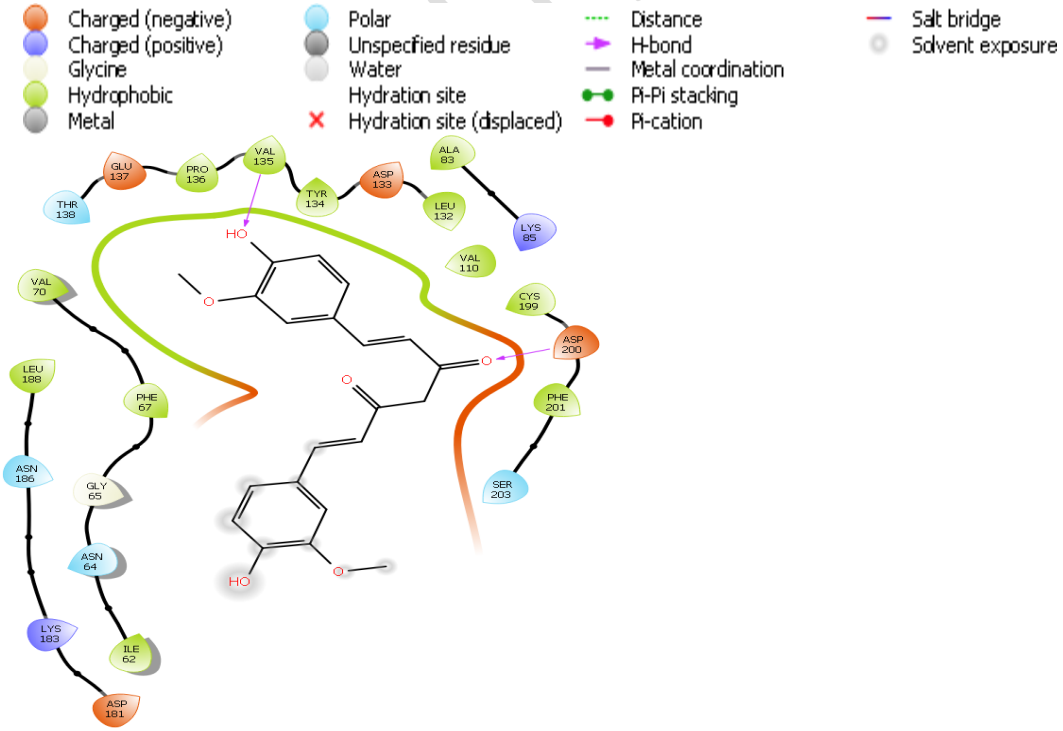

Fig 14: Binding pose of Curcumin with human GSK3β

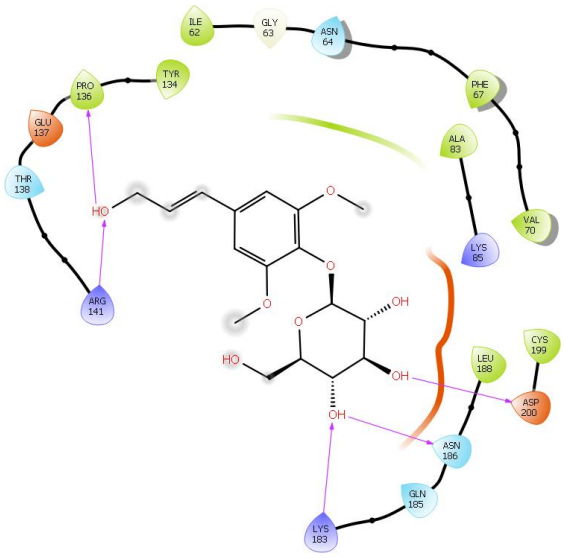

Fig 15: Binding pose of Lilacin with human GSK3β

Table 3: ADMET-TOX PROPERTIES OF PHYTOCHEMICALS FROM AFRAMOMUM MELEUGATA LEAVES

Compound Names	M.W (g/mol)	donorHB	accptHB	QPlogPo/w	QPlogBB	QPlogKhsa	HOA	ROF
alpha-Cadinol	222.37	1	0.75	4.048	0.151	0.696	3	0
Alpha-Caryophyllene	204.36	0	0	5.185	1.047	0.993	1	1
alpha-Gurjunene	204.36	0	0	5.191	1.111	0.968	1	1
alpha-Muurolene	204.36	0	0	5.568	1.059	0.983	1	1
alpha-Selinene	204.36	0	0	5.268	1.008	0.949	1	1
Aromadendrene	204.36	0	0	5.214	1.051	0.973	1	1

beta-Cadinene	204.36	0	0	5.549	1.014	0.979	1	1
beta-Caryophyllene oxide	220.35	0	2	2.487	0.093	0.378	3	0
beta-Caryophyllene	204.36	0	0	5.126	1.039	0.961	1	1
beta-Chamigrene	204.36	0	0	4.989	0.998	0.921	3	0
beta-Cubebene	204.36	0	0	5.518	1.082	1.005	1	1
beta-Elemene	204.36	0	0	5.697	0.995	0.948	1	1
beta-Guaiene	204.36	0	0	5.292	1.113	0.997	1	1
beta-Maaliene	204.36	0	0	4.974	1.085	0.922	3	0
beta-Patchoulene	204.36	0	0	6.551	1.078	0.896	1	1
beta-Selinene	204.36	0	0	5.306	1.011	0.955	1	1
Copaene	204.36	0	0	5.449	1.084	0.959	1	1
Curcumin	368.39	2	7	2.828	-2.246	0.007	2	0
Cyperene	204.36	0	0	4.861	1.074	0.884	3	0
E-Nerolidol	222.37	1	0.75	4.913	-0.2	0.782	3	0
Elemol	222.37	1	0.75	4.204	0.066	0.657	3	0
Elixene	204.36	0	0	5.637	1.048	0.984	1	1
gamma-Cadinene	204.36	0	0	5.554	1.062	0.993	1	1
gamma-Gurjunene	204.36	0	0	5.303	1.063	0.961	1	1
gamma-Muurolene	204.56	0	0	5.586	1.062	0.995	1	1
Germacrene D	204.36	0	0	5.474	1.053	0.995	1	1
Gingerol	294.39	1	4.2	3.743	-1.473	0.301	3	0
Humulene Epoxide	220.35	0	2	2.614	0.134	0.435	3	0
Ibuprofen	206.28	1	2	3.497	-0.436	0.057	3	0
Isoaromadendrene Epoxide	220.354	0	2	2.504	0.155	0.383	3	0

Isolimonene	136.24	0	0	4.018	0.796	0.388	3	0
Ledene	204.35	0	0	5.296	1.115	0.987	1	1
Lilacin	372.37	5	12.45	-0.319	-2.304	-0.949	2	0
Linalool	154.25	1	0.75	3.14	0.015	0.135	3	0
Longiborneol	222.37	1	1.7	3.336	0.265	0.424	3	0
Myrtenol	152.24	1	1.7	2.11	0.103	-0.124	3	0
Myrtenyl Acetate	194.27	0	2	2.899	-0.061	0.207	3	0
Octadecenoic acid	282.47	1	2	5.847	-0.9	0.606	3	1
Pinocarvyl Acetate	194.27	0	2	2.977	0.14	0.232	3	0
Spathulenol	220.35	1	0.75	3.931	0.25	0.67	3	0
T-Muurolol	222.37	1	0.75	4.091	0.178	0.703	3	0
trans-Sabinol	152.24	1	1.7	2.271	0.141	-0.07	3	0

M.W: *Molecular Weight of compounds* (range: 130.0 – 725.0)
DonorHB: *Hydrogen Bond donor* (range: 0.0 --6.0)
AccptHB: *Hydrogen Bond acceptor* (range: 2.0 – 20.0)
QPlogPo/w: *octanol/water partition coefficient* (range: - 2.0 – 6.5)
HOA: *Human Oral Absorption*. 1, 2, or 3 for low, medium, or high.
QPlogBB: *Prediction of blood-brain barrier penetration* (range: -3.0 -- 1.2);
QPlogKsha: *Prediction of binding to human serum albumin* (range: -1.5 to 1.5)
ROF: *Rule of Five Violation* (range: maximum is 4)

DISCUSSION

Age-dependent accumulation of amyloid beta (1–42) protein leads to self-association and soluble oligomer formation [54]. Amyloid beta oligomers bind specifically and saturate neurons triggering a variety of changes that result in inhibition of synaptic plasticity [35,57] and concomitant hyperphosphorylation of tau proteins with increased activity of acetyl and butyryl cholinesterases leading to neuronal death. Currently there is no cure for Alzheimer's disease [55] as scientists are working in developing a multi-therapeutic approach in the development of a possible drug for AD cure.

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In this work we used an *in-silico* technique to screen the various phytochemicals from the

leaves medicinal plant *Aframomum melegueta* and to see if any of the phytochemicals present in this plant leaves would have a better binding affinity than that Rivastigmine (a standard AChE inhibitor used commercially in Alzheimer's disease treatment) against acetylcholinesterase, butyrylcholinesterase and GSK-3 beta which all proteins that exacerbate the neuronal loss in Alzheimer's disease.

From the molecular docking result, Curcumin and Lilacin are shown to have a higher binding poses than the co-crystallized ligands of either Acetylcholinesterase, Butyrylcholinesterase or GSK-3 beta and the standard drug (Rivastigmine).

The ADMET/Tox (Absorption, Distribution, Metabolism, Excretion and Toxicity) properties of the phytochemicals from the leaves of *Aframomum melegueta* are displayed in Table 3, however we will be commenting of the ADMET/Tox properties of our lead compounds (Curcumin and Lilacin).

According to the Lipinski's rule of five, for a molecule to be drug-like, it should have:

Not more than 5 hydrogen bond donors; curcumin and lilacin contains 2 and 5 hydrogen bond donors respectively thus making both compounds obey this first rule.

→ Not more than 10 hydrogen bond acceptors; curcumin and lilacin have 7 and 12.45 hydrogen bond acceptor respectively making only curcumin obey the second rule.

→ The molecular weight of the compound should not be more than 500; curcumin and lilacin having a molecular weight of 368.385 and 372.371 respectively.

→ A partition co-efficient Log P (a measure of lipophilicity) of less than 5; curcumin and lilacin having 2.828 and -0.319, thus making them obey the rule.

→ The last law states that the compound will be a useful compound for drug development once it doesn't violate more than one of the rules.

Pardridge [43] also stated that the ability of compounds to pass the blood brain barrier must be considered when screening and designing drugs against neurodegenerative diseases, two out the factors to consider are the molecular weights of the compounds (range 179 – 380 Da) and the log BB (range: -3.0 -- 1.2).

The results from Table 3 shows that the molecular weights and the log BB values of curcumin and lilacin falls within the accepted range and will pass through the blood brain barrier into the brain and elicit their pharmacological function (inhibit acetylcholinesterase, butyrylcholinesterase and GSK3 beta).

CONCLUSION

Plant phytochemical extracts have showed important role in treating and preventing human diseases particularly those that are of increased prevalence such as Alzheimer's disease. Here, based on this *in silico* studies, we propose the inhibiting activity of the phytochemical constituents from the leaves of the plant *Aframomum melegueta* particularly the lead compounds curcumin and lilacin which showed the highest docking scores against acetylcholinesterase, butyrylcholinesterase and GSK-3 beta suggesting potent compounds in Alzheimer's disease therapy. The pharmacokinetics studies also showed that curcumin and lilacin have drug-like properties and will most likely pass through the blood brain barrier into the brain.

However, further i*n vitro* blood barrier models are needed to be performed to confirm the ability of curcumin and lilacin to pass through the blood brain barrier and also *in vivo* experiments should be carried out to establish the pharmacological activities of curcumin and lilacin in the development of Alzheimer disease multi-dimensional therapy.

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NOTE:

The study highlights the efficacy of "medicinal plant" which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

REFERENCES

[1] M. Abdellatif, A. Ali, A. Ali, M. Hussien, Computational studies by molecular docking of some antiviral drugs with COVID-19 receptors are an approach to medication for COVID-19. Open Chemistry, 19(1), 245-246, 2021.

[2] S.A. Adefegha, G. Oboh, Acetylcholinesterase (AChE) inhibitory activity, antioxidant properties and phenolic composition of two Aframomum species .*J Basic Clin Physiol Pharmacol*, 2012.

[3] D. Atmani, C. Nassima, A. Dina, B. Meriem, D. Nadjet, B. Hania, Flavonoids in Human Health: From Structure to Biological Activity.*Current Nutrition & Food Science*,5: 225-237, 2009.

[4] Butt, S., Badshah, Y., Shabbir,M. and Rafiq, M. (2020) Molecular Docking Using Chimera and Autodock Vina Software for Nonbioinformaticians, JMIR Bioinform Biotech 1(1):e14232,DOI: 10.2196/14232.

[5] Castello, M. A. and Soriano, S. (2013). "Rational heterodoxy: cholesterol reformation of the amyloid doctrine," *Ageing Research Reviews*, vol. 12, no. 1, 282–288.

[6] Cho, I.A., Osuagwu, A.N., Agbor, R.B., Okpako, E.C. and Ekanem, B.E. (2012**).** Phytochemical Composition of Aframomum melegueta and Piper guineense Seeds**.**

[7] Cummings, J.L., Morstorf, T and Zhong, K. (2014**).** "Alzheimer's disease drug development pipeline: few candidates, frequent failures". *Alzheimer's Research & Therapy*. **6** (4):37.

[8] Darvesh S. (2016). Butyrylcholinesterase as a Diagnostic and Therapeutic Target for Alzheimer's Disease. *Current Alzheimer research*, *13*(10), 1173–1177. https://doi.org/10.2174/1567205013666160404120 54

[9] Eketjall, S., Janson, J., Kaspersson, K., Bogstedt, A., Jeppsson, F. and Falting, J. (2016). a novel,orally active BACE1 inhibitor with high potency and permeability and markedly slow off- rate kinetics. *J Alzheimers Dis*. 50: 1109–23.

[10] Friesner, R. A., Murphy, R. B., Repasky, M. P., Frye, L. L., Greenwood, J. R., Halgren, T. A. and Mainz, D. T. (2006). Extra precision glide: Docking and scoring incorporating amodel of hydrophobic enclosure for protein-ligand complexes. Journal of medicinal chemistry, 49(21), 6177- 6196.

[11] Frisardi, V., Solfrizzi, V., Imbimbo, P.B., Capurso, C., D'Introno, A. and Colacicco, A.M. (2010). Towards disease-modifying treatment of Alzheimer's disease: drugs targeting beta- amyloid. *Curr Alzheimer Res*.7: 40–55.

[12] Gatz, M., Reynolds, C.A., Fratiglioni, L., Johansson, B., Mortimer, J.A., Berg, S., Fiske, A. and Pedersen, N.L. (2006). "Role of genes and environments for explaining

Alzheimer disease". *Archives of General Psychiatry*. **63** (2): 168–74.

[13] Gómez De Barreda, E., Perez, M., Gomez Ramos, P., De Cristobal, J., Martin-Maestro, P., Moran, A., et al. (2010). Tau-knockout mice show reduced GSK3-induced hippocampal degeneration and learning deficits. *Neurobiol. Dis.* 37, 622–629. doi: 10.1016/j.nbd.2009.11.017 **63**(2):168–174.

[14] Greenwood, J. R., Calkins, D., Sullivan, A. P. and Shelley, J. C. (2010). Towards the comprehensive, rapid, and accurate prediction of the favorable tautomeric states of drug- like molecules in aqueous solution. Journal of computer-aided molecular design, 24(6-7),591- 604.

[15] Grossberg, G.T. (2003). Cholinesterase inhibitors for the treatment of Alzheimer's disease: Getting on and staying on. *Curr Therapeut Res*.64:216–235.

[16] Grozinger, L., Amos, M., Gorochowski, T.E. *et al.* (2019). Pathways to cellular supremacy in biocomputing. *Nat Commun* **10,** 5250

[17] Haake, A., Nguyen, K., Friedman, L., Chakkamparambil, B. and Grossberg, G. T. (2020). An update on the utility and safety of cholinesterase inhibitors for the treatment of Alzheimer's disease. *Expert opinion on drug safety*, *19*(2), 147–157.

[18] Hampel, H., Caraci, F., Cuello, A. C., Caruso, G., Nisticò, R., Corbo, M., Baldacci, F., Toschi, N., Garaci, F., Chiesa, P. A., Verdooner, S. R., Akman-Anderson, L., Hernández, F., Ávila, J., Emanuele, E., Valenzuela, P. L., Lucía, A., Watling, M., Imbimbo, B. P., Vergallo, A., … Lista, S. (2020). A Path Toward Precision Medicine for Neuroinflammatory Mechanisms in Alzheimer's Disease. *Frontiers in immunology*, *11*, 456.

[19] Holmes, C., Boche, D., Wilkinson, D., Yadegarfar, G., Hopkins, V., Bayer, A., Jones, R.W., Bullock, R., Love, S., Neal, J.W., Zotova, E. and Nicoll, J.A. (2008). "Long-term effects of Abeta42 immunisation in Alzheimer's disease: follow-up of a randomised, placebo-controlled phase I trial". *Lancet*. **372** (9634): 216–23.

[20] Hooper, C., Killick, R., and Lovestone, S. (2008). The GSK3 hypothesis of Alzheimer's disease. *J. Neurochem.* 104, 1433–1439. doi: 10.1111/j.1471-4159.2007.05194.x

[21] Hsu, D and Marshall, G.A. (2017). "Primary and Secondary Prevention Trials in Alzheimer Disease: Looking Back, Moving Forw*ard".* Current Alzheimer Research.14 **(4)**: 426–440.

[22] Huang,Y. and Mucke,Y.(2012). "Alzheimer mechanisms and therapeutic strategies," *Cell*, vol. 148, no.6, 1204–1222.

[23] Ilic, N.M., Dey, M., Poulev, A., Logendra, S. and Kuhn, P.E. (2014). Anti-inflammatory Activity of Grains of Paradise Extract. *J Agric Food Chem*. 62:10452-10457.

[24] Inegbenebor, U. (2009**).** Effect of alligator pepper (Zingiberaceae aframomum melegueta) on first trimester pregnancy in Sprague Dawley rats **.** *Niger J Physiol Sci*.

[25] Jeon, S. G., Song, E. J., Lee, D., Park, J., Nam, Y., Kim, J. I., & Moon, M. (2019). Traditional Oriental Medicines and Alzheimer's Disease. *Aging and disease*, *10*(2), 307–328. https://doi.org/10.14336/AD.2018.0328

[26] Kabir, M. T., Uddin, M. S., Mamun, A. A., Jeandet, P., Aleya, L., Mansouri, R. A., Ashraf, G. M., Mathew, B., Bin-Jumah, M. N. and Abdel-Daim, M. M. (2020). Combination Drug Therapy for the Management of Alzheimer's Disease. *International journal of molecular sciences*, *21*(9), 3272. https://doi.org/10.3390/ijms21093272

[27] Khan, M.T., Orhan, I. and Enol, S.S. (2009). Cholinesterase inhibitory activities of some flavonoid derivatives and chosen xanthone and their molecular docking studies. *Chem Biol Interact* 181, 383–389.

[28] Kitchen, D.B., Decornez, H., Furr, J.R. and Bajorath, J. (2004). "Docking and scoring in virtual screening for drug discovery: methods and applications". *Nature Reviews. Drug Discovery*. **3** (11): 935–49.

[29] Klyubin, I., Wang, Q., Reed, M.N., Irving, E.A. and Upton, N. (2011). Protection

[30] Against amyloid- beta mediated rapid disruption of synaptic plasticity and memory by memantine. *NeurobiolAging.* 32: 614–623.

[31] Knippers, R.H.M., Gallois, S. and van Andel, T. (2021). Commercialization of *Aframomum* spp. in Africa: a Systematic Review of Literature and Supporting Botanical Vouchers. *Econ Bot* **75,** 76–91 https://doi.org/10.1007/s12231-021-09517-4.

[32] Lahiri, D.K., Farlow, M.R., Greig, N.H. and Sambamurti, K. (2002). Current drug targets for Alzheimer's disease treatment. *Drug Develop Res*.261–280.

[33] Leis, S., Schneider, S. and Zacharias, M. (2010). In silico prediction of binding sites on proteins. Current medicinal chemistry, 17(15), 1550–1562.

[34] Lengauer, T. and Rarey, M. (1996). "Computational methods for biomolecular docking". *Current Opinion in Structural Biology*. **6** (3): 402–6.

[35] Lesne, S.E., Sherman, M.A., Grant, M., Kuskowski, M. Schneider, J.A. (2013). Brain amyloid- beta oligomers in ageing and Alzheimer's disease. Brain 136:1383.

[36] Martorana, A., Esposito, Z. and Koch, G. (2010). "Beyond the cholinergic hypothesis: do current drugs work in Alzheimer's disease?". *CNS Neuroscience & Therapeutics*. **16** (4): 235–45.

[37] Mudher, A and Lovestone, S. (2002). "Alzheimer's disease-do tauists and baptists finally shake hands?".*Trends in Neurosciences*. **25** (1): 22–6.

[38] Muyllaert, D., Kremer, A., Jaworski, T., Borghgraef, P., Devijver, H., Croes, S., et al. (2008). Glycogen synthase kinase-3β, or a link between amyloid and tau pathology? *Genes Brain Behav.* 7, 57–66. doi: 10.1111/j.1601-183x.2007.00376.x

[39] Nwozo, S.O and Oyinloye, B.E. (2011). Hepatoprotective effect of aqueous extract of Aframomum melegueta on ethanol-induced toxicity in rats. *Acta Biochim Pol 58*: 355-358.

[40] Ono K., Hasegawa K. and Yamada M. (2004). Curcumin has potent anti-amyloidogenic effects for Alzheimer"s beta-amyloid fibrils in vitro. *J Neurosci Res.*75:742-750

[41] Osuntokun, O. T., Olumekun, V. O., Ajayi, A. O., Omotuyi, I. O. and Olonisakin, A. (2020). Assessment of in-vitro Antioxidant/Enzymes Inhibitory Potentials Aframomum melegueta [Roscoe] K. Schum (Grains of Paradise) Leaf, Stem Bark, Seed Bark and Seed Extracts. *Archives of Current Research International*, *20*(2), 40-57. https://doi.org/10.9734/acri/2020/v20i230176

[42] Owokotomo, I., Ekundayo, O., and Oguntuase, B. (2014). Chemical Constituents of the Leaf, Stem, Root and Seed Essential Oils of Aframomum melegueta (K. Schum) from South West Nigeria. *International Research Journal of Pure and Applied Chemistry, 4*, 395-401.

[43] Pardridge W. M. (2020). Treatment of Alzheimer's Disease and Blood-Brain Barrier Drug Delivery. *Pharmaceuticals (Basel, Switzerland)*, *13*(11), 394. https://doi.org/10.3390/ph13110394

[44] Sastry, G. M., Adzhigirey, M., Day, T., Annabhimoju, R., & Sherman, W. (2013). Protein and ligand preparation: parameters, protocols, and influence on virtual screening enrichments. Journal of computer-aided molecular design, 27(3), 221-234.

[45] Selkoe, D.J. and Hardy, J. (2016). The amyloid hypothesis of Alzheimer's disease at 25 years.*EMBO Mol Med*. **8**: 595–608.

[46] Sharma, K. (2019). Cholinesterase inhibitors as Alzheimer's therapeutics (Review) Molecular Medicine Reports, 20, 1479-1487. https://doi.org/10.3892/mmr.2019.10374

[47] Solfrizzi, V., Panza, F., Frisardi, V., Seripa, D., Logroscino, G., Imbimbo, B.P. and Pilotto, A. (2011). "Diet and Alzheimer's disease risk factors or prevention: the current evidence". *Expert Review of Neurotherapeutics*. **11** (5): 677–708.

[48] Stahl, S.M. (2000). "The new cholinesterase inhibitors for Alzheimer's disease, Part 2: illustrating their mechanisms of action". *The Journal of Clinical Psychiatry*. **61** (11): 813–4.

[49] Stern, Y. (2006). "Cognitive reserve and Alzheimer disease". Alzheimer Disease and Associated Disorders. **20** (3 Suppl 2): S69–74.

[50] Suresh, P.S., Kumar, A., Kumar, R. and Singh, V.P. (2008). "An in silico [correction of insilico approach to bioremediation: laccase as a case study". *Journal of Molecular Graphics & Modelling*. **26** (5): 845–9.

[51] Viswanathan, A. and Greenberg, S.M. (2011). Cerebral amyloid angiopathy in the elderly. *Ann Neurol*.**70**: 871–80.

[52] Wilkinson, D.G., Francis, P.T., Schwam, E. and Payne-Parrish, J. (2004). Cholinesterase inhibitors used in the treatment of Alzheimer's disease: the relationship between pharmacological effects and clinical efficacy. *Drugs Aging*. 21:453–478.

[53] World Health Organization (2006). Neurological Disorders: Public Health Challenges**.** Switzerland: World Health Organization. pp. 204–07. www.nia.nih.gov/Alzheimers

[54] Yang, T., Hong, S., O'Malley T., Sperling, R. and Walsh, D.M. (2013) New ELISAs with high specificity for soluble oligomers of amyloid beta-protein detect natural amyloid-beta oligomers in human brain but not CSF. *Alzheimers Dement* 9: 99–112.

[55] Yiannopoulou, K. G., & Papageorgiou, S. G. (2020). Current and Future Treatments in Alzheimer Disease: An Update. *Journal of central nervous system disease*, *12*, 1179573520907397. https://doi.org/10.1177/1179573520907397

[56] Yiannopoulou, K.G. and Papageorgiou, S.G. (2013). Current and future treatments for Alzheimer's disease. *Ther Adv Neurol Disord*. 6:19–33.

[57] Zempel, H., Luedtke, J., Kumar, Y., Biernat, J. and Dawson, H., (2013). Amyloid-beta oligomers induce synaptic damage via Tau-dependent microtubule severing by TTLL6 and spastin. *EMBO J.* 32: 2920–2937.