TOXICOLOGY STUDY OF TURMERIC SPICES USED IN ANIMAL PRODUCTS USING MOLECULAR DOCKING TECHNIQUES

[1*]Oshibanjo D.O., [2]Barki L. J., [3]A. Z. Sanusi, [4]Ohikhobo O J. and [1]Mfon J.S
[1]Department of Animal Production, Faculty of Agriculture, University of Jos, Plateau State, Nigeria
[2]Department of Pharmacology and Toxicology, University of Jos, Plateau State, Nigeria
[3]Department of Animal Science, Federal University Dutsin-Ma, Kastina State, Nigeria
[4]Nigerian Institute of Leather and Science Technology, Owan East Cluster Center, Edo State
**Corresponding author's email: oshibanjoo@unijos.edu.ng*

ABSTRACT
This study was aimed to evaluate the toxicological properties of turmeric spices used in animal products through molecular docking techniques. Fifteen phytocompounds of turmeric, sourced from ethnobotanical databases, were prepared using the Ligprep module for molecular docking. The crystal structure of high blood pressure human adenosine A2A receptor complexed with a G protein-mimetic nanobody (6OS2) was employed molecular docking using the Glide ligand docking in Schrödinger Suite 2023. Toxicity assessments were conducted using the Protox-II web servers. Fifteen compounds extracted from the turmeric plant were compared with standard drug (Puerarin) for regulating high blood pressure. Among these, only nine compounds exhibited docking scores superior to the standard drug. The co-crystalized drug Puerarin bound to specific amino acid residues, namely VAL141, ILE 210, ILE124, LEU 217, TYR 127, CYS121, VAL 62, and PHE 66 through hydrophobic interactions, ARG 140 and ARG 139 through positive charge bonds and ASP 125 through negative charge interactions. Additionally, THR213, SER214, SER 136, and THR 148 were involved in polar interactions while those of turmeric phytocompounds are bound to ASP 125, THR 148, VAL 144, LUE 128, ILE 124, CYS 121 and PHE 66 through hydrophobic interactions. The toxicity classes of various compounds ranged from 4 to 5 with LD50 of 832, 2250, 1000, 2480, 2980, 2300 and 1330 respectively, for Puerarin, 4-Hydroxybenzaldehyde, Vanillin, Eucalyptol, Caffeic Acid, (E)-3-hydroxy-1,7-bis(4-hydroxyphenyl)hept-6-ene-1,5-dione, (5S,6S,9R)-9-hydroxy-9-methyl-3-propan-2-ylidene-6-prop-1-en-2-yl-1-oxaspiro[4.4]nonan-2-one. In conclusion, the identified phytocompounds including 4-hydroxybenzaldehyde, Vanillin, Eucalyptol, Caffeic Acid, (E)-3-hydroxy-1,7-bis(4-hydroxyphenyl)hept-6-ene-1,5-dione,(5S,6S,9R)-9-hydroxy-9-methyl-3-propan-2-ylidene-6-prop-1-en-2-yl-1-oxaspiro[4.4]nonan-2-one exhibited higher docking scores suggesting potential for hypertensive prophylaxis.

Keywords: Toxicology, turmeric, Animal Products, molecular docking and spices

INTRODUCTION

Animal products are derived from the body of an animal and can be processed into foodstuffs or animal feed. Common examples of animal products include milk, eggs, honey, and various food products prepared from meat or fish. In contrast, spices, seasonings and flavourings play a pivotal role in augmenting the taste and aroma of meat products (Sanusi *et al.,* 2022). Spices and herbs have been utilised across cultures for centuries to enhance the flavour and aroma of foods. Spices consists of aromatic flavourings made from plants parts while herbs fragrant leaves from herbaceous plants (Sanusi *et al.,* 2022). The famous spice author Rosen Garten describes a spice as a substance that enriches or alters the quality of another substance, such as modifying the taste of food to impart zest or pungency, a piquant or lasting flavour, or a relish. The common spices used in processed meat include paprika, chilli, pimento, mace, ginger, nutmeg, cloves, cinnamon, thyme, cardamom, cumin, coriander seeds, garlic, ginger, black pepper and turmeric (Sanusi *et al.,* 2023).

Turmeric (*Curcuma longa* Linn. Syn C. domestica Valeton) is extensively used as a spice, food preservative and colouring agent particularly in the Indian subcontinent (Aggarwal *et al*., 2007; Chattopadhyay et al., 2004). This spice has traditionally been associated with numerous medicinal properties dating back to the time of Ayurveda (1900 B.C). Turmeric has been credited with numerous therapeutic activities for a wide range of diseases, including conditions affecting skin, pulmonary, gastrointestinal systems, aches, pains, wounds, sprains, and liver disorders (Aggarwal *et al*., 2007). Turmeric contains a wide variety of phytochemicals, including but not limited to curcumin,

demethoxycurcumin, bisdemethoxycurcumin, zingiberene, curcumenol, curcumol, eugenol, tetrahydrocurcumin, triethylcurcumin, turmerin, turmerones, and turmeronols (Chattopadhyay et al., 2004). Extensive research within the last half century has proven that most of these activitiesattributed to turmeric are credited to curcumin, which holds diferuloylmethane, demethoxycurcumin, and bisdemothycurcumin as its three major analogues.).
However, there are some evidences to suggest that turmeric extracts can be toxic (Deshpande et al., 1998; Kandarkar *et al.*, 1998). Hence in the present study we have adopted in silico methods to predict the toxicity of 13 compounds from turmeric.

MATERIALS AND METHODS

Materials: Computer (laptop), Schrödinger Suite 2023 13.5, Web servers (Protox-II) and selected bioactive compound of turmeric which was obtained from Pubchem site (https://pubchem.ncbi.nlm.nih.gov)

Ligand Preparation

The Ligprep module from Schrödinger Suite 2023 13.5 was utilized to prepare a total of 13 phytocompounds extracted from turmeric, as identified from ethnobotanical databases in preparation for molecular docking.

Protein Preparation

The crystal structures of high blood pressure was obtained from the RCSB protein data bank (PDB) with PDB ID 6OS2 of the crystal structure of the human adenosine A2A receptor in complex with a G protein-mimetic nanobody.

Receptor Grid Generation

Using the receptor grid construction tool in Schrödinger Maestro 13.5, the scoring grid was defined and supported by the co-crystalized ligand GK.

Molecular Docking

Molecular docking of the prepared ligands and proteins were performed using the glide ligand docking in Schrödinger Suite 2023.

Toxicity properties

Toxicity properties was carried out using the Web servers (Protox-II).

RESULTS AND DISCUSSION

Fifteen selected compounds from the turmeric plant were assessed alongside one standard drug to test for their efficacy against high blood pressure. Among them, only nine compounds exhibited docking scores that were higher than that of the standard drug. Subsequent analysis and visualization using the Schrodinger software revealed that the co-crystalized drug Puerarin bound to specific amino acid residues, namely VAL141, ILE 210, ILE124, LEU 217, TYR 127, CYS121, VAL 62, PHE 66, forming hydrophobic bonds. ARG 140 and ARG 139 formed positively charged bonds, ASP 125 formed negative charges, while THR213, SER214, SER 136, and THR 148 exhibited polar interactions. Conversely, turmeric phytocompounds exhibited diverse binding profiles with amino acid residues. For instance, 4-**Hydroxybenzaldehyde** obtained formed negative charges with ASP 125, and positive charges with THR 148 along with hydrophobic VAL 144, LUE 128, ILE 124, CYS 121, PHE 66. **Vanillin** displayed positive charges with ARG 140 and negative charges with ASP 125 Charge, while also forming hydrophobic bonds with VAL 62, VAL 144, CYS 121, ILE 124, and PHE 66. **Eucalyptol** displayed negative charges with ASP 125 and positive charges with ARG 139 and ARG 140 alongside hydrophobic interactions with VAL 144, CYS 121, VAL 62, ILE 124, PHE 66, and LEU 128. These interactions underscore the potential binding affinities of the turmeric compounds with the target protein.

For **Puerarin (ID: 5281807):** Docking Score: -4.14. This suggests that puerarin is predicted to interact with the target protein, and the docking score of -4.14 indicates a reasonably strong binding affinity. The amino acid residues THR 148 and CYS 121 are likely involved in the binding process.
For **Bisdemethoxycurcumin (ID: 5315472):** Docking Score: -4.36. Bisdemethoxycurcumin has a docking score of -4.36, which is even lower than that of puerarin, suggesting a potentially stronger binding. The amino acid residues THR 148 and ARG 139 are implicated in the binding interaction.
Monodemethylcurcumin (ID: 5469426): Docking Score: -4.05. Monodemethylcurcumin has a docking score of -4.05, indicating a moderate-to-strong binding affinity. The amino acid CYS 121 is

implicated in the binding interaction. **Curcumenone (ID: 153845):** Docking Score: -4.24. Curcumenone has a docking score of -4.24, suggesting a strong binding affinity. The amino acid ARG 139 is implicated in the binding interaction. The rest of the ligands have similar number of basic amino acids for this protein. Hydrophobic interactions are important in molecular docking because they can contribute to the binding affinity between the ligand and receptor. Charge (negative) interactions are also important in molecular docking, as they can form ionic bonds with positively charged regions on the receptor. Charge (positive) interactions can also play a role in molecular docking, as they can form ionic bonds with negatively charged regions on the receptor. Polar bonding is important in molecular docking because it can contribute to the formation of hydrogen bonds between the ligand and receptor.

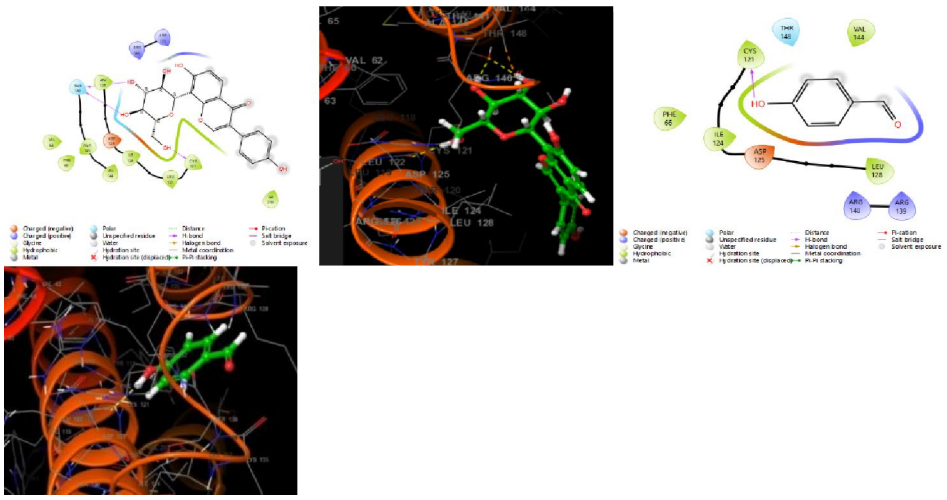

Plate 1. 2D and 3D of Puerarin with high blood pressure

Plate 2. 2D and 3D of 4-Hydroxybenzaldehyde

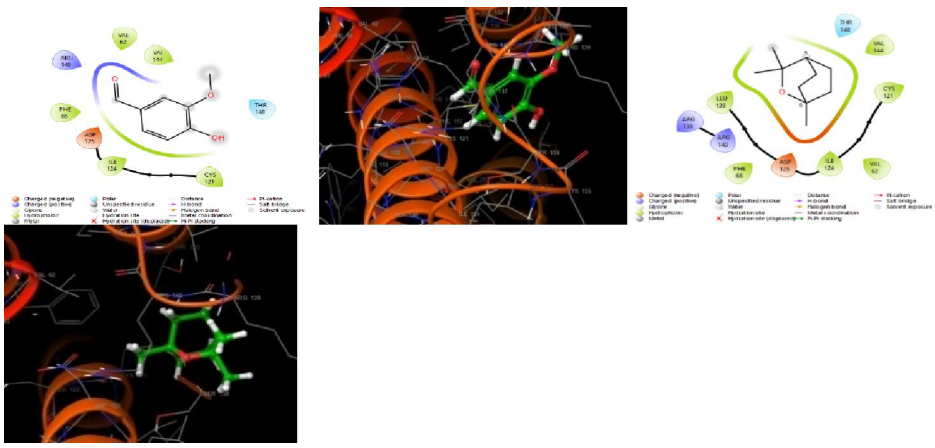

Plate 3. 2D and 3D of Vanillin with high blood pressure

Plate 4. 2D and 3D of Eucalyptol

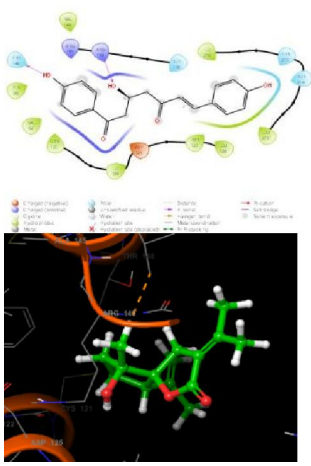

Plate 5. 2D and 3D of (E)*

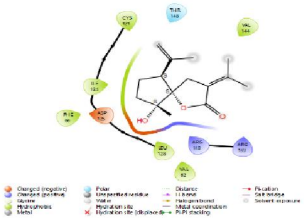

Plate 6. 2D and 3D of 5S*

*(E)-3-hydroxy-1,7-bis(4-hydroxyphenyl)hept-6-ene-1,5-dione

*(5S,6S,9R)-9-hydroxy-9-methyl-3-propan-2-ylidene-6-prop-1-en-2-yl-1-oxaspiro[4.4]nonan-2-one

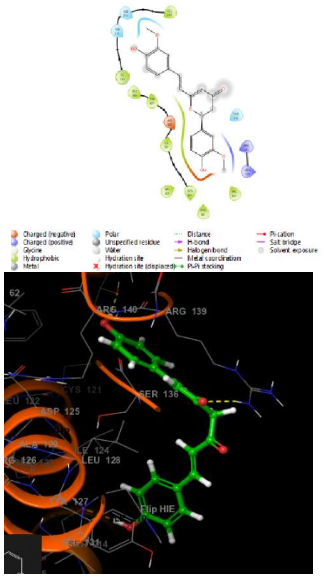

Plate 7. 2D and 3D of Cyclocurcumin

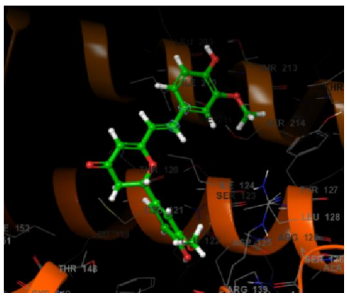

Plate 8. 2D and 3D of Bisdemethoxycurcumin

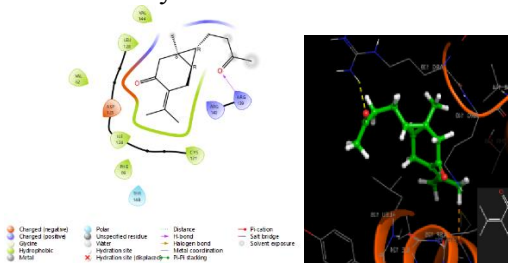

Plate 9. 2D and 3D of Curcumenone

Table 1: Docking score, LD_{50} and toxicity class

Bioactive name	Predicted LD_{50} (mg/kg)	Predicted Toxicity Class	ID number	Docking score	Amino acid affinity
Puerarin	832	4	5281807	-4.14	THR 148 and CYS 121
Curcumin	-	-	969516	-3.93	
Demethoxycurcumin	-	-	5469424	-4.00	
Bisdemethoxycurcumin	-	-	5315472	-4.36	THR 148 and ARG 139
Didemethyl Curcumin	-	-	5469425	-3.82	
Monodemethylcurcumin	-	-	5469426	-4.05	CYS 121
5′-methoxycurcumin	-	-	10250249	-3.80	-
Cyclocurcumin	-	-	69879809	-4.70	-
α-turmerone	-	-	160512	-3.93	-
Curcumenone	-	-	153845	-4.24	ARG 139
4-Hydroxybenzaldehyde	2250	5	126	-5.23	CYS 121
Vanillin	1000	4	1183	-4.86	THR 148
Eucalyptol	2480	5	2758	-4.57	THR 148
Caffeic Acid	2980	5	689043	-4.14	THR 148
(E)-3-hydroxy-1,7-bis(4-hydroxyphenyl)hept-6-ene-1,5-dione	2300	5	1.2E+08	-4.75	THR148 and AGR 139
(5S,6S,9R)-9-hydroxy-9-methyl-3-propan-2-ylidene-6-prop-1-en-2-yl-1-oxaspiro[4.4]nonan-2-one	1330	4	1E+08	-4.60	-

The docking score is a numerical value that represents the predicted strength of the interaction between a ligand and a target protein. A lower docking score typically suggests a more favorable and stronger binding interaction (Agu *et al*., 2023 and Furlan *et al.,* 2018).Amino acid affinity specifies which amino acids in the target protein are involved in the binding interaction with the ligand. The Protein Data Bank (PDB) entry 6OS2 refers to the crystal structure of the human adenosine A2A receptor in complex with a G protein-mimetic nanobody. The adenosine A2A receptor is a G protein-coupled receptor (GPCR) that plays a role in various physiological processes, including the regulation of blood pressure Sumirtanurdin *et al.* (2020).

THR 148 (Threonine at position 148): Threonine at position 148 might be involved in the binding pocket of the adenosine A2A receptor, contributing to ligand binding and receptor activation.

ARG 139 (Arginine at position 139): Arginine at position 139 could participate in interactions with ligands or other proteins, potentially influencing the conformational changes associated with G protein coupling. **CYS 121 (Cysteine at position 121):** Cysteine at position 121 might contribute to the structural stability of the receptor or be involved in disulfide bond formation.

The adenosine A2A receptor is part of the adenosine receptor family and is implicated in the regulation of blood flow and blood pressure. Its activation can lead to vasodilation, influencing blood vessel diameter and blood pressure (Hu *et al*., 2023). It is important to note that while the crystal structure provides valuable insights into the three-dimensional arrangement of amino acids in the receptor, the specific interactions and roles of these amino acids in blood pressure regulation may vary depending on the ligands and signaling pathways involved.

The toxicity result obtained from Protox-II. 4-Hydroxybenzaldehyde showed activity as a carcinogen but that intense. Myrcene showed very intense activity on antioxidant response element and heat shock factor response element. Cinnamic and geranic acid showed mild activity on the hepatic system. Oleic acid and 9,12-Octadecadienoic acid, (E,Z)- showed intense activity on PPAR-Gamma, nrf2/ARE and HSE. Ferulic acid show intense activity on compromising the immunity. Caffeic acid showed intense activity on carcinogenicity and androgen receptor. Linolenic acid showed intense activity on PPAR-Gamma. All these are responsible for either the short term or long toxicities. Also, the various toxicity class 4, 5, 4, 5, 5, 5 and 4 with LD_{50} of 832, 2250, 1000, 2480, 2980, 2300 and 1330 respectively.

CONCLUSION

The phytocompounds 4-Hydroxybenzaldehyde, Vanillin, Eucalyptol, Caffeic Acid, (E)-3-hydroxy-1,7-bis(4-hydroxyphenyl)hept-6-ene-1,5-dione, (5S,6S,9R)-9-hydroxy-9-methyl-3-propan-2-ylidene-6-prop-1-en-2-yl-1-oxaspiro[4.4]nonan-2-one of interest have higher docking score of -5.23, -4.86, -4.57, -4.14, -4.75, and -4.60 compared to the standard drug with a docking score of -4.14. This result suggests their potential efficacy in prophylactic treatment against hypertension by targeting the blood pressure-related protein (6OS2). . Further in silico research into other medicinal plants in handling hypertension and to conduct in vitro and in vivo tests tovalidate the antihypertensive activity of compounds derived from turmeric plants.

REFERENCES

Agu, P. C., Afiukwa, C. A., Orji, O. U., Ezeh, E. M., Ofoke, I. H., Ogbu, C. O., Ugwuja, E. I., and Aja, P. M. (2023). Molecular docking as a tool for the discovery of molecular targets of nutraceuticals in diseases management. Scientific Reports, 13(1), 1-18. https://doi.org/10.1038/s41598-023-40160-2

Deshpande, U. R., Gadre, S. G., Raste, A. S., Pillai, D., Bhide, S. V., and Samuel, A. M. (1998). Protective effect of turmeric (Curcuma longa L.) extract on carbon tetrachloride-induced liver damage in rats. Indian journal of experimental biology, 36(6), 573–577.

Furlan, V, Konc., J and Bren, U. (2018). Inverse Molecular Docking as a Novel Approach to Study Anticarcinogenic and Anti-Neuroinflammatory Effects of Curcumin. Molecules; 23(12):3351. https://doi.org/10.3390/molecules23123351

Hu, M., Yan, H. and Li, H. (2023). Use of network pharmacology and molecular docking to explore the mechanism of action of curcuma in the treatment of osteosarcoma. Scientific Reports, 13(1), 1-13. https://doi.org/10.1038/s41598-023-36687-z

Kandarkar, S. V., Sawant, S. S., Ingle., A.D., Deshpande, S. S and Maru, O.R. (1998). Subchronic oral hepatotoxicity of turmeric in mice-Graded levels of raw and cooked turmeric rhizome (Curcuma longa) on broiler chicks 207 histopathological and ultra clinical studies. Indian. J. Exp. Biol, 36:675-679

Mir, S. A., Madkhali, Y., Firoz, A., Al Othaim, A., Alturaiki, W., Almalki, S. G., Algarni, A., and Alsagaby, S. A. (2023). Inhibitory Potential of the Ocimum sanctum Phytochemicals on Bruton's Tyrosine Kinase, a Well-Known Drug Target for Treatment of Chronic Lymphocytic Leukemia: An In Silico Investigation.Molecules (Basel, Switzerland), 28(8),3287.
https:// doi.org/10.3390/molecules28083287

Sanusi, A. Z., Garba, M.G., Jibia, Z.S., Salisu, U. S., Aljameel K.M., Sabo. M.N., Babba H.U., Yetunde, H.K., Jibir, M., Mikailu, M., Garba., S and Saidu, S. S. (2022). Evaluation of spices used in meat products in Sokoto metropolis, Nigeria. Nigerian J. Anim. Sci., 23 (3): 278-285

Sumirtanurdin, R., Sungkar, S., Hisprastin, Y., Sidharta, K. D., and Nurhikmah, D. D. (2020). Molecular Docking Simulation Studies of Curcumin and Its Derivatives as Cyclin-Dependent Kinase 2 Inhibitors. Turkish Journal of Pharmaceutical Sciences, 17(4), 417–423. https://doi.org/10.4274/tjps.galenos.2019.55822

Tainter, D. R. andand Grenis, A. T. (eds.) (1993). In spices and seasonings. New York, N. Y.: VCH Publishers, Inc.