Proceedings of Science and Mathematics
Faculty of science, universiti teknologi malaysia

https://science.utm.my/procscimath/
Volume 27 (2024) 86-95

In Silico Evaluation of *Curcuma longa* Phytochemicals Interactions on Hypothalamic-Pituitary Adrenal (HPA) Axis Pathway Protein in Traditional Malay Medicine

Nadhirah Rodzuan[a], Farah Wahida Md Yusoff[b], Norhissam Mustafa[c] and Razauden Mohamed Zulkifli[a,b*]
[a]Department of Biosciences, Faculty of Science, Universiti Teknologi Malaysia (UTM), 81310, Skudai, Johor, Malaysia
[b]Center of Research for Fiqh Science and Technology, Universiti Teknologi Malaysia, 81310 Skudai, Johor, Malaysia
[c]Akademi Tahfiz Medik Antarabangsa
*Corresponding author: razauden@utm.my

Abstract
Curcuma longa (turmeric) has been extensively used in traditional Malay medicine for postnatal care across various cultures and is believed to possess therapeutic properties that may be beneficial for postpartum depression (PPD). The current mechanism of *C. longa* for PPD treatment has deficient scientific evidence, requiring exploring phytochemical and target protein interactions for therapeutic studies. This study investigates the role of *C. longa* in regulating the hypothalamic-pituitary-adrenal (HPA) axis through molecular docking and molecular dynamic simulations, followed by network analysis. Bisdemethoxycurcumin (BDMC) phytochemical selected by screening through ADME analysis exhibited stable binding interactions with glucocorticoid receptor (GR) with docking score – 7.5 kcal/mol. This binding suggests that BDMC can effectively activate GR, suppressing CRH and ACTH synthesis. Consequently, BDMC may suppress cortisol production through a negative feedback mechanism, thereby regulating the HPA axis. The protein-pathway mapping revealed that BDMC's interaction with target proteins could significantly impact various biological mechanisms, including hormone balance, stress response, and mood regulation. These findings highlight BDMC as a promising candidate for regulating the HPA axis and treating PPD. These can facilitate the development of new treatments targeting the underlying physiological signalling pathways in PPD.
Keywords: Postpartum depression; *Curcuma longa*; molecular docking; molecular dynamic simulation

Introduction

Postpartum depression (PPD), commonly known as “meroyan” in traditional Malay medicine, is a condition caused by emotional instability due to hormonal changes that affect women following childbirth (Alimuddin et al., 2023). It can lead to depression and aggressive behavior in women. The rate of PPD incidence ranges from 3.9% to 22% in Malaysia (Hairol et al., 2021). In Malay postnatal care, traditional medicinal plants have been intensively utilized by traditional midwives for many years as a postnatal remedy to improve women’s recovery and after-delivery health that could trigger postpartum depression in women. These can help restore emotional and physical energy for women after delivering by utilizing indigenous medicinal plant (Mohamad et al., 2022).

Curcuma longa plant (turmeric) is one of the medicinal plants widely used in postnatal care and has many health benefits, including treating postpartum depression. It is a perennial herbaceous plant that is classified as a member of the Zingiberaceae family. It is one of the medicinal plants utilized for years to prevent various health conditions due to its various therapeutic properties. *C. longa*, widely used in postnatal care, has been associated with treating ‘meroyan air’, a type of PPD caused by hormone imbalance. Traditionally, turmeric’s mother rhizome part is used due to its unique phytochemical contents and benefits. Recent studies also show that *C. longa* exhibits antidepressant actions through several mechanisms, such as monoamine inhibition, the

neural plasticity hypothesis, the inflammatory mechanism, neurodegeneration including hypothalamic-pituitary-adrenal axis (Matias et al., 2021; Seo et al., 2015).

The hypothalamic pituitary adrenal (HPA) axis is a complex system compromising the endocrine pathways that interact with various feedback mechanism that includes three main components, which are the anterior pituitary gland, adrenal gland, and hypothalamus. It is closely related to the development of postpartum depression, as pregnancy, birth, and lactation can significantly alter the function of the HPA axis. The HPA axis acts as a regulator to control the production of cortisol, a glucocorticoid steroid hormone synthesized in the adrenal cortex that is crucial to regulating stress response. When the HPA axis is activated, the hypothalamus releases corticotropin-releasing hormone (CRH) that stimulates the anterior pituitary gland to secrete adrenocorticotropic hormone (ACTH). This, in turn, leads to the release of cortisol, which has many biological functions to regulate body mechanisms. However, excessive cortisol production can be harmful. During pregnancy, placental secretion of cortisol elevates the mother's cortisol levels leading to hypercortisolemia that induces the development of PPD in women afterbirth (Hantsoo et al., 2023; Stickel et al., 2021). Through glucocorticoid receptors (GR), cortisol negatively affects the anterior pituitary and the hypothalamus.

Materials and methods

Interview session with a traditional Malay practitioner

A qualitative interview was conducted face-to-face with a registered traditional Malay practitioner, Mr. Noorhissam Mustafa, an expert in traditional Malay medicinal studies at Batu Pahat, Johor. The information gathered through these interview sessions was used as an outline for classifying phytochemicals from the C. longa and identifying target proteins in the HPA axis pathways to treat PPD disease by performing comprehensive literature studies about their interactions between phytochemicals and target proteins.

Classification and preparation of phytochemicals

Ten phytochemicals from the *C. longa* plant focussing on the rhizome part of the plant were collected from the public database. The IMPPAT (Indian medicinal plants, phytochemistry and therapeutics) database (https://cb.imsc.res.in/imppat/) (Mohanraj et al., 2018) were used to search the phytochemicals in the plant. The phytochemicals identified were classified based on their percentage of composition obtained through published research papers. Phytochemicals with the highest percentage were further screened using the SwissADME database (http://www.swissadme.ch/). PubChem database was used to retrieve the structures of phytochemical compounds that have been selected through the classification process in .sdf format. The phytochemicals' energy was minimized before the molecular docking study.

Retrieval of target protein related to HPA axis pathway

The target proteins used in this study were glucocorticoid receptor (GR), corticotropin-releasing hormone receptor 1 (CRHR1) and peptidyl-prolyl cis-trans isomerase (FKBP5). The three-dimensional (3D) structures of target proteins, GR with PDB ID: 1P93, CRHR1 with PDB ID: 3EHS and FKBP5 with PDB ID: 5OMP were downloaded from RCSB Protein Data Bank in .pdb format. Upon obtaining the PDB file of the targeted proteins, energy minimization of the protein was performed using the YASARA energy minimization server (https://www.yasara.org/minimizationserver.htm) to optimize the protein structure before molecular docking.

Molecular docking

The molecular docking of the phytochemicals and the target protein was conducted using AutoDock Vina in the PyRx software to dock the phytochemicals into the binding site of the target protein (Kondapuram et al., 2021). The BIOVIA Discovery Studio software was used for the visualization of interactions between the target proteins and phytochemicals (Design, 2014). After docking, the conformations with the best binding affinity scores were selected for further analysis.

Molecular Dynamic simulation

The conformation with the best binding affinity score was further analyzed using molecular dynamic (MD) simulations for 20 ns. This MD simulation was performed through GROMACS with the 2023.3 software package (Abraham et al., 2015) with CHARMM36m used as its force field (Matsubara et al., 2022). Prior to the MD simulation, the topology files were prepared in the CHARMM-GUI server (https://www.charmm-gui.org/) using solution builder tools. The simulation used sodium and calcium ions to neutralize the complex charges and was conducted at a temperature of 300 K and under the pressure of 1 bar. The root-mean-square deviation (RMSD), the root-mean-square fluctuation (RMSF) and hydrogen bonds (HBs) were used for further analysis (Halder et al., 2023).

Protein-protein interaction (PPI) analysis

A protein-protein interaction (PPI) network for disease and drug mapping targets was constructed using Cytoscape 3.10.2 software (Otasek et al., 2019; Shannon et al., 2003). The protein-protein interactions in phytochemical compounds with target proteins were expanded by using the STRING database plugin (https://string-db.org/) in the Cytoscape software to obtain the related proteins, and its network visualization was generated (Szklarczyk et al., 2023). The STRING enrichment table network was displayed in the table panel, and Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathways were screened from the enrichment table results. The minimum threshold value for STRING enrichment analysis was set at $p < 0.05$ and FDR < 0.01, indicating the significance and strength of the enrichment obtained for the network (Mohanty et al., 2024)

Results and discussion

Classification of phytochemicals compounds in *Curcuma longa* and target protein in HPA axis

The chemical composition of *C. longa* mostly consists of two main components, curcuminoids and essential oils, which are widely used in treating many diseases. Ten phytochemical components were identified in rhizomes of C. longa and selected based on a comprehensive review of scientific literature. Following that, these phytochemicals were further classified based on their composition percentages. Table 1 presents the phytochemicals identified in *C. longa* rhizomes.

Table 1: The phytochemicals in *C. longa* with its chemical composition.

Phytochemicals 1	Percentage (%)	References
curcumin	57	(Dosoky et al., 2019; Krishnakumar et al., 2015; Naz et al., 2010; Setzer et al., 2021; Singh et al., 2022)
ar-turmerone	36.02	
α-turmerone	13.6–31.5	
bis-demethoxycurcumin	23	
demethoxycurcumin	20	
beta turmerone	19.07	
eucalyptol	3.22	
α-phellandrene	2.41	
β-sesquiphellandrene	0.98	
α-zingiberene	0.34	

The *C. longa* phytochemicals were further screened based on their compound properties using the SwissADME tool to predict the pharmacokinetic properties and drug-likeness of the compound. Bisdemethoxycurcumin, eucalyptol, ar-turmerone, α-turmerone and β-turmerone were selected for docking with target proteins due to it best pharmacokinetic properties (Table 2). The absorption of these phytochemicals through the gastrointestinal (GI) and blood-brain barrier (BBB) was an important aspect

that was measured to determine the efficacy of the phytochemical in the body. Properties such as lipophilicity, solubility and molecular weight were also analyzed to select the best phytochemicals as they influenced the absorption into the body.

Table 2: ADME analysis of phytochemicals in *C. longa.*

Phytochemicals	GI absorption	Solubility	MW	Log P	BBB
Ar-turmerone	High	Moderately soluble	216.32	3.84	Yes
Eucalyptol	High	Soluble	47.12	2.67	Yes
α-turmerone	High	Soluble	218.33	3.59	Yes
Bisdemethoxycurcumin	High	Moderately soluble	308.33	2.83	Yes
β-turmerone	High	Moderately soluble	218.33	3.7	Yes

Molecular docking between phytochemicals and target proteins

The five phytochemical compound structures that were selected through screening were obtained from the PubChem database (https://pubmed.ncbi.nlm.nih.gov/). Bisdemethoxycurcumin, eucalyptol, ar-turmerone, α-turmerone and β-turmerone undergo energy minimization prior to the molecular docking process were docked into three target proteins, GR (PDB ID: 1P93), CRHR1 (PDB ID: 3EHS) and FKBP5 (PDB ID: 5OMP) using PyRx, Autodock Vina feature to analyze the phytochemicals interactions with the proteins. The binding score of docking between the phytochemicals and target proteins is represented in Table 3. Bisdemethoxycurcumin has shown the best docking interaction with all the target proteins among all the phytochemicals. The binding of bisdemethoxycurcumin with GR protein was - 7.5 kcal/mol, which is considerably higher than with CRHR1 (- 6.8 kcal/mol) and FKBP5 (- 6.9 kcal/mol). These suggest that bisdemethoxycurcumin potentially interacts with target proteins to regulate the HPA axis, which could lead to PPD.

Table 3: The binding affinity score of docking between phytochemicals and target proteins.

Ligand	GR (PDB ID: 1P93)	CRHR1 (PDB ID: 3EHS)	FKBP5 (PDB ID: 5OMP)
Bisdemethoxycurcumin	-7.5	-6.8	-6.9
α-turmerone	-7.2	-5.8	-5.2
β-turmerone	-7.1	-5.7	-5.1
ar-turmerone	-7.0	-5.7	-5.1
Eucalyptol	-5.9	-4.1	-5.5

In order to explore the interaction of bisdemethoxycurcumin as a ligand with each of these proteins, the docking structure was analyzed using BIOVIA discovery software. The visualization of the 2D and 3D interaction of bisdemethoxycurcumin with each protein is shown in Figure 1. A complex is defined as strong when its structure contains more hydrogen bonds and few hydrophobic interactions, salt bridges, and pi-pi interactions. Bisdemethoxycurcumin formed 3 hydrogen bonds with GR protein at residue TRP610 with a distance of 2.79 Å, 2.88 Å and ARG614 with a distance of 3.05 Å (Figure 1A, 1B). Besides, it also exhibits the pi-cation interaction with ARG611 and pi-alkyl interaction with PRO541, ALA607 and VAL543 between the benzene ring of the ligand. These interactions contribute to the high affinity, indicating a strong binding with the protein.

The binding interaction of bisdemethoxycurcumin with CRHR1 and FKBP5 shows no hydrogen bond was formed between bisdemethoxycurcumin with CRHR1 and FKBP5. Instead, bisdemethoxycurcumin has established pi-pi interactions with CRHR1 protein, including pi-alkyl interaction on ILE51 and VAL97, pi-stacked interaction with PHE71 and pi-pi T-shaped interaction with TYR99 (Figure 1C, 1D,). Meanwhile, bisdemethoxycurcumin with FKBP5 formed pi-alkyl interactions with Leu363 and Leu328 and pi-cation interaction with Lys397 (figure 1E, 1F). These docking forms significant pi–pi stacking interactions that are important in stabilizing the protein complex and relevant for molecular recognition processes (Lanzarotti et al., 2020). Thus, this interaction influences the high binding affinity of bisdemethoxycurcumin with these proteins compared to other phytochemical compounds.

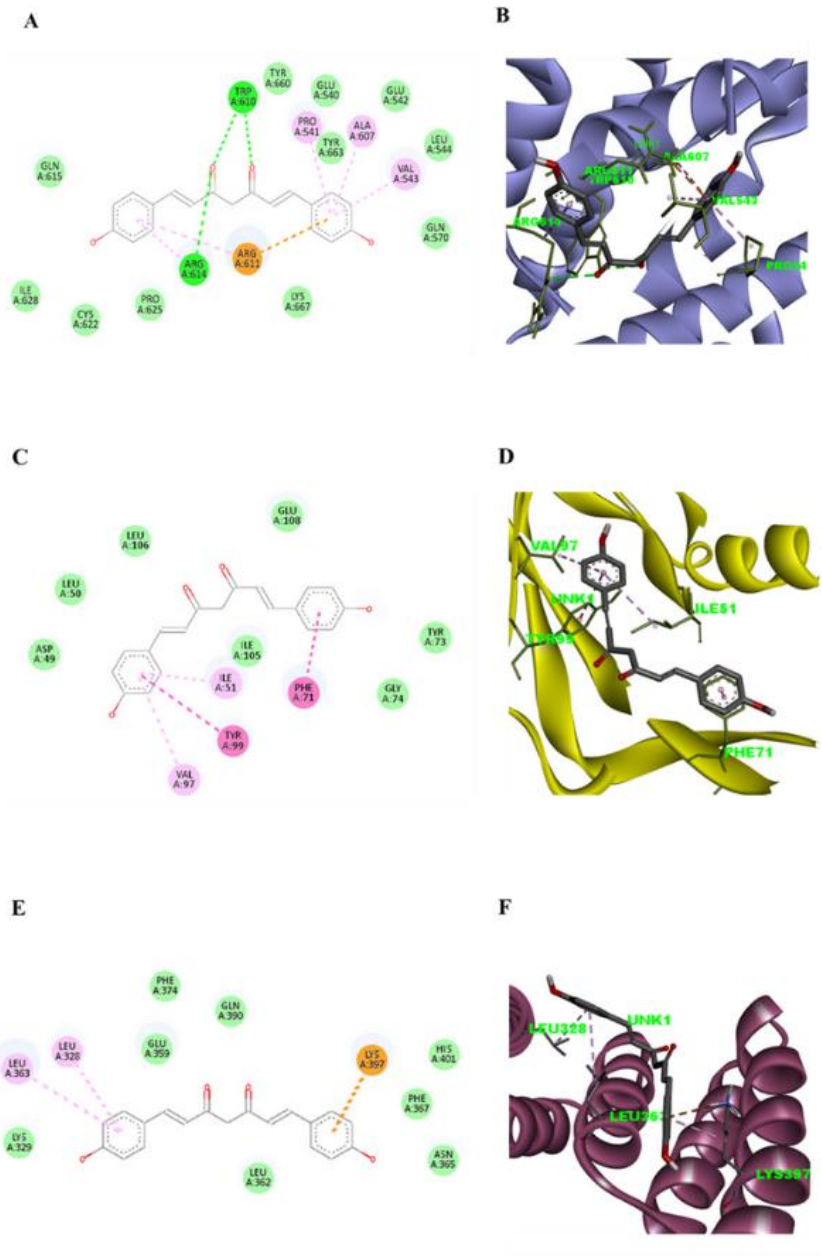

Figure 1 The molecular interactions of bisdemethoxycurcumin with target proteins (A) Molecular Interaction with GR protein (B) 3D interaction with GR protein (C) Molecular Interaction with CRHR1 protein (D) 3D interaction with CRHR1 protein (E) Molecular Interaction with FKBP5 protein (F) 3D interaction with FKBP5 protein

Molecular Dynamic Simulation Analysis

Following the docking analysis, MD simulation was conducted for the best complex bisdemethoxycurcumin with GR protein. The ligand-protein complexes undergo a simulation of 20 ns to assess the hydrogen bonding, root-mean-square deviation (RMSD) and root-mean-square fluctuation (RMSF) (Figure 2). The RMSD simulation plot shows two sharp increases within the first 8 ns, indicating initial structural changes as the complex equilibrated into favourable conformation. The RMSD plot with a mean value of 1.071 nm was eventually stabilized with several fluctuations, suggesting that the complex has maintained its binding to the receptor in a stable condition. The structural stability is demonstrated by a low RMSD value, suggesting that the structure closely resembles its initial structure (Arnittali et al., 2019). Since a lower RMSD value indicates a relatively stable complex structure, it is concluded that bisdemethoxycurcumin can stably bind with GR protein to perform its biological function in regulating the HPA axis.

The RMSF that represents the changes of each residue in the protein from its initial position during the simulation was also observed to determine the flexibility of different residue regions in GR protein following the binding of bisdemethoxycurcumin (Ghahremanian et al., 2022). It also stated that a low RMSF value indicates less movement and minimizes the fluctuation of protein structures. The mean RMSF value of the simulation was 0.123 nm, suggesting that most regions of the protein maintain a relatively stable conformation throughout the simulation. The RMSF graph plotted also shows the least fluctuation and stayed in a range of 0.1 nm – 0.2 nm with a spike at the early and end of the residues representing its conformational arrangement of the protein prior to ligand binding. The fluctuation indicates the movement of residue arrangement in the protein upon binding bisdemethoxycurcumin. Thus, it is suggested that this phytochemical has a stable binding with GR, which involves the changes in residue during the binding into the active site.

Since hydrogen bonds play a crucial role in stabilizing ligand-protein interactions and the overall structure of the complex, hydrogen bonding was assessed by observing the number of hydrogen bonds formed in the protein complex during the simulation. Initially, the maximum number of hydrogen bonds observed was four, which decreased to two by the end of the simulation. The molecular interaction of the complex shows that three hydrogen bonds were formed, but only two persisted throughout the simulation. However, the number of pairs within 0.35 nm suggests the regular appearance of three hydrogen bonds during the simulation, indicating potential hydrogen bond interactions. This is due to the dynamics of protein-ligand interactions that are influenced by intrinsic protein flexibility and external factors, which can lead to breaking these interactions during simulations. (Shukla & Tripathi, 2020). Therefore, the formation of hydrogen bonds is critical in providing a stable foundation for biological systems.

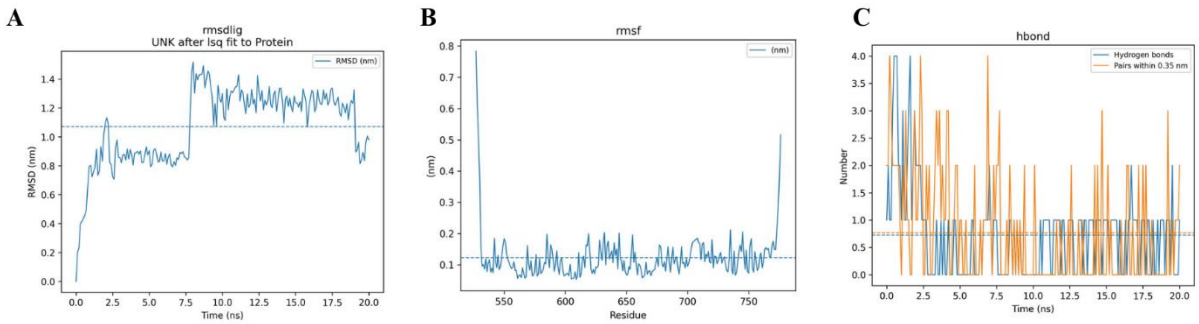

Figure 2 The analysis of MD simulation (A) The graph of RMSD against time (B) The graph of RMSF against time (C) The number of hydrogen bonds and atoms within 0.35 nm against time.

Protein-Protein Interaction analysis

The network visualization of PPI between BDMC compound with targeted protein generated, shown in Figure 3, presents the involvement of 24 proteins interconnected with the target protein, NR3C1,

CRHR1, and FKBP5 in the HPA axis pathway. proteins. The target proteins show multiple secondary connections with other proteins related to them that are important to the networks. These interactions involve proteins such as HSD11B2, CYP11B1, POMC, MRAP, and AVPR1B that occur in the metabolic pathway of cortisol synthesis from cholesterol (Ahmed et al., 2019). The protein shows a significant link with HPA axis pathway regulation involved with the target protein that affects mood regulation, stress response, and neuroendocrine functions. The HPA axis pathways play an important role in maintaining the stress response in humans involved in the central nervous systems and endocrine systems by balancing the hormones. PPD is a condition influenced by hormonal, genetic, and neurobiological factors. The proteins such as MC5R, UCN2, UCN3, CRHR2, OXTR, OXT, POMC, MRAP, and PTH2 are involved in play roles in various pathways that regulate stress response, hormone levels, and mood. Dysregulation in these proteins can lead to depressive symptoms, including PPD.

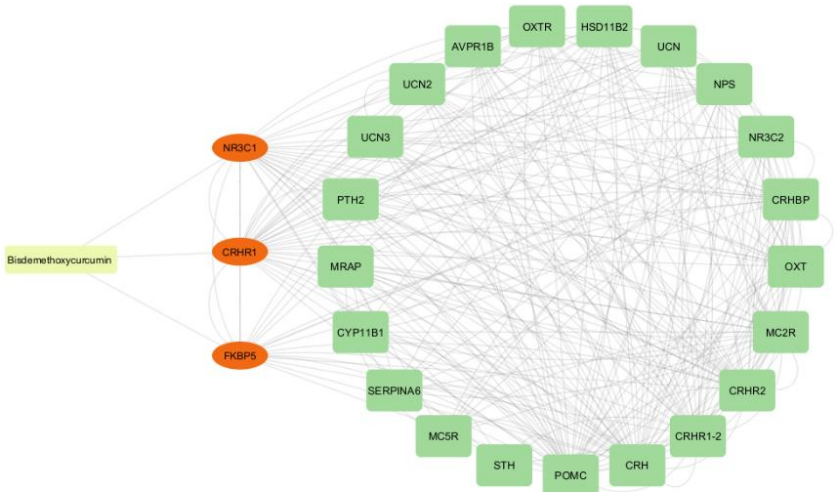

Figure 3 The protein-protein analysis of targeted protein with bisdemethoxycurcumin

The KEGG pathway analysis explored the connection between target proteins and phytochemical networks within various physiological pathways. Figure 4 shows these interactions, revealing that the proteins are involved in four key pathways: the cAMP signalling pathway, neuroactive ligand-receptor interactions, Cushing syndrome, and cortisol production and secretion.

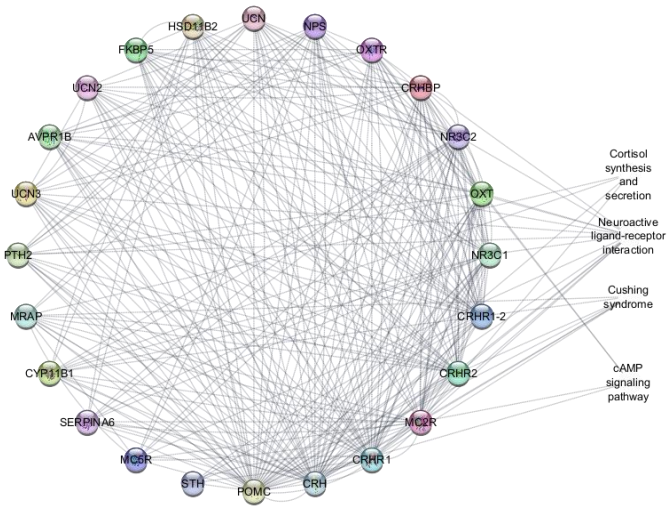

Figure 4 The protein pathway interaction involves with the targeted protein and bisdemethoxycurcumin.

Table 4: The genes in the pathway related to the target protein and compound.

KEGG term	Pathway	FDR value	p-value	Related Proteins
hsa04080	Neuroactive ligand-receptor interaction	1.00E-16	2.98E-19	OXT, NR3C1, PTH2, UCN2, CRH, UCN, OXTR, MC2R, CRHR2, AVPR1B, UCN3, CRHR1, POMC, MC5R
hsa04934	Cushing syndrome	1.05E-07	6.25E-10	CRH, CYP11B1, MC2R, CRHR2, CRHR1, MRAP, POMC
hsa04927	Cortisol synthesis and secretion	1.50E-04	1.38E-06	CYP11B, MC2R, MRAP, POMC
hsa04024	cAMP signalling pathway	0.0096	1.10E-04	OXT, OXTR, C2R, POMC

Table 4 lists the proteins related to the pathway that are related to the target proteins. Some related proteins not specifically mentioned in the table may form secondary connections within these proteins. Several of these proteins have partially been confirmed by previous studies, potentially contributing to the progression of depression. It is reported that the 11β-hydroxylase (HSD11B2) that is responsible for metabolizing the conversion of 11-deoxycortisol into cortisol involved in the molecular synthesis of cortisol can be inhibited to lower the cortisol production (Pierscionek et al., 2014). High cortisol levels can impact brain areas like the hippocampus and prefrontal cortex, contributing to depression in PPD. Besides, the cAMP signalling pathway helps repair brain cell damage in the hippocampus during depression and supports these neurons' survival, growth, and development. It also boosts the production of CRH, a hormone that plays a role in stress response, by activating a protein called CREB. CRH, in turn, increases the levels of BDNF, which supports brain cell health and helps reduce brain damage by increasing the production of stress-related hormones such as glucocorticoids (Adachi et al., 2018). Thus, it plays a role in treating depression by enhancing the health and survival of neurons by maintaining the HPA axis and managing stress response. Cushing syndrome happens due to HPA axis dysfunction and also leads to depression, which can be studied to prevent PPD by regulating the HPA axis.

Conclusion

In this study, *Curcuma longa* provides promising insight into traditional medicine in treating PPD by regulating the HPA axis through multiple targets, particularly by activating the negative feedback mechanism of the HPA axis. The molecular docking studies and dynamic simulations revealed that bisdemethoxycurcumin shows significant interactions with the GR, CRHR1, and FKBP5, indicating a potential regulatory role in the stabilizing the hypothalamic-pituitary-adrenal axis dysregulation associated with PPD. Lastly, bisdemethoxycurcumin shows protein interactions with target proteins through the cAMP signalling pathway, neuroactive ligand-receptor interactions, Cushing syndrome, and cortisol production and secretion, suggesting a correlation of this pathway that can be utilized for future application in treating PPD. Nonetheless, further research involving *in vitro* studies is necessary to validate the efficacy of *Curcuma longa* in modulating GR activity and to regulate cortisol production and to validate the mechanism of Curcuma longa in the treatment of PDD.

Acknowledgement

We would like to acknowledge PuRNAMA for financial assistance awarded for presenting this paper.

References

Abraham, M. J., Murtola, T., Schulz, R., Páll, S., Smith, J. C., Hess, B., & Lindahl, E. (2015). GROMACS: High performance molecular simulations through multi-level parallelism from laptops to supercomputers. *SoftwareX, 1–2*, 19–25.

Adachi, N., Suzuki, S., Matsuoka, H., Fushimi, S., Ono, J., Ohta, K. ichi, Hirai, Y., Miki, T., & Koshimizu, H. (2018). Corticotropin-releasing hormone-binding protein is up-regulated by brain-derived neurotrophic factor and is secreted in an activity-dependent manner in rat cerebral cortical neurons. *Journal of Neurochemistry, 146*(1), 99–110.

Arnittali, M., Rissanou, A. N., & Harmandaris, V. (2019). Structure of biomolecules through molecular dynamics simulations. *Procedia Computer Science, 156*, 69–78.

Design, L. (2014). Pharmacophore and ligand-based design with Biovia Discovery Studio®. *BIOVIA*. California.

Dosoky, N. S., Satyal, P., & Setzer, W. N. (2019). Variations in the volatile compositions of Curcuma species. *Foods, 8*(2).

Fadzil, F., Shamsuddin, K., & Wan Puteh, S. E. (2016). Traditional postpartum practices among Malaysian mothers: A review. *The Journal of Alternative and Complementary Medicine, 22*(7), 503–508.

Ghahremanian, S., Rashidi, M. M., Raeisi, K., & Toghraie, D. (2022). Molecular dynamics simulation approach for discovering potential inhibitors against SARS-CoV-2: A structural review. *Journal of Molecular Liquids, 354*, 118901.

Hairol, M. I., Ahmad, S., Sharanjeet-Kaur, S., Wee, L. H., Abdullah, F., & Ahmad, M. (2021). Incidence and predictors of postpartum depression among postpartum mothers in Kuala Lumpur, Malaysia: A cross-sectional study. *PLOS ONE, 16*(11), e0259782.

Hantsoo, L., Jagodnik, K. M., Novick, A. M., Baweja, R., di Scalea, T. L., Ozerdem, A., McGlade, E. C., Simeonova, D. I., Dekel, S., Kornfield, S. L., Nazareth, M., & Weiss, S. J. (2023). The role of the hypothalamic-pituitary-adrenal axis in depression across the female reproductive lifecycle: Current knowledge and future directions. *Frontiers in Endocrinology, 14*.

Kondapuram, S. K., Sarvagalla, S., & Coumar, M. S. (2021). Docking-based virtual screening using PyRx tool: Autophagy target Vps34 as a case study. *Molecular Docking for Computer-Aided Drug Design: Fundamentals, Techniques, Resources and Applications*, 463–477.

Krishnakumar, I. M., Kumar, D., Ninan, E., Kuttan, R., & Maliakel, B. (2015). Enhanced absorption and pharmacokinetics of fresh turmeric (Curcuma longa L) derived curcuminoids in comparison with the standard curcumin from dried rhizomes. *Journal of Functional Foods, 17*, 55–65.

Matias, J. N., Achete, G., dos Santos Campanari, G. S., Guiguer, É. L., Araújo, A. C., Buglio, D. S., & Barbalho, S. M. (2021). A systematic review of the antidepressant effects of curcumin: Beyond monoamines theory. *Australian & New Zealand Journal of Psychiatry, 55*(5), 451–462.

Matsubara, D., Kasahara, K., Dokainish, H. M., Oshima, H., & Sugita, Y. (2022). Modified protein-water interactions in CHARMM36m for thermodynamics and kinetics of proteins in dilute and crowded solutions. *Molecules, 27*(17).

Mohamad, T. A. S. T., Islahudin, F. H., Jasamai, M., & Jamal, J. A. (2022). Traditional practitioner's knowledge of Malay post-partum herbal remedies in Malaysia. *Archives of Pharmacy Practice*.

Mohanraj, K., Karthikeyan, B. S., Vivek-Ananth, R. P., Chand, R. P. B., Aparna, S. R., Mangalapandi, P., & Samal, A. (2018). IMPPAT: A curated database of Indian medicinal plants, phytochemistry, and therapeutics. *Scientific Reports, 8*(1), 1–17.

Mohanty, D., Padhee, S., Sahoo, C., Jena, S., Sahoo, A., Chandra Panda, P., Nayak, S., & Ray, A. (2024). Integrating network pharmacology and experimental verification to decipher the multitarget pharmacological mechanism of *Cinnamomum zeylanicum* essential oil in treating inflammation. *Heliyon, 10*(2).

Naz, S., Ilyas, S., Parveen, Z., & Javad, S. (2010). Chemical analysis of essential oils from turmeric (*Curcuma longa*) rhizome through GC-MS. *Asian Journal of Chemistry, 22*, 3153–3158.

Otasek, D., Morris, J. H., Bouças, J., Pico, A. R., & Demchak, B. (2019). Cytoscape automation: Empowering workflow-based network analysis. *Genome Biology, 20*(1).

Pierscionek, T., Adekunte, O., Watson, S., Ferrier, I. N., & Alabi, A. (2014). Role of corticosteroids in the antidepressant response. *ChronoPhysiology and Therapy, 4*, 87–98.

Seo, H. J., Wang, S. M., Han, C., Lee, S. J., Patkar, A. A., Masand, P. S., & Pae, C. U. (2015). Curcumin as a putative antidepressant. *Expert Review of Neurotherapeutics, 15*(3), 269–280.

Setzer, W. N., Duong, L., Poudel, A., & Mentreddy, S. R. (2021). Variation in the chemical composition of five varieties of *Curcuma longa* rhizome essential oils cultivated in North Alabama. *Foods, 10*(2).

Shannon, P., Markiel, A., Ozier, O., Baliga, N. S., Wang, J. T., Ramage, D., Amin, N., Schwikowski, B., & Ideker, T. (2003). Cytoscape: A software environment for integrated models of biomolecular interaction networks. *Genome Research, 13*(11), 2498–2504.

Shukla, R., & Tripathi, T. (2020). Molecular dynamics simulation of protein and protein–ligand complexes. *Computer-Aided Drug Design*, 133–161.

Singh, K., Srichairatanakool, S., Chewonarin, T., Prommaban, A., Samakradhamrongthai, R. S., Brennan, M. A., Brennan, C. S., & Utama-Ang, N. (2022). Impact of green extraction on curcuminoid content, antioxidant activities, and anti-cancer efficiency (in vitro) from turmeric rhizomes (*Curcuma longa* L.). *Foods, 11*(22), 3633.

Stickel, S., Eickhoff, S. B., Habel, U., Stickeler, E., Goecke, T. W., Lang, J., & Chechko, N. (2021). Endocrine stress response in pregnancy and 12 weeks postpartum – Exploring risk factors for postpartum depression. *Psychoneuroendocrinology, 125*, 105122.

Szklarczyk, D., Kirsch, R., Koutrouli, M., Nastou, K., Mehryary, F., Hachilif, R., Gable, A. L., Fang, T., Doncheva, N. T., Pyysalo, S., Bork, P., Jensen, L. J., & Von Mering, C. (2023). The STRING database in 2023: Protein-protein association networks and functional enrichment analyses for any sequenced genome of interest. *Nucleic Acids Research, 51*(D1), D638–D646.