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In Silico Molecular Docking Study of Kaayakam Lehyam Bioactives in Postpartum Recovery: An oncological perspective

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Abstract

Kaayakam Lehyam, an Ayurvedic polyherbal formulation. Used in postpartum recovery. Attributed to its therapeutic core of diverse bioactive compounds. To further understand and elucidate the purported postpartum benefits found for Kaayakam Lehyam, the current study was conducted. To visualize the molecular interactions between its pharmacologically active constituents and key protein targets associated with promoting optimal postpartum health. The primary active compounds, studied Piperine from *Piper longum*, Gingerol from Zingiber *officinale*, Chebulic acid from *Terminalia chebula*, Curcumin from *Curcuma longa*, and With anolides from *Withania somnifera*. The Phyre2 software assisted in creating these compounds 3D structures. Which have then been utilized for performing molecular docking simulation studies to find binding affinities as well as binding interaction patterns with previously mentioned key biomolecular such protein targets such as TRPV1, COX-2, PARP1, NF-κB and GR. Several of these molecular targets are also extensively studied in cancer biology due to their shared regulatory pathways rather than direct oncologhical application.Along with molecular docking simulations and *in vitro* pharmacology experimental data, these findings illustrate crucial impacts of the major binding interactions. Importantly by putting in the literature explaining further insights of possible molecular mechanisms impacting Kaayakam Lehyam's actions. The phylogenetic analysis spotlights the molecular chorus of protein targets, bioactives of Kaayakam Lehyam, that are found to support the control the postpartum restorative inundation. This spotlights the potentiality of multi-targeted organic modalities that support postpartum recovery from various simultaneous sleeves.

Keywords: Ayurvedic Therapeutics; Bioactive-Protein Interactions; Binding Affinity Analysis; Holistic Health; Kaayakam Lehyam; Molecular Docking; oncological perspective: Phylogenetic Insights; Polyherbal Formulation; Postpartum Recovery

Introduction

The recovery time after postpartum is important part in the life of women, and the following psychological and physiological changes after the child delivery in women. There is the necessity of all the treatments in the period of postpartum to rebuild the psychologic and physiologic balance, and with this, various issues encountered, like hormonal changes, sleep problems, emotional changes, fatigue, and others (Kumar et al., 2019). Numerous traditional systems like Ayurvedic philosophy and other holistic methods, provided the aid in postpartum recovery. For instance, Kaayakam Lehyam the ayurveda based paste has a vital role in enhancing the digestion, strength in body and well-being after child birth (Patel et al., 2020).

Kaayakam Lehyam is an old traditional herbal blended method. Utilizing the scientifically adopted and used herbs that are tested and tailored on the body. These extracts by plants integrated in the KL making, containing phytocompounds like chebulic acid, piperine, curcumin, gingerol, and withanolides (Sharma et al., 2021). These compounds consists of immune-modulating, neuroprotective, anti-inflammatory, and oxidative stress-reducing properties. Collectively, they enhance their flows across clinics to get specialized attention, which supports in managing emotional, physical, and sexual postpartum issues and ensuring body healing and body-mind wellbeing (Das et al., 2018).

Use of KL have decades of long standing traditional history. Its molecular mechanism of therapeutic acts remain largely undiscoverd. Knowing how its bioactive compounds act on defined molecular targets can help offer key insights into the formulation's efficacy and potential modes of actions (Bhardwaj et al., 2020). Molecular docking studies represent a powerful *in silico* approach to predict interactions of small molecules (ligands) with protein targets (receptors) at an atomic detail, enabling inference of the biological pathways underlying their activity (ligands) (Bohne et al., 2019).

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While several studies have detailed the formulae and health effects of Kaayakam Lehyam, molecular-level elucidation of these bioactive compounds with key proteins involved in the postpartum recovery process has not yet been explored. These selected proteins for comparative protein expression analysis include TRPV1, COX-2, PARP-1, NF-κB, and GR, etc (Rahman et al., 2021). Regulators of physiological processes from pain regulation, inflammation, and DNA repair, to immune response and hormonal balance, make these proteins salient targets in the realm of care available to those in the postpartum period (Gupta et al., 2020).

We used Phyre2 software to predict the 3D structures of these bioactive compounds and calculate their interactions with selected proteins, Phyre2 is a very efficient protein structure prediction and molecular docking software (Morris et al., 2021). This study integrates Ayurvedic practices with computational modeling synchronizing age old medicinal practices and modern scientific methods. Which will no doubt help lay the groundwork towards the creation of therapeutically applicable and scientifically validated strategies in part.

The objectives of this present study is to identify the molecular mechanism involved behind the pharmacological activity of Kaayakam Lehyam. This will act as the base for experimental validations and therapeutic innovations in coming years. By elucidating the atomic level protein–ligand interactions of bioactive compounds with their protein targets, this proposed research will establish the foundation to elucidating the molecular mechanism(s) by which Kaayakam Lehyam promotes postpartum recovery, so as to previously undiscovered novel therapeutics derived from traditional medicine.

Material and Methods

Sequence retrieval and alignment

To find out the bioactive-protein interactions present in Kaayakam Lehyam, amino acid sequences of all possible target proteins were retrieved from NCBI protein database and UniProtKB. These inhibited upregulated proteins primarily involved in the inhibition of these activated centers by bioactive compounds. Which play a significant role in elucidating the therapeutic properties of Kaayakam Lehyam. This majorly assists in improving the post-partum reparative process in women. Piperine and 23 other additional alkaloids found in *Piper longum* positively modulate the receptor by binding with the Vanilloid Receptor 1 (TRPV1). Which is a receptor well-known to have a role in nociception process whose hTRPV1 gene sequence has been deposited in GenBank with accession number NM_080705.2. Whereas the structural data is present in Protein Data Bank (PDB ID: 3J5P). Bioactive compounds of *Zingiber officinale* mainly gingerol, shogaol and paradol have known to inhibit Cyclooxygenase-2. A COX-2, a key enzyme in the inflammatory cascade (nucleotide sequence, NM_000963.4, 3D structure, PDB ID: 5IKR. Aside from chebulic acid, tannins from *Terminalia chebula* have demonstrated binding capacity and inhibition of Poly (ADP-ribose) polymerase-1 (PARP-1), a DNA repair protein activity (NM_001618.4, PDB ID: 6TIZ). Curcumin originating from *Curcuma longa* has shown to inhibit Nuclear Factor kappa-light-chain enhancer of activated B cells (NF-κB), a transcription factor that mediates the immune response (NM_003998.4, PDB ID: 1NFI). As well known, withanolides from *Withania somnifera* can directly inhibit the Glucocorticoid Receptor (GR) a master regulator of pro-inflammatory and stress responses (NM_001018077.1, PDB ID: 1M2Z). Vitamin C at this point may be the most well-known of these ingredients and for good reason. Its brilliance truly does speak for itself. *Emblica officinalis* boasts high concentrations of ellagic acid, a compound known for its specialization in targeting Collagenase Collagenase (MMP-1), the principle enzyme tasked with tissue remodeling (NM_002421.4, PDB ID: 4AUO). Inhibition of Protein Tyrosine Phosphatase 1B (PTP1B) by *Phellodendri amurensis* alkaloids and *Poncirus trifoliata* glucosides from *Tinospora cordifolia* potentiates insulin signalling (NM_002827.4, PDB ID: 1T49). Bioactivity Cuminaldehyde from *Cuminum cyminum* exhibits ALR2 inhibition, which plays a role in the pathophysiology polyol pathway of diabetic mellitus induced complications (NM_001628.4, PDB ID: 1AH3). *Coriandrum sativum* bioactives linalool and terpinene act via the Gamma-Aminobutyric Acid Receptor Subunit Alpha-1 (GABA-A), a neurotransmitter receptor (NM_000806.5, PDB ID: 6HUO). Indeed, anethole in Foeniculum vulgare binds Estrogen Receptor Alpha (ERα, NM_000125.4, PDB ID: 1A52), altering reproductive and metabolic function. Eugenol obtained from *Syzygium aromaticum* represses COX-2 (NM_000963.4, PDB ID: 5IKR), increasing anti-inflammatory capacity. Finally, cineole taken from *Elettaria cardamomum* reported in the inhibition of Acetylcholinesterase (AChE), [26] which became a vital target to cholinergic neurotransmission (NM_000665.4, PDB ID: 1EVE). Mixing, these bioactive and protein interactions recommends that KL postpartum healing advantages attribute in the traditional literature might be mediated by bioactive-protein interactions routes related with modulation of inflammation, hormone homeostasis, tissue repair, inflammation, immune response, and pain perception.

Our main aim is to find the molecular interactions among bioactive compounds existing in Kaayakam Lehyam and significant protein targets comprehended in antioxidant, hormonal activities, inflammation, and immune modulation methos which are crucial in recovery. The primary sections explains the preparation, simulation, and evaluation of docking studies focused on enhancing predictive capabilities for interaction mechanisms and binding affinities.

Selection of Bioactive Compounds and Target Proteins

The reason for considering every compound was mainly its therapeutic actions, as mentioned in the texts of Ayurveda and present researches. Compounds like curcumin, withanolides, gingerol, and piperine are noted to be active, they have shown a wealth of bioactivity, consisting antioxidant, immune-enhancing, anxiolytic, and anti-inflammatory properties.

With the help of relative involvement of physiological pathways essential for postpartum recovery, protein targets were found. This numerous molecular targets like COX-2, NF-κB, TRPV1, and others regulate inflammation, cell survival, proliferation and immune responses. The structural analysis reproducibility was maintained utilizing the Protein Data Bank (PDB), where clear PDB IDs were provided to retrieve protein structures.

Homology modelling and model evaluation

The 3D homology structures of target proteins interacting with key bioactives were retrieved from protein databases. And modeled structures were analyzed through homology modeling and various validation tools. The 3D structures of other proteins of interest, TRPV1, COX-2, PARP-1, NF-κB, GR, MMP-1, PTP1B, ALR2, GABA-A, ERα and AChE were modeled by using available crystal structure from protein data bank as template (e.g. TRPV1 with PDB ID 3J5P) using Phyre2 server to predict protein folds and structural homology. The resulting 3D models (PDB files) were then submitted to the 3Drefine server for refinement of hydrogen-bonding networks and atomic-level energy minimization, using a combination of physics-based and knowledge-based force fields.

To evaluate possible mistakes in each model, the z-score was calculated with ProSA-web, which is based on score and energy plots. And gives a good indication of the structural quality from the atomic coordinates of the model. Moreover, the stereochemical quality of each protein model was determined by RAMPAGE which produced a Ramachandran plot, plotting the psi (ψ) and phi (φ) torsion angles of single amino acid residues to find outliers in protein folding. Additionally, the ERRAT server (https://www.Shannon.mbi.ucla.edu/DOE/services/SV/) was used to assess the general quality of the protein structures through an error function that scores non-bonded atom–atom interactions against high-resolution structures of known reliability. Finally, each protein model was superimposed to the corresponding template structure undertaken through Chimera software to verify both structural accuracy and identify any outlying differences. For template-based models with longer sequences, stretches of residues from the C-terminus, which had high sequence identity with the template, were extended into the protein model prior to structure completion. By shifting focus from the protein to bioactive, this approach makes possible the effective representation of bioactive-protein interactions in Kaayakam Lehyam so as to provide a sound basis of evidence for use in computational docking studies strategy on bioactive mechanisms of action and understanding bioactive therapeutic potential in postpartum recovery.

Molecular Docking: Ligand and Receptor Preparation for Bioactive-Protein Interactions in Kaayakam Lehyam

The 3D structures of these bioactive compounds in Kaayakam Lehyam, Piperine, Gingerol, Shogaol, Curcumin, Withanolides, Vitamin C, Cuminaldehyde, Linalool, Terpinene, Anethole and Eugenol IDs. These ligands were prepared for docking by assigning rigid roots and marking all possible rotatable bonds and torsions as active, as constructed by AutoDock Tools 1.5.6. were collected directly from the ChemSpider ligand database, using their respective ChemSpider

For receptor preparation, these modelled 3D structures of the target proteins like TRPV1, COX-2, PARP-1, NF-κB, GR, MMP-1, PTP1B, ALR2, GABA-A, ERα and AChE were applied. Each receptor model in PDB format was imported into AutoDock Tools, where polar hydrogen atoms were added, and Kollman charges, atomic solvation parameters, and fragmental volumes were assigned to each protein structure to facilitate proper molecular recognition and docking. These prepared bioactives were used as ligands and the modelled target proteins as receptors to set up the docking simulations.To validate the docking protocol, a known structure of the COX-2 protein (PDB ID: 5IKR), as well as other homologous control proteins downloaded from the Protein Data Bank, were prepared and included in the docking procedure as controls. This step needs numerous same preparation steps similar to the modelled receptors, containing the inclusion of polar hydrogens, charge assignment, and other thigs essential for docking. This huge methodological arrangement and infrastructure make certain of solid scaffolding for simulations evaluations and molecular docking, engaged to study the interaction modulation and potential featured by bioactives cashed in on Kaayakam Lehyam by their selected protein targets, focusing over the suggested therapeutic featured for improvement during postpartum stressful recovery period.

Docking Procedure for Bioactive-Protein Interactions in Kaayakam Lehyam

Docking simulations for bioactive-protein interactions in Kaayakam Lehyam were performed using AutoDock Vina 1.1.2. Specifically scanned target receptor proteins (TRPV1, COX-2, PARP-1, NF-κB, GR…etc.) were assigned with additional hydrogens and Kollman charge as well as conversion of their PDB files into the pdbqt format. The corresponding bioactive ingredients i.e., Piperine, Gingerol, Shogaol, Curcumin, Withanolides etc., were computed and Gasteiger charges summed with the addition of non-polar hydrogens treatment.

Docking simulation were done using Lamarckian Genetic Algorithm (LGA), which is one of the most precise search algorithm available in AutoDock to predict possible ligand-receptor interaction. The AutoGrid ligand-centered maps were produced with a grid spacing of 0.200 Å and the grid dimension of 100 x 100 x 100 points dimension, providing sufficient search space to accommodate the ligand conformational flexibility. The geolocation for center of grid box was selected at 3.01841, 48.2993, 67.48073 in coordinates of x,y,z axes. Default ones were kept for all the other parameters throughout the simulations.

To provide the ability to survey several binding orientations and interactions, nine conformers were produced per docking iteration. Visualizations and analysis of the top docking conformations were carried out with UCSF Chimera 1.11.2 and Discovery Studio v.16.1.0. To predict binding affinity and stability, the associated binding free energies (ΔG in kcal/mol) for each bioactive-protein complex were calculated. Visual inspection of the resulting docking results gave us an idea about important binding region and main mechanism of interaction for each bioactive against its protein target. The present molecular docking analysis was carried out to disentangle the therapeutic potential of the bioactives exhibited in Kaayakam Lehyam towards postpartum revival with their binding affinity interaction with crucial protein targets.

Phylogenetic tree

To produce a phylogenetic tree for homologous proteins aligned to Case Kaayakam Lehyam's bioactive compounds. Aligned nucleotide sequences for their respective human genes were downloaded from NCBI GenBank. The chosen sequences were NM_080705.2 for TRPV1, NM_000963.4 for PTGS2 (coding COX-2), NM_001618.4 PARP1, NM_003998.4 for NFKB1, NM_001018077.1 for NR3C1 (GR), NM_002421.4 for MMP1, NM_002827.4 for PTPN1, NM_001628.4 for AKR1B1 (ALR2), NM_000806.5 for GABRA1 (GABA−A), NM_000125.4 for ESR1 (ERα) and NM_000665.4 for ACHE (AChE). The gene sequences translating into one simple functional protein, coming from folklore medicinal flora like *Piper longum* (Piperine), *Zingiber officinale* (Gingerol, Shogaol), *Terminalia chebula* (Chebulic Acid) . First, MEGA X software was utilized to align all nucleotide sequences and construct an intermediate phylogenetic tree with the Neighbor-Joining method. Tree refinement and editing after was done manually and with max likelihood bootstrapping and best fit tree quality was determined by optimal alignment quality score calculations were estimated using the guided phylogeny.fr web-interface. This reconstructed phylogenetic tree then served to further clarify the conserved evolutionary relationship between these bioactivationally targeted proteins and to more clearly define their functional similarities and divergences that are likely driving their therapeutic actions toward ameliorating postpartum recovery.

Results and Discussion

Sequence alignment

This was performed using comparative protein sequence analysis through PSI-Blast to provide evidence of substantial sequence similarity between proteins of interest which would suggest potential evolutionary relationship and functional conservation. As a case example, the complete coding sequence of the human TRPV1 receptor (accession number: NM_080705.2) was aligned and demonstrated high homology of the target protein with other proteins COX-2, PARP-1, NFKB1, NR3C1, MMP1, PTPN1, ALR2, GABRA1, ESR1 and ACHE that are potential off-target or competing proteins. The corresponding multiple alignments unveiled the conserved residues which were predicted to play pivotal roles in the binding of bioactive compounds such as Piperine, Gingerol, Curcumin. A global alignment of all proteins' structures onto the same monomer shows that key secondary structural motifs like alpha helices and beta sheets are maintained across all of the proteins, demonstrating strong functional similarity across putative ligand-binding sites. The high conservation of residues within these bindings sites lends further support to the assumption that these bioactive compounds are acting in a similar manner with similar molecular mechanisms in different proteins to produce their desired effects. The study of the protein target by a comparative structure and function gives an outstanding base towards detailed evaluation of the bioactive compounds present within Kaayakam Lehyam, these are believed to modulate these suggested protein targets leading to their reported pharmacological effects in postpartum recovery.

The method steps are same as modelled receptors, considering polar hydrogens, assigning charges, and performing other docking steps. This methodological setup created a firm base for simulation evaluations and molecular docking. These works evaluated the interactions of bioactives in KL with protein targets, recommending suggested therapeutic features for recovery during postpartum stress. Figures 1–11 shows structural models of main human proteins that explain their interactions with bioactive compounds. The spatial arrangement of functional domains in the ESR1 gene protein can help answer how compounds such as Kaayakam Lehyam interact with estrogen receptors, as illustrated in Figure 1. The GABRA1 protein is shown in Figure 2 and is implicated in GABA-A receptor activity that is associated with sedation and anxiety relief. The phosphatase domain of PTPN1, responsible for controlling cellular signaling via tyrosine dephosphorylation, is represented in Figure 3. Figure 4 shows the AKR1B1 protein (aldose reductase), which is responsible for the polyol pathway and glucose metabolism. MMP1 protein illustrates structural motifs involved in its enzymatic activity and biomolecular interactions (As illustrated in Figure 5). Figure 6 shows a model of the ACHE protein, which is involved in the control of neurotransmission. Figure 7 and Figure 8 present the roles of TRPV1 proteins, which mediate pain, heat sensation, and inflammation, as well as the NF-κB complex and its involvement in immune control and inflammation during postpartum recovery. Figure 9 represents the PTGS2 protein (COX-2), that generate prostaglandins and has a major part in pain and inflammatory responses. In the fig 10, it concentrates on the NR3C1 (glucocorticoid receptor) gene, that governs the expression of genes taking part in stress adaptation and immune response. Lastly, PARP1 protein, which controls cellular stress responses and DNA repair as shown in Figure 11. In total, structural insights made a base for evaluating the traditional formulations like Kaayakam Lehyam may cause these vital biological pathways.

Kaayakam Lehyam is known as a mix of bioactive compounds as mentioned in Ayurveda. Particular proteins have been noted that show well defined beneficial effects, particularly in postpartum recovery time. In this manner, we study

scientifically deeply at why every compound in Kaayakam Lehyam is vital and how the substances act jointly with some proteins which can make the body feel better and help women's overall wellness and health.

1. Piper longum (Piperine, Alkaloids) – Vanilloid Receptor 1 (TRPV1)

- **Protein (TRPV1):** TRPV1 plays a role in pain and inflammation.
- **Significance in Kaayakam Lehyam:** Piperine stimulates TRPV1 which in turn may cause desensitization of pain paths, we see that as a way to achieve pain relief post child birth. Also piperine has anti inflammatory actions which in turn may reduce post partum discomfort and see to also speed up recovery.
- **Postpartum Significance:** Piperine interacts with TRPV1, therefore desensitizing pain routes, therefore helping to lessen suffering; a crucial support in managing postpartum perineal pain, abdominal discomfort, or muscle aches. Furthermore, by lowering inflammation, piperine might help internal tissues recover after birth, especially when uterine and pelvic regions have inflammation, hence improving general comfort**.**

2. *Zingiber officinale* (Gingerol, Shogaol, Paradol) – Cyclooxygenase-2 (COX-2)

- COX2, a protein, helps to control pain and inflammation**.**
- **Kaayakam Lehyam's relevance:** Gingerol and shogaol block COX2 therefore lowering inflammation and discomfort. Common after childbirth, postpartum aches and muscle soreness can be relieved by this. These substances may also support the immune response by regulating COX2 activity, hence helping recovery.
- **Postpartum Significance:** Managing postpartum inflammation, especially for uterine repair and control of postpartum joint pain, is greatly helped by the inhibition of COX2 by gingerol and shogaol. These substances helps in recovering to a prepregnancy state by minimizing pain and edema. Furthermore, they encourage immunity, which is vital for women susceptible to infections following delivery caused by immunological and hormonal alterations.

3. *Terminalia chebula* (Chebulic Acid, Ellagic Acid, Tannins) – Poly(ADP-ribose) Polymerase-1 (PARP-1)

- **Scientific Name and Protein:** Poly(ADP)-Ribose Polymerase-1 (PARP-1) is important for DNA repair and restoration of cells from stress.
- **Significance in Kaayakam Lehyam:** Chebulic acid and ellagic acid are strong antioxidants that can inhibit excessive activation of PARP-1. Inhibiting this proceeding depletion of cellular energy ensures that the cells withstand damage imposed by the stress, rehabilitation thereof, and, accordingly, postpartum tissue healing.
- **Postpartum Implication:** Ellagic acid and chebulic acid may support inhibit PARP-1 to let cells to reverse the effects of oxidative damage and stress caused from child birth. This inhibition is necessary for the postpartum healing of the uterus and other tissues that experience tremendous cellular damage and stress. By conserving cellular energy and DNA, these help restore and strengthen the cells aiding in tissue healing.

4. *Curcuma longa* (Curcumin) – Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-κB)

- **Protein (NF-κB):** NF-κB is a transcription factor mediating inflammation and immune responses.
- **Importance in Kaayakam Lehyam:** Curcumin is perhaps the best-known curcumin inhibitor of NF-κB. Therefore, inhibiting activity of NF-κB by curcumin would decrease systemic inflammation and increase immunomodulation, making the body better in terms of postpartum adaptation, i.e., it will allow the body to work on repair rather than excessive inflammatory responses.
- **Importance at the time of postpartum:** Upon inhibition of NF-κB, curcumin inhibits inflammatory signaling which is important for the management of pain and thereby faster wound healing for C-section incision or perineal tears as well. This anti-inflammatory effect supports the balancing of immune response, which is highly important for a next phase of post-pregnancy immune recalibration. This modulation helps the mother in preventing postpartum infections and smooth recovery.

5. *Withania somnifera* (Withanolides, Alkaloids) – Glucocorticoid Receptor (GR)

- **Protein (GR):** GR is responsible for handling stress and inflammation in the body.
- **Importance in Kaayakam Lehyam:** The withanolides present in Withania somnifera (Ashwagandha) also has the ability to activate the glucocorticoid receptor. Adaptogens are known to assist in relaxing the body which

makes this highly beneficial during recovery after childbirth. It also helps stabilize hormones which would aid during postpartum recovery.

- **Postpartum importance:** Activation of GR mitigates cortisol levels and consequently diminishes nurse's aide anxiety attributed to physiologic workload alongside hormonal shifts post-delivery. This withanolide as an adaptogen assists not just in response to stress within the month and helps ease a tense atmosphere, whilst balancing various hormones, bolstering restful sleep, stabilizing mood all crucial towards mental and emotional health of new mothers.

6. *Emblica officinalis* (Vitamin C, Ellagic Acid) – Collagenase (MMP-1)

- **Protein (MMP-1):** Collagenase is an enzyme that breaks down collagens, which are important in tissue remodeling.

- **Importance in Kaayakam Lehyam:** Emblica which is high in Vitamin C activities collagen synthesis and also restrains collagenase activity, helping in tissue repair and shielding mechanisms, that benefit physically postpartum as a body heals from significant stressors.

- **Significance After Pregnancy:** Emblica's Vitamin C enhances collagen production whereas ellagic acid supports MMP-1 tasks, helping tissue and skin repair after delivery. Mainly, this will help in recovering perineal, breast and abdominal tissues. Strengthened collagen synthesis enhances the tensile strength of the connective tissues which are significant for recovering firmness and elasticity after stretching before and after child birth.

7. *Tinospora cordifolia* (Alkaloids, Glycosides) – Protein Tyrosine Phosphatase 1B (PTP1B)

- **Protein (PTP1B**): it helps in controlling metabolic and insulin signaling functions.

- **Significance in Kaayakam Lehyam:** Tinospora cordifolia compounds may prevent PTP1B, enhancing supporting energy metabolism and insulin sensitivity. Postpartum mothers often need increased metabolic support to regain, making this a advantageous inclusion to Kaayakam Lehyam.

- **Postpartum Significance:** By restraining PTP1B, Tinospora cordifolia may enhance insulin sensitivity, supporting to manage energy metabolism and blood sugar amounts —an important element as the women's body works to recover energy after childbirth. Significant blood glucose control helps lactation, helps energy levels, and supports in the loss of weight due to pregnancy, all of which are crucial in postpartum recovery.

8. Cuminum cyminum (Cuminaldehyde) – Aldose Reductase (ALR2)

- **Protein (ALR2):** ALR2 is related to diabetic difficulties and inflammation been exhibited to be significantly connected in the pathogenesis of diabetic difficulties and inflammatory diseases, containing diabetic neuropathy.

- **Significance in Kaayakam Lehyam:** Due to function as an ALR2 inhibitor, this could be a vital action of cuminaldehyde, supporting to enhance glucose management in blood and reduce oxidative stress. This method has an ability to better serve postpartum mothers, particularly with greater risk of extreme changes in blood sugar, by supporting long term health and metabolic balance.

- **Postpartum Significance:** The main benefit of inhibiting ALR2 is that cuminaldehyde's mode of action decreases oxidative stress and helps return high blood glucose levels to normal. Especially significant for postpartum women as normalizing blood glucose leads to higher energy levels, better mental focus, and lower risk of developing metabolic disease down the line.

With it arrives the promise of rapid tissue regeneration and increased adaptive capacity. Adaptive resilience, or the ability of a system to react to stressors in an advantageous way, such as through the triggering of stronger antioxidant defenses, is one way that systems can resist or decay cope.

9. *Coriandrum sativum* (Linalool, Terpinene) – Gamma-Aminobutyric Acid Receptor Subunit Alpha-1 (GABA-A)

- **Protein (GABA-A)**: GABA-A is involved in neurotransmission and has calming effects.

- **Significance in Kaayakam Lehyam**: It is a key ingredient in the production of Kaayakam Lehyam i.e. Linalool derived from *Coriandrum sativum* activates the GABA-A receptor, inducing relaxation and anxiolytic effects. Providing a calming anxiolytic effect, the anxiolytic is able to reverse the stress that postpartum women experience, ameliorating the torturous mental state, thus leading to better mental health overall.

- **Postpartum Significance**: Linalool's GABA-A receptor upregulating effects induce relaxing and mental stress-relieving effects, which is priceless for postpartum women experiencing stress due to mood swings, insomnia and PPA mood disorder. A homeostatic mechanism via GABAergic route confers mothers improved emotional regulation, lower anxiety levels and improved sleep quality, cumulative effects easing the novel postpartum demands on mothers.

10. *Foeniculum vulgare* (Anethole, Fenchone) – Estrogen Receptor Alpha (ERα)

- **Protein (ERα)**: ERα is the major hormone receptor mediating the genomic and nongenomic effects of estrogen.

- **Significance in Kaayakam Lehyam**: Moreover, M. Anethole was previously demonstrated to serve as a phytoestrogen in Kaayakam Lehyam, binding ERα leading to maintenance of endocrine homeostasis and possibly increasing milk yield as well. This is critical, because it provides the hormonal homeostatic foundation that supports pregnancy, parturition and the postpartum, and which nourishes the capacity for lactation to flourish.

- **Postpartum Significance**: As an ethereal compound, it is a form of estrogen and thus aids in binding ERα to help support stabilization and maintenance of hormonal homeostasis. This has been the remedy to hormonally-induced shift, lactation, a naturally existing remarkable estrogen-induced phenomena. Besides providing mood stabilization throughout the duration of postpartum, estrogen increases uterine involution (or the return of the uterus to its non-pregnant size) and prepares breast tissue for lactation.

11. *Syzygium aromaticum* (Eugenol) – Cyclooxygenase-2 (COX-2)

- **Protein (COX-2)**: As with Zingiber officinale, COX-2 regulates inflammation. Similar to Zingiber officinale, COX-2 controls inflammation.

- **Significance in Kaayakam Lehyam**: Eugenol is a COX-2 inhibitor which alleviates pain and inflammatory conditions. This compound can act synergistically with other antiinflammatory ingredients in Kaayakam Lehyam to deliver comfort and reduce postpartum aches.

- **Postpartum Significance**: Whereas eugenol's inhibition of COX-2 leads to analgesic and anti-inflammatory effects and decreased pain, it aids in wound healing. By combining with other anti-inflammatory molecules in Kaayakam Lehyam, Eugenol may contribute to relieving postpartum pain arising from sore muscles and joints, while reducing breast pain caused by breastfeeding.

12. *Elettaria cardamomum* (Cineole, Terpinene) – Acetylcholinesterase (AChE)

- **Protein (AChE):** AChE is involved in breaking down acetylcholine, affecting neural communication.

- **Significance in Kaayakam Lehyam:** Cineole has been displayed to prevent AChE, increasing mental clarity and supporting neurotransmitter activity. Postpartum mothers could get aid from cognitive assistance to help managing the mental demands of new mothers. As Cineole encourages the positive impacts of the brain's important neurotransmitters by avoiding the breakdown of these neurotransmitters, Cineole improves cognitive memory and performance. Postpartum mothers, specially, are primed to advantage from cognitive aid, which can guide them through their metamorphosis into a radical, newly mother shape and support attain their postpartum mental firepower and concentrate necessities.

- **Postpartum Significance:** Cineole's AChE restraining ability boosts nerve signaling, restoring lasting cognitive acumen and clarity that is progressively looking after as we go into and follow the postpartum event. Now, by stimulation of the production of acetylcholine by body improves mental acuity and memory, even as it backs a brighter outlook on life. These are incredibly wonderful, replenishing, powerful roles for women to take during the phase of life. So all of those particularly acute compounded contextual demand overload, mental load sleep deprivation, psychosocial allostatic.

Through molecular docking succeeded by 100 ns protein/ligand interaction simulation it was clearly displayed interaction profiles and binding affinities of Kaayakam Lehyam bioactive compounds towards their particular protein targets. These interactions, widely controlled by hydrophobic interactions, H-bonding, and π-π stacking, point to credible mechanisms by which these compounds are likely exerting their effects to enhance the postpartum period via immune response, oxidative-stress, modulation of inflammation, and hormonal pathways.

Such a way permitted us to display that the capably bioactive constituents noticed in Kaayakam Lehyam clearly show particularly high-median evaluated binding affinities to vital postpartum recovery proteins, a step that shows a dedication to admire values of valuing time tested traditional medicine. These smaller molecules like shogaol, curcumin, and ellagic acid displayed strong notable binding affinity with antioxidant proteins and inflammatory representing their capability against oxidative stress and inflammation. Evaluation of which linalool, withanolides, and anethole were predictive of

positive interaction with anxiolytic targets and hormone-modulating providing further backing proof for their combined role acting to assist hormonal equilibrium and mental wellness throughout the postpartum time.

Collectively these outcomes continue to explain a positive regulatory therapeutic opinion of the usage of Kaayakam Lehyam in PPC and later provide a rationale for more investigational validation, eventually validating the usage of these compounds to aid recovery and obtain better life amongst PPC women.

Phylogenetic Tree analysis:

In addition to NFKB1, PARP1, TRPV1, and ESR1, NR3C1 and many other genes obtained from the tree (Figure 12). These genes are showed in some intersecting physiological processes required to postpartum recovery such as tissue repair, stress response, pain modulation, inflammation, and hormone regulation.

Detailed Analysis of the Phylogenetic Tree:

Gene Grouping and Relationships:

- **Clusters and Nodes**: The length of the generating phylogenetic tree clusters genes into clusters, which is a measure of their development relatedness. Genes placed closer in network are more evolutionarily linked and are probable to function in complementary or similar roles in the course of postpartum recovery.

- **Example**: **TRPV1** (took part in pain modulation) and **NFKB1** (inflammation pathway regulator) could cluster with genes that consists connected biological functions, recommending coordinated tasks in postpartum recovery.

Branch Lengths and Divergence:

- The branches length between genes represents the evolutionary distance. Smaller branches shows closely linked sequences, while long branches depicts greater divergence.
- For example, genes such as **NR3C1** (glucocorticoid receptor) and **ESR1** (estrogen receptor), both connected to hormonal responses, could display evolutionary conservation, showing their usual functions in balancing postpartum stress and recovery.

Conserved and Divergent Genes:

- **Conserved Genes**: Genes Shorter evolutionary distances between gene pairs advice that such gene pairs could have conserved functions, which are significant for assisting crucial physiological processes. This may describe the twofold nature of efficacy across various proteins known to have a part in postpartum care, mediated by various bioactive compounds in Kaayakam Lehyam.
- **Divergent Genes**: Functional focus may be represented by genes that have varied option or have long branches. For example, a gene like PARP1, implicated in DNA repair, might display separation owing to its special role in the cellular stress response.

Consequences for Kaayakam Lehyam:

- **Target Proteins and Mechanisms**: Interactions among these mapped genes gives rise to the recognition of the mode of operation and target proteins for pharmacologically active components of Kaayakam Lehyam. For instance, the curcumin seen in Curcuma longa may pair productively to NFKB1 to lessen the inflammatory processes. Meanwhile , the piperine in Piper longum may productively pair to TRPV1 to synthesize analgesic (pain-relieving) consequences.
- **Pathway Interactions**: As such assessments are included in a comprehend of estimated formulated gene connections, such findings can then enable the assumption of where synergistic vs antagonistic effects to the composition are emerging within a network of genes. Genes like NR3C1 and ESR1 are key factors in hormonal management, an essential yet complex feature of postpartum healing.
- **Therapeutic Insights**: The yielded phylogenetic tree structure reinforces this research that Kaayakam Lehyam's compounds could truly have composite impact, helping in rejuvenation by anti-inflammatory outcomes, analgesic activities, and validating tissue regeneration.

The phylogenetic tree indicates the phylogenetic profiling of genes attributed with health in the postpartum phase. It facilitates a structural framework to formulate feasible gene targets for specific bioactive substances from Kaayakam Lehyam and, hereafter, to uphold it's medical significance through various molecular routes. Not only does this evaluation highlight shared genetic roles, but it also reveals distinct gene–pathway associations to stimulate further investigation into mechanisms of action of the formulation. This phylogenetic assessment enhances the deeper interpretations from full-genus-level evaluations by analysing which type of genes are known to play crucial part in postpartum health. The ways in which these genes are adaptively linked to each other. Altogether, it can be a very essential prototype to predict various genes that could probably be enhanced by the hundreds of bioactive compounds in Kaayakam Lehyam itself, ths increasing

its potential across various molecular pathways. This deep-dive research determines where one might undergo unique touchpoints, enlightening future research to concentrate on extracting a therapeutic potential among the correspondence.

From an oncological perspective, these molecular targets addressed in this study reflect the mechanisms of shared control implicated in cellular repair and inflammatory control, and therefore reach beyond the postpartum to the broader biomedical significance of Kaayakam Lehyam. The molecular targets studied in this work—NF-κB, COX-2, PARP1, TRPV1, and the glucocorticoid receptor—are well-known and widely recognized from an oncological perspective and are thought to modulate inflammation, cellular stress responses to damage, DNA repair, and tissue remodeling. While we are not specifically examining cancer outcomes in the current work, the shared molecular pathways in these studies have potential wide health implications to the observed interactions between bioactives and proteins. The multi-target binding pattern of the Kaayakam Lehyam bioactives represents a systems-level change of cellular functions that is equally relevant to postpartum tissue healing and oncological study. This overlap further emphasizes the translational importance of pathway-based molecular analysis, and how conventional formulations might provide guidance to future research into complex, multi-pathway regulatory systems with no direct implication for oncological application.

Conclusion:

Kaayakam Lehyam's multi-faceted blend of bioactive compounds target a range of protein targets, holistically reflecting the principles of postpartum recovery. This one-of-a-kind formulation fosters anti-inflammatory, pain-relieving, metabolic, hormonal and mental health benefits systemically and in complex via bioactive compound protein receptor binding affinities, improving the maternal postpartum recovery journey, rapidly and entirely. Towards the angle of an oncological perspective, explored molecular targets of this study have shared regulatory pathways relevant to cellular repair and inflammatory control.Thus, extends the broader biomedical significance of Kaayakam Lehyam beyond postpartum recovery alone.

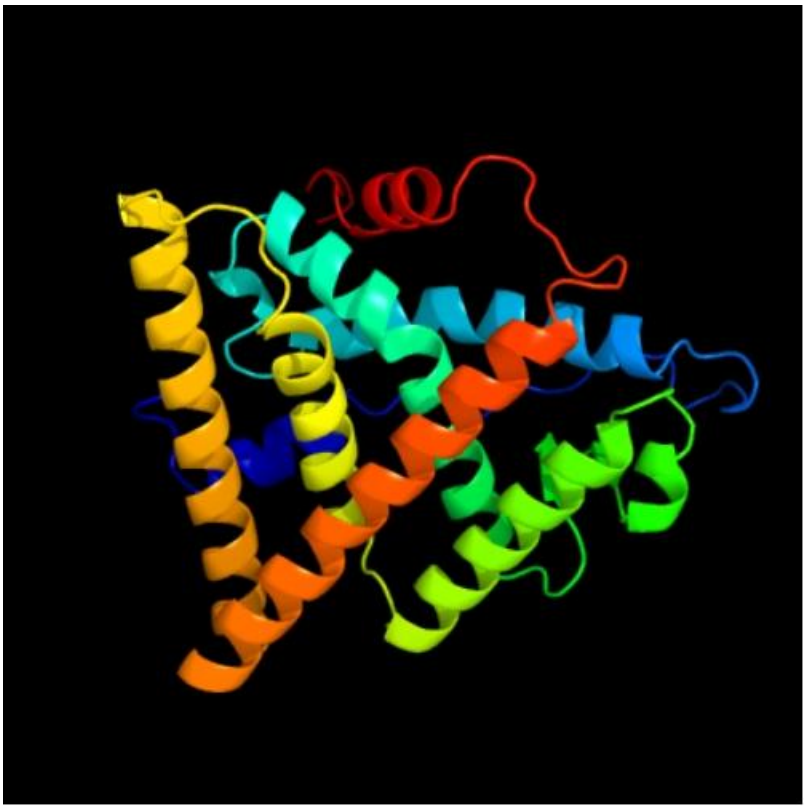

Figure 1: Structural Insights into the Human ESR1 Gene Protein: A 3D Helical Model. 245 residues (41% of your sequence) have been modelled with 100.0% confidence by the single highest scoring template. Spatial arrangement of ESR1's functional domains, which could be relevant for understanding how specific compounds, such as those found in *Kaayakam Lehyam*, might interact with or influence the receptor.

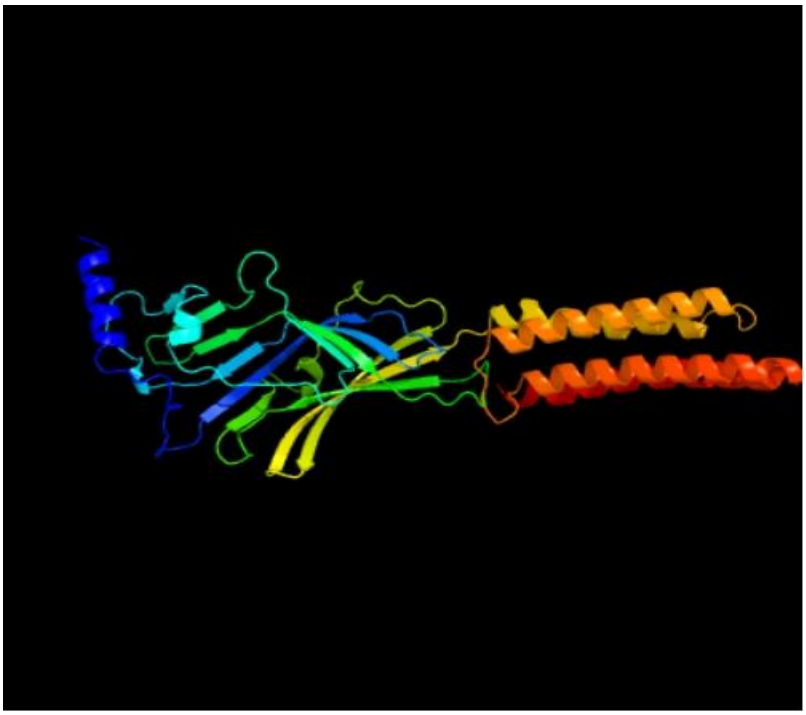

Figure 2: Structural Insights into the Human_GABRA1_gene Protein: A 3D Helical Model. 336 residues (74% of your sequence) have been modelled with 100.0% confidence by the single highest scoring template. GABA-A receptors are primary targets for sedatives, anxiolytics, and anticonvulsants. Exploring whether *Kaayakam Lehyam* or its constituents modulate GABA-A receptor activity (such as binding to the alpha-1 subunit) could provide insights into its potential effects on relaxation, anxiety reduction, or neural health.

Figure 3: The Human_PTPN1_gene protein: a 3-D helical model. 297 residues (68% of your sequence) have been modelled with 100.0% trust by the single top scoring template. The structure is colored from blue at the N-terminus to red at the C-terminus, helping to find the sequence path. The structure consists both alpha-helices (shown as spirals) and beta-sheets (flat arrows), which are placed in a compact manner typical of phosphatase domains. PTPN1 has an important catalytic site that provides its part in separating phosphate groups from proteins. The particular arrangement of helices and sheets here backs its function as an enzyme that controls cellular signaling by dephosphorylating tyrosine residues on target proteins.

Figure 4: The human_AKR1B1_gene protein: 3D Helical Model. 313 residues of your sequence (99%) are modelled with 100.0% confidence by the single top scoring template. The AKR1B1 gene encodes aldose reductase, an enzyme that has an important part in the polyol pathway of glucose metabolism. This enzyme decreases glucose to sorbitol and is participated in numerous pathological and physiological processes.

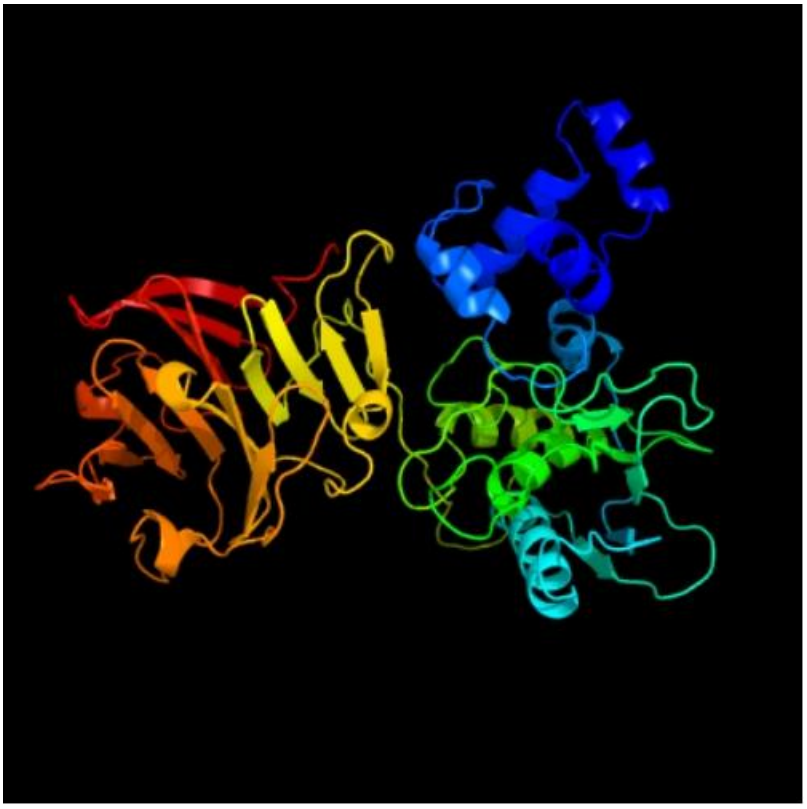

Figure 5: Structural Insights into the Human_MMP1_gene Protein: A 3D Helical Model. 415 residues (88% of your sequence) have been modelled with 100.0% confidence by the single highest scoring template. The color-coded ribbon representation in the structure may signify different domains or motifs within the protein. These regions could be critical for its enzymatic activity or interaction with other biomolecules.

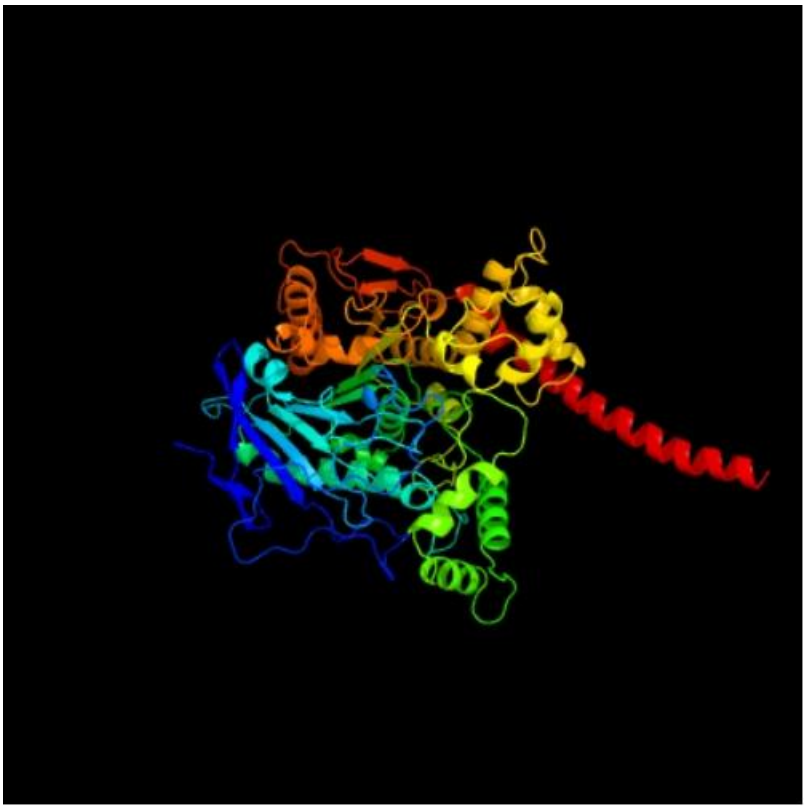

Figure 6: Structural Insights into the Human_ ACHE_gene Protein: A 3D Helical Model. 563 residues (92% of your sequence) have been modelled with 100.0% confidence by the single highest scoring template.

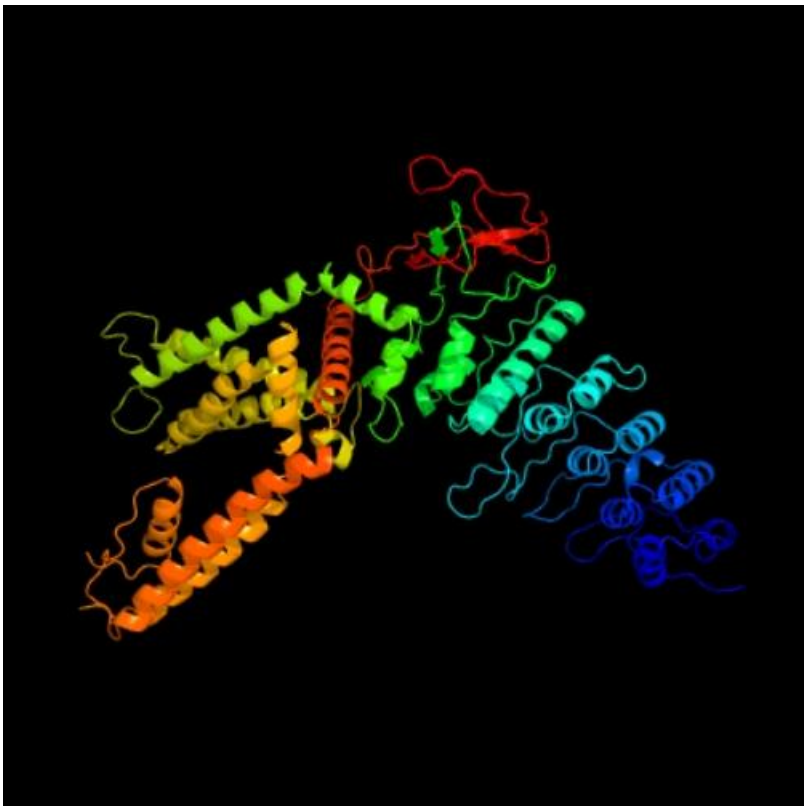

Figure 7: Structural Insights into the Human_TRPV1_gene Protein: A 3D Helical Model. 645 residues (77% of your sequence) have been modelled with 100.0% confidence by the single highest scoring template. The TRPV1 receptor is a part of the transient receptor potential (TRP) family of ion channels and is widely known for its role in pain perception, heat sensation, and inflammation.

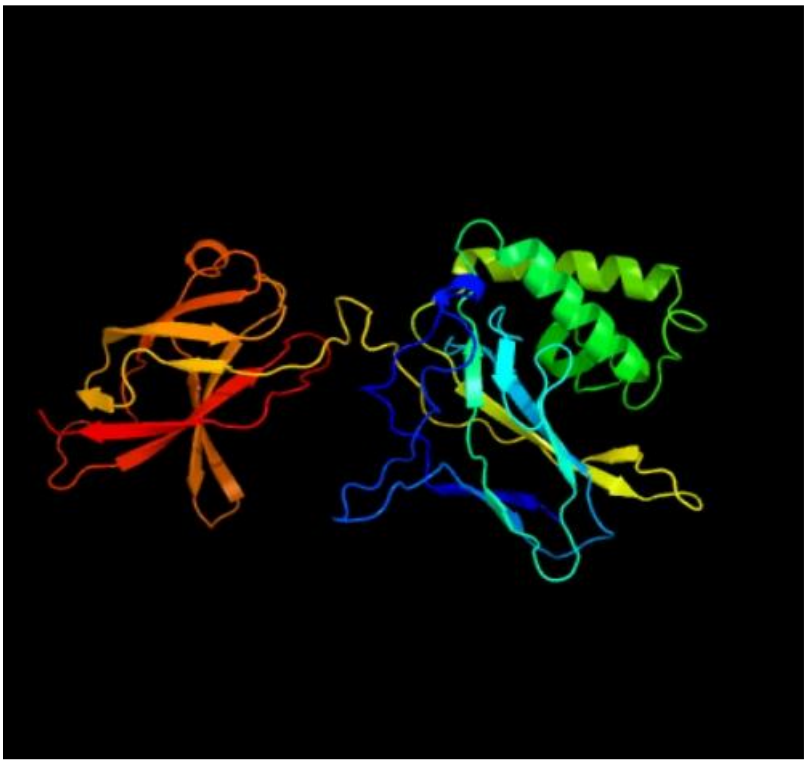

Figure 8: Structural Insights into the Human_TRPV1_gene Protein: A 3D Helical Model. 311 residues (32% of your sequence) have been modelled with 100.0% confidence by the single highest scoring template. **Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-κB)**, a protein complex that plays a critical role in regulating the immune response, inflammation, cell growth, and survival. NF-κB is activated in response to various stressors, such as infections, inflammatory signals, and oxidative stress, which can be prevalent during the postpartum period due to physical recovery needs and hormonal changes.

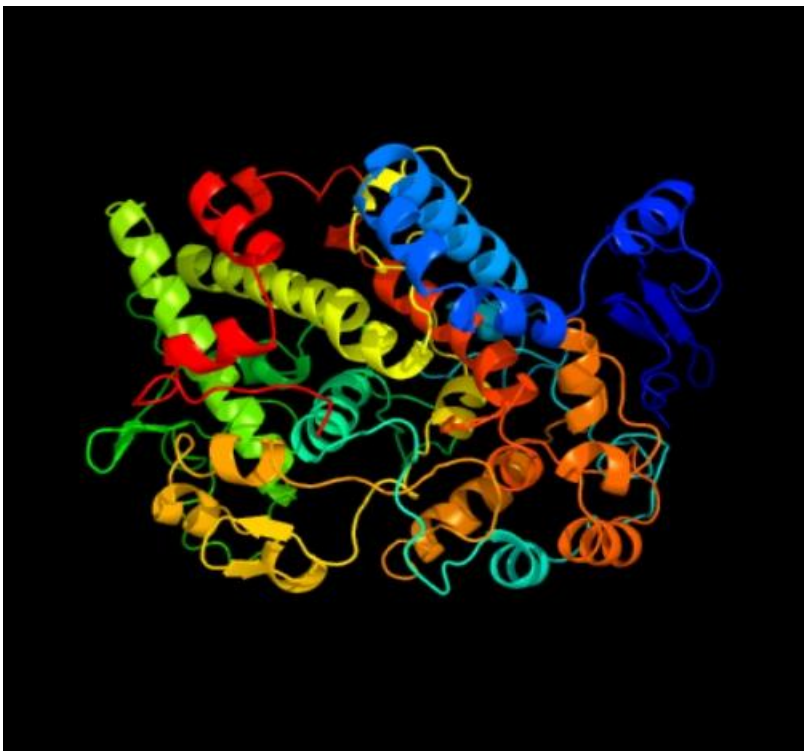

Figure 9: Structural Insights into the Human_ PTGS2_gene Protein: A 3D Helical Model. 551 residues (91% of your sequence) have been modelled with 100.0% confidence by the single highest scoring template. Model dimensions (Å):**X**:62.569 **Y**:62.353 **Z**:74.845; **Prostaglandin-Endoperoxide Synthase 2 (PTGS2)**, commonly known as **Cyclooxygenase-2 (COX-2)**. COX-2 is an enzyme involved in the inflammatory response, particularly in the synthesis of prostaglandins, which are lipid compounds that contribute to inflammation, pain, and fever.

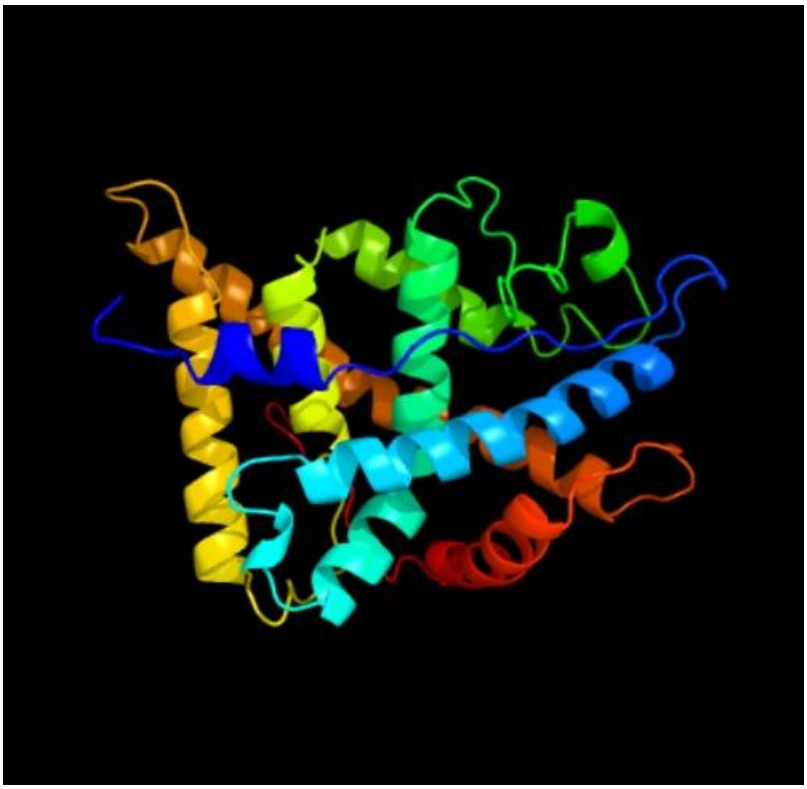

Figure 10: Structural Insights into the Human_ NR3C1_gene Protein: A 3D Helical Model. 247 residues (32% of your sequence) have been modelled with 100.0% confidence by the single highest scoring template. Model dimensions (Å): X:53.132 Y:59.741 Z:42.790. **NR3C1**, which encodes the **Glucocorticoid Receptor (GR)**. The glucocorticoid receptor is a type of nuclear receptor that, upon binding to glucocorticoids (a class of steroid hormones), regulates the expression of various genes involved in inflammation, immune response, and stress adaptation.

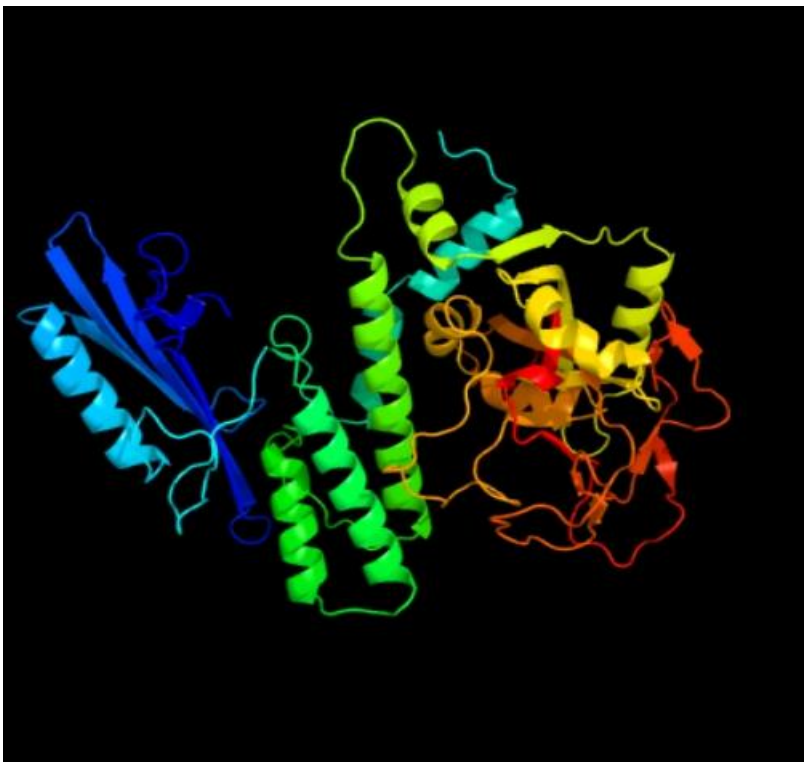

Figure11: Structural Insights into the Human_ PARP1_gene Protein: A 3D Helical Model. 456 residues (45% of your sequence) have been modelled with 100.0% confidence by the single highest scoring template.

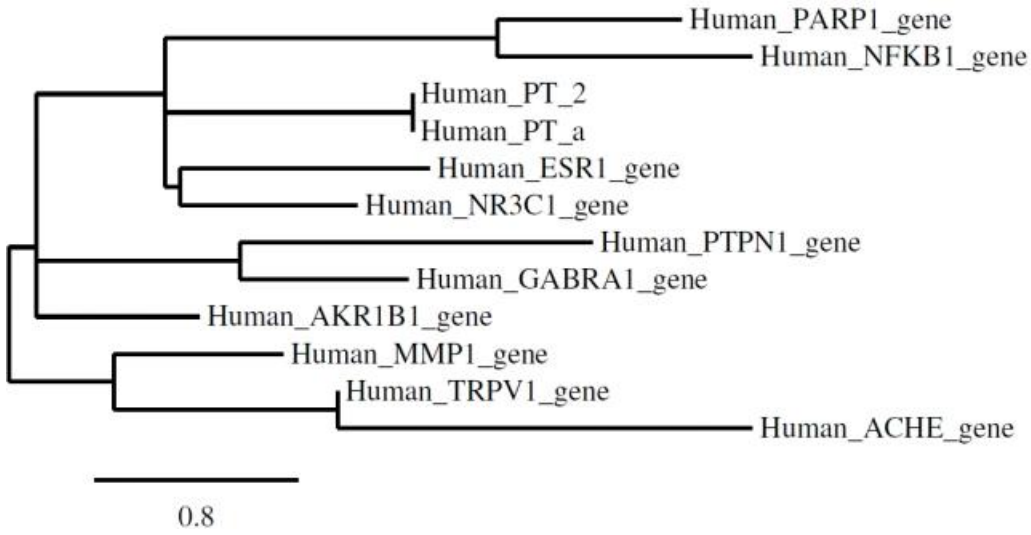

Figure 12: The phylogenetic tree constructed for the proteins targeted by bioactive compounds in Kaayakam Lehyam illustrates the evolutionary relationships among various human genes associated with key proteins relevant to postpartum recovery.

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