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Formulation Development, Physicochemical Evaluation, and *in silico* Analysis of a Polyherbal Roll-on Balm for Pain Management

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ABSTRACT

Conventional management of localized pain relies heavily on systemic therapeutics, which carry substantial gastrointestinal and systemic risks. Topical polyherbal systems offer a high-compliance alternative but frequently lack structural optimization and mechanistic validation**.** This study engineered, physically optimized, and computationally validated a natural polyherbal roll-on balm containing essential oils of peppermint, eucalyptus, clove, rosemary, and camphor designed for synergistic multi-target pain and inflammation management. Four lipid-based formulations (F1–F4) were fabricated via controlled thermomechanical blending. High-resolution molecular docking elucidated the thermodynamic binding landscapes of principal phytoconstituents against core nociceptive and inflammatory targets, including COX-1/2, 5-LOX, TRPM8, TRPV1, and TRPA1. Optimized formulation F1 and F3 demonstrated exceptional optical clarity, structural homogeneity, and robust physical stability under accelerated stress. The matrices precisely matched skin physiological pH (4.7–5.0), inducing zero dermal irritation or microbial bioburden while exhibiting a distinct biphasic warming-to-cooling sensory profile. Computational profiling successfully rationalized this synergy: eugenol exhibited highly potent binding affinity within the catalytic pocket of COX-2 (-6.9 kcal/mol), while menthol anchored robustly to the cold-sensing TRPM8 receptor via targeted Arg842 hydrogen bonding (-6.7 kcal/mol), providing a definitive structural rationale for the balm's multi-pathway analgesia.

Keywords: Topical Drug Delivery; Polyherbal biomatrix; Multi-target Analgesia; Molecular docking; Thermodynamic stability; Essential oils.

Abbreviations

COX – Cyclooxygenase; 5-LOX – 5-Lipoxygenase; TRP – Transient Receptor Potential; TRPA1 – Transient Receptor Potential Ankyrin 1; TRPM8 – Transient Receptor Potential Melastatin 8; TRPV1 – Transient Receptor Potential Vanilloid 1; PDB – Protein Data Bank; ΔG – Gibbs Free Energy (Binding Free Energy).

1. INTRODUCTION

Topical drug delivery systems offer an opportunity for targeted delivery of therapeutic agent to the site of action thereby reducing systemic exposure and associated adverse effects for the treatment of localized pain[18]. These systems can be very beneficial in conditions like headache, muscular pain and inflammatory discomfort where quick action is essential and patient compliance is crucial. Roll-on balms have become novel and convenient dosage forms, which allows for accurate dosing, minimisation of product waste and hygienic application using a roller-ball applicator[8].

Roll-on delivery systems are specially designed to dispense low viscosity, volatile, and lipophilic formulations in a controlled way[4]. The use of a rolling ball not only ensures even spreading across the surface of the skin, but also increases the contact with the nerve receptors in the skin and makes this product more acceptable to the patient because of the non-messy, portable and non-touchy design[10]. These properties make roll-on balms very well suited for use on the go with pain relief and aromatherapeutic uses[14].

Analgesic, anti-inflammatory, counter-irritant and vasomodulatory are the long-standing medicinal properties of essential oils utilized in traditional and complementary medicine[1]. Menthol is abundant in peppermint oil and is known to produce analgesia selectively by TRPM8, which is a cold-sensitive transient receptor potential (TRP) channel that produces a cold sensation and modulates pain sensation[5]. Eugenol, the major component of clove oil, and camphor, the main component of camphor oil act as counter-irritants and local anesthetics by inhibiting the TRP vanilloid-1 (TRPV1) heat and nociception channel[6]. Eucalyptus oil has anti-inflammatory and decongestant properties, while rosemary oil has antioxidant and circulatory-stimulating effects, making them more therapeutic[16].

In addition to the sensory and analgesic mechanisms, headache relief roll-ons stimulate the olfactory pathways and peripheral sensory receptors. TRP also affect thermal perception and cause desensitization of pain-transmitting neurons, which is a symptom relief. Furthermore, some of the constituents of essential oil are known to inhibit the cyclooxygenase (COX) enzymes, which explain their anti-inflammatory activity[11].

Although herbal roll-on formulations are in common use, there is a limited number of systematic formulation development with the help of mechanistic validation and physicochemical evaluation. Hence, this study focused on the formulation and testing of a polyherbal roll-on balm with a combination of the essential oils of peppermint, eucalyptus, clove, rosemary and camphor in the carrier base of coconut and

olive oils. The study also combines molecular docking results with the rationalization of the interaction of molecules of interest with the molecular targets responsible for the pain effect, followed by the complete evaluation of the physicochemical properties, stability and skin compatibility of the molecules to guarantee their safety and efficacy.

2. MATERIALS AND METHODS

2.1 Materials

The formulation development was made using peppermint oil (*Mentha piperita*), eucalyptus oil (*Eucalyptus globulus*), clove oil (*Syzygium aromaticum*), rosemary oil (*Rosmarinus officinalis*), camphor (*Cinnamomum camphora*), coconut oil and olive oil. All essential oils of pharmaceutical grade were obtained from good commercial suppliers. The carrier oils, coconut oil and olive oil were chosen because of their emollient and chemical stability properties along with ability to be applied topically. Where necessary distilled water was used. All glassware and containers were cleaned, dried and sterilized before use.

2.2 Methods

Four formulations (F1-F4) were prepared with different amounts of essential oils and base oil. Different concentrations of the essential and carrier oils were formulated to assess their effect on physical stability, sensory performance and skin compatibility. Formulation was prepared in different proportion and final product of 3 mL were prepared as shown in Table 1, which was further packed in roll-on form[20,22].

Table 1: Quantitative composition of essential oil blends and carrier lipid bases for formulations.

Ingredient	F1 (mL/g)	F2 (mL/g)	F3 (mL/g)	F4 (mL/g)
Peppermint oil (mL)	1.05	0.75	0.45	0.45
Eucalyptus oil (mL)	0.69	0.60	0.24	0.30
Clove oil (mL)	–	0.75	0.90	0.90
Rosemary oil (mL)	0.06	–	0.45	0.39
Camphor (g)	0.30 g	0.30 g	0.60 g	0.66 g
Base (Coconut oil) (mL)	0.90	–	–	–
Base (Olive oil) (mL)	–	0.60	0.36	0.30

2.2.1 Preparation of Herbal Roll-On Balm

The controlled melting and mixing method were used to prepare the herbal roll-on balms. The measured amount of camphor and then carrier oil (coconut or olive oil) were transferred to a clean dry beaker (Figure 1). The mixture was heated at a controlled temperature, not exceed 40 °C, on a heating mantle until camphor was completely dissolved. To avoid the degradation of volatile oils, overheating was avoided. It was then taken out of the hot plate and cooled down slightly to avoid volatilization and degradation of essential oils. Pre-measured amounts of essential oils (peppermint, eucalyptus, clove and rosemary) were then added, one by one, under constant stirring with a glass rod and mixing it evenly. Stirred until a clear and homogeneous solution was formed. The prepared formulation was then allowed to cool to room temperature and then placed in sterile 3 mL roll-on bottles on which a roller-ball applicator is fitted and a cap is placed. The filled containers were kept in airtight conditions until they could be further evaluated[12].

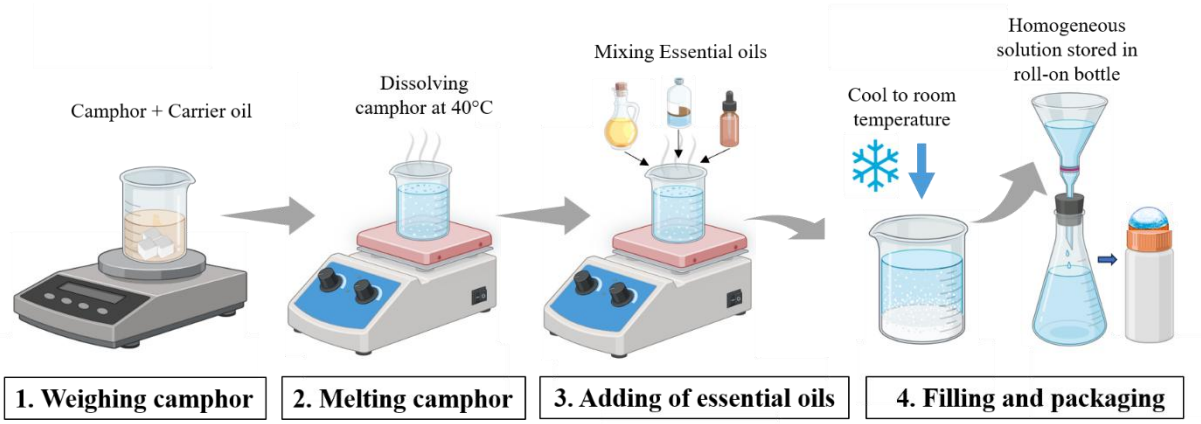

Figure.1 Schematic representation of step wise herbal roll-on balm preparation

2.3 Evaluation of Herbal Roll-On Balm

The herbal roll-on balm formulations prepared, were evaluated systematically for their physicochemical characteristics, sensory performance, skin compatibility, microbial safety, and short-term stability[17,23].

2.3.1 Organoleptic Properties: The organoleptic properties were visually checked for colour, clarity and physical appearance of all formulations. Smell was tested through a light smelling test and texture was tested by touching the outside to see how smooth and even it is.

2.3.2 Homogeneity: Formulations were checked for homogeneity by visual inspection and by rubbing a small sample of formulation between the fingers to check for the presence of lumps, phase separation or any particulate matter.

2.3.3 pH Determination: The pH of the developed roll-on formulations was obtained by making a 10% w/v dispersion in distilled water. The mixture was stirred well to ensure phase equilibrium. The pH of the aqueous phase was then measured using a calibrated digital pH meter and confirmed by comparing the color changes with a standard reference chart with standard pH indicator paper strips to be compatible with the physiological pH of the human skin.

2.3.4 After-Feel and Skin Sensation: After-feel characteristics of the formulation were evaluated on the forearm such as emolliency, greasiness and residue. Sensory performance was measured by recording sensation such as cooling and warming effects a few minutes after application

2.3.5 Removal Test: Ease of removal was determined by washing the area to which the material was applied under running tap water. The time required and effort needed for complete removal of the formulation were noted.

2.3.6 Irritancy and Patch Test: A skin patch test was performed on a selected area of skin with a small amount of the formulation applied and for 24 hours observation for erythema, itching, edema and inflammation were observed.

2.3.7 Microbial Growth Test: The test for microbial contamination was done by the agar streak plate method. The formulations were then placed on nutrient agar plates and allowed to incubate for 24 hours at 37 °C. The plates were checked for microbial growth and were compared with the control plates.

2.3.8 Stability Studies: Short term stability studies were carried out with the formulations by storing them under refrigerated conditions at 4 °C, room temperature and accelerated conditions at 47 °C. Changes in physical appearance, clarity, homogeneity and odor were assessed at 24, 48 and 72 hours for all samples.

2.4 Molecular Docking

To gain insight into the mechanism of action of the selected phytoconstituents obtained from a herbal roll-on balm, molecular docking analysis was included. The interaction of the main essential oil compounds with molecular targets related to pain perception and inflammation, such as TRP channels and COX enzymes was rationalized through docking studies with previously reported computational studies[7].

The main component of peppermint oil, menthol, has been reported to interact with the cold-sensing transient receptor potential melastatin 8 (TRPM8) channels. According to docking studies, menthol is found to be hydrogen bonding with hydroxyl group and residue R842 inside the cavity of the channel, thus validating its possible role in inducing cooling effect and modulating nociceptive signaling. This interaction accounts for the swift topical sensory analgesic effect (Figure 2)[9].

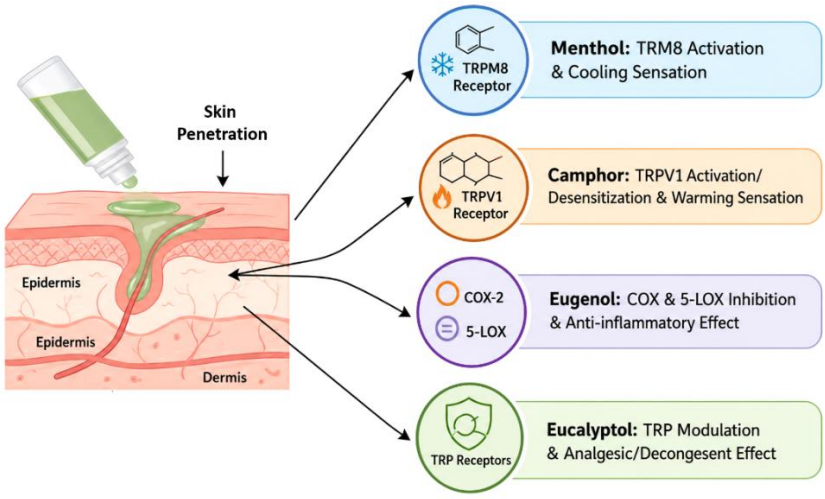

Figure. 2. Mechanism of Analgesic Action of Herbal Roll-On Balm

The binding affinity of camphor to COX-1 enzymes has been found to be contributed by hydrogen bonding and hydrophobic interaction with an active site of enzymes. These interactions indicate the anti-inflammatory and analgesic activity may be due in part to its inhibitory effect on prostaglandin synthesis. Moreover, camphor is known to modulate TRPV1 channels, which also helps potentiate its counter-irritant and warming sensory activity[19].

The main component of eucalyptus oil, eucalyptol (1,8-cineole) has been known to interact with TRPV1 and TRPA1 receptors. Based on the docking, eucalyptol is proposed to influence the nociceptive signaling by inhibiting the activation of receptor, which is responsible for anti-nociceptive and decongestant activities observed in topical formulations[15].

The main compound in clove oil, eugenol, has high binding affinity to COX-2 and 5-Lipoxygenase (5-LOX) enzymes[2,3]. During the molecular modeling studies, it was noted that eugenol binds to the catalytic binding pockets of these enzymes through the establishment of hydrogen bonds and hydrophobic interactions, which are responsible for its anti-inflammatory and analgesic effect[7].

All these insights based on docking are together a pharmacological basis for the use of essential oils in the present formulation, in a synergistic combination. Docking analysis, although not directly proving efficacy, provides supporting evidence for the observed sensory and analgesic activity of the herbal roll-On balm, by correlating the formulation constituents with known molecular targets associated with pain and inflammation.

3. RESULT AND DISCUSSION

In the present study, a polyherbal roll-on balm was successfully formulated and evaluated for its physical stability and non-irritation properties with multi-target localized pain management action. The formulation strategy focused on patient compliance, aesthetic acceptability, sensory response, physical stability, and detailed in-silico docking interactions, to elucidate the mechanism of action of the molecules.

3.1 Physicochemical, Sensory, and Safety Evaluation.

The organoleptic evaluation showed good appearance for all the formulations. The essential oils successfully dispersed in the lipid bases or carriers (coconut oil and olive oil) gave smooth and easy spreading matrices with a pleasant aromatic profile. No batch was outside the range of pH 4.7 - 5.0 to prevent localised contact dermatitis, disruption of microflora and irritation of the skin, which falls within the vital range of the acidic mantle of human skin (4.5 - 5.5).

Formulations F1 (coconut oil based) and F3 (olive oil based) were found to have the best physical homogeneity, transparency and structural clarity, indicating optimal solubilisation of camphor and essential oils in the carrier matrices. On the other hand, F2 showed some turbidity and some slight lumping, which was due to increasing level of clove oil and the lipid phase incompatibility at high thermal limits. The patch test confirmed that no dermal irritation (erythema, edema, or inflammation) was observed and no microbial growth was found in all batches, indicating topical safety. Accelerated stability testing (4 ˚C, ambient temperature, 47 ˚C for 72 hours) showed that there was no phase separation or organoleptic degradation of F1 and F3.

The optimized formulations (F1/F3) provide a natural alternative without any synthetic ingredients when compared with the commercial formulations (Amrutanjan balm). They present a higher concentration

of active essential oil without synthetic additives (such as methyl salicylate and artificial preservatives) and a different sensory transition from initial warming to prolonged cooling (Table 2).

Table 2. Comparative Evaluation of Phytoconstituents, Sensory Profiles and Safety Parameters of Developed Roll-on Formulations (F1/F3) versus Marketed Commercial Product.

Parameter	Developed Roll-On (F1/F3)	Marketed Product (Amrutanjan)
Actives	Essential oils	Synthetic + natural
Menthol (%)	15–35	~8
Camphor (%)	10–22	~10
Methyl salicylate	Absent	Present
Sensory profile	Warm → Cool	Cooling
Irritation	Not observed	Mild (reported)
Application	Roll-on	Balm / roll-on

3.2 In-Silico Molecular Docking and Thermodynamic Interaction Analysis

Molecular docking was performed on the major inflammatory/anti-inflammatory and nociceptive targets to provide a mechanistic basis for the biphasic sensory pattern and anti-inflammatory/analgesic activity. The structural binding landscapes, 3D conformational poses and 2D interaction maps (Figures 3-5), along with binding affinities (ΔG) and active site residue profiles explain the multi-target synergistic therapeutic profile.

3.2.1 Modulation of Cyclooxygenase and Lipoxygenase Pathway (COX-1, COX-2, 5-LOX)

Modulation of the inflammatory cascade was computationally validated on COX-1, COX-2 and 5-LOX enzymes. The eugenol exhibited the highest binding energy (ΔG=-6.9 kcal/mol) in the catalytic domain of COX-2 (PDB ID: 1PXX). The binding pose (Figure 3A, B) in the 3D model is well anchored through conventional H-bonds between HIS90 (2.84 Å) and the phenolic hydroxyl group, as well as between TYR355 (3.01 Å) and the catalytic residues, and hydrophobic π-alkyl contacts between LEU359 and VAL523. Menthol (ΔG=-6.0 kcal/mol) was firmly bound to COX-2 by a direct hydrogen bond to GLU465 (2.28 Å) and an alkyl contact with ALA462 and ILE466. In this case, Eucalyptol (ΔG=-5.9 kcal/mol) formed ASN224 hydrogen bonds (2.95 Å), and Camphor (ΔG=-5.7 kcal/mol) was stabilized only by hydrophobic interactions with VAL523, ILE257 and LEU352.

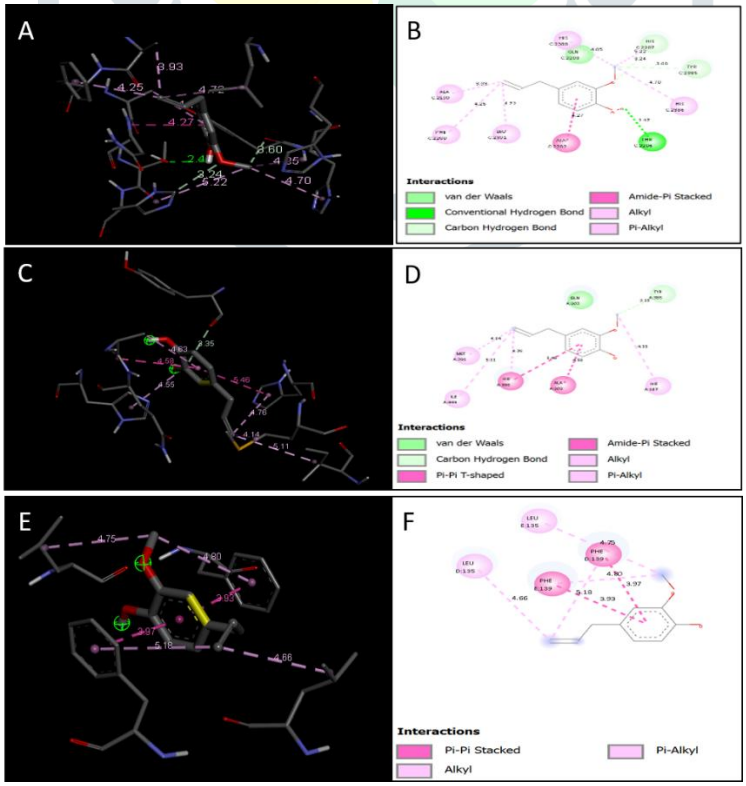

Figure. 3 BIOVIA Discovery Studio illustrations of potential three- and two-dimensional interactions between COX-2 protein (PDB ID: 1PXX) and phytoconstituent Eugenol (3A, B); COX-1 protein (PDB ID: 3N8X) and phytoconstituent Eugenol (3C, D); 5-LOX protein (PDB ID: 6VG1) and phytoconstituent Eugenol (3E, F).

Eugenol (ΔG=-6.2 kcal/mol) made a key hydrogen bond with the active site residue ARG120 (3.12 Å) as well as hydrophobic packing with VAL349 and LEU352 (Figure. 3C, D). Eucalyptol (ΔG=-5.8 kcal/mol) interacted with TYR385 (2.90 Å). Camphor (ΔG=-5.3 kcal/mol) and Menthol (ΔG=-5.4 kcal/mol) had hydrophobic contacts with LEU214, VAL220, ILE345 and VAL349 with no hydrogen bonding.

Strong binding affinities of Eugenol (ΔG=-6.4 kcal/mol) and Eucalyptol (ΔG=-6.2 kcal/mol) confirmed the dual inhibition of leukotrienes pathway in 5-LOX. Hydrogen bonding was observed between eugenol and GLN363 (2.92 Å) and HIS367 (3.10 Å), with π-alkyl stacking between eugenol and LEU414 and PHE421, respectively (Figure. 3E, F). The molecule Eucalyptol formed ASN554 (2.88 Å) in direction hydrogen bonding. The Camphor (ΔG=-6.0 kcal/mol) and Menthol (ΔG=-6.0 kcal/mol) both were stabilized within the binding cavity through the formation of hydrophobic interaction clusters namely LEU368, ILE406, VAL604, and ALA608.

3.2.2 Thermosensory modulation and nociceptive modulation (TRP channels: TRPM8, TRPV1, TRPA1)

The fast biphasic (warming-to-cooling) sensory perception seen clinically is directly reflected in the thermodynamic binding profiles against TRP channels. Camphor and Menthol showed significant binding affinities towards the cold-sensing TRPM8 receptor (PDBID: 9B6D) with binding energies of ΔG=-6.9 kcal/mol and ΔG=-6.7 kcal/mol respectively. The menthol is deeply embedded at the transmembrane binding pocket and makes a hydrogen bond with GLN94 (3.04 Å) and extensive hydrophobic interactions with ILE741, VAL748 and TYR745 (Fig. 4A, B). Strong hydrophobic interactions with TYR745, PHE748 and LEU744 stabilized Camphor (Fig. 4C, D). The two groups of interest were the hydrogen bond (between eugenol and TYR745 (2.98 Å)) and hydrophobic interaction (between Eucalyptol and LEU802 and VAL805).

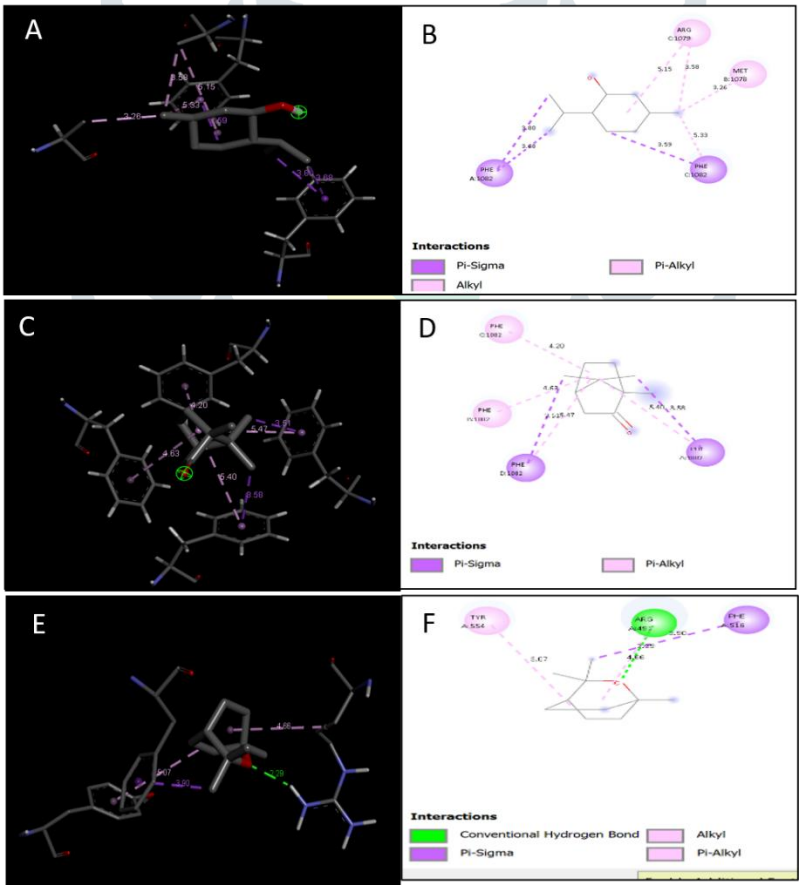

Figure 4. Three-dimensional and two-dimensional interactions that occur between the TRPM8 protein (PDB ID: 9B6D) and phytoconstituents Menthol and Camphor (4A, B, C, D); TRPV1 protein (PDB ID: 9OGK) and phytoconstituent Eucalyptol (4E, F).

The counter-irritant warming sensation is mediated by modulation of heat- and pain-sensing TRPV1 (PDB ID: 9OGK). Menthol, Eucalyptol, Eugenol and Camphor were all found to bind inside the channel complex. The hydrogen bonding of the key residues THR511 and TYR511 with Eucalyptol, THR550 with Eugenol and the non-covalent hydrophobic interactions of Camphor with LEU515, VAL518, ILE569 and

LYS572, Eugenol with LEU515, VAL518, ILE569, and LYS572, and Menthol with LEU515, VAL518, ILE569 and LYS572 were responsible for the stabilization. The four phytoconstituents showed the best interaction with the TRPV1 with the binding affinity, but eucalyptol showed the highest binding affinity of −5.9 kcal/mol (Figure. 4E, F). The bond distance between the ligand and TYR511 was 3.02 Å, with the ligand forming a hydrogen bond with TYR511, which resulted in a stable conformation of the ligand within the binding pocket of TRPV1, supported by hydrophobic interactions with LEU553, PHE512, and LEU515.

TRPA1 Channel Interaction: Within the nociceptive TRPA1 channel (PDB ID: 7YKR), Menthol (ΔG=-5.0 kcal/mol) formed dual hydrogen bonds with ASP1066 (2.15 Å) and ILE1058 (1.88 Å). Camphor (ΔG=-5.4 kcal/mol) formed a strong hydrogen bond with SER509 (2.44 Å), and Eugenol (ΔG=-5.5 kcal/mol) engaged ILE1058 (2.27 Å) (Figure. 5A, B). The stabilization of eucalyptol (ΔG=-5.9 kcal/mol) was achieved through a large number of van der Waals contacts (LYS744, ASP1066, LEU1062, GLU1054, ILE715, PRO714, HIS1059, GLN1063), but no hydrogen vectors were oriented in a direction that could be considered as pointing in a preferred direction (Figure. 5C, D).

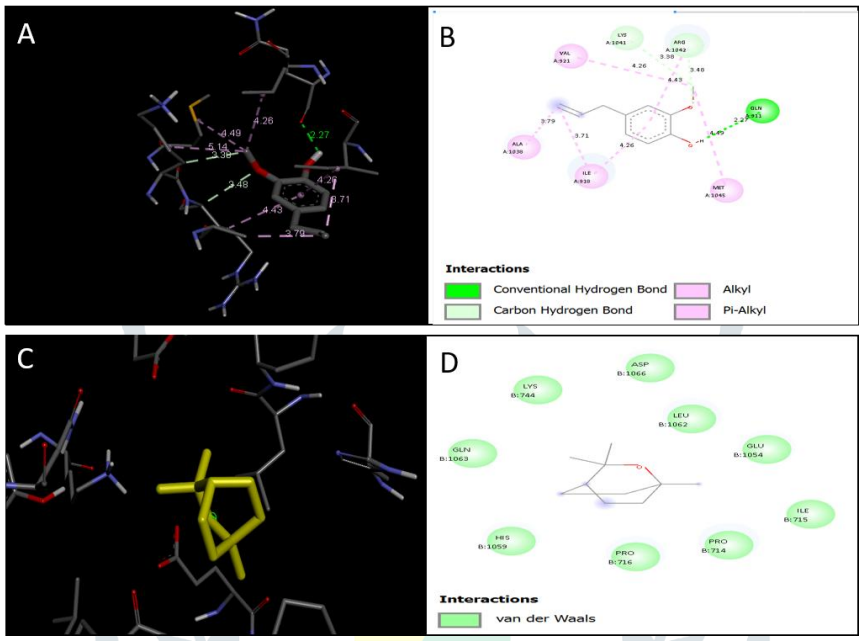

Figure 5. Three dimensional and two-dimensional interaction profile between TRPA1 channel protein (PDB ID: 7YKR) and phytoconstituents: (A, B) Eugenol, (C, D) Eucalyptol.

3.3 Integrated Synergy and Mechanistic Synthesis

The computational results directly complement the physical profile of the roll-on balm according to its sensory properties. The high binding affinity of Camphor (ΔG=-6.9 kcal/mol) and Menthol (ΔG=-6.7 kcal/mol) to TRPM8 and TRPV1 channels suggests a molecular mechanism for the ability of these channels to generate both warming and cooling sensations. It is a thermal modulation counter-irritation, which is also known as counterirritant therapy. At the same time, Eugenol has a high binding affinity (ΔG=-6.4 kcal/mol at COX-2; ΔG=-6.9 kcal/mol at 5-LOX) to block the synthesis of pro-inflammatory prostaglandins and leukotrienes systematically.

The multi-target interaction profile (sensory receptor gating + enzymatic pathway inhibition of inflammation) supports the therapeutic synergy of the polyherbal roll-on balm as an effective, non-invasive tool for local pain treatment.

4. CONCLUSION

In this study, the formulation of a non-irritating, physically stable polyherbal roll-on balm, in both coconut oil (F1) and olive oil (F3) carrier matrices, has been shown to be successful, for targeted, localized pain management. The optimized delivery system showed structural integrity, homogeneity and organoleptic properties under the accelerated thermal stress (4 ˚C to 47 ˚C) over 72 hours. Biocompatibility tests validated physiological skin alignment, ranging from pH 4.7 - 5.0 and no dermal contamination of microorganisms were found, with zero erythema and edema. Moreover, a clear sensory profile was achieved, providing a natural and synthetic free formulation alternative to available commercial sensory profiles.

Mechanically validation of therapeutic efficacy of the formulation was performed by multi-target in-silico molecular docking with synergistic dual mechanism of action. Rapid transitions from warming to cooling were found to be mediated by thermosensory modulation: camphor (ΔG=-6.9 kcal/mol) and menthol (ΔG=-6.7 kcal/mol) high-affinity binding in the transmembrane domains of TRPM8 and TRPV1 channels, respectively, provides a molecular explanation for the fast warming and cooling effects of counter-irritants. In contrast, among the evaluated phytoconstituents, eucalyptol showed the most favorable docking profile towards TRPV1 having a binding affinity of −5.9 kcal/mol with a hydrogen bond with TYR511, a residue involved in the stabilization of the ligand in the receptor. These extra H-bond interactions are present with LEU553, PHE512 and LEU515 which further stabilizes the protein-ligand complex. Eugenol showed strong enzymatic suppression properties, including selective catalytic binding to COX-2 (ΔG=-6.9 kcal/mol) as a result of the interactions with HIS90 and TYR355 and also dual 5-LOX inhibition (ΔG=-6.4 kcal/mol), which would block the downstream cascade of pro-inflammatory mediators at the site of administration.

The research brings together the classical formulation science of phytochemicals and the current computational profiling of targets to provide a structure and thermodynamic rationale for the synergy of polyherbal formulations in the treatment of topical analgesics. Overall, the produced roll-on balm is a platform of non-invasive, localized inflammatory and nociceptive care with great potential for future in-vivo pharmacokinetic studies and clinical translation.

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