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Original Article

Bridging Molecules and Models: A Study of Curcumin-Eugenol Anti-Inflammatory Synergy

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ABSTRACT

A vital part of the body's ability to repair damaged tissues and protect itself from outside threats is inflammation, a basic biological response to potentially dangerous stimuli. However, when persistent or dysregulated, it can contribute to the development of chronic diseases. This study employs both in vitro and in silico approaches to investigate the anti-inflammatory properties of curcumin and eugenol. The compounds were first characterized using Thin-Layer Chromatography (TLC), followed by assessing their anti-inflammatory properties using assays for protein denaturation and anti-proteinase, with indomethacin serving as the standard control. A 1:1 combination of curcumin and eugenol exhibited significantly greater inhibition of protein denaturation (81.32%) and proteinase activity (69.67%) compared to the individual compounds and the standard drug. This combination also demonstrated the lowest IC_{50} values in both assays, indicating enhanced potency. Molecular docking studies targeting COX-2, JAK, NF-κB, and STAT3 revealed stronger binding affinities for curcumin, while eugenol showed complementary interactions, supporting a synergistic, multi-target mechanism of action. These findings underscore the therapeutic potential of this phytochemical combination as a natural, beneficial, and safer alternative to conventional anti-inflammatory agents.

Keywords: Inflammation, molecular docking, COX-2, JAK, NF-κB, and STAT3

INTRODUCTION

Inflammation, a fundamental biological response to harmful stimuli like damaged cells or irritants, is a crucial component of the body's innate immune defense. It initiates healing and tissue repair. While acute inflammation is a self-limiting and protective mechanism, chronic inflammation has been linked to the pathogenesis of various ailments, such as cardiovascular disease, rheumatoid arthritis, diabetes, inflammatory bowel disease, and neurodegenerative disorders. The inflammatory response requires a cascade of biochemical events triggered by the immune system. These include the release of cytokines, chemokines, prostaglandins, and reactive oxygen species (ROS), which collectively contribute to the clinical symptoms of inflammation— heat, swelling, pain, redness and loss of function. The cellular participants of inflammation, such as neutrophils, lymphocytes and macrophages are recruited to the site of injury to neutralize the offending agent and promote recovery. [1] Despite the essential nature of inflammation in host defense, persistent or uncontrolled inflammation is harmful and contributes to the occurence of chronic diseases. This has prompted the search for therapeutic agents that can modulate the inflammatory process without eliciting severe side effects. Widely used anti-inflammatory treatments like NSAIDs, steroids, and biologics offer symptomatic relief, but their prolonged use is linked to adverse effects. However, their long-term use is correlated with a wide variety of negative consequences such as gastrointestinal toxicity, cardiovascular obstacles, immunosuppression and increased susceptibility to infections. [2] NSAIDs, for instance, suppress cyclooxygenase enzymes (COX-1 and COX-2), this consequence to reduced prostaglandin synthesis but also compromises gastric mucosal protection and renal function. Corticosteroids act broadly on inflammatory gene expression but may cause metabolic disturbances, osteoporosis, and adrenal suppression when used chronically. Biologic agents, though highly effective, are often expensive and may result in immune suppression. Given these challenges, there is growing interest in exploring natural anti-inflammatory agents derived from plants that are effective, affordable, and possess fewer side effects.

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

[3] A polyphenolic compound termed curcumin is extracted from the rhizome of Curcuma longa, or turmeric. It has been extensively researched for its anti-inflammatory properties, anticancer, antioxidant, and neuroprotective properties. Mechanistically, curcumin modulates several signaling pathways, including JAK/STAT, NF-κB, and MAPK, thereby inhibiting the expression of inflammatory mediators as inducible nitric oxide synthase (iNOS), COX-2, IL-6, and TNF-α. [4] Eugenol is a phenolic substance primarily extracted from clove oil (*Syzygium aromaticum*). It has proven to have an analgesic, antioxidant, anti-inflammatory and antimicrobial activities. Eugenol is known to inhibit prostaglandin synthesis, reduce leukocyte migration, and modulate ion channels and receptor-mediated pathways, including NMDA and histamine receptors, which play a vital role in inflammation and pain perception. [5] Both curcumin and eugenol possess membrane-stabilizing properties and free radical scavenging abilities, making them ideal candidates for the development of novel anti-inflammatory therapies. Importantly, they are both generally regarded as safe (GRAS) substances and have been employed by traditional medicine over decades. While curcumin and eugenol have been studied extensively in isolation, there is limited evidence evaluating their combined effects. Recent findings suggest that eugenol may enhance the bioavailability of curcumin by improving membrane permeability and inhibiting its metabolic degradation. This synergistic interaction could potentiate their anti-inflammatory efficacy by targeting multiple mechanisoms contributed in the inflammatory reaction. The combined administration of curcumin and eugenol may offer several advantages: reduced required dosage of each compound, enhanced therapeutic efficacy, minimized side effects, and a broader spectrum of action. Such combinations are particularly relevant in the context of chronic inflammatory diseases where long-term management is necessary. [6] The present research aims to evaluate the anti-inflammatory synergy of curcumin and eugenol using *in vitro* models such as protein denaturation assays and *in silico* molecular docking studies. These approaches aim to elucidate molecular interactions and medicinal benfits of this phytochemical combination.

Fig:1.1 *Curcuma longa* (turmeric)

Fig:1.2 *Syzygium romaticum* (Eugenol)

METHODS

2.1 Thin-Layer Chromatography (TLC) of Curcumin and Eugenol

TLC was performed using silica gel pre-coated plates as the stationary phase, activated by heating at 110°C for 30 minutes to enhance adsorption. A baseline was marked 1 cm from the bottom for consistent sample application and accurate Rf measurement. Sample solutions, including curcumin and eugenol extracts (10 mg/mL in ethanol) and standard solutions (curcumin in methanol, eugenol in ethanol at 0.5 mg/mL), were applied in 1–2 µL volumes using capillary tubes. After solvent evaporation, plates were placed in pre-saturated chambers with mobile phases for development: n-hexane and ethyl acetate (7:3) for curcumin, and hexane-acetone (9:3) for eugenol. The solvent ascended to three to fourths of the plate's height, followed by drying and visualization under UV light at 254 nm and 365 nm. Curcumin appeared as a yellow fluorescent spot, while eugenol displayed dark spots, ensuring reliable phytochemical analysis. [7]

2.2 *In vitro* evaluation of anti-inflammatory activity.

The *in vitro* evaluation of anti-inflammatory activity of curcumin and eugenol was done by protein denaturation method (albumin method) and anti-proteinase method.

2.2.1 *In vitro* protein denaturation method.

The *in vitro* protein denaturation method, often referred to as the albumin method, was employed to assess their ability to inhibit protein denaturation, a signature of inflammation. This method was carried out for three primary test samples: Curcumin, Eugenol, and a fixed combination containing both Curcumin and Eugenol in equal proportions. For comparative purposes and to establish a standard reference, Indomethacin, a familiar non-steroidal anti-inflammatory drug (NSAID), was utilized as the standard control. [8]

2.2.2 *In vitro* Anti-Proteinase Action

To elucidate the anti-inflammatory mechanisms of the selected compounds, an *in vitro* anti-proteinase action assay was conducted. This assay investigated the ability of the test samples to inhibit the activity of proteinases, enzymes that degrade connective tissue and produce inflammatory mediators. Similar to the protein denaturation method, Curcumin, Eugenol, and a combination of Curcumin and Eugenol (in equal proportions) were evaluated. The most prevalent reference substance employed for direct comparison was indomethacin. [9] The assay involved preparing a reaction mixture with a proteinase, a substrate, and varying concentrations of the test compounds and the standard. The enzymatic cleavage of the substrate was measured, typically through the liberation of a chromogenic product that could be quantified spectrophotometrically. An anti-proteinase agent inhibited this activity, reducing the measured product. The percentage inhibition of proteinase activity was determined for each concentration. This assay helps understand how Curcumin, Eugenol, and their combination might mitigate inflammation by preventing tissue breakdown and reducing inflammatory peptide generation. The data contribute to a comprehensive understanding of their *in vitro* anti-inflammatory profile.

2.3 Molecular Docking Studies

Molecular docking is a computer technique that predicts the intensity of a contact, typically represented as a docking score or binding affinity (Glide score in this work), as well as the preferred orientation of a molecule (ligand) when coupled to a target protein (receptor). This method is essential to contemporary drug discovery since it clarifies possible molecular pathways prior to the costly and drawn-out in vivo studies. In this study, we selected four key protein targets involved in inflammation:

- **Cyclooxygenase-2 (COX-2)** – a key enzyme in prostaglandin biosynthesis and a well-established target of NSAIDs.
- **Janus kinase (JAK)** – involved in cytokine receptor signaling and immune modulation.
- **Nuclear Factor kappa B (NF-κB)** – a central transcription factor regulating expression of pro-inflammatory cytokines.
- **Signal Transducer and Activator of Transcription 3 (STAT3)** – another transcription factor involved in inflammation and immune response.

The Glide scores (expressed in negative kcal/mol) indicate the binding strength—**the more negative the score, the stronger and more favorable the binding**.

RESULTS

3.1 Thin-Layer Chromatography (TLC) of Curcumin, and Eugenol

Thin layer chromatography of Curcumin and Eugenol was performed.

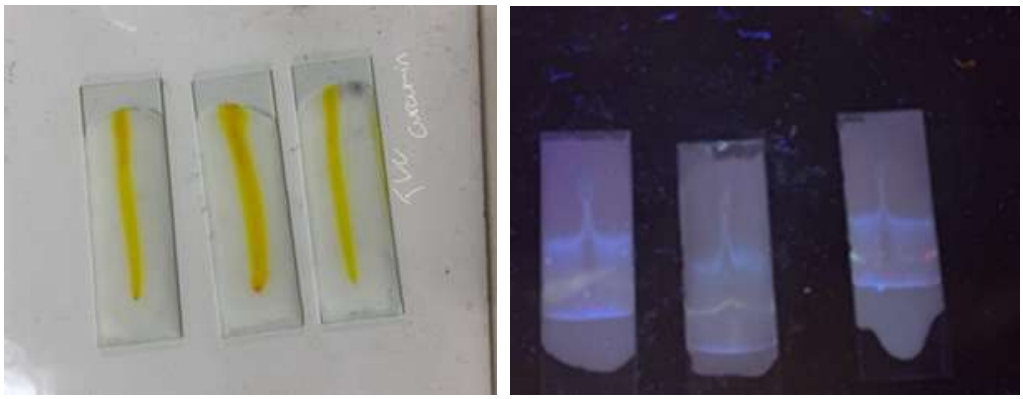

Fig 3.1 TLC of Curcumin and Eugenol

The Retention factor of Eugenol was found to be 0.6 and Curcumin was found to be 0.95.

3.2 *In Vitro* denaturation method (Albumin method)

The *in-vitro* denaturation method was carried out for Curcumin, Eugenol and a combination containing the two in equal proportions while Indomethacin was used as standard. The results are characterized below.

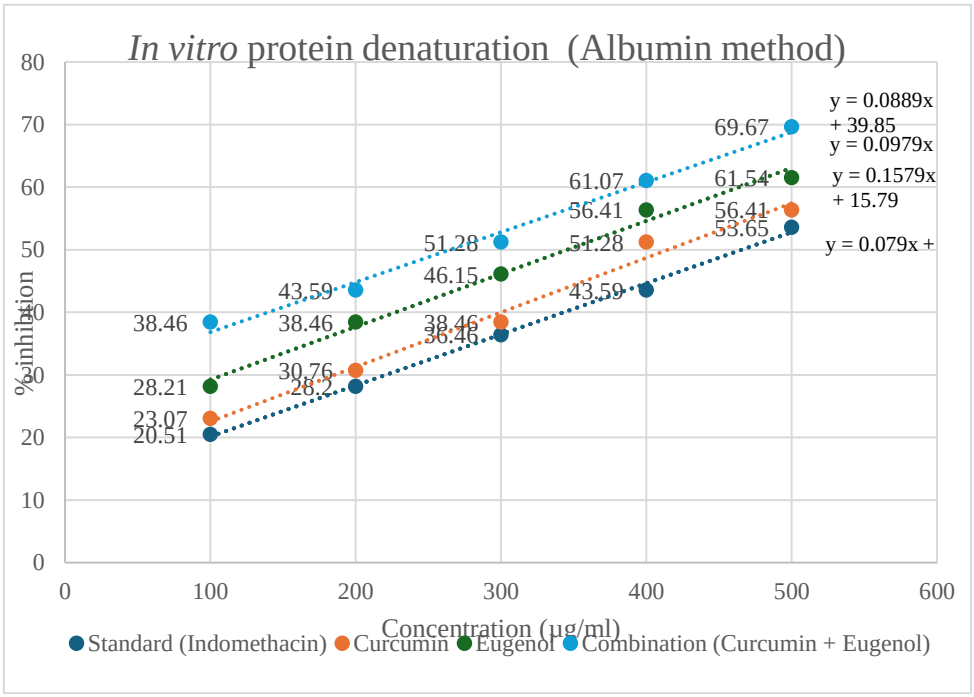

Figure 3.2 Percentage inhibition of *In Vitro* protein denaturation

Table 3.2 Percentage inhibition of *In Vitro* protein denaturation

Concentration (µg/mL)	Standard (Indomethacin) %	Curcumin %	Eugenol %	Combination (Curcumin + Eugenol) %
100	26.31	31.58	40.21	48.74
200	34.21	47.37	50	57.63
300	42.10	55.89	57.89	65.16
400	52	65.79	68.42	75.05
500	59.89	71.05	76.68	81.32

IC_{50} (µg/mL)	Standard (Indomethacin)	Curcumin	Eugenol	Combination (Curcumin + Eugenol)
	400.89	216.6	200	114.13

Table 3.2 IC_{50} values of *In Vitro* protein denaturation

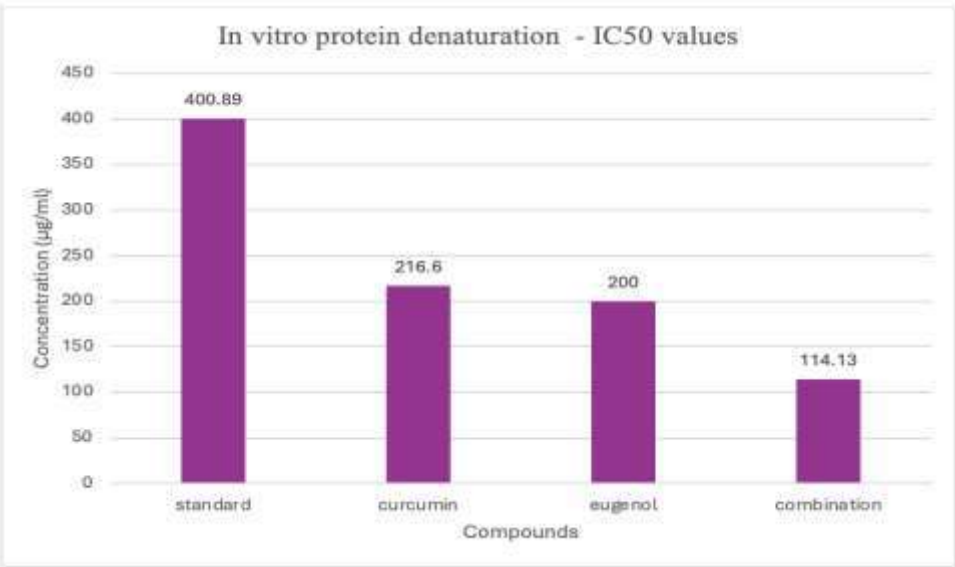

Figure 3.3 IC_{50} values of *In Vitro* protein denaturation

3.3 *IN VITRO* anti-proteinase action

The *in-vitro* anti-proteinase method was carried out for Curcumin, Eugenol and a combination containing the two in equal proportions while Indomethacin was used as standard. The results are characterized below

Table 3.3 Percentage inhibition of In Vitro anti-proteinase

Concentration (µg/mL)	Standard (Indomethacin)	Curcumin	Eugenol	Combination (Curcumin + Eugenol)
100	20.51	23.07	28.21	38.46
200	28.2	30.76	38.46	43.59
300	36.46	38.46	46.15	51.28
400	43.59	51.28	56.41	61.07
500	53.65	56.41	61.54	69.67

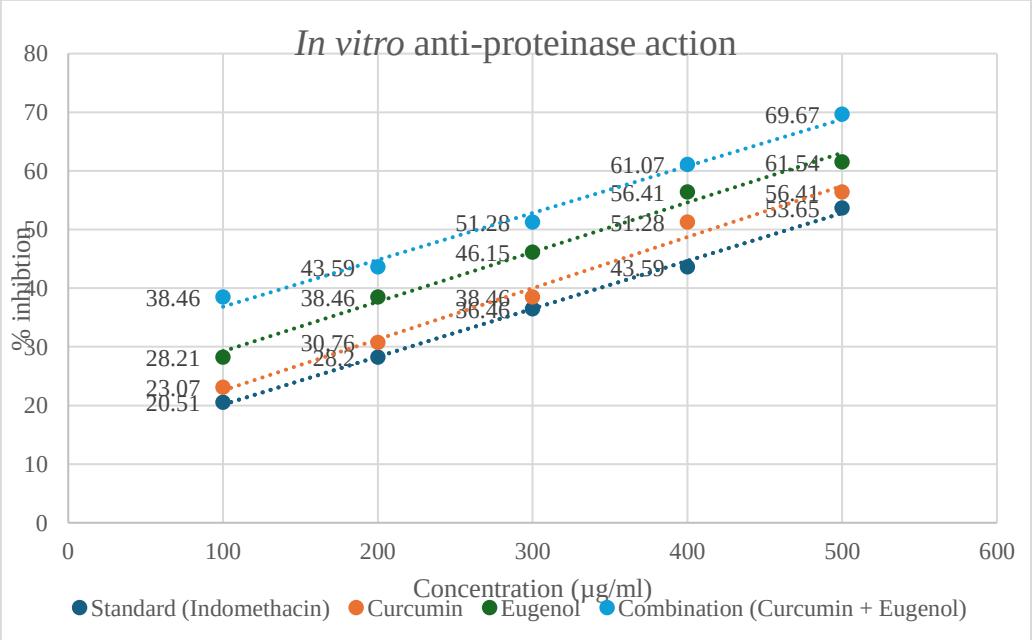

Figure 3.4 Percentage inhibition of *In Vitro* anti-proteinase

Table 3.4 IC50 values of In Vitro anti-proteinase activity

IC_{50} (µg/mL)	Standard (Indomethacin)	Curcumin	Eugenol	Combination (Curcumin + Eugenol)
	483.34	450.21	312.58	280.23

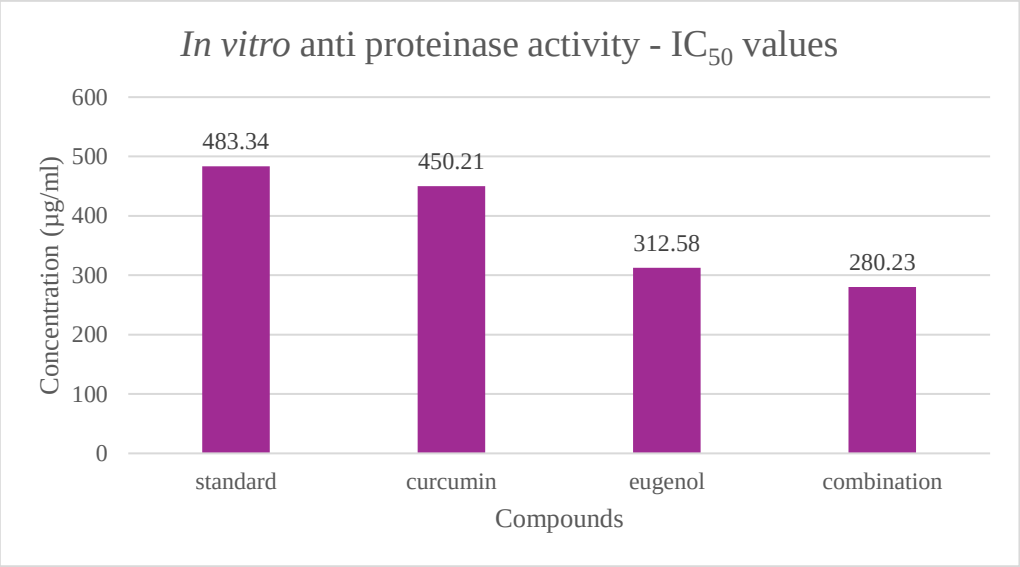

Figure 3.5 IC_{50} values of *In Vitro* anti-proteinase activity

3.4 Molecular Docking

The target proteins selected were COX-2, JAK, NFK, STAT3 and the glide score for Curcumin and Eugenol are displayed below. The higher the negative value, the greater the binding affinity. Curcumin shows comparatively better binding affinity.

Table 3.5 Glide scores

Compound	Glide Score	
	Curcumin	Eugenol
COX-2	-8.9	-6.8
JAK	-7.9	-5.4
NFK	-7	-5.3
STAT3	-7	-5.5

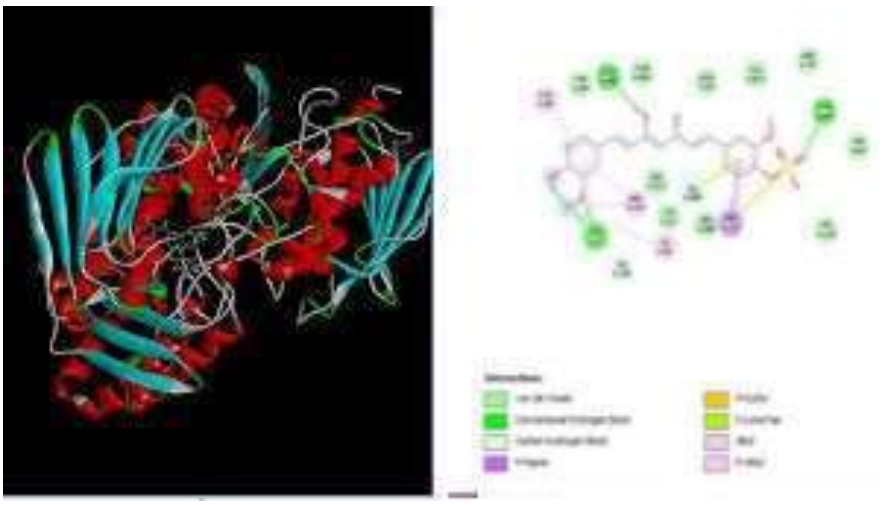

Figure 3.6 Curcumin with COX

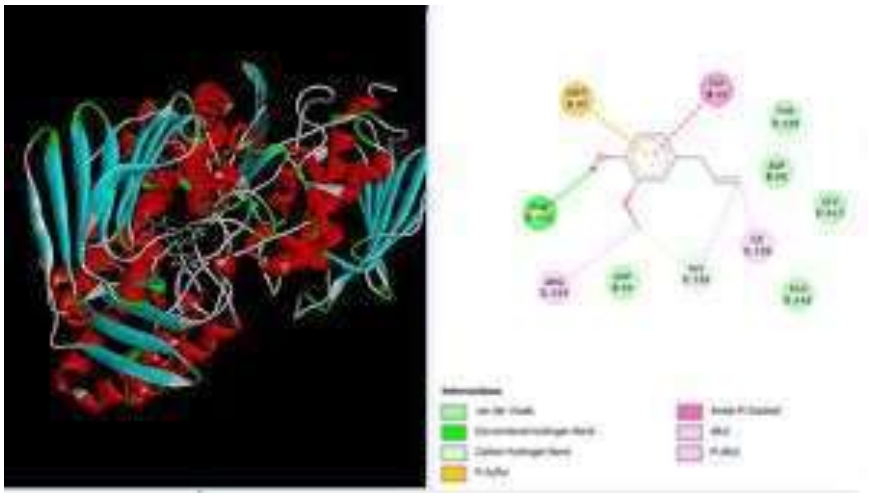

Figure 3.7 Eugenol with COX

DISCUSSION

The current research work assessed the synergistic anti-inflammatory potential of curcumin and eugenol through *In Vitro* experiments and molecular docking analyses. Curcumin, a polyphenolic compound from Curcuma longa, modulates inflammation pathways. Eugenol, a phenolic compound in clove oil, inhibits prostaglandin synthesis and suppresses pro-inflammatory cytokines. The study hypothesized that curcumin and eugenol's distinct mechanisms of action may synergize for enhanced therapeutic benefits. *IN VITRO* assays measured key inflammatory markers, and also includes research on molecular docking elucidated their interaction patterns and binding affinities with inflammatory mediators. The study aimed to establish the anti-inflammatory efficacy of the combination and provide mechanistic insights for novel anti-inflammatory therapeutics leveraging phytochemical synergy. Thin-layer chromatography (TLC) confirmed the identity and purity of curcumin and eugenol before biological assays. The technique separated the compounds, as indicated by distinct retention factor (Rf) values. Curcumin had an Rf of 0.95, while eugenol had an Rf of 0.6. Curcumin traveled farther, suggesting lower polarity. Eugenol migrated shorter, indicating higher polarity. These results match the compounds' chemical structures and properties. Curcumin, a polyphenolic diketone, is less polar than eugenol, which has a methoxy-substituted phenol group that increases its solubility in polar solvents. The TLC solvent system determined compound mobility, and the compounds were differentiated based on their affinities for the stationary and mobile phases. The distinct Rf values validated the isolation and characterization of both compounds. They also confirmed the absence of contamination or overlapping spots, indicating sufficient purity for *IN VITRO* biological assays. Ensuring the chemical identity and separation of curcumin and eugenol was crucial for reliable experimental results, especially when investigating their anti-inflammatory effects. The Rf values aligned with previous literature, reinforcing the method's reproducibility and validity. TLC remains a rapid and cost-effective technique for preliminary compound identification in phytochemical analysis. Though less precise than HPLC or spectroscopic methods, it confirms compound presence and guides further experiments. The TLC findings support the structural differentiation between curcumin and eugenol, ensuring the biological relevance of subsequent assays as the compounds are chemically distinct and resolved. Inflammation disrupts protein structure and function, making protein denaturation inhibition a key anti-inflammatory marker. This reseach evaluated the anti-inflammatory potential of curcumin and eugenol individually and in combination using a protein denaturation assay. Both curcumin and eugenol inhibited protein denaturation dose-dependently, suggesting their anti-inflammatory potential. Interestingly, their combined effect was significantly enhanced compared to their individual effects and the standard anti-inflammatory drug, indomethacin, across all tested concentrations. At the highest concentration (500 µg/mL), the combination achieved an impressive inhibition rate of 81.32%, higher than eugenol alone (76.68%), curcumin alone (71.05%), and indomethacin (59.89%). The concentration is

indicated by the IC_{50} value is required to reduce 50% of protein denaturation, was significantly lower for the combination (114.13 µg/mL) compared to curcumin (216.6 µg/mL), eugenol (200 µg/mL), and indomethacin (400.89 µg/mL), indicating a more potent inhibitory effect and a strong synergistic interaction between curcumin and eugenol. These findings highlight the superior efficacy of the curcumin and eugenol combination in stabilizing proteins against denaturation, a crucial mechanism in mitigating the inflammatory response and supporting their potential therapeutic application in managing inflammatory conditions. Proteinase enzymes are crucial mediators in tissue degradation and the body's inflammatory response. They break down extracellular matrix components, allowing immune cells to infiltrate affected tissues. This activity is essential for immune defense and tissue remodeling, but dysregulation can result in tissue damage and ongoing inflammation. Inhibition of proteinase enzymes offers anti-inflammatory benefits in conditions with excessive inflammation. In this assay, curcumin and eugenol showed notable inhibitory activity against proteinase enzymes individually. However, they were more effective when combined. At 500 µg/mL, the combination of curcumin and eugenol inhibited proteinase activity by 69.67%, significantly surpassing eugenol alone (61.54%), curcumin alone (56.41%), and even indomethacin (53.65%), a widely used NSAID. The superior inhibitory effect suggests the potential advantage of using these natural compounds together. The IC_{50} value, a measure of the concentration required to reduce 50% of enzyme activity, was lower for the combination at 280.23 µg/mL. This indicates higher potency compared to curcumin (450.21µg/mL), eugenol (312.58 µg/mL), and indomethacin (483.34 µg/mL). A smaller dose is needed to achieve the desired inhibitory effect, potentially reducing side effects and improving safety in therapeutic applications. Curcumin and eugenol interact synergistically, enhancing their anti-inflammatory effects by suppressing enzymatic degradation pathways. This suggests promising prospects for developing combined curcumin-eugenol formulations as natural and safer alternatives to traditional pharmaceutical drugs. To gain deeper perception into the molecular mechanisms underlying the observed synergistic outcome, comprehensive *in silico* molecular docking studies were conducted targeting pivotal proteins involved in inflammatory pathways. The selected targets—Cyclooxygenase-2 (COX-2), Janus kinase (JAK), Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-κB) and Signal Transducer and Activator of Transcription 3 (STAT3)—were chosen based on their well-documented roles in mediating inflammatory responses, cytokine signaling, and regulating the pro-inflammatory genes transcription. Curcumin exhibited superior binding affinities across targets compared to eugenol, as evidenced by lower Glide scores, indicating a stronger predicted binding. Curcumin's enhanced binding efficiency is attributed to its polyphenolic structure, facilitating extensive hydrogen bonding and hydrophobic interactions within protein active sites, likely contributing to its potent inhibitory effects on inflammatory mediators. Eugenol, despite lower binding affinities, retains significant bioactivity due to its simpler aromatic structure, enabling modulatory interactions that influence protein function differently from curcumin. Eugenol's known bioactivity may impact specific enzymatic activities and signaling pathways, albeit to a lesser extent. The simultaneous presence of curcumin and eugenol orchestrates a broad-spectrum modulation of the inflammatory signaling network. Curcumin's robust inhibition of key transcription factors and kinases, coupled with eugenol's potential to diminish enzyme activities or disrupt ROS signaling, suggests a complementary mode of action. This dual-target approach enhances anti-inflammatory efficacy *in vitro*. The synergistic effect underscores the potential therapeutic advantage of multi-target strategies in managing inflammatory conditions. The synergistic anti-inflammatory potential of curcumin and eugenol surpasses their individual efficacy. Comprehensive anti-inflammatory assays show significantly lower IC_{50} values and higher inhibition percentages with the combined treatment. This suggests a synergistic interaction, not a simple additive effect.

CONCLUSION

Empirical evidence from *in vitro* assays, together with protein denaturation and proteinase inhibition studies, consistently confirms the potent anti-inflammatory synergy of the curcumin-eugenol combination. Molecular docking analyses elucidate their

cooperative interactions. This study underscores the remarkable synergistic efficacy of curcumin and eugenol, establishing a solid foundation for *in vivo* investigations and advanced formulation development. These insights open new avenues for developing novel phytotherapeutic interventions. Future research can optimize therapeutic formulations and explore clinical applications of curcumin and eugenol in inflammation management. This combination holds promise for advancing anti-inflammatory therapeutics with a natural, effective, and safe profile.

REFERENCE

1. Medzhitov R. Origin and physiological roles of inflammation. Nature. 2008 Jul 24;454(7203):428–35. doi:10.1038/nature07201. PMID: 18650913.
2. Abbas AK, Lichtman AH, Pillai S. Basic Immunology: Functions and Disorders of the Immune System. 6th ed. Philadelphia: Elsevier; 2019.
3. Vane JR, Botting RM. Mechanism of action of anti-inflammatory drugs. Int J Tissue React. 1998;20(1):3–15. PMID: 9654425.
4. Koeberle A, Werz O. Multi-target approach for natural products in inflammation. Drug Discov Today. 2014 Dec;19(12):1871–82. doi: 10.1016/j.drudis.2014.06.014. PMID: 24997384.
5. Aggarwal BB, Harikumar KB. Potential therapeutic effects of curcumin, the anti-inflammatory agent, against neurodegenerative, cardiovascular, pulmonary, metabolic, autoimmune and neoplastic diseases. Int J Biochem Cell Biol. 2009 Jan;41(1):40–59. doi: 10.1016/j.biocel.2008.06.010. PMID: 18662800.
6. Gupta SC, Patchva S, Aggarwal BB. Therapeutic roles of curcumin: lessons learned from clinical trials. AAPS J. 2013 Jan;15(1):195–218. doi:10.1208/s12248-012-9432-8. PMID: 23288627.
7. Daniel AN, Sartoretto SM, Schmidt G, Caparroz-Assef SM, Bersani-Amado CA, Cuman RK. Anti-inflammatory and antinociceptive activities of eugenol essential oil in experimental animal models. Rev Bras Farmacogn. 2009;19(1):212–17. doi:10.1590/S0102-695X2009000100036.
8. Akella S, Akella R, Jampana A. A review on synergistic anti-inflammatory activity of natural products: curcumin and eugenol. J Pharmacogn Phytochem. 2022;11(3):1674–80.
9. Yadav RNS, Agarwala M. Phytochemical analysis of some medicinal plants. J Phytol. 2011;3(12):10–14.
10. Sakat S, Juvekar AR, Gambhire MN. In vitro antioxidant and anti-inflammatory activity of methanol extract of Oxalis corniculata Linn. Int J Pharm Pharm Sci. 2010;2(1):146–55.
11. Shinde UA, Phadke AS, Nair AM, Mungantiwar AA, Dikshit VJ, Saraf MN. Membrane stabilizing activity—a possible mechanism of action for the anti-inflammatory activity of Cedrus deodara wood oil. Fitoterapia. 1999 Jun;70(3):251–57. PMID: 10353184.

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