



Propolis and Hydroxyapatite Combination for Dentin Repair: Bioactivity, Target Prediction, and SAR Analysis

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ABSTRACT

Preserving dental pulp vitality remains a critical challenge in restorative dentistry, particularly when managing deep carious lesions with potential pulpal exposure. In this study, a biofunctional approach is introduced by combining propolis, a natural resinous compound rich in bioactive flavonoids, with hydroxyapatite (HA), a bioceramic material known for its mineralization and osteoinductive properties. The aim is to develop a dual-function pulp capping system capable of both suppressing inflammation and promoting dentin regeneration. Using a structure–activity relationship (SAR)-based prediction model, key compounds in propolis were identified to exhibit strong affinity for pro-inflammatory mediators such as TNF- α and IL-1 β , while also modulating angiogenic factors like VEGFA. Hydroxyapatite enhances these effects by facilitating ionic and hydrogen bonding with functional groups of propolis, thereby improving the stability and delivery of these compounds at the pulp–dentin interface. Network pharmacology analysis further reveals the composite's multi-target potential, with regulatory effects across immune and regenerative pathways, including the NF- κ B and TGF- β 1 signaling cascades. This study demonstrates that the molecular synergy between HA and propolis supports a bioactive microenvironment conducive to pulp healing, with significant implications for the rational design of next-generation biomaterials for vital pulp therapy.

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Keywords: Propolis, Hydroxyapatite, Pulp capping, Inflammatory, TNF- α , Network pharmacology

Introduction

Dental caries remains a pervasive global health issue, representing the most prevalent chronic disease affecting both children and adults.^{1,2} As a multifactorial infection primarily caused by acidogenic bacteria, it progressively demineralizes the calcified structures of the tooth, ultimately compromising the structural integrity of enamel and dentin. Recent global epidemiological data underscore the alarming prevalence of untreated caries in permanent teeth, with more than 2 billion cases reported in 2019 alone, reflecting a 46% increase since 1990 and confirming dental caries as the most widespread human condition worldwide.^{3,4} Despite advances in restorative dentistry, maintaining pulp vitality in teeth affected by deep carious lesions remains a formidable clinical challenge. Pulp capping, a conservative and biologically driven treatment modality, seeks to preserve the vitality of the dental pulp by controlling inflammation and promoting reparative dentinogenesis.⁵

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Among various pulp capping materials, hydroxyapatite (HA) has garnered significant attention due to its chemical similarity to native mineralized tissues, exceptional biocompatibility, and osteoinductive properties.^{6,7} HA can form a strong physicochemical bond with dentin, facilitating tissue integration and promoting healing. However, despite these advantages, its intrinsic limitations, including poor antimicrobial activity, lack of bioresponsiveness under inflammatory conditions, and limited capacity to modulate immune responses, hinder its ability to induce optimal pulp healing and complete dentin bridge formation.⁸ To address these shortcomings, there is growing interest in incorporating natural bioactive compounds with immunomodulatory and antimicrobial properties. Propolis, a resinous substance produced by *Apis mellifera*, emerges as a promising adjunct in pulp therapy. Rich in flavonoids, phenolic acids, and waxes, propolis exhibits broad-spectrum biological activities, including antioxidant, anti-inflammatory, antibacterial, antifungal, and tissue-regenerative effects.^{9–11} It has been shown to modulate both innate and adaptive immune responses by downregulating pro-inflammatory cytokines such as TNF- α and TLR2, while upregulating anti-inflammatory mediators including IL-10 and TGF- β 1.^{12–14} Additionally, the physicochemical interaction of propolis with dentin, characterized by the formation of protective crystalline deposits within dentinal tubules, suggests its potential to enhance sealing ability and promote localized repair. Despite promising findings from in vivo and in vitro studies, a comprehensive mechanistic understanding of how the combination of hydroxyapatite and propolis synergistically contributes to dentin regeneration remains limited. In this context, computational pharmacology tools offer an innovative avenue for unraveling the complexity of multi-component systems. Structure–Activity Relationship (SAR) modeling enables the prediction and optimization of bioactive molecular structures relevant to dentin repair.^{15,16} To our

knowledge, no previous study has systematically integrated SAR modeling and network pharmacology to elucidate the synergy of hydroxyapatite and propolis in the context of dentin pulp regeneration. Therefore, this study aims to investigate the therapeutic synergy between hydroxyapatite and propolis in dentin regeneration through a multi-dimensional approach. By integrating SAR-based bioactivity prediction with network pharmacology analysis, key protein targets and biological pathways involved in pulp healing are identified. This strategy is expected to provide mechanistic insights and establish a theoretical framework for the rational development of next-generation pulp capping materials with enhanced clinical efficacy.

Materials and Methods

Propolis Profiling

The bioactive profiling of propolis was initiated by identifying key phytochemical constituents based on previous studies, specifically referring to Zhang et al.¹⁷ Each compound reported in the literature was systematically searched using the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>) to retrieve its molecular identity. For each compound, the SMILES (Simplified Molecular Input Line Entry System) notation was extracted to enable standardized digital representation for in silico modeling.¹⁸ Additionally, 3D molecular structures were downloaded in SDF or MOL formats and subsequently optimized using molecular modeling software to ensure accurate conformational geometry. This step ensured the readiness of each compound for downstream computational analyses involving activity prediction and target mapping.

Prediction of Bioactive Compound Activity Using Structure-Activity Relationship (SAR) Approach

To predict the potential biological activities of the propolis-derived compounds, the PASS (Prediction of Activity Spectra for Substances) tool was utilized through the WAY2DRUG platform (<http://www.pharmaexpert.ru/passonline/predict.php>). The PASS algorithm uses structure-activity relationship (SAR) modeling to compare the chemical structure of input compounds with a large training set of bioactive substances. Output values are expressed as Pa (Probability to be Active), ranging from 0 to 1. A Pa score > 0.7 indicates a high likelihood of the compound possessing a specific biological activity, such as anti-inflammatory effects. For screening purposes, a Pa cutoff value of 0.5 was employed, balancing sensitivity with specificity in activity prediction. Compounds such as apigenin, chrysin, ferulic acid, isoferulic acid, caffeic acid, and tectochrysin were identified as top candidates based on their high predicted scores in anti-inflammatory, TNF inhibition, NF-κB suppression, wound healing, and angiogenesis-related activities.

Protein Target Prediction

Following SAR-based activity screening, molecular target prediction was performed using the Similarity Ensemble Approach (SEA) (<https://sea.bkslab.org/>), a chemoinformatics tool that maps compounds to putative protein targets based on chemical similarity. SMILES representations were input into the SEA platform, and the resulting targets were filtered using a p-value < 0.05 and a minimum Tanimoto coefficient > 0.4. The predicted targets were then functionally annotated using the DAVID Bioinformatics Resources (<https://david.ncifcrf.gov/>),¹⁹ focusing on Gene Ontology (GO) biological processes and KEGG pathway classifications, with statistical significance set at p < 0.05. This enabled the identification of enriched pathways, notably those involved in inflammatory responses, NF-κB signaling, angiogenesis, and tissue regeneration, all of which are critical in the application of propolis for therapeutic pulp capping.

Network Pharmacology Analysis

To further elucidate the molecular mechanisms and interactions among the predicted protein targets, a network pharmacology approach was undertaken. Protein-protein interaction (PPI) analysis was conducted using the STRING database version 12.0 (<https://string-db.org/>), with

the species set to *Homo sapiens* and a minimum required confidence score of 0.9 to ensure high reliability of the interactions. The resulting interaction dataset was exported in TSV format and visualized in Cytoscape version 3.10, a platform for biological network integration and analysis. Topological metrics, particularly degree centrality, were calculated to identify hub proteins with pivotal roles in the network. Proteins such as TP53, TNF, STAT3, IL6, and SRC exhibited the highest degree scores, indicating their regulatory significance in inflammation, angiogenesis, and dentinogenesis. These insights provide a systems-level understanding of how propolis may exert therapeutic effects through multi-target modulation, supporting its potential use in regenerative dental therapies.

Results and Discussion

Propolis Profile

To elucidate the molecular constituents underpinning the pharmacological potential of propolis, a systematic compound profiling was conducted based on literature-reported phytochemicals, as referenced in Zhang et al. (2023).¹⁷ Rather than relying on direct chromatographic or spectrometric identification, this profiling utilized secondary data obtained from previously published LC-MS analyses. Each compound was subsequently traced and validated through the PubChem database, from which molecular identities were retrieved, including canonical SMILES strings and three-dimensional structural formats (SDF or MOL files), enabling their readiness for in silico modeling workflows.

A total of 20 bioactive compounds were compiled, spanning multiple phytochemical classes such as flavonoids (e.g., quercetin, apigenin, chrysin, kaempferol), phenolic acids (e.g., caffeic acid, ferulic acid, chlorogenic acid), and diterpenoid derivatives (e.g., artemisinin). These compounds are consistently associated with a broad range of pharmacological functions, notably anti-inflammatory, antioxidant, and tissue-regenerative activities, properties critical for the envisioned application of propolis in dental pulp capping therapies.

Each compound's molecular fingerprint was standardized via SMILES encoding to ensure compatibility with cheminformatics tools for downstream structure-based activity prediction and protein target mapping. Notably, compounds such as apigenin, chrysin, caffeic acid, ferulic acid, and tectochrysin, all of which are polyphenolic in nature, are recognized for their robust radical-scavenging capacity and their modulatory effects on key signaling pathways, including NF-κB and TNF-α.

In addition to organic phytochemicals, hydroxyapatite (HA), as illustrated in Figure 1, a calcium phosphate biomineral (CID 14781), was included in the profiling as a reference scaffold relevant to bone and dentin regeneration. Although HA is inorganic, its integration into the analysis offers comparative insight into potential synergistic interactions between propolis compounds and mineral matrices during therapeutic pulp capping.

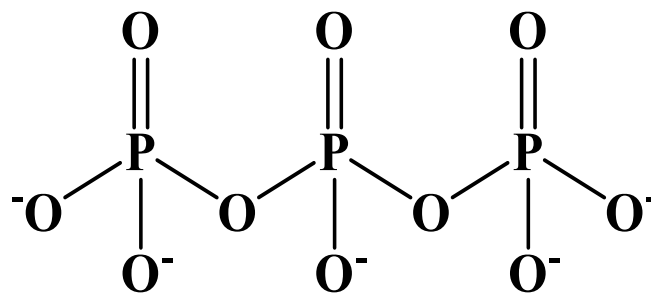


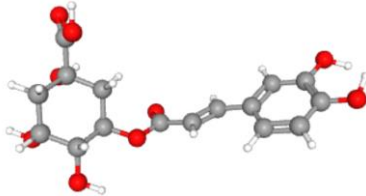
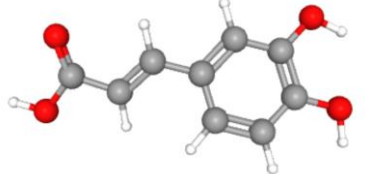
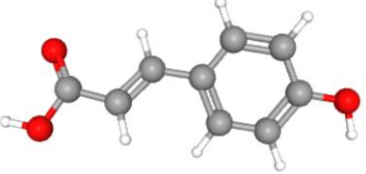
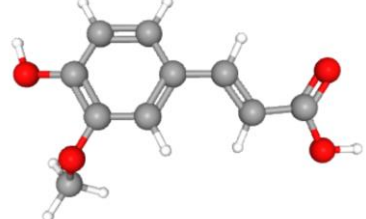
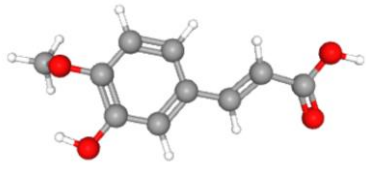
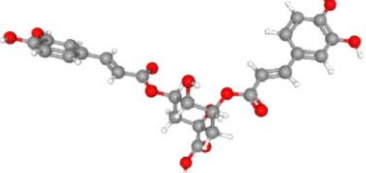
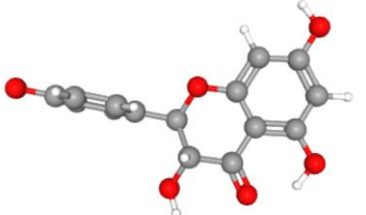
Figure 1: Structure of hydroxyapatite (HA)

A summary of all identified compounds, including their PubChem CIDs, SMILES representations, and structural availability, is presented in Table 1. This molecular-level inventory forms the foundation for subsequent computational modeling efforts, including SAR-based activity prediction, protein target mapping, and network pharmacology elucidation.

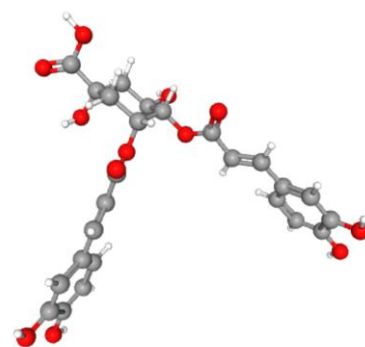
By aligning literature-reported chemical constituents with public compound databases and computational tools, this profiling approach bridges traditional phytochemical knowledge with modern systems pharmacology methodologies. This strategy facilitates not only high-

throughput screening of therapeutic activities but also enables mechanistic inference at the molecular network level, advancing our understanding of propolis as a multifunctional bioactive material.

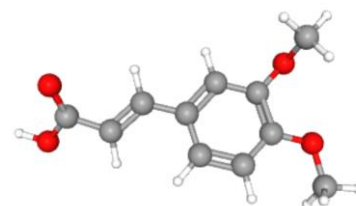
Table 1: Propolis bioactive compounds¹⁷

Compound Name	CID	Canonical_SMILES	3D Structure
chlorogenic acid	1794427	<chem>C1C(C(C(CC1(C(=O)O)O)OC(=O)C=CC2=C(C=C(C=C2)O)O)O)O</chem>	
caffeic acid	689043	<chem>C1=CC(=C(C=C1C=CC(=O)O)O)O</chem>	
p-coumaric acid	637542	<chem>C1=CC(=CC=C1C=CC(=O)O)O</chem>	
ferulic acid	445858	<chem>COC1=C(C=CC(=C1)C=CC(=O)O)O</chem>	
isoferulic acid	736186	<chem>COC1=C(C=C(C=C1)C=CC(=O)O)O</chem>	
isochlorogenic acid A	6474310	<chem>C1C(C(C(CC1(C(=O)O)O)OC(=O)C=CC2=C(C=C(C=C2)O)O)O)OC(=O)C=CC3=CC(=C(C=C3)O)O</chem>	
aromadendrin	122850	<chem>C1=CC(=CC=C1C2C(C(=O)C3=C(C=C(C=C3O)O)O)O)O</chem>	

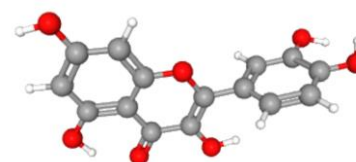
isochlorogenic acid C 6474309 C1C(C(C(CC1(C(=O)O)O)OC(=O)C=CC2=C(C(=C(C=C2)O)O)OC(=O)C=CC3=CC(=C(C=C3)O)O)O



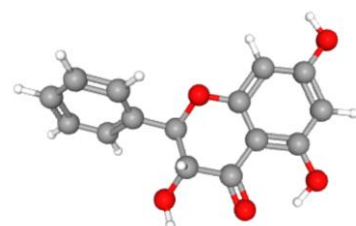
3,4-dimethoxycinnamic acid 717531 COC1=C(C=C(C=C1)C=CC(=O)O)OC



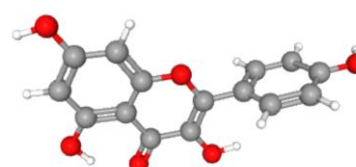
quercetin 5280343 C1=CC(=C(C=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O



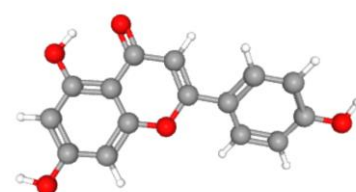
pinobanksin 73202 C1=CC=C(C=C1)C2C(C(=O)C3=C(C=C(C=C3O2)O)O)O



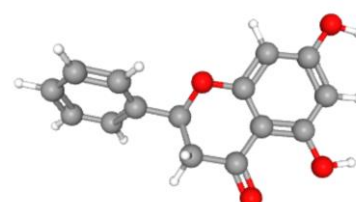
kaempferol 5280863 C1=CC(=CC=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O)O

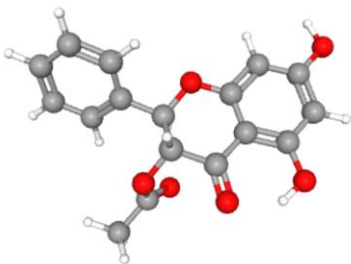
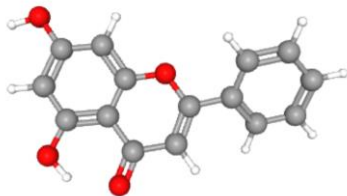
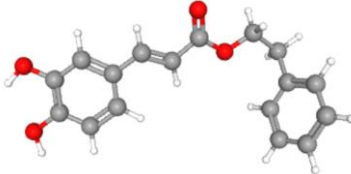
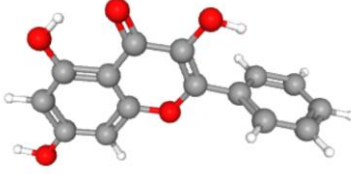
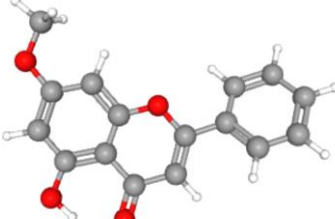
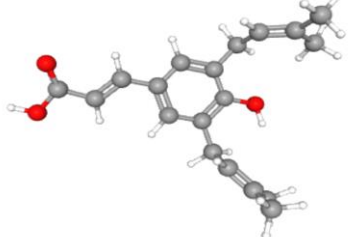


apigenin 5280443 C1=CC(=CC=C1C2=CC(=O)C3=C(C=C(C=C3O2)O)O)O



pinocembrin 68071 C1C(OC2=CC(=CC(=C2C1=O)O)O)C3=CC=CC=C3



pinobanksin acetate	3-O-	148556	<chem>CC(=O)OC1C(OC2=CC(=CC(=C2C1=O)O)O)C3=CC=CC=C3</chem>	
chrysin		5281607	<chem>C1=CC=C(C=C1)C2=CC(=O)C3=C(C=C(C=C3O2)O)O</chem>	
caffeic acid phenethyl ester		5281787	<chem>C1=CC=C(C=C1)CCOC(=O)C=CC2=CC(=C(C=C2)O)O</chem>	
galangin		5281616	<chem>C1=CC=C(C=C1)C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)O</chem>	
tectochrysin		5281954	<chem>COC1=CC(=C2C(=C1)OC(=CC2=O)C3=CC=CC=C3)O</chem>	
artepillin C		5472440	<chem>CC(=CCC1=CC(=CC(=C1O)CC=C(C)C)C=C(C(=O)O)O)C</chem>	

Analysis of Bioactive Compound Activity in Propolis Using Structure–Activity Relationship (SAR) Approach

To predict and rationalize the potential pharmacological roles of propolis-derived bioactive compounds, a computational Structure, Activity Relationship (SAR) approach was employed using the Prediction of Activity Spectra for Substances (PASS) platform. This AI-driven tool enables high-throughput screening of small molecules by evaluating their structural similarity to a comprehensive database of known pharmacologically active compounds, encompassing over 4,000 types of biological activity.

Each compound, previously profiled and structurally standardized via SMILES encoding, was subjected to activity prediction using the WAY2DRUG PASS online interface. The output included Pa values

(Probability “to be active”) for a wide spectrum of biological functions, with Pa > 0.5 considered moderately predictive and Pa > 0.7 indicating high confidence in biological relevance. This threshold balances specificity and sensitivity, which is particularly important in evaluating multifunctional natural products.

The analysis revealed a distinct activity pattern across the propolis-derived compounds. Notably, pinobanksin 3-O-acetate, artepillin C, and apigenin emerged as top candidates with strong predicted anti-inflammatory activity (Pa = 0.83, 0.73, and 0.64, respectively) and TNF expression inhibition (Pa = 0.64–0.82). These activities are highly relevant to inflammation resolution and immune modulation, key processes in tissue regeneration and pulp capping.

Furthermore, several compounds showed moderate to high predicted probabilities for inhibiting the transcription factor NF- κ B, a central regulator in the inflammatory cascade. Tectochrysin (Pa = 0.43), chrysin (Pa = 0.44), and ferulic acid (Pa = 0.41) are characterized by conjugated ring systems and hydroxyl groups, features commonly linked with NF- κ B inhibition through suppression of I κ B kinase activity.

Predictions also indicated angiogenic stimulation and wound-healing activity, two essential components of reparative dentinogenesis. Apigenin and tectochrysin exhibited Pa values > 0.5 for angiogenesis

stimulation, supporting their potential to promote neovascularization in damaged pulp tissue. Conversely, pinobanksin 3-O-acetate and artemillin C had lower wound healing scores, suggesting more targeted rather than broad-spectrum bioactivity.

By averaging activity scores across five therapeutic axes, anti-inflammatory, TNF inhibition, NF- κ B inhibition, wound healing, and angiogenesis, compound ranking and prioritization were established. As shown in Table 2, apigenin, chrysin, ferulic acid, isoferulic acid, caffeic acid, and tectochrysin emerged as top multifunctional agents, displaying consistent and robust activity profiles.

Table 2: Prediction Score Bioactive Compound Activity in Propolis

Compound Name	Prediction Score					
	Anti-inflammatory	TNF expression inhibitor	Transcription factor NF- κ B inhibitor	Wound healing agent	Angiogenesis stimulant	Average
apigenin	0.64	0.61	0.46	0.26	0.55	0.50
chrysin	0.64	0.58	0.44	0.26	0.55	0.49
ferulic acid	0.60	0.82	0.41	0.19	0.40	0.48
isoferulic acid	0.60	0.82	0.41	0.19	0.40	0.48
caffeic acid	0.65	0.77	0.34	0.23	0.41	0.48
tectochrysin	0.61	0.60	0.43	0.23	0.52	0.48
pinobanksin 3-O-acetate	0.83	0.64	0.52	0.00	0.30	0.46
p-coumaric acid	0.64	0.74	0.31	0.26	0.34	0.46
3, 4-dimethoxycinnamic acid	0.60	0.77	0.32	0.21	0.35	0.45
artepillin C	0.73	0.70	0.51	0.00	0.22	0.43
aromadendrin	0.77	0.52	0.42	0.00	0.34	0.41
kaempferol	0.68	0.48	0.29	0.17	0.40	0.40
galangin	0.69	0.45	0.27	0.18	0.40	0.40
caffeic acid phenethyl ester	0.54	0.60	0.24	0.26	0.34	0.40
quercetin	0.69	0.50	0.33	0.00	0.42	0.39
pinobanksin	0.71	0.50	0.40	0.00	0.33	0.39
chlorogenic acid	0.60	0.52	0.22	0.18	0.32	0.37
isochlorogenic acid A	0.66	0.55	0.21	0.00	0.34	0.35
pinocembrin	0.68	0.48	0.27	0.00	0.30	0.35
isochlorogenic acid C	0.66	0.59	0.19	0.00	0.27	0.34

These findings underscore the polypharmacological nature of propolis constituents. Rather than acting on a single molecular target, these compounds exert a constellation of overlapping effects, making them ideal for complex pathophysiological conditions such as inflamed or necrotic dental pulp. This is especially relevant given the increasing demand for therapeutic materials that not only exhibit biocompatibility but also provide bioactivity capable of directing cellular responses.

Importantly, the SAR-based predictions align with previously reported *in vitro* and *in vivo* findings, supporting the translational value of this *in silico* approach. For example, apigenin and chrysin have been widely documented to downregulate proinflammatory cytokines and enhance collagen matrix formation, while caffeic acid derivatives are known to synergize with mineral matrices to promote odontogenic differentiation. Taken together, the SAR analysis offers a robust theoretical framework for understanding and leveraging the multifaceted biological actions of propolis-derived compounds. These insights serve as a critical foundation for subsequent studies involving target-based molecular docking, network pharmacology analysis, and ultimately, *in vivo* functional validation.

Protein Target Prediction

In order to gain deeper insight into the molecular interactions governed by the bioactive compounds in propolis, a comprehensive protein target prediction was conducted using the Similarity Ensemble Approach (SEA). This methodology enables high-resolution mapping of chemical entities to their most probable protein targets by comparing the structural fingerprints of the compounds with a reference database of ligand–target associations. All SMILES-encoded molecules derived from the previous profiling phase were submitted to the SEA platform, with the filtering parameters set to a p-value threshold below 0.05 and a Tanimoto coefficient greater than 0.4, thereby ensuring a high-confidence target identification landscape.

The SEA-based predictions yielded a total of 926 unique protein targets corresponding to the propolis-derived compounds (Table S1). In parallel, 16 protein targets were associated with hydroxyapatite (HA), which was included in the analysis due to its relevance to dental mineralization and tissue regeneration. Remarkably, 12 protein targets were shared between propolis and hydroxyapatite, including several high-impact regulators such as MAPK3, TP53, TNF, IL6, IL1B, and NOS2 (Figure 2). These overlapping targets suggest converging molecular pathways through which both organic (propolis) and

inorganic (HA) agents may co-modulate biological responses related to inflammation, apoptosis, and regenerative signaling. This intersection reinforces the hypothesis that combining propolis with hydroxyapatite may offer synergistic benefits in therapeutic applications such as pulp capping.

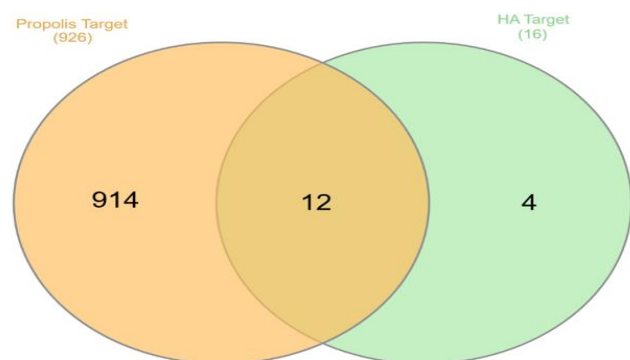


Figure 2: Venn Diagram of Propolis and Hydroxyapatite Targets

The remaining 914 protein targets were exclusively linked to propolis, demonstrating its polypharmacological richness and potential for extensive network engagement within biological systems. To further characterize these targets functionally, an annotation analysis was performed using the DAVID Bioinformatics Resources. This analysis revealed significant enrichment in several biological processes central to the therapeutic mechanism of action of propolis. Most notably, the inflammatory response emerged as the dominant functional category, with 94 targets associated with cytokine regulation, chemokine signaling, and immune cell recruitment (Table 3). Additionally, a large subset of targets was found to participate in the transcriptional regulation of NF- κ B activity, a master regulator of inflammatory gene expression, indicating that propolis may exert its anti-inflammatory effects through upstream interference with nuclear transcription pathways. Other enriched processes included angiogenesis, vascular endothelial cell migration, odontogenesis, wound healing, and bone mineralization, all of which are intricately linked to tissue repair and regeneration.

Table 3: Functional Analysis Target Protein Propolis p-value < 0.05

Terminology	Count	FDR	Genes
inflammatory response	94	1.06E-35	IL22, CD40, CXCL8, CXCL1, IL27, HMGB1, TNF, CXCL2, LTB4R, PYCARD, IKBKB, IL36A, TBK1, IL18RAP, IL36B, ADORA3, PROK2, AKT1, OLR1, IKBKG, CCR4, CCR2, HAVCR2, PTGDR, CHUK, IL1R1, SPHK1, IL13, IL18, CYBA, FOS, MIF, FOXP3, IL17RA, TNFRSF1A, IL1A, IL23A, IL1B, TLR9, CRH, TLR4, IDO1, TLR2, PTGER4, CRP, CEBPB, PTGER2, PTGER3, ALOX15, GATA3, HDAC9, RELA, C3, PRDX5, CCL8, IRAK2, CXCR3, CCL5, CCL4, CCL3, CCL2, NLRP3, IL36RN, S1PR3, CD14, NDP, CCR1, TGFB1, VCAM1, NOS2, PLA2G2A, STAT3, NR1H4, IL36G, SELE, NFKB1, IL1F10, NFKB2, P2RX7, AIMP1, CXCL10, IL6, CD40LG, IL5, AXL, FASN, NOX4, PTX3, HAMP, MYD88, IL17B, IL17A, NFE2L2, NFKBIB
positive regulation of NF- κ B transcription factor activity	30	7.12E-12	CD40, TFRC, PLA2G1B, CAMK2A, NOD2, TNF, RELA, IKBKB, PYCARD, IL18RAP, IRAK2, TNFSF11, NLRP3, IKBKG, CHUK, SPHK1, STAT3, IL18, IRAK3, AR, CD40LG, TRAF6, IL1B, CLOCK, TLR4, MYD88, HSPA1B, LTF, HSPA1A, TLR2
positive regulation of angiogenesis	29	4.65E-08	MIR30A, CD40, CXCL8, SERPINE1, HSPB1, ETS1, FGF2, HIF1A, C3, TERT, CXCR3, KDR, PTK2B, CYP1B1, HMOX1, PLCG1, NOS3, SPHK1, STAT3, MIR27B, MIR21, F3, SIRT1, PGF, RHOB, VEGFA, IL1A, IL1B, NFE2L2
positive regulation of blood vessel endothelial cell migration	16	1.37E-07	MIR30A, CD40, TGFB1, NOS3, MIR200A, HSPB1, HMGB1, ETS1, FGF2, SIRT1, HIF1A, VEGFA, KDR, AKT1, PLCG1, NFE2L2
vascular endothelial growth factor receptor signaling pathway	13	2.00E-07	FOXC2, SRC, FLT4, HSPB1, MAPK14, PGF, PTK2, VEGFA, AXL, KDR, PTK2B, FYN, BCAR1
osteoblast differentiation	19	5.82E-04	MSX2, MYOC, NR1I3, NOG, HSPE1, GLI1, MAPK14, FGF2, SND1, RUNX2, SOX2, FASN, CAT, CCL3, SNAI1, AKT1, ALPL, CTNNB1, SNAI2
wound healing	14	6.62E-041	NOTCH2, SMAD2, TGFB2, CDKN1A, SMAD3, SDC4, ALOX15, NOG, FN1, IGF1, FGF2, ERBB2, RAF1, PPARA
bone mineralization	9	0.033	SMPD3, MMP13, COL1A2, ALOX15, ALPL, PTGS2, HIF1A, IFITM5, RUNX2

odontogenesis of dentin-containing tooth	9	0.033	NGFR, HDAC2, TGFB1, HDAC1, TRAF6, BAX, CTNNB1, TP63, RUNX2
TNF signaling pathway	45	6.42E-18	CEBPB, CSF2, XIAP, CXCL1, PIK3R1, NOD2, PTGS2, TNF, CXCL2, RELA, ICAM1, IKBKB, MAPK9, SOCS3, CASP7, MAPK8, CASP8, CASP3, CCL5, CCL2, AKT1, MAPK1, IKBKG, DNML, MAPK3, JUN, EDN1, VCAM1, CHUK, IL15, MMP3, FOS, MAPK14, SELE, MMP9, NFKB1, TNFRSF1A, NFKBIA, CXCL10, IL6, CREB1, IL1B, FAS, BIRC2, BIRC3
MAPK signaling pathway	72	1.76E-16	RET, FLT3, FLT4, HSPB1, ARRB1, FASLG, ARRB2, FGF2, ELK1, TNF, IGF1R, IKBKB, RPS6KA3, FGF7, RPS6KA2, CASP3, KDR, AKT1, NRTN, IKBKG, PRKACA, PDGFRB, CHUK, IL1R1, PLA2G4A, FOS, NGF, DUSP6, PGF, TNFRSF1A, IL1A, TRAF6, IL1B, DDIT3, MAPT, RAF1, SOS1, MET, TP53, CACNA1A, EGFR, CACNA1H, RELA, MAPK9, MAPK8, ERBB2, MAPK1, CD14, MAP2K5, MAPK3, NGFR, HSPA8, TGFB2, JUN, TGFB1, GADD45B, HSPA1L, EGF, GADD45A, BDNF, IGF1, MAPK14, NFKB1, NFKB2, VEGFA, TAOK3, GDNF, FAS, GRB2, MYD88, HSPA1B, HSPA1A
NF-κB signaling pathway	40	3.11E-16	CD40, CXCL8, XIAP, CXCL1, PTGS2, TNF, CXCL2, RELA, ICAM1, IKBKB, PLA2, CCL4, TNFSF11, IKBKG, PLCG1, CD14, LYN, VCAM1, SYK, GADD45B, PARP1, CSNK2A1, CHUK, IL1R1, GADD45A, NFKB1, TNFRSF1A, NFKB2, NFKBIA, CXCL12, CD40LG, LCK, TRAF6, IL1B, BCL2, TLR4, MYD88, BIRC2, BIRC3, BCL2L1

Several key molecular targets identified in this analysis play central roles in multiple biological contexts. For instance, TNF and TP53 are not only pivotal in regulating inflammation and apoptosis but also serve as convergence points for stress signaling and immune modulation. Similarly, MAPK1 and MAPK3 are integral components of the MAPK signaling cascade, mediating cellular responses to proinflammatory stimuli and growth factors. The identification of IL6, STAT3, and RELA further underscores the breadth of signaling axes engaged by propolis-derived compounds, as these targets are involved in both proinflammatory amplification and tissue remodeling.

In addition to their involvement in inflammatory pathways, several targets were also implicated in promoting odontogenic and angiogenic responses. Proteins such as VEGFA, FGF2, KDR, and TGFB1 are directly involved in neovascularization, endothelial cell proliferation, and extracellular matrix remodeling, events that are critical for restoring vascular integrity in damaged pulp tissue. The concurrent modulation of immune, vascular, and mineralization pathways suggests that propolis functions not as a single-pathway inhibitor but rather as a multi-layered bioactive agent capable of orchestrating complex biological repair processes.

Overall, the SEA-based protein target prediction provides a systems-level perspective on how propolis may exert its therapeutic actions through a multi-target, multi-pathway mechanism. By identifying a large repertoire of biologically relevant targets, many of which overlap with those of hydroxyapatite, this analysis offers compelling molecular evidence for the rational use of propolis in combination with mineral-based scaffolds for regenerative endodontic therapies. The expansive target landscape also sets the stage for integrative analyses through network pharmacology, which may uncover key hub proteins and regulatory circuits driving the observed bioactivities.

The integration of propolis with hydroxyapatite (HA, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) offers a compelling synergistic strategy for pulp capping applications, not only reinforcing biological properties but also facilitating molecular-level interactions that enhance material functionality. The intrinsic presence of phosphate (PO_4^{3-}) and calcium ions (Ca^{2+}) in HA enables the formation of ionic and coordination bonds with functional groups found in the bioactive constituents of propolis, such as phenolic hydroxyl ($-\text{OH}$), carbonyl ($-\text{C}=\text{O}$), and carboxylate ($-\text{COO}^-$) moieties. These interactions are presumed to promote the surface adsorption of

active compounds onto HA and contribute to increased stability and bioavailability in the inflammatory microenvironment of the dental pulp.

Caffeic acid phenethyl ester (CAPE), one of the major active components in propolis, contains aromatic hydroxyl groups and a phenethyl side chain, which are capable of forming hydrogen bonds and engaging in van der Waals interactions with hydroxyl and phosphate groups on the HA surface. This interface not only supports molecular docking to pivotal targets such as tumor necrosis factor- α (TNF- α) and vascular endothelial growth factor A (VEGFA), but also reinforces downstream biological effects. Notably, CAPE's binding to TNF- α may attenuate pro-inflammatory signaling via the NF- κ B pathway, while VEGFA activation promotes neovascularization essential for pulp regeneration.

Moreover, HA contributes to dentin remineralization through sustained release of Ca^{2+} and PO_4^{3-} ions, further enhancing its osteoinductive properties.²⁰ Calcium ions are hypothesized to influence Ca^{2+} -dependent kinase pathways that drive odontoblastic differentiation.²¹ When combined with propolis, which exhibits regulatory effects on inflammatory mediators such as TLR2 and IL-1 β , as evidenced by network pharmacology analysis, the resulting hybrid system offers a dual-mode therapeutic mechanism: inflammation suppression and tissue regeneration promotion. This integrated biofunctional platform positions the propolis–HA composite as a promising candidate for next-generation pulp therapy.

Hydroxyapatite, despite its biomimetic mineral composition, exhibits inherent porosity that compromises its mechanical strength, limiting its standalone efficacy in pulp capping applications. To address this, nanostructured hydroxyapatite has been developed to enhance surface area and bioactivity, facilitating better cellular interactions and biological responses. However, pure hydroxyapatite remains insufficient in promoting essential regenerative processes such as angiogenesis and fibroblast proliferation due to its limited ability to stimulate key growth factors like VEGF.²² Furthermore, hydroxyapatite lacks intrinsic antibacterial and anti-inflammatory properties, which are critical for resolving pulpal inflammation. The incorporation of bioactive agents such as epigallocatechin-3-gallate (EGCG) has been shown to enhance the antibacterial potential of hydroxyapatite and

improve its viscosity, making it more suitable for pulp capping applications.²³

Propolis reduces the secretion of several pro-inflammatory cytokines such as IL-1 β , IL-6, and TNF- α , which play critical roles in immune cell migration and the inflammatory response.²⁴ It also promotes VEGF expression, which is pivotal for neovascularization and pulp vitality, thereby enhancing reparative dentin formation.²⁵ When combined with HA, propolis synergistically elevates anti-inflammatory and pro-regenerative markers such as IL-10, TGF- β , and VEGF, while concurrently downregulating pro-inflammatory cytokines like IL-8, thus supporting an optimal environment for pulp tissue repair and dentinogenesis.¹³

Network Pharmacology Analysis

To comprehensively unravel the systems-level mechanisms through which propolis exerts its therapeutic actions, a network pharmacology approach was employed. Protein-protein interaction (PPI) data were retrieved from the STRING database (v12.0) with a stringent confidence threshold (score > 0.9), ensuring high-fidelity interaction mapping. The resulting interaction network was visualized using Cytoscape v3.10 (Figure 3), where topological analysis, particularly degree centrality, was applied to identify hub proteins. Degree centrality reflects the number of direct interactions a protein maintains within the network, highlighting its regulatory influence. Among the identified nodes, TP53 (degree = 88), TNF (66), STAT3 (57), IL6 (56), and SRC (55) emerged as the top five hub proteins, underscoring their central roles in orchestrating biological responses modulated by propolis.

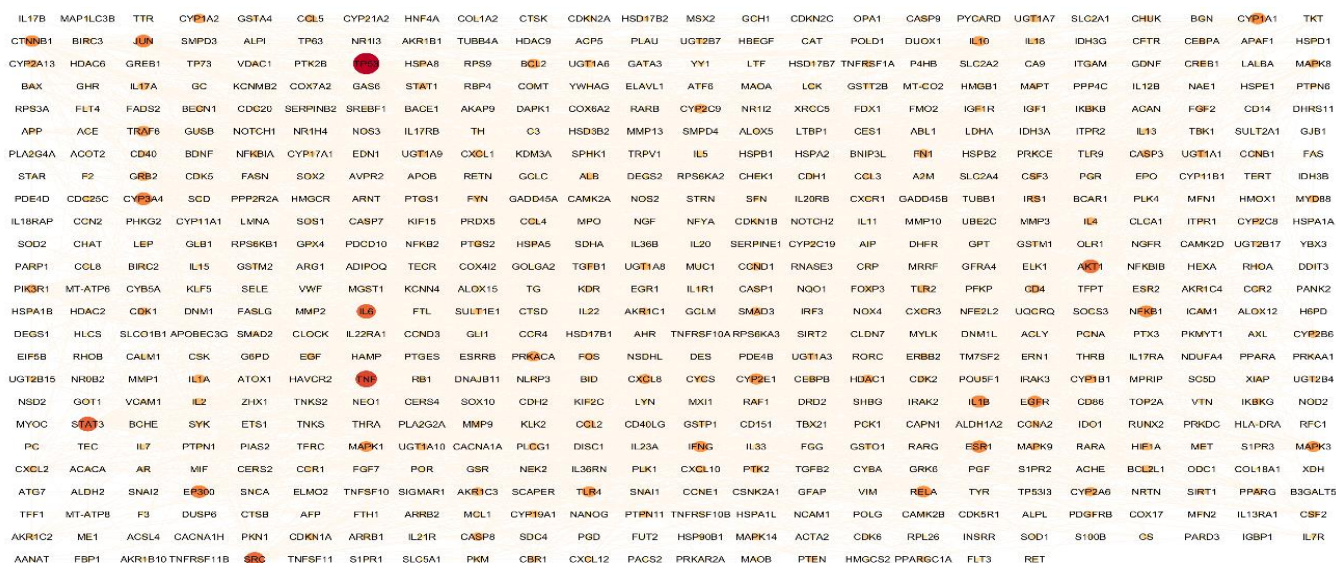


Figure 3: Protein-Protein Interaction Network of Propolis Targets. The larger and darker the color of the node, the more proteins that can be regulated by that node.

TP53, a master regulator of cellular stress responses and apoptosis, anchors the network and suggests that propolis may enhance tissue regeneration by modulating damage control and cell cycle pathways. TNF, a principal mediator of inflammation, exhibited extensive interconnectivity, aligning with the compound's predicted anti-inflammatory activity. STAT3 and IL6, pivotal elements of the JAK/STAT signaling cascade, indicate that propolis may stimulate regenerative responses through immunomodulation and cytokine regulation. Meanwhile, SRC, a key non-receptor tyrosine kinase involved in cell adhesion, migration, and angiogenesis, links propolis activity to vascular remodeling and tissue integration processes.

Further functional network analysis revealed that the identified protein targets collectively participate in three interrelated biological modules: inflammatory response, vascular endothelial cell migration, and odontogenesis. As illustrated in the network visualization (Figure 4), these modules are spatially and functionally interconnected, indicating that propolis does not act through isolated molecular events but instead modulates overlapping biological pathways. This finding supports the modern pharmacological paradigm of polypharmacology, wherein a single compound exerts therapeutic effects through the simultaneous engagement of multiple molecular targets within integrated biological systems.

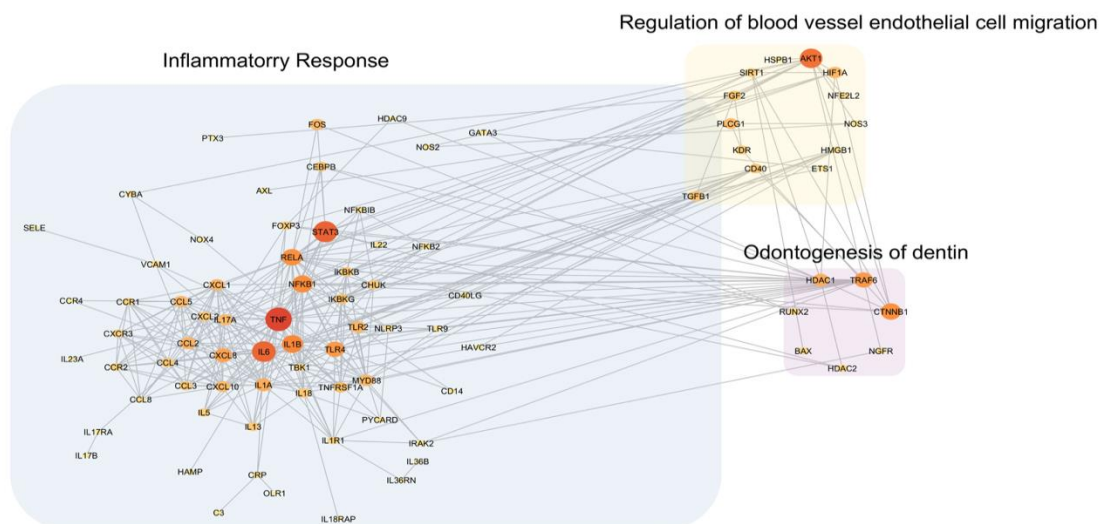


Figure 4: Protein-Protein Interaction Network of Propolis Targets.

Notably, propolis was found to modulate key angiogenic and regenerative regulators, including VEGFA, FGF2, TGF β 1, and KDR, suggesting an active role in promoting neovascularization, a process essential for pulp vitality and reparative dentin formation. The simultaneous targeting of pro-inflammatory pathways (e.g., via RELA, NF κ B1, and CXCL8), endothelial activation (e.g., ICAM1, NOS3), and odontogenic differentiation (e.g., RUNX2, BMP2) reveals propolis as a multifaceted therapeutic agent capable of orchestrating complex regenerative events. Within the context of regenerative endodontics, this capability is especially pertinent, as it implies that propolis functions not merely as a biocompatible adjunct but as a biologically active agent that facilitates the reconstruction of pulp and dentin microarchitecture via activation of angiogenic and odontogenic signaling axes. Overall, the network pharmacology approach employed in this study reveals the systemic complexity of propolis action by linking small-molecule phytochemicals to intricate protein interaction landscapes. The identification of key hub proteins such as TP53, TNF, STAT3, IL6, and SRC as central regulators strengthens the conceptual framework that propolis acts as a molecular orchestrator within both inflammatory and regenerative environments. This methodology not only demonstrates the power of *in silico* modeling in deciphering the mechanistic landscape of natural products, but also provides a robust scientific rationale for the clinical optimization of propolis in pulp capping formulations and broader regenerative therapeutic applications.

Conclusion

This study highlights the synergistic potential of combining propolis and hydroxyapatite as a bioactive pulp capping system with dual functionalities: immunomodulation and tissue regeneration. The chemical interactions between hydroxyapatite's phosphate and calcium ions and the hydroxyl, carbonyl, and carboxyl groups of propolis constituents, particularly caffeic acid phenethyl ester (CAPE), enhance compound stability and enable site-specific delivery. Through Structure–Activity Relationship (SAR) analysis and network pharmacology, this composite system demonstrates significant modulation of inflammatory mediators (e.g., TNF- α , TLR2, IL-1 β) and activation of regenerative pathways (e.g., VEGFA, TGF- β 1). These findings support the development of a targeted, multifunctional biomaterial capable of sustaining pulp vitality, attenuating inflammation, and promoting reparative dentinogenesis. Thus, the propolis–hydroxyapatite hybrid represents a promising, molecularly informed platform for next-generation vital pulp therapies.

Conflict of Interest

The authors declare no conflict of interest

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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