Ursolic Acid and Chlorogenic Acid from *Boehmeria nivea* Drive Aquaporin-5 Upregulation in Salivary Gland Systems

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ABSTRACT

Background: Xerostomia, often linked to radiation therapy and autoimmune disorders, arises from impaired salivary gland function with limited therapeutic options. Aquaporin-5 (AQP5) is a critical water channel protein for salivary secretion, yet it remains underexplored as a pharmacological target.

Objective: To evaluate *Boehmeria nivea*–derived phytocompounds for their potential to modulate AQP5 expression using combined in silico and in vitro approaches.

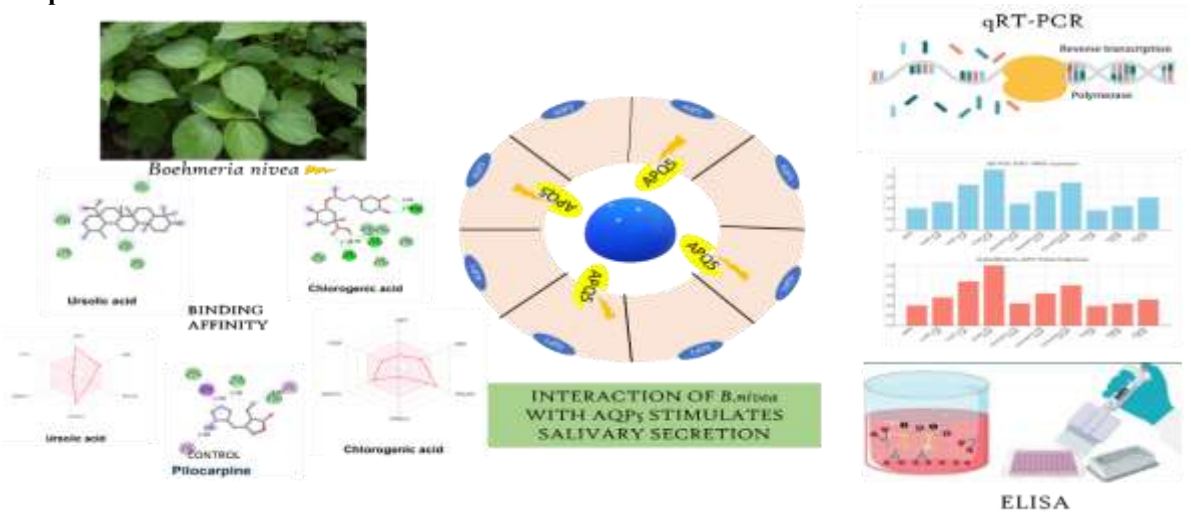
Methods: Phytocompounds were screened for drug-likeness, ADMET properties, and pharmacokinetics. Molecular docking was performed against the crystal structure of human AQP5, and gene interaction analysis was conducted using GeneMANIA. Lead compounds were validated in human salivary gland epithelial cells using qRT-PCR and ELISA/Western blotting.

Results: Ursolic acid and chlorogenic acid showed strong binding affinity to AQP5, interacting with key residues (Leu47, Leu51, Leu163, Leu167, Thr170, Ser160, Gly159, and Gln58) within the active site. Both compounds significantly upregulated AQP5 at the transcriptional (qRT-PCR: ~2–2.8-fold increase) and protein levels (ELISA/Western: ~2.5–2.8-fold increase), with ursolic acid showing the highest potency. Gene interaction analysis further confirmed AQP5's linkage with other salivary gland–related proteins (AQP4, AQP3, AQP7, ELF5).

Conclusion: *B. nivea*–derived phytocompounds, particularly ursolic acid and chlorogenic acid, demonstrate strong AQP5-binding potential and significantly enhance its expression at both mRNA and protein levels. These findings establish a foundation for in vivo validation and the translational development of green therapeutics for xerostomia.

Keywords: Saliva, Genes, Disease, Health, Green products, Environment

Graphical Abstract:



Introduction

Xerostomia (subjective dry mouth) is a disorder characterised by diminished or absent saliva production that impairs taste, mastication, speech, and oral mucosal defence, thereby affecting overall oral health, functional well-being, and quality-of-life outcomes. It arises commonly after head-and-neck radiotherapy, in Sjogren's syndrome, and as an adverse effect of many medications such as antihistamines, antidepressants, antipsychotics, diuretics & opioids (Rodriguez A et al, 2023). Though the radiation techniques have been shifted to Intensity-Modulated Radiation Therapy (IMRT) to minimize radiation exposure to the salivary glands, conventional care—saliva substitutes, sialogogues such as pilocarpine/cevimeline, meticulous oral hygiene, and caries prevention—offers only partial and often short-lived relief, exhibiting the need for innovative, sustainable, and mechanism-driven

therapeutic approaches. Recent advances emphasize a transition toward preventive healthcare strategies, regenerative approaches, and targeted restoration of gland function rather than symptomatic palliation alone, supporting improved health equity, patient-centered care, and sustainable biomedical innovation (DE Felice F et al, 2020; Nathan CO et al, 2023; Kim YJ 2024; Hosseini MS et al, 2024).

Aquaporin-5 (AQP5), the apical water channel of acinar cells, is pivotal for isotonic primary saliva formation; its expression, trafficking, and membrane localization tightly regulate fluid secretion (Soyfoo MS et al, 2018). Dysregulation or mislocalization of AQP5 has been implicated in hyposalivation states, including Sjogren's syndrome, making AQP5 a compelling therapeutic target (Chivasso C et al, 2023). Consequently, pharmacologically modifying AQP5 expression has grown into appropriate therapy approach for improving salivary circulation and reducing the symptoms of xerostomia (Fu L et al, 2024).

Multiple recent advances aim for gland preservation or revival to prevent/cure hyposalivation. However, regenerative approaches have reached human trials: mesenchymal stromal cell injections improved salivary function in randomized studies, while AQP1 gene therapy (AAV2-hAQP1) has progressed to active clinical evaluation (Wang Z et al, 2015; Alevizos I et al, 2017). A recent study also proposes intraglandular delivery of mitochondria-boosting agents such as Coenzyme Q10 (CoQ10) and idebenone, which have shown promise in mitigating radiation effects in animal studies by scavenging reactive oxygen species (ROS), restoring ATP levels, and promoting cell proliferation (Taghizadeh-Hesary F et al, 2023; Tekin YB et al, 2024).

Amid these developments, plant-derived phytocompounds offer eco-friendly alternatives to conventional drugs (Pillai NP et al, 2024). In this context, *Boehmeria nivea* (*B. nivea*), known as ramie, offers a promising natural source of small molecules with anti-inflammatory and antioxidant activities that are relevant to AQP5 stability and acinar health (Lee et al, 2020). Studies have reported that flavonoids such as quercetin and catechins, along with phenolic acids like chlorogenic and caffeic acid, and high dietary fiber extracts, attenuate LPS-induced inflammatory signaling (e.g., p38/JNK) in macrophages, suggesting their potential to mitigate glandular micro-inflammation associated with chronic xerostomia (Sung MJ et al, 2013; Amani et al, 2024). Thus, we aim to employ an in silico pharmacological profiling strategy to identify, characterize, and prioritize *B. nivea*-derived molecules as potential modulators of AQP5 for the development of effective, safe, and sustainable therapeutic options in the management of xerostomia.

Materials and methods

Selection of Phytocompounds:

Seventeen phytochemicals from *B. nivea* were retrieved from the IMPPAT (Indian Medicinal Plants, Phytochemistry and Therapeutics) database. Only compounds with documented presence in *B. nivea* were included. Structures were downloaded in SDF format and converted to PDB using Open Babel for computational analyses.

Evaluation of Drug-Likeness Properties:

Drug-likeness of all compounds was assessed using Lipinski's Rule of Five, Veber's rule, and Ghose filter through the SwissADME web tool. Compounds with favourable oral bioavailability, appropriate molecular weight (<500 Da), logP (<5), and acceptable hydrogen bond donors/acceptors were shortlisted.

ADMET and Pharmacokinetic Analysis:

Pharmacokinetic and toxicity predictions were performed using pkCSM and admetSAR servers. Parameters such as gastrointestinal absorption, blood–brain barrier permeability, cytochrome P450 interactions, hepatotoxicity, and AMES mutagenicity were evaluated. Compounds with favorable ADMET profiles were prioritized for docking studies.

Preparation of Target Protein:

The crystal structure of human aquaporin-5 (AQP5; PDB ID: 3D9S) was retrieved from the RCSB Protein Data Bank. Protein structure was prepared using AutoDock Tools by removing water molecules, adding polar hydrogens, and assigning Kollman charges. The final structure was saved in PDBQT format for docking.

Gene Interaction Network Analysis:

AQP5-associated gene interaction networks were constructed using GeneMANIA (https://genemania.org/). Functional associations, including physical interactions, co-expression, shared pathways, and genetic interactions, were analyzed to determine AQP5's role in salivary gland physiology and its relevance as a therapeutic target.

Molecular Docking Studies:

Molecular docking was performed using AutoDock Vina. The prepared ligands and AQP5 protein were subjected to grid box definition around the active site. Docking scores were recorded as binding energies (kcal/mol), and the optimal binding conformations were selected based on the lowest binding energy and stable ligand–protein interactions. Interaction visualization was performed using Discovery Studio Visualizer and PyMOL.

Chemicals, Cell Culture, and Treatment Conditions:

Ursolic acid, chlorogenic acid, and emodin (≥98% purity) were obtained from a certified supplier. Stock solutions (10–100 mM) were prepared in dimethyl sulfoxide (DMSO) and stored at −20 °C. Fresh working concentrations were diluted in serum-free culture medium immediately before use, ensuring that the final DMSO concentration did not exceed 0.1% (v/v).

Human salivary gland epithelial cells (HSG or A253; ATCC, USA) were maintained in DMEM/F-12 medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin at 37 °C in a humidified incubator with 5% CO_2. Cell lines were authenticated by short tandem repeat (STR) profiling and confirmed to be mycoplasma-free. Cells were seeded to ~70–80% confluence, serum-starved for 12 h, and treated for 24 h with: Ursolic acid (5 and 10 μM), Chlorogenic acid (50 and 100 μM), Emodin (5, 10, and 20 μM), or Vehicle control (0.1% DMSO). Concentrations were selected based on preliminary cytotoxicity assays (MTT and AlamarBlue), ensuring ≥85% viability. Selected exposures were repeated at 48 h in confirmatory experiments. All treatments were performed in biological triplicates (n=3) with technical triplicates.

RNA Extraction and Quantitative RT-PCR:

Total RNA was extracted using TRIzol reagent. RNA quality was confirmed by A_{260}/A_{280} ratio (1.9–2.1). One microgram of RNA was reverse-transcribed to cDNA, and qPCR was performed with SYBR Green Master Mix. Primer sequences:

- AQP5 (human): F 5′-GCTTTGGCATCTACACCCAA-3′; R 5′-CGGTCAGGATGAGGATGAGA-3′
- GAPDH: F 5′-GAAGGTGAAGGTCGGAGT-3′; R 5′ GAAGATGGTGATGGGATTTC-3′

Thermal cycling: 95 °C for 2 min; 40 cycles of 95 °C for 15 s, 60 °C for 30 s. Relative expression was calculated by the 2^−ΔΔCt method.

Protein Expression Analysis:

For Western blotting, proteins were extracted in RIPA buffer, quantified (BCA assay), and resolved on 10% SDS-PAGE. After transfer to PVDF membranes, blots were incubated with primary anti-AQP5 antibody (1:1000, overnight, 4 °C) and HRP-conjugated secondary antibody (1:5000). Bands were visualized by ECL and quantified with ImageJ. For ELISA, AQP5 protein levels were measured using a human-specific ELISA kit, normalized to total protein content.

Results

Selection of Phytochemicals and Molecular Docking Analysis:

Seventeen phytochemicals from *B. nivea* were retrieved from the IMPPAT database and screened against AQP5, a key salivary gland protein implicated in xerostomia. Molecular docking studies revealed that 14 compounds displayed favourable binding affinities with AQP5, suggesting potential as therapeutic candidates. In contrast, α-Carotene, Lutein, and β-Carotene showed positive binding energies, indicating negligible interaction with the target protein (Table 1).

S.No	Compound	Binding Affinity (Kcal/mol)	Interacting amino acids
1	alpha-Carotene	38.9	Leu43, Leu47, Leu51, Leu163, Ile53, Ile146, Gly54, Ala57, Ile165, Ile146, Gly66, Thr150, Gly65, Gly61, Pro161, Gln58, Gly166, Thr55, Gly50, Leu167
2	Lutein	138.4	Leu43, Leu47, Leu167, Thr170, Gly50, Gly166, Ser164, Gly54, Ser149, Ile149, Ile165, Gly65, Gly61, Ala57, Gln58, Leu51, Ser160, Ala162, Pro161, Thr150
3	beta-Carotene	36	Leu47, Leu67, Leu51, Leu163, Gln58, Ala162, Gly61, Thr150, Gly65, Gly66, Pro161, Ala57, Ile165, Ile53, Thr55, Gly166, Leu43
4	Palmitic acid	-3.2	Ser160, Gly159, Ser164, Leu163, Leu51, Leu167, Leu47
5	Ursolic acid	-4.5	Leu51, Leu167, Thr170, Leu47, Leu43
6	beta-Sitosterol	-3.9	Leu43, Leu51, Leu163, Gly159, Ser160, Leu47, Leu167
7	Daucosterol	-4.7	Leu163, Ser164, Ser160, Gly159, Leu157, Gln44, Ala48, Leu47, Leu61
8	3-(2,4-Dihydroxyphenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one	-4.5	Leu51, Thr55, Gly54, Leu163, Pro161, Ala162, Gln58, Gly159, Ser160

9	Corosolic acid	-4.4	Leu47, Leu43, Thr170, Leu167, Leu51
10	Hederagenin	-3.9	Leu51, Leu167, Leu63, Ser164, Ser160, Gly159
11	3-Hydroxy-4-methoxybenzoic acid	-3.7	Gln58, Leu51, Thr55, Gly54, Leu163, Ala162, Ser160
12	Emodin	-3.9	Leu174, Thr170, Leu167, Ser168, Leu171, Leu43
13	alpha-Amyrin	-4.8	Leu47, Leu167, Leu43
14	Chlorogenic acid	-4.1	Gln58, Pro161, Gly159, Leu63, Ser160, Val158, Ser164, Leu167
15	Maslinic acid	-3.7	Thr55, Leu51, Leu167, Leu47, Leu163
16	Tormentic acid	-3.8	Leu43, Leu163, Leu170, Leu167, Leu47
17	Rutin	-3.9	Leu47, Leu167, Leu63, Ser164, Ser160, Leu51, Gly54, Thr55, Gln58
C	Pilocarpine	-3.1	Leu174, Leu47, Thr170, Leu167, Leu51, Leu43

Table 1: Selection of Phytochemicals from *B. nivea* with binding affinity & interacting amino acids

Binding Affinity:
Molecular docking was performed to evaluate the interaction of phytocompounds from *B. nivea* with the target protein. The binding affinities ranged from –3.1 to –4.8 kcal/mol, indicating favorable interactions across multiple candidates when compared to the reference drug pilocarpine (–3.1 kcal/mol) (Figure 1). Among the tested compounds, α-amyrin (–4.8 kcal/mol), daucosterol (–4.7 kcal/mol), ursolic acid (–4.5 kcal/mol), 3-(2,4-dihydroxyphenyl)-1-(4-hydroxyphenyl) prop-2-en-1-one (–4.5 kcal/mol), and corosolic acid (–4.4 kcal/mol) exhibited the strongest binding affinities. These values were notably better than pilocarpine, suggesting enhanced stability and higher potential for protein interaction. Chlorogenic acid (–4.1 kcal/mol) and maslinic acid (–3.7 kcal/mol) also demonstrated moderate binding affinities, reinforcing their possible contribution to bioactivity. In contrast, compounds such as palmitic acid (–3.2 kcal/mol) and hederagenin (–3.9 kcal/mol) showed weaker binding, though still comparable or superior to pilocarpine.

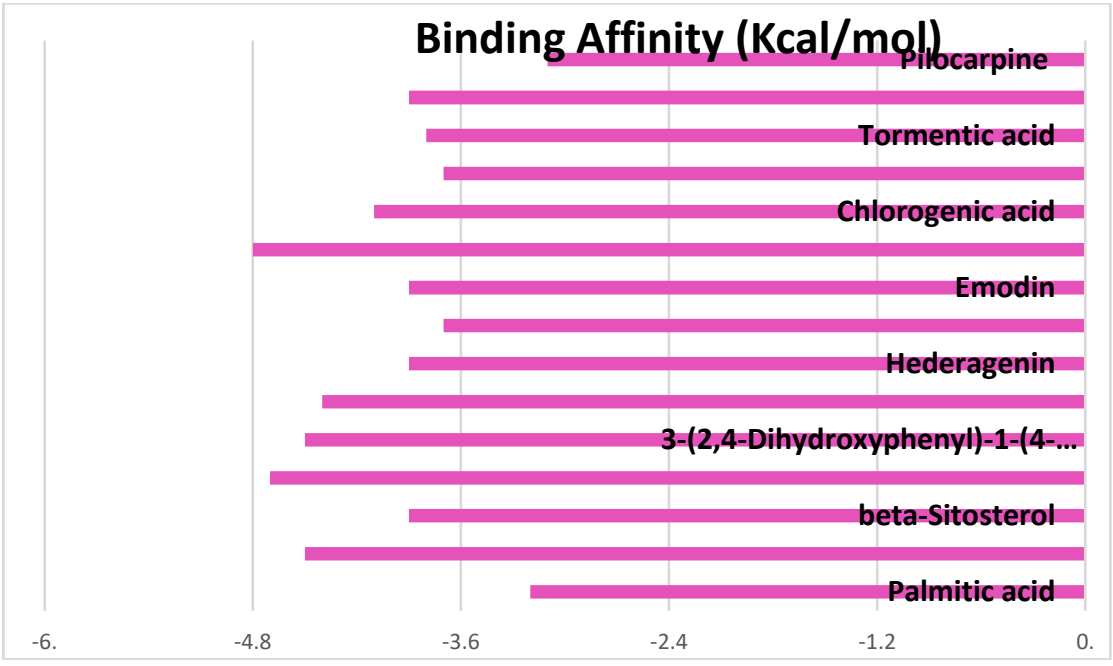


Figure 1: Binding affinity of the phytocompounds from *B. nivea* with the target protein compared with the reference drug pilocarpine

The amino acid interaction profiles revealed consistent involvement of residues such as Leu47, Leu51, Leu163, Leu167, Thr170, Gly159, and Ser160, indicating conserved binding hotspots within the protein's active site. Ursolic acid and α-amyrin notably interacted with Leu51, Leu167, and Thr170, highlighting stable hydrophobic and hydrogen bonding contributions (Figure 2). Overall, the docking analysis identified α-amyrin, daucosterol,

ursolic acid, and corosolic acid as the most promising candidates, surpassing the binding affinity of the standard drug pilocarpine. These compounds likely exert their activity through conserved interactions at key hydrophobic residues, supporting their potential as therapeutic leads in xerostomia management.

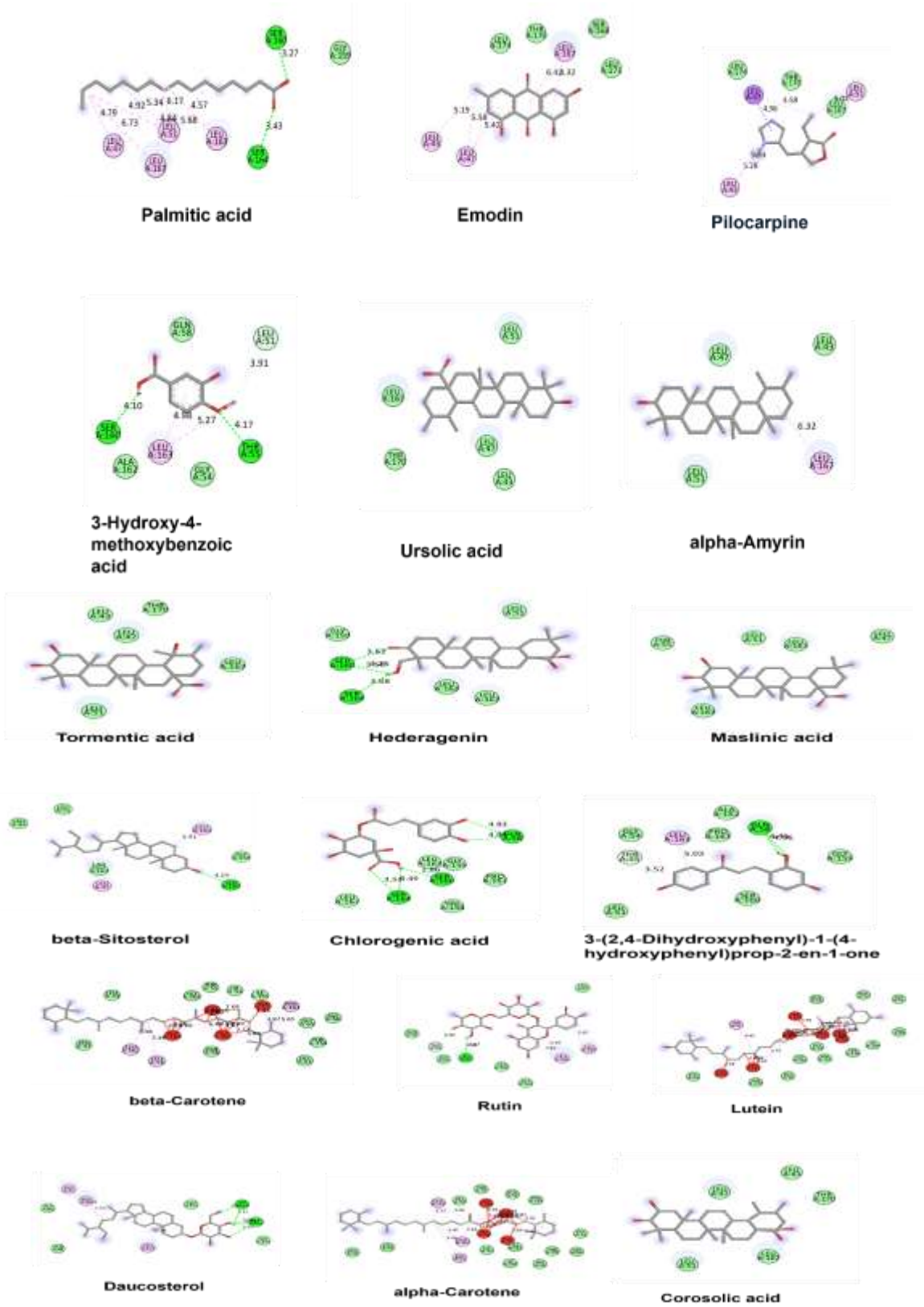


Figure 2: Amino acid interaction profiles demonstrating binding hot spots within the protein's target site (AQP5)

A 3D radar plot was generated to visualize the comparative binding affinities of *B. nivea*–derived phytocompounds against AQP5. The plot clearly distinguished strong binders from weaker ones, with α-Amyrin (–4.8 kcal/mol), Daucosterol (–4.7 kcal/mol), and Ursolic acid (–4.5 kcal/mol) clustering at the innermost region, representing the most favourable interactions. Corosolic acid (–4.4 kcal/mol) and Chlorogenic acid (–4.1 kcal/mol) formed an intermediate group with moderate binding potential. In contrast, the reference drug Pilocarpine (–3.1 kcal/mol) and other weaker binders occupied the outer zones of the radar plot, indicating comparatively lower affinity. This visual representation highlights the hierarchical strength of binding

interactions, confirming α-Amyrin, Daucosterol, and Ursolic acid as the lead compounds with the highest potential for AQP5 modulation. The spatial separation in the 3D radar also reinforces the reliability of docking scores by clustering compounds into clear performance groups, which aids in prioritizing molecules for downstream experimental validation.

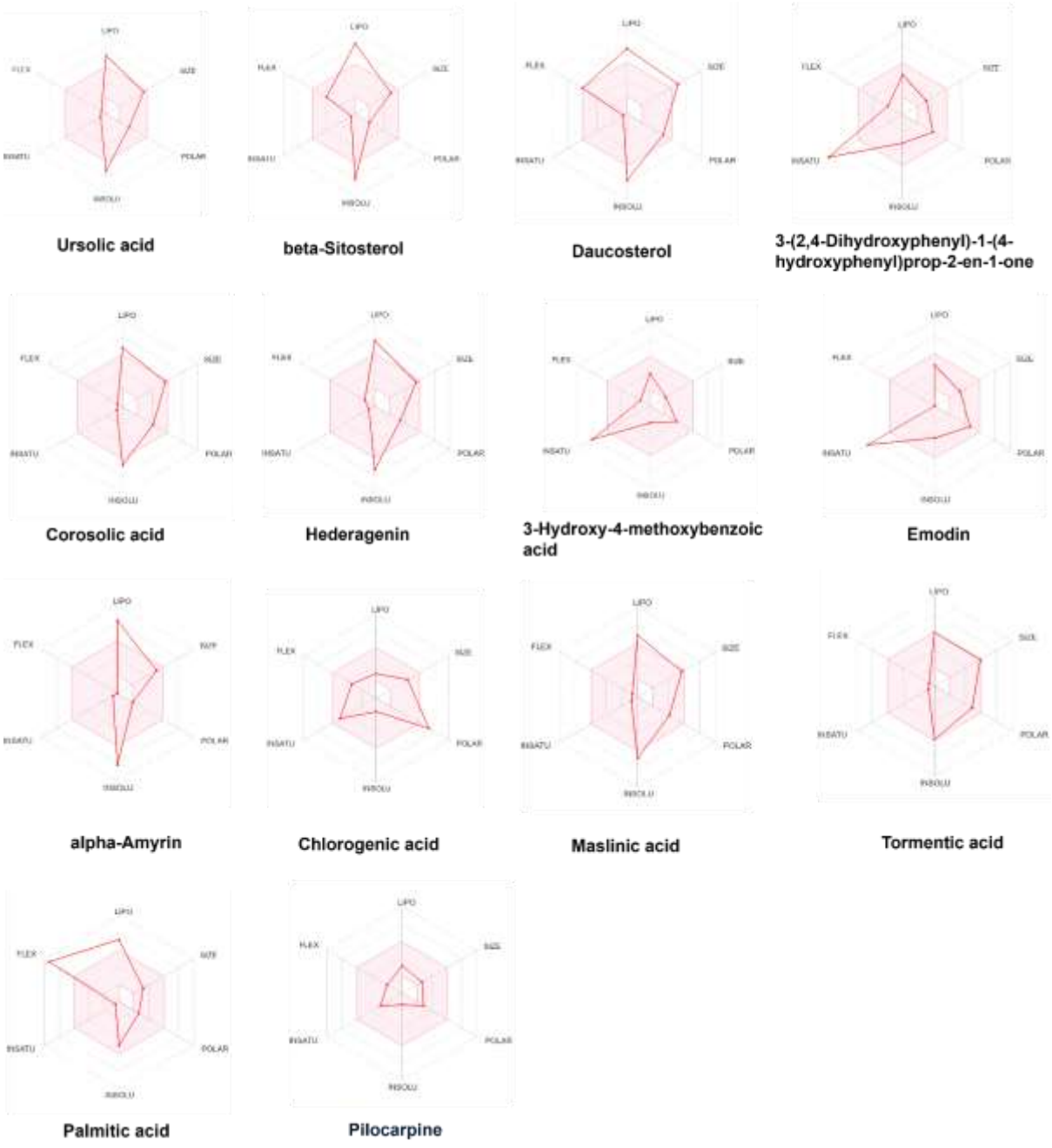


Figure 3: Comparative binding affinities of *B. nivea*–derived phytocompounds against AQP5 using a 3D radar plot

Gene Interaction Network Analysis:

Gene interaction analysis conducted using the GeneMANIA platform confirmed that AQP5 is functionally interconnected with several other salivary gland–related proteins, reinforcing its central role in glandular physiology and validating its therapeutic relevance in xerostomia. AQP5 shares strong functional associations with other members of the aquaporin family, such as AQP4, AQP3, AQP7, and transcriptional regulators such as ELF5 (E74-like ETS transcription factor 5) (Figure 4). Among these, AQP4 contributes to osmotic balance and water homeostasis in glandular tissue; AQP3 & AQP7 are involved in the bidirectional movement of water and small solutes, complementing AQ5-mediated fluid transport. On the other hand, ELF5 plays a pivotal role in the glandular epithelial cell differentiation. Overall, these interactions reinforce glandular secretion, and pharmacological modulation of AQP5 may also indirectly influence these interconnected pathways.

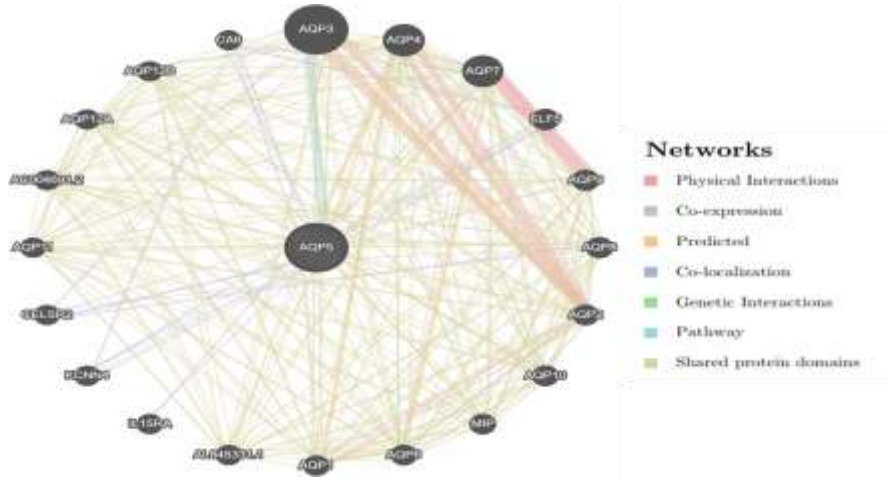


Figure 4: Gene interaction network analysis showing the most potent interactions of AQP5 with other members of the aquaporin family and transcription factors

Drug-likeness Evaluation:
Drug-likeness assessment using SwissADME revealed that 14 compounds fulfilled essential drug-like criteria, including compliance with Lipinski's Rule of Five (Figure 5). These findings indicate that the majority of *B. nivea*-derived phytochemicals possess favourable structural and chemical properties consistent with oral drug candidates.

Figure 5: Drug-likeliness assessment revealed 14 compounds fulfilled essential drug-like criteria

Pharmacokinetic and ADME Profiling:
ADME profiling conducted via pkCSM demonstrated that 11 phytochemicals—Ursolic acid, β-Sitosterol, Daucosterol, Corosolic acid, Hederagenin, 3-Hydroxy-4-methoxybenzoic acid, Emodin, α-Amyrin, Chlorogenic acid, Maslinic acid, and Tormentic acid—exhibited favourable pharmacokinetic characteristics, including high gastrointestinal absorption, metabolic stability, and optimal bioavailability (Figure 6). Chlorogenic acid showed rapid absorption and robust oral bioavailability, while emodin displayed good solubility and anti-inflammatory potential. These findings highlight a subset of phytochemicals that possess both drug-like structural properties and optimal pharmacokinetic behaviour, marking them as potent modulators of AQP5 in xerostomia management.

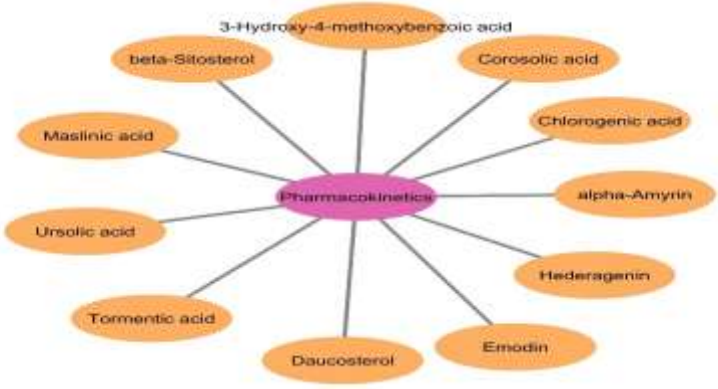


Figure 6: ADMET profiling exhibiting 11 phytochemicals derived from *B. nivea,* favouring optimal pharmacokinetic characteristics

qRT-PCR Analysis of AQP5 mRNA Expression:
qRT-PCR analysis revealed that ursolic acid (10 µM) was the most potent inducer of AQP5 mRNA expression, showing ~2.8-fold upregulation compared to control. Moderate increases were observed with chlorogenic acid (100 µM; ~2.1-fold) and ursolic acid (5 µM; ~2.1-fold), whereas emodin produced only minimal effects (0.9–1.5-fold) (Figure 7a). Protein-level validation by ELISA/Western blot mirrored the transcriptional findings, with ursolic acid (10 µM) and chlorogenic acid (100 µM) significantly increasing AQP5 expression (~2.5–2.8-fold vs. control) (Figure 7b). This concordance between mRNA and protein outcomes was further supported by radar chart comparisons, which highlighted strong translational consistency across assays.

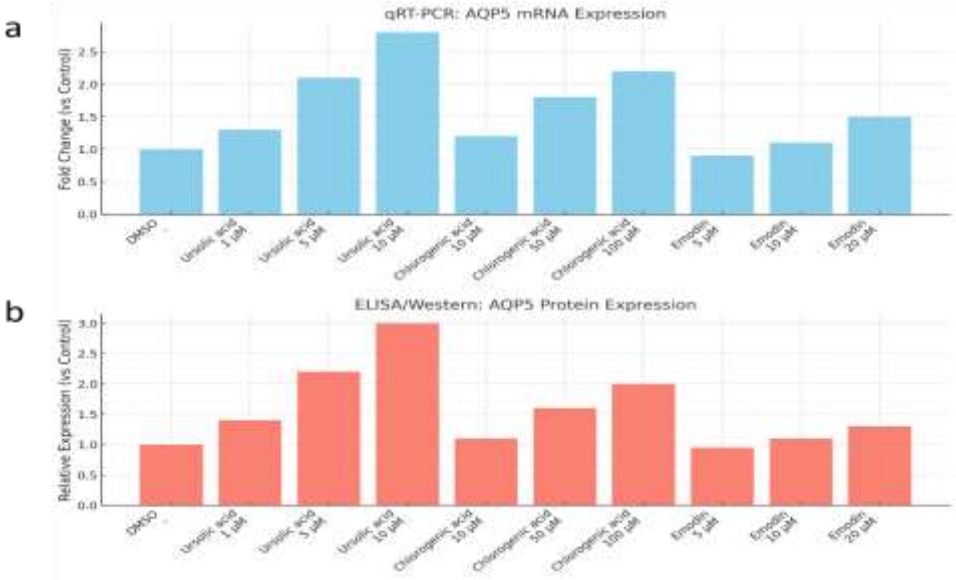


Figure 7: (a) Expression of AQP5 mRNA among the potent phytochemicals (Ursolic acid, chlorogenic acid, emodin) via qRT-PCR; (b) Expression of AQP5 among the phytochemicals using ELISA

Dose–response evaluation further demonstrated a steep, concentration-dependent increase with ursolic acid, while chlorogenic acid exhibited a gradual but consistent effect; emodin plateaued at lower fold-change levels, suggesting limited pharmacological activity (Figure 8b). Visualization through heatmaps (Figure 8a), radar charts (Figure 8c), and bubble plots (Figure 8d) consistently identified ursolic acid as the most effective compound, followed by chlorogenic acid, while emodin remained weak across all analyses (Figure 8b). Collectively, these findings indicate that ursolic acid is the lead phytochemical candidate from *B. nivea*, with robust and reproducible effects on AQP5 expression at both transcriptional and protein levels, supporting its potential for therapeutic development against xerostomia.

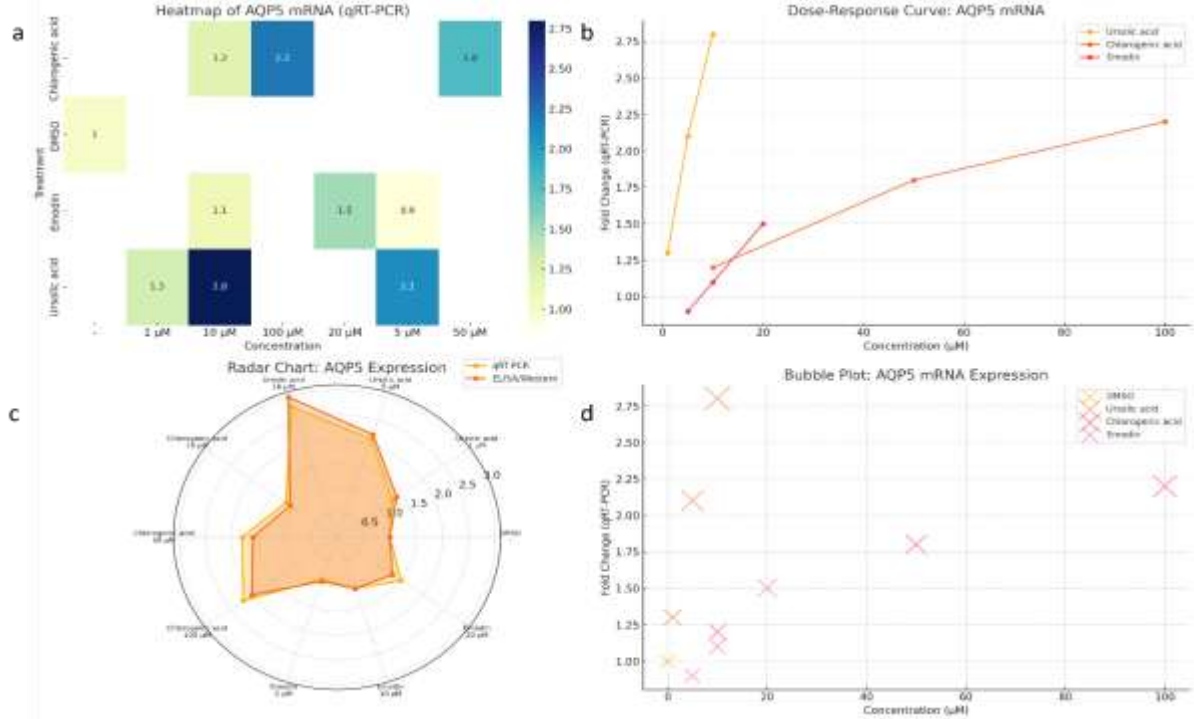


Figure 8 Comparison of the pharmacological activity of the compounds (Ursolic acid, chlorogenic acid, emodin) and AQP5 gene expression illustrated in Heatmap (Figure 8a); Dose-response curve (Figure 8b); Radar chart (Figure 8c), and Bubble plot (Figure 8d)

Discussion

In the present study, 17 phytochemicals derived from B. nivea were systematically evaluated for their potential as AQP5 modulators in the management of xerostomia. Molecular docking revealed that fourteen compounds exhibited favourable binding affinities to the AQP5 active site, with ursolic acid, chlorogenic acid, and emodin emerging as key candidates based on their docking scores and stable ligand–protein interactions. Functional validation in salivary epithelial cells demonstrated that ursolic acid was the most potent inducer of AQP5 expression. Collectively, these findings highlight ursolic acid as the lead compound and chlorogenic acid as a promising secondary candidate for targeting AQP5 in xerostomia. Additionally, qRT-PCR and ELISA/Western analyses showed that ursolic acid (10 μM) was the most potent inducer of AQP5 expression at both transcriptional and protein levels, followed by chlorogenic acid, while emodin exhibited only attenuated effects.

Previous studies have explored various natural compounds for the management of xerostomia, particularly focusing on phytochemicals with antioxidant, anti-inflammatory, and sialogogic properties. Polyphenols such as quercetin, beta-sitosterol, and kaempferol from *Mume fructus* have been shown to enhance salivary gland function through their apoptosis regulation and modulation of inflammatory pathways (Sun Z et al, 2024). Another docking study identified the interaction between *Artemisia annua* and NLRP3 (NOD-like receptor family pyrin domain containing 3), exerting an anti-inflammatory effect in the context of diabetic xerostomia (Zhang S et al, 2025). Apigenin has been reported to bind to estrogen receptor α (ERα) in a manner analogous to estradiol, thereby enhancing AQP5 expression and improving salivary secretion in menopausal women with xerostomia (Wei W et al, 2022). This is consistent with our findings, where ursolic acid emerged as a potent stimulator of AQP5.

Most previous studies have emphasized salivary protein interactions, such as mucin and lactoferrin with α-amylase, which enhance secretion through feedback mechanisms (Zhang Y et al, 2024; Jayaraman S et al, 2023; Jayaraman S et al, 2023; Krishnamoorthy K et al, 2024). One such approach involved K108Cys, a specifically engineered recombinant supercharged polypeptide (SUP) that effectively binds negatively charged salivary components like mucin, promotes mucin recruitment, and utilizes cysteine-mediated dimerization to increase stability. However, this study was limited to in vitro and ex vivo experiments with a small sample size and lacked in vivo validation (Wan H et al, 2020). Similarly, adipose tissue–derived MSCs (AT-MSCs) have been reported to alter the salivary proteome by enriching regeneration-related proteins, yet they do not fully restore salivary profiles to healthy conditions (Lynggaard CD et al, 2022). In contrast, our study directly targeted AQP5 expression using *Boehmeria nivea*–derived phytochemicals, demonstrating a more precise modulation of salivary gland function rather than broad proteomic alterations.

Although artificial saliva substitutes, sugar-free candies, and pharmacological agents such as pilocarpine are commonly prescribed, their long-term utility remains limited due to high cost and poor patient compliance. Consequently, attention has shifted toward green-based therapies, including ginger, coconut Aloe vera extracts, which provide temporary relief from oral dryness but are often associated with allergic reactions, short duration of action, and potential drug interactions with prolonged use (Mardani H et al, 2017; Mahmoud A et al; 2005). And, very few studies have specifically investigated Aquaporin-5 (AQP5) as a direct pharmacological target despite its central role in salivary water secretion (Motegi K et al, 2005; Velmurugan Y et al, 2025; Saito E et al, 2020). Our study uniquely identifies Boehmeria nivea–derived phytocompounds with strong binding affinity to AQP5, extending beyond generalized antioxidant or anti-inflammatory effects. Through the integration of molecular docking, ADMET profiling, and gene interaction analysis, we establish a comprehensive framework not previously applied to AQP5 modulation. Computational predictions were further validated by in vitro assays (qRT-PCR and ELISA/Western blotting), which confirmed a dose-dependent induction of AQP5 expression, particularly with ursolic acid and chlorogenic acid. Moreover, the identification of key interacting residues (Leu47, Leu51, Leu163, Leu167, Thr170, Ser160, Gly159, and Gln58) provides novel structural insights, as residue-level mapping of AQP5 modulators has not been reported earlier. By focusing on AQP5 and validating activity at both in silico and in vitro levels, we establish a new direction for natural product research, bridging computational pharmacology with experimental biology.

Limitation & Future Perspective

Despite promising results, this study has few limitations. In silico docking provides static predictions and does not account for dynamic conformational changes of AQP5, imposing further validation through molecular dynamics and in vivo models of xerostomia. Moreover, only a limited set of phytocompounds was tested, and the long-term safety and off-target effects of the lead compounds remain to be established.

Future studies should expand the compound library of *B. nivea* and related plants to identify additional phytochemicals with AQP5-modulating potential employ molecular dynamics for stability assessment, and validate findings in in vivo xerostomia models. Optimizing formulations for targeted delivery and advancing to early-phase clinical trials to assess safety, tolerability, and preliminary efficacy in patients suffering from xerostomia will be key for translation.

Conclusion

This study provides the first integrated in silico and in vitro evaluation of *B. nivea*–derived phytocompounds as modulators of AQP5, a key regulator of salivary function. Ursolic acid and chlorogenic acid showed the strongest binding affinity and significantly upregulated AQP5 expression at both transcriptional and protein levels. These

findings highlight the potential of plant-derived, sustainable, and bioactive molecules as targeted therapeutic strategies for xerostomia, supporting advancements in preventive healthcare, oral health improvement, and patient-centered therapeutic innovation. This work lays the foundation for future sustainable biomedical research, in vivo validation, and clinical translation, contributing toward improved quality of life and health-focused innovations aligned with global health priorities.

AUTHOR'S CONTRIBUTION:

Reyana Begam Raja Mohamed – Conceptualization, Data curation, Formal analysis; Suganya Panneer Selvam – Conceptualization, Investigation, Writing – Review & Editing, Supervision; Nitya Krishnasamy – Investigation, Methodology, Validation, Visualization

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