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Exploring the Antioxidant, Anti-inflammatory, Antibacterial, and Antibiofilm Potential of *Matricaria pubescens* Extracts Through *In Vitro* and Computational Molecular Interactions

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Abstract

Matricaria pubescens, a medicinal plant endemic to the Algerian Sahara, is traditionally used for treating various ailments. This study explored its potential as a source of natural antioxidants and antibacterial agents, with particular relevance to antimicrobial resistance. The ethanolic extract, rich in polyphenols and flavonoids, demonstrated strong antioxidant and anti-inflammatory activities, as well as notable antibacterial effects, with *Staphylococcus aureus* being the most sensitive strain. Both ethanolic and aqueous extracts effectively inhibited bacterial and fungal biofilms, with inhibition rates reaching up to 89.73% and 70.14%, respectively. Computational analysis further confirmed the strong binding affinities of *M. pubescens* bioactive compounds to key molecular targets, supporting the *in vitro* findings. These results highlight the pharmacological potential of *M. pubescens*, reinforcing its traditional use and positioning it as a promising candidate in the search for alternative therapies against antimicrobial resistance.

Keywords: *Matricaria pubescens*, Antioxidant, Anti-inflammatory, Antibacterial, Antibiofilm, Molecular interactions

1. Introduction

Antibiotic resistance has emerged as one of the greatest threats to global public health, driven largely by the overuse, misuse, and inappropriate prescription of antimicrobial agents (Machado *et al.,* 2023; Guedes *et al.*, 2024). Pathogenic bacteria have developed a wide range of mechanisms to counteract the effects of antibiotics, significantly reducing their efficacy and making infections increasingly difficult to manage (Abd El Aty *et al.,* 2023; Sett *et al.,* 2024). Among these resistance mechanisms, bacterial biofilm formation is particularly concerning. Biofilms are complex and highly protective structures that enable bacteria to persist in hostile environments, evade immune defenses, and exhibit increased tolerance to antimicrobial treatments (Prima *et al.,* 2023; Tue *et al.*, 2024).

The rapid rise of antimicrobial resistance necessitates the urgent exploration of alternative strategies. Medicinal plants, owing to their vast diversity of bioactive compounds, offer a promising source for the development of new broad-spectrum antimicrobial agents (Ahmed-Gaid *et al.,* 2025a; Hanoun *et al.,* 2025a; Hanoun *et al.,* 2025b). Unlike conventional antibiotics that typically target a single bacterial process, plant extracts contain multiple phytochemicals capable of acting on different cellular pathways, thereby limiting bacterial adaptation and reducing the risk of resistance (Seukep *et al.,* 2023).

Matricaria pubescens (Fig. 1) is an endemic North African plant species belonging to the Asteraceae family. It is locally known as *guartoufa* or *Ouazouaza*, names derived from its slightly pungent and spicy taste, a distinctive characteristic noted by Saharan communities (Kherraz *et al.,* 2019a; Ahmed-Gaid *et al.,* 2025b). According to the Integrated Taxonomic Information System, *M. pubescens* is classified within the kingdom *Plantae*, phylum *Angiosperms*, class *Eudicots*, order *Asterales*, family *Asteraceae*, and genus *Matricaria* (Elhasnaoui *et al.,* 2025). Traditionally, this plant has been

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widely used to treat a variety of ailments, including rheumatic and muscular pain, scorpion stings, dysmenorrhea, dehydration, coughs, allergies, eye disorders, toothaches, and cardiovascular, digestive, and genitourinary conditions (Chenna *et al.*, 2025). Its broad therapeutic potential is attributed to its rich phytochemical composition. Our previous research identified 32 phytochemical compounds in *M. pubescens* extracts using LC-MS/MS, which exhibit significant antimicrobial, antioxidant, anti-inflammatory, antidiabetic, and antihyperlipidemic activities (Kherraz *et al.*, 2019b; Amssayef *et al.*, 2020; Chenna *et al.*, 2024).

This study aimed to evaluate the activity of the aqueous and ethanolic extracts of *M. pubescens* against pathogenic strains. It focuses on examining the biological characteristics of these two extracts, with an emphasis on evaluating their antimicrobial, anti-biofilm, antioxidant, and anti-inflammatory properties through *in vitro* and *in silico* methods using combined in vitro and computational assays by targeting human peroxiredoxin 5 (1H2D), TyrRS from *S. aureus* (1JIJ), and cyclooxygenase 2 (1CX2).

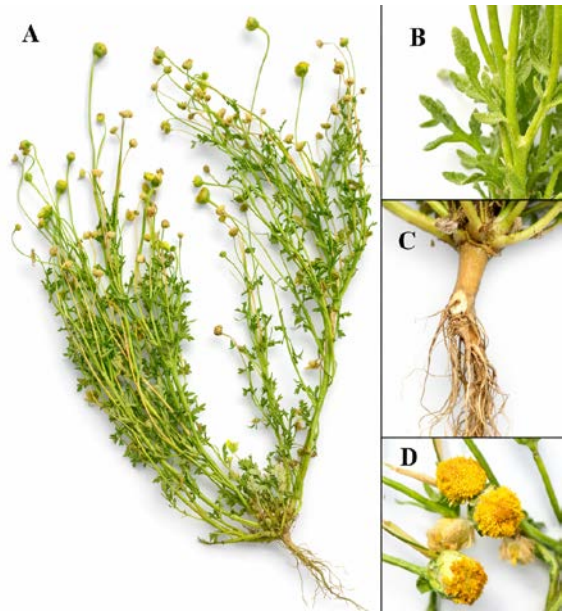

Figure 1. Parts of *M. pubescens*: (A) Whole plant; (B) Leaves; (C) Root; (D) Flower.

2. Materials and methods

2.1. Plant material

The aerial parts (leaves, stem, and flowers) of M. pubescens were collected during flowering in April 2022 from Elhamraya, EL-Oeud region (Algerian Sahara). The plant was identified by Tarek Hamel, a botany professor at Badji Mokhtar Annaba University. A plant specimen was deposited at the Laboratory of Botany, Algiers, Algeria, with voucher number Hv644 (Metrouh-Amir *et al.*, 2015). After the collection, the plant was cleaned, dried in the dark at room temperature, and ground with a mechanical grinder to obtain fine powder

2.2. Preparation of extracts

The aerial parts of *M. pubescens* (10 g) were macerated in 100 ml of each solvent (ethanol and distilled water) at room temperature for 24 hours. After filtration, the solution was evaporated to dryness under reduced pressure using a rotary evaporator (IKA® RV10) connected to a Buchi V-700 vacuum pump at 40°C. The weighed dry residues of the aqueous and ethanolic extract of *M. pubescens* were stored at 4°C in the dark until phytochemical analysis (Yilmaz *et al.*, 2023).

2.3. Total Phenolic and Flavonoid Contents

Total phenolic content was determined using the Folin–Ciocalteu method (Uysal et al., 2017). Briefly, 0.25 ml of extract solution (1 mg/ml) was mixed with 1 ml of diluted Folin–Ciocalteu reagent (1:9, v/v) and shaken vigorously. After 3 min, 75 µL of Na_2CO_3 (0.1%) was added, and the absorbance was recorded at 760 nm following 2 h incubation at room temperature. Results were expressed as mg gallic acid equivalents per g of extract (mg GAE/g).

Total flavonoid content was measured using the $AlCl_3$ colorimetric assay (Ak et al., 2020). One ml of extract solution (1 mg/ml) was mixed with 1 ml of 2% $AlCl_3$ in methanol. A blank was prepared with methanol instead of $AlCl_3$. After 10 min of incubation at room temperature, the absorbance was measured at 415 nm. Flavonoid content was expressed as mg rutin equivalents per g of extract (mg RE/g).

2.4. Antioxidant Activity Assays

2.4.1. DPPH Radical Scavenging Assay

DPPH activity was determined following Mahomoodally et al. (2021). One ml of extract (1 mg/ml) was added to 4 ml of 0.004% DPPH solution in methanol. After 30 min of incubation in the dark, absorbance was read at 517 nm. Results were expressed as mg Trolox equivalents per g of extract (mg TE/g).

2.4.2. $ABTS^+$ Radical Scavenging Assay

$ABTS^+$ solution was prepared by mixing 7 mM ABTS with 2.45 mM potassium persulfate and incubating in the dark for 12–16 h (AL-Hmadi et al., 2023). The solution was then diluted with methanol to an absorbance of 0.700 ± 0.02 at 734 nm. 1 ml of extract (1 mg/ml) was mixed with 2 ml of $ABTS^+$ solution, and the absorbance was measured at 734 nm after 30 min. Results were expressed as mg TE/g.

2.4.3. CUPRAC Assay

CUPRAC activity was assessed according to Ak et al. (2020). A reaction mixture containing 0.5 ml extract (1 mg/ml), 1 ml $CuCl_2$ (10 mM), 1 ml neocuproin (7.5 mM), and 1 ml ammonium acetate buffer (1 M, pH 7.0) was incubated for 30 min at room temperature. Absorbance was measured at 450 nm, and results were expressed as mg TE/g.

2.5. Anti-Inflammatory Activity

Protein denaturation inhibition was tested following Rahman et al. (2012) using bovine serum albumin (BSA).

- Test solution (500 µL): 450 µL of 0.5% BSA + 50 µL extract (from 2.5 mg/ml stock).
- Control solution (500 µL): 450 µL 0.5% BSA + 50 µL distilled water.

- Product control (500 µL): 450 µL distilled water + 50 µL extract.
- Standard solution (500 µL): 450 µL 0.5% BSA + 50 µL diclofenac sodium (variable concentrations).

Absorbance was measured at 660 nm, and the percentage inhibition of protein denaturation was calculated using the formula

% inhibition = [(Abs Negative control - Abs Sample)/Abs Negative control] x 100

2.6. Antimicrobial activity

The antimicrobial efficacy of the extracts was evaluated using the agar well diffusion technique against various pathogenic bacteria and fungi, including three Gram-negative bacteria: *Acinetobacter baumannii, Klebsiella pneumoniae, and Pseudomonas aeruginosa*; two Gram-positive bacteria: *Enterococcus faecium* and *Staphylococcus aureus*; as well as two yeast species from the genus Candida: *Candida albicans* and *Candida tropicalis*. After aseptically creating wells of eight millimeters in diameter on Muller Hinton and Sabouraud agar using sterilized glass beads, these wells were loaded with 100 µl of the test samples dissolved in dimethyl sulfoxide (DMSO) at a concentration of 5 mg/ml each (Bouyahya *et al.*, 2017).

A microbial suspension was prepared from an 18-24-hour pure culture and adjusted to an optical density between 0.08 and 0.1 at 600 nm, corresponding to (1 x 10^8 CFU/ml) according to the McFarland scale (Boughendjioua 2017). DMSO was used as the negative control, while the positive controls consisted of antibiotics (amoxicillin-clavulanic acid (AMC30) and amikacin (AK10) and an antifungal agent (amphotericin B (AM-B)). After 24 hours of incubation at 37°C, the antimicrobial effect was measured by evaluating the diameter of the zone of inhibition (DZI).

2.7. Determination of minimum inhibitory concentration (MIC)

Minimum inhibitory concentrations (MICs) of the extracts were established by microdilution in 96-well curved-bottom microplates. In each microcupule, 100µl of decimal dilutions of the extracts in DMSO were introduced, followed by the addition of 100µl of microbial inoculum prepared in Muller Hinton broth for bacteria and Sabouraud broth for yeast (at 10^8 CFU/ml according to the McFarland scale). The positive control comprised a microbial suspension without any additional substances, while the antibiotics to which the strains were sensitive served as the antibacterial reference. After incubation from 18h to 24h, the MIC is determined from the first well where no visible growth is observed with the naked eye (Rahmoun *et al.*, 2010).

2.8. Inhibition of biofilm formation

To illustrate the power of extracts to inhibit biofilm formation in our isolates, the standard method of Cristal Violet staining on a 96-well microplate was adopted, using extracts at MIC concentrations (Musk *et al.*, 2005). Absorbance was measured at 570 nm using a microplate reader.

Inhibition of biofilm formation was calculated using the following formula:

%= [(Abs of negative control - Abs of test) / Abs of negative control] x100

2.9. Computational Study

The phytochemical compounds of *M. pubescens*, recently identified in our previous study (Chenna *et al.*, 2024), were utilised in the in-silico investigation. The phytochemical profile comprised the following compounds: (1) Quinic acid, (2) Fumaric acid, (3) Aconitic acid, (4) Gallic acid, (5) Protocatechuic acid, (6) Chlorogenic acid, (7) Protocatechuic aldehyde, (8) Tannic acid, (9) 4-Hydroxybenzoic acid, (10) Caffeic acid, (11) Vanillin, (12) *p*-Coumaric acid, (13) Ferulic acid, (14) Coumarin, (15) Salicylic acid, (16) Luteolin-7-O-glucoside, (17) Rutin, (18) Isoquercitrin, (19) Hesperidin, (20) *o*-Coumaric acid, (21) Rosmarinic acid, (22) Cosmosiin, (23) Astragalin, (24) Nicotiflorin, (25) Quercetin, (26) Naringenin, (27) Luteolin, (28) Genistein, (29) Kaempferol, (30) Apigenin, (31) Chrysin, and (32) Acacetin.

The potential antioxidant, antibacterial, and anti-inflammatory effects of *M. pubescens* compounds were evaluated through computational modelling and interaction assays. The crystal structures of human peroxiredoxin 5 (1H2D), TyrRS from *S. aureus* (1JIJ), and cyclooxygenase-2 (1CX2) were retrieved from the RCSB Protein Data Bank, and their active sites were targeted to assess molecular interactions. The 3D structures of the identified phytochemicals were obtained from the PubChem database or drawn using ChemDraw. All compounds and receptors were prepared using the CHARMm force field, as previously described (Badraoui et al., 2023; Boudjema et al., 2024; Rahmouni et al., 2024), by removing water molecules and adding polar hydrogens and Kollman charges. Bond interactions and binding scores were analysed following established protocols (Jedli et al., 2022; Mhadhbi et al., 2023; Chira et al., 2024). The choice of 1H2D, 1JIJ, and 1CX2 was based on their known roles in antioxidant, antibacterial, and anti-inflammatory pathways (Aldarhami et al., 2023; Boudjema et al., 2024).

2.10. Statistical Analysis

All analyses were performed in three technical replicates (n = 3), and results are expressed as mean ± standard deviation (mean ± SD). Comparisons between means were conducted using one-way ANOVA followed by Tukey's post hoc test, with differences considered significant at $p < 0.05$. Statistical analyses were carried out using GraphPad Prism version 9.0.0 (GraphPad Software, San Diego, CA, USA).

3. Results and discussion

The extraction of phytochemical compounds from the aqueous and ethanolic extracts of *M. pubescens* was performed by maceration for 24 hours, as shown in Table 1. It was observed that the aqueous extract yielded a higher amount compared to the ethanolic extract (17.54% *vs.* 7.37%, respectively). Additionally, the results of total polyphenol and total flavonoid assays revealed that the ethanolic extract contains a higher concentration of total polyphenols and flavonoids (17.67±0.29 mg GAE/g and 21.79±0.02 mg RE/g, respectively) compared to the

aqueous extract (12.01±0.20 mg GAE/g and 0.16±0.04 mg RE/g, respectively). These results align with the studies conducted by Metrouh-Amir *et al.,* (2015), who reported that the ethanolic extract is richer in polyphenols and flavonoids than the aqueous extract.

Table 1. Extraction yield, total polyphenol, and total flavonoid contents of aqueous (AEMP) and ethanolic (EEMP) extracts of *M. pubescens*.

Extract	Yield	Total polyphenol content (mg GAE/g)	Total flavonoid content (mg RE/g)
EEMP	7.37%	17.67±0.29[a]	21.79±0.02[a]
AEMP	17.54 %	12.01±0.20[b]	0.16±0.04[b]

Values are presented as mean ± SD of three technical replicates (n = 3). Means in a column with different letters are significantly different ($p < 0.05$, one-way ANOVA followed by Tukey's post hoc test). GAE: Gallic acid equivalent; RE: Rutin equivalent.

The antioxidant activity of *M. pubescens* extracts was evaluated *in vitro* using three methods to determine their radical scavenging ability by DPPH and ABTS test, and their reducing power by the CUPRAC method. Results are shown in fig. 2.

DPPH and ABTS are among the most widely used stable free radicals for testing the anti-free radical activity of antioxidants, due to their rapidity and simplicity. The principle of this technique is based on the reduction of the violet-colored DPPH radical and the blue-colored ABTS to non-radical compounds. The discoloration of the mixture is proportional to the concentration of the antioxidant (Karagecili *et al.,* 2023). Our results showed that the aqueous and ethanolic extracts of *M. pubescens* exhibit potent antiradical activity (DPPH: 21±0.77; 14.19±0.70mg ET/g and ABTS: 76.15±1.10; 64.88±1.41mg ET/g, respectively), with strong antiradical activity in the aqueous extract.

The reducing power of *M. pubescens* aqueous and ethanolic extracts was assessed using the CUPRAC method. This method is based on the ability of antioxidants to reduce divalent cupric ions (Cu^{2+}) to monovalent cupric cations (Cu^{+}) (AL-Hmadi *et al.,* 2023). The results obtained show that the ethanolic and aqueous extracts of *M. pubescens* exhibit potent reducing power (74.37±1.01 mg ET/g; 42.08±0.23 mg ET/g, respectively), with a higher reducing power for the ethanolic extract.

These results indicated that the aqueous and ethanolic extracts of *M. pubescens* possess strong antioxidant activity, enabling them to neutralize free radicals due to their richness in phytochemical compounds. These compounds can neutralize free radicals by donating an electron or a hydrogen atom to various reactive species of oxygen, nitrogen, or chlorine. This reduces molecular damage caused by oxidative stress and helps preserve human health (Altay *et al.,* 2022; Karatas *et al.,* 2022; Chenna *et al.,* 2024). These findings align with the antioxidant activity results of *M. pubescens* reported in previous studies (Kherraz *et al.,* 2019; Metrouh-Amir and Amir 2023).

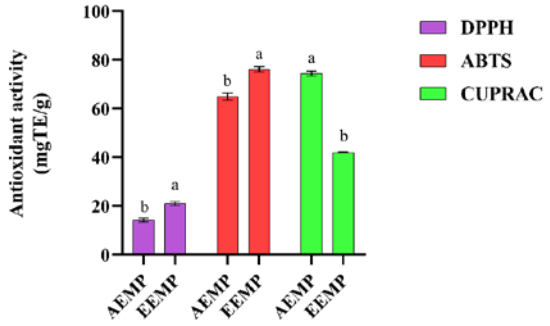

Figure 2. Antioxidant activity of aqueous (AEMP) and ethanolic (EEMP) extracts of *M. pubescens*. Data are expressed as mean ± SD from three technical replicates (n = 3). Different superscript letters (a, b) within the same method indicate significant differences between samples ($p < 0.05$, one-way ANOVA followed by Tukey's post hoc test).

The results of the *in vitro* evaluation of the anti-inflammatory properties of the extracts are shown in Fig. 3. The studied extracts showed strong dose-dependent inhibitory activity on heat-induced albumin denaturation. The Aqueous and ethanolic extracts of *M. pubescens* were particularly active, protecting BSA against heat denaturation by 26.57 ± 4.45% and 40.18 ± 2.40%, respectively, at the lowest concentration (125 µg/ml). At the highest concentration (2.5 mg/ml), a protective effect of 78.49 ± 3.58% and 84.17 ±5.01% was obtained with Aqueous and ethanolic extracts of *M. pubescens*, respectively, compared with diclofenac, which exerted an inhibitory activity of 40.45±0.15% at the lowest dose and better activity of 95±0.08% at the high dose. These findings are consistent with those reported by Bouden *et al.,* (2017). The protective effect of these extracts against BSA denaturation is due to their richness in polyphenols and flavonoids such as caffeic acid, chlorogenic acid, génistéine and luteolin 7-O-glucoside, which were previously identified in these extracts and exhibit remarkable anti-inflammatory properties through various mechanisms (Al-Khayri *et al.,* 2022, Ahammed *et al.,* 2023; Chenna *et al.,* 2024).

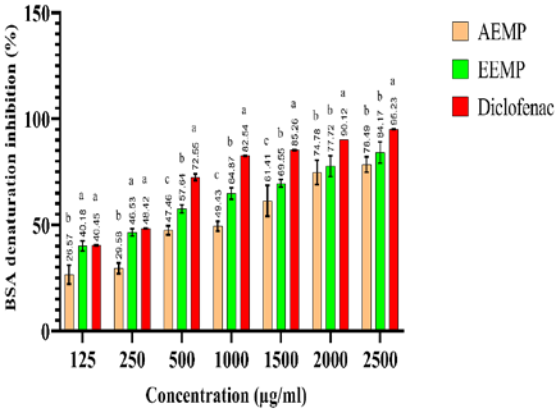

Figure 3. In vitro anti-inflammatory activity of aqueous (AEMP) and ethanolic (EEMP) extracts of *M. pubescens*, and diclofenac, determined by the BSA denaturation assay. Percentage inhibition of protein denaturation was measured spectrophotometrically at 660 nm. Data are expressed as mean ± SD from three technical replicates (n = 3). Different superscript letters (a, b, c) within the same concentration indicate significant differences between samples ($p < 0.05$, one-way ANOVA followed by Tukey's post hoc test).

The results of the antimicrobial activity of these extracts are summarized in Table 2. The results were evaluated against established reference criteria (Bouyahya *et al.,* 2017).

- A weak interaction (zone of inhibition ≤ 12 mm);
- An intermediate interaction (12 mm < zone of inhibition ≤20 mm);
- A sensitive interaction (zone of inhibition >20 mm).

The data in Table 2 revealed inhibition zone diameters ranging from 12 to 14 mm for the aqueous extract and from 12.5 to 18 mm for the ethanolic extract. The minimum inhibitory

concentrations vary between 7.81 and 500 µg/ml. However, it is important to note that *E. faecium* showed clear resistance to both extracts, while *S. aureus* was identified as the most sensitive strain to the extracts. For the fungal strains, *C. albicans* was the only one showing sensitivity to the ethanolic extract, with an inhibition zone diameter of 13 mm and an MIC of 500 µg/ml, in contrast to *C. tropicalis*, which was resistant.

The results regarding the sensitivity of the strains to reference antimicrobials showed significant resistance among the examined strains, with the exception of *P. aeruginosa*, which exhibited sensitivity to amikacin. The inhibition zone diameter was measured at 19 mm, with MIC of 250 µg/ml.

The extracts of *M. pubescens* had an effect on both Gram-positive and Gram-negative bacteria, as well as on *C. albicans*, with a particularly notable effect observed with the ethanolic extract. Our results align with previous studies on the antimicrobial effects of these plant extracts, which confirm that the ethanolic extract demonstrated antimicrobial activity against various microbial strains, including *S. aureus*, *E. coli*, *P. aeruginosa*, and *C. albicans* (Makhloufi *et al.,* 2012; Maiza *et al.,* 2014; Nadji *et al.,* 2022). A previous study revealed the presence of chlorogenic, caffeic, p-coumaric, and tannic acids in both ethanolic and aqueous extracts of *M. pubescens*. These compounds are likely responsible for the antimicrobial effects observed, as they contribute to inhibiting bacterial proliferation by disrupting microbial cell walls and interfering with cellular processes (Chenna *et al.,* 2024; Kalinowska *et al.,* 2024).

Table 2. Antimicrobial activity of aqueous and ethanolic extracts of *M. pubescens*

Stains	Plant extracts				Antibiotics/ Antifungal			Control DMSO
	AEMP		EEMP		Code	DIZ (mm)	MIC (µg/ml)	
	DIZ (mm)	MIC (µg/ml)	DIZ (mm)	MIC (µg/ml)				
Baumannii	12±0.2	250	16±0.2	62.5	AMC30	R	R	R
					AK10	R	R	
					AM-B	/	/	
K. pneumoniae	13.5±0.2	125	14±0.2	125	AMC30	R	R	R
					AK10	R	R	
					AM-B	/	/	
P. aeruginosa	12±0.2	500	12.5±0.2	500	AMC30	R	R	R
					AK10	19±0.2	250	
					AM-B	/	/	
E. faecium	R	R	R	R	AMC30	R	R	R
					AK10	20±0.2	250	
					AM-B	/	/	
S. aureus	14±0.2	15.63	18±0.2	7.81	AMC30	R	R	R
					AK10	R	R	
					AM-B	/	/	
C. albicans	R	R	13±0.2	500	AMC30	/	/	R
					AK10	/	/	
					AM-B	R	R	
C. tropicalis	R	R	R	R	AMC30	/	/	R
					AK10	/	/	
					AM-B	R	R	

Data are expressed as mean ± SD from three technical replicates (n = 3). **AEMP:** Aqueous Extract; **EEMP:** Ethanolic Extract; **AK10:** Amikacin; **AMC30:** Amoxicillin/Clavulanic Acid; **AM-B:** Amphotericin B; **DMSO:** Dimethyl Sulfoxide; **DIZ:** Diameter of Inhibition Zone; **MIC:** Minimum Inhibitory Concentration; **R**: resistant.

The results from the crystal violet staining indicated a notable reduction in biofilm formation among the analyzed bacterial and fungal strains (Fig. 4). Bacterial biofilm inhibition rates ranged from 35.36% to 89.73% with the aqueous extract and from 50.07% to 80.82% with the ethanolic extract, while fungal biofilm inhibition was recorded at 70.14%. Additionally, these results highlighted the remarkable efficacy of the extracts compared to the standard antibiotic, which showed only a 45.43% inhibition rate for *P. aeruginosa*.

Our results align with previous studies demonstrating that these extracts, rich in phenolic compounds such as

chlorogenic acid, protocatechuic acid, and vanillin, as well as flavonoids like quercetin and rutin, exhibit high antibiofilm activity (Salim *et al.,* 2023; Chenna *et al.,* 2024). These compounds reduce biofilm biomass, cell viability, and exopolysaccharide levels in biofilms, achieving more than 70% inhibition against *S. aureus* and *P. aeruginosa.* They affect bacterial adhesion and virulence gene expression and have significant antimicrobial activity against spoilage yeasts, disrupting biofilm formation and adhesion. This highlights their potential in preventing biofilm-associated infections (Kostić *et al.,* 2020; Kimani *et al.,* 2021; Ivanov *et al.,* 2022).

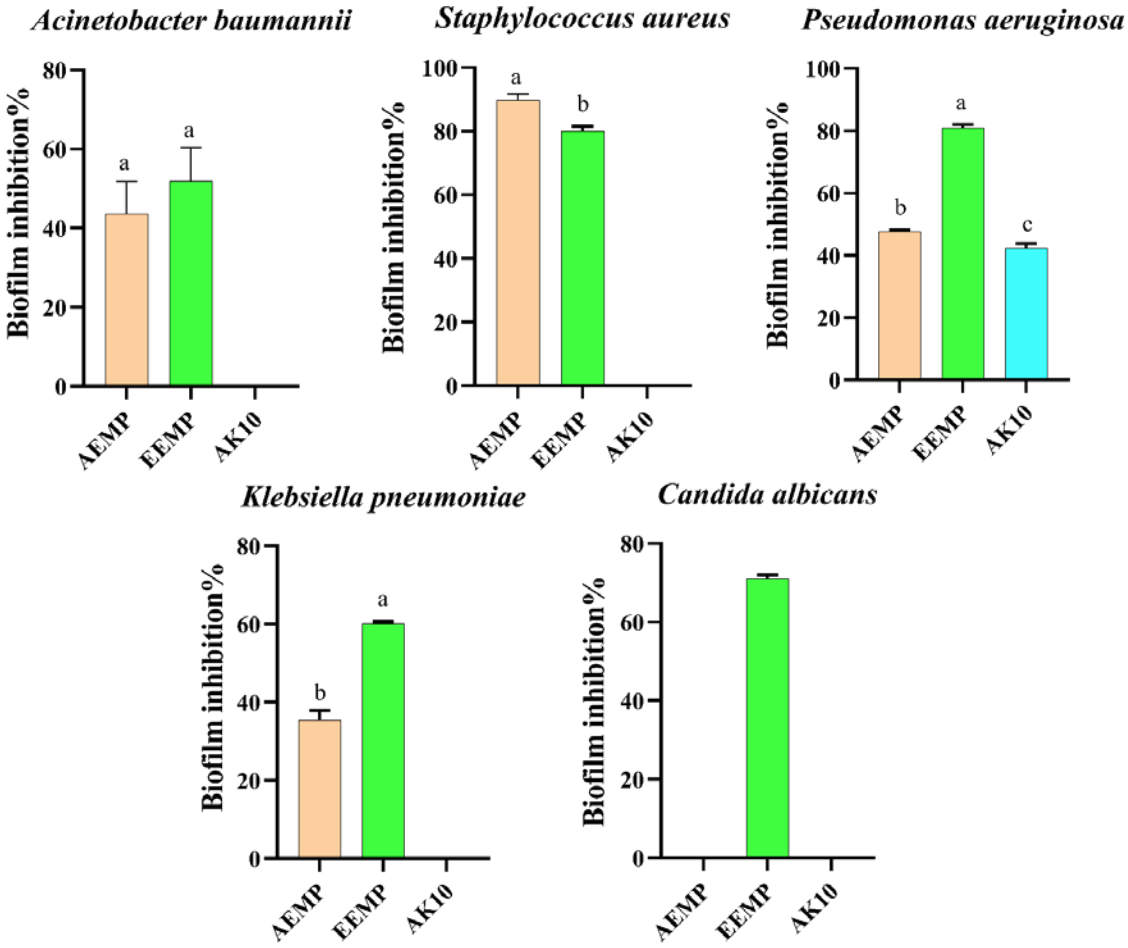

Figure 4. Inhibition of biofilm formation by aqueous (AEMP) and ethanolic (EEMP) extracts of *M. pubescens*, and amikacin (AK10), was evaluated using the crystal violet staining assay. Biofilm biomass was quantified by measuring absorbance at 570 nm. Data are expressed as mean ± SD from three technical replicates (n = 3). Different superscript letters (a, b, c) indicate significant differences between samples ($p < 0.05$, one-way ANOVA followed by Tukey's post hoc test).

In view of the potent antioxidant, anti-inflammatory, antibacterial, and anti-biofilm activities of the aqueous and ethanolic extracts of *M. pubescens*, we further investigated these effects through computational modelling and interaction assays using the phytochemicals previously identified. UHPLC-ESI-MS/MS analysis revealed 32 phytochemical compounds in total, with the ethanolic extract containing 30 and the aqueous extract 17 (Chenna et al., 2024) (Table 3), which aligns with the total polyphenol and flavonoid contents reported in Table 1.

M. pubescens compounds bound human peroxiredoxin 5 (1H2D), TyrRS from *S. aureus*(1JIJ), and cyclooxygenase 2 (1CX2) with acceptable affinities that reached –8.6, –11.2, and –12.6 kcal/mol for 1HD2, 1JIJ, and 1CX2, respectively (Table 3). Interestingly, all the 32 identified compounds within the *M. pubescens* extract possessed negative binding affinities for the different targeted receptors, which support their potential bioactivities. It has been shown that variation in binding affinities depends on the 3D chemical structure and geometry of the ligands (Chira *et al.,* 2024; Kraiem *et al.,* 2024). 4-OH, Benzoic acid was found to have the best binding affinities for the three targeted receptors (Fig. 5-6). The three compounds that have the best binding scores were further analyzed for bond category, molecular interactions, and deep embedding (Table 4). These compounds were predicted to establish good molecular interactions.

The molecular interactions included conventional H-bonds associated with a network of hydrophobic bonds. As previously described, such a rich network of bonds contributes to the stability of the ligand-receptor complex (Badraoui *et al.,* 2023; Ben Saad *et al.,* 2023; Rahmouni *et al.,* 2024). The established interactions concerned several key residues and deep embedding (<2.5 Å), which have been commonly reported to be associated with potential bioactivities, including anti-inflammatory, antiproliferative, antioxidant, and antimicrobial effects (Zammel *et al.,* 2021; Jedli *et al.,* 2022). Overall, our computational modeling results showed that the

antioxidant, antibacterial, and anti-inflammatory effects of *M. pubescens* compounds are thermodynamically possible. In the current study, these biological effects had already been reported using *in vitro* analyses. These findings supported the health promotion and promising benefits of natural-derived compounds and their phytotherapeutic potential (Akacha *et al.*, 2022; Bédoui *et al.*, 2024; Kraiem *et al.*, 2024).

Importantly, our work presents the first comparative analysis of aqueous versus ethanolic *M. pubescens* extracts for antimicrobial and antibiofilm efficacy using both *in vitro* and *in silico* approaches. While previous studies have generally reported antimicrobial activity, few have explored resistance profiles or directly compared multiple solvent extracts. In the context of rising antimicrobial resistance, the observed sensitivity of *S. aureus* and *P. aeruginosa* to plant-based extracts underscores the potential of traditional medicinal plants as alternative or complementary antimicrobial agents. Notably, few prior investigations have examined the antibiofilm activity of *M. pubescens*, particularly against both Gram-negative and Gram-positive bacteria, as well as yeasts. Our findings provide new evidence of its potent biofilm-inhibitory effects, especially from the ethanolic extract, which significantly reduced biofilm formation in *S. aureus* and *P. aeruginosa*. By integrating *in vitro* bioactivity assays with *in silico* modeling, this study delivers a deeper and more predictive insight into the therapeutic potential of *M. pubescens*, filling critical gaps left by previous investigations focused solely on phytochemical content or general antimicrobial activity. Given the growing concern over biofilm-associated infections and their role in antibiotic resistance, these results highlight *M. pubescens* particularly its ethanolic extract as a promising natural source of bioactive compounds for managing resistant pathogens and biofilm-related complications in clinical and food industry contexts.

Table 3. Binding energy and root mean square deviation (RMSD) of the compounds identified in *M. pubescens* extracts while complexed with human peroxiredoxin 5 (1H2D), TyrRS from *S. aureus* (1JIJ), and cyclooxygenase 2 (1CX2).

Compounds	UHPLC-ESI-MS/MS analysis			Binding energy (kcal/mol)			RMSD (lower-upper)		
	Rt	EEMP	AEMP	1H2D	1JIJ	1CX2	1H2D	1JIJ	1CX2
Quinic acid	3.0	+	+	–5.7	–7.2	–6.6	0.0-19.47	0.0-4.50	0.0-7.82
Fumaric aid	3.9	-	+	–4.4	–5.5	–4.8	0.0-26.63	0.0-6.01	0.0-29.82
Aconitic acid	4.0	-	+	–5.4	–5.5	–5.1	0.0-4.29	0.0-28.96	0.0-50.03
Gallic acid	4.4	+	+	–5.6	–7.3	–6.5	0.0-26.48	0.0-3.93	0.0-29.52
Protocatechuic acid	6.8	+	+	–4.9	–6.7	–6.0	0.0-27.59	0.0-26.64	0.0-23.77
Chlorogenic acid	8.4	+	+	–7.1	–8.6	–8.9	0.0-22.15	0.0-26.15	0.0-29.77
Protocatechuic aldehyde	8.5	+	+	–5.5	–7.1	–6.3	0.0-9.35	0.0-26.61	0.0-29.77
Tannic acid	9.2	+	-	–6.1	–6.9	–6.7	0.0-24.22	0.0-25.16	0.0-26.48
4-OH Benzoic acid	10.5	+	+	–8.6	–11.2	–12.6	0.0-26.31	0.0-6.47	0.0-32.85
Caffeic acid	12.1	+	-	–5.5	–7.3	–7.0	0.0-28.57	0.0-22.90	0.0-30.84
0Vanillin	13.9	+	+	–5.1	–6.2	–6.2	0.0-27.36	0.0-23.19	0.0-31.05
p-Coumaric acid	17.8	+	-	–5.2	–6.4	–7.1	0.0-27.84	0.0-25.62	0.0-24.56
Ferulic acid	18.8	+	+	–5.6	–6.9	–6.9	0.0-27.60	0.0-23.54	0.0-11.93
Coumarin	20.9	+	-	–5.2	–6.9	–7.6	0.0-28.80	0.0-23.74	0.0-29.08
Salicylic acid	21.8	+	+	–5.6	–6.7	–6.4	0.0-16.9	0.0-24.98	0.0-29.74
luteolin 7-O-glucoside	23.7	+	+	–7.4	–10.1	–10.2	0.0-25.27	0.0-3.54	0.0-30.29
Rutin	25.6	+	-	–7.9	–9.5	–9.1	0.0-30.51	0.0-21.38	0.0-32.79
isoquercitrin	25.6	+	-	–8.0	–8.6	–8.2	0.0-29.40	0.0-22.47	0.0-32.74
Hesperidin	25.8	+	-	–7.7	–10.5	–9.6	0.0-23.18	0.0-3.58	0.0-35.04
o-Coumaric acid	26.1	+	-	–5.9	–6.8	–6.7	0.0-27.55	0.0-24.96	0.0-27.71
Rosmarinic acid	26.6	+	-	–6.2	–7.6	–7.6	0.0-7.07	0.0-37.29	0.0-36.04
Cosmosiin	28.2	+	+	–7.3	–10.0	–10.1	0.0-30.57	0.0-21.67	0.0-32.36
Astragalin	30.4	+	-	–8.0	–7.5	–8.1	0.0-29.64	0.0-19.21	0.0-31.20
Nicotiflorin	30.6	+	-	–7.8	–8.7	–9.9	0.0-30.54	0.0-22.05	0.0-10.04
Quercetin	35.7	+	-	–6.8	–9.9	–8.9	0.0-8.65	0.0-2.77	0.0-30.06
Naringenin	35.9	+	-	–6.5	–9.4	–8.8	0.0-27.39	0.0-3.38	0.0-11.89
Luteolin	36.7	+	+	–7.0	–9.8	–9.4	0.0-28.55	0.0-3.75	0.0-10.29
Genistein	36.9	+	-	–6.5	–9.0	–8.9	0.0-20.73	0.0-24.14	0.0-8.42
Kaempferol	37.9	+	-	–6.6	–9.4	–8.5	0.0-7.11	0.0-24.06	0.0-32.63
Apigenin	38.2	+	+	–6.6	–9.4	–9.0	0.0-21.76	0.0-8.41	0.0-9.80
Chrysin	40.5	+	+	–6.4	–9.2	–9.3	0.0-30.13	0.0-23.36	0.0-32.80
Acacetin	40.7	+	+	–6.6	–9.5	–9.1	0.0-28.95	0.0-25.38	0.0-25.63

AEMP: Aqueous Extract of *M. pubescens*, **EEMP:** Ethanolic Extract of *M. pubescens*, +/-: detected/ not detected, Human peroxiredoxin 5 (1H2D), TyrRS from *S. aureus* (1JIJ), Cyclooxygenase 2 (1CX2).

Table 4. Number of Hydrogen bonds and closest interacting residues for the compounds identified in *M. pubescens* that possessed the best binding scores with human peroxiredoxin 5 (1H2D), TyrRS from *S. aureus* (1JIJ), and cyclooxygenase 2 (1CX2).

Compound (Affinity)	No. H-Bond	Closest Interacting Residues	
		Residue (Letters & ID)	Distance to closest Interacting Residue (Å)
Human peroxiredoxin 5 (pdb id: 1HD2)			
4-OH Benzoic acid	6	Glu27, Glu27, Lys63, Glu13, Glu27, Val70, Lys93, Gly92, Arg95, Glu27, Val69	Lys63:HZ1 (1.730)
Astragalin	4	Asn21, Arg86, Gly82, Gly17, Arg86, Arg95, Glu16, Glu16	Gly82:O (1.898)
isoquercitrin	8	Arg86, Leu96, Gly82, Ala90, Val94, Glu91, Arg95, Glu16, Arg95, Glu16, Glu16	Val94:O (2.117)
TyrRS from *S. aureus* (pdb id: 1JIJ)			
4-OH Benzoic acid	5	Asp40, Asp80, Asp40, Asp80, Gly38, Gln196, Tyr170, Tyr36, Gln190, His47, Lys84, Lys84, His47, Asp80, Ala239	Tyr36: OH (2.248)
Hesperidin	6	Lys84, Lys84, Lys84, Asp40, Thr75, Pro222, His50, Gly193, Gly49, Gly49, Gln196, Tyr36, Asp177, Asp195, Gly38, Leu70, Tyr36	Asp40:OD2 (2.164)
luteolin 7-O-glucoside	5	Asp40, Gln174, Asp177, Asp40, Ser82, Gly38, Lys84	Asp40:HN (1.961)
Cyclooxygenase-2 (pdb id: 1CX2)			
4-OH Benzoic acid	9	Glu465, Glu465, Asp157, Lys468, Asp157, Cys47, Met48, Pro154, Cys41, Gln42, Lys468, Met48, Gly135, Ala156, Val155; Ala156, Cys36, Pro153, Cys36, Ala156, Arg44, Lys468	Pro154:O (1.927)
luteolin 7-O-glucoside	4	Tyr122, Lys468, Tyr122, Asn43, Arg44, Glu465, Arg44, Lys468, Arg44, Leu152, Arg469	Asn43:OD1 (2.707)
Cosmosiin	6	Asn43, Lys468, Lys468, Gln42, Asn43, Asn43, Glu465, Asn43, Arg44, Lys468, Lys468, Leu152, Arg469	Gln42:OE1 (2.182)
Bold residues: interacting with Conventional H-Bonds			

Ala: Alanine, **Arg:** Arginine, **Asn:** Asparagine, **Asp:** Aspartic acid, **Cys:** Cysteine, **Gln:** Glutamine, **Glu:** Glutamic acid, **Gly:** Glycine, **His:** Histidine, **Leu:** Leucine, **Lys:** Lysine, **Met:** Methionine, **Pro:** Proline, **Ser:** Serine, **Thr:** Threonine, **Tyr:** Tyrosine, **Val:** Valine, **pdb**: Protein Data Bank

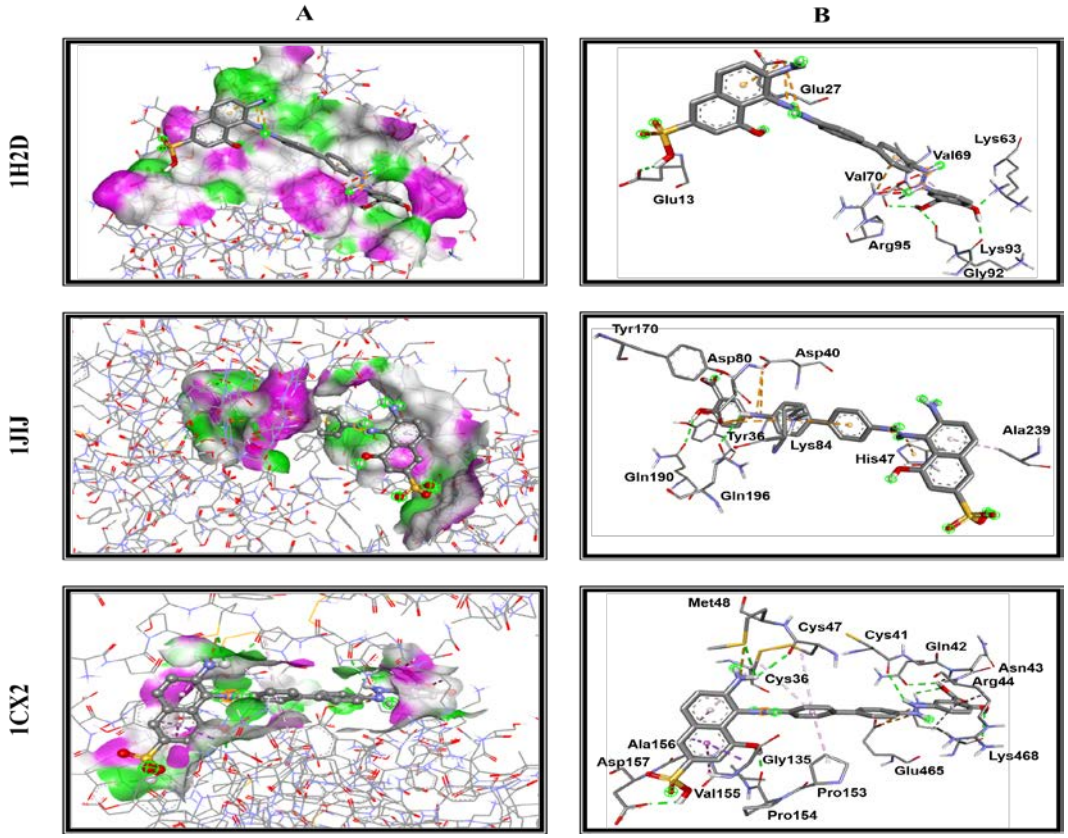

Figure 5. 3D illustration of A) the H-bond acceptor/donor attributes and B) the resulting interactions for 4-OH Benzoic acid, which possessed the best binding score for each of human peroxiredoxin 5 (1H2D), TyrRS from *S. aureus* (1JIJ), and cyclooxygenase 2 (1CX2) macromolecules.

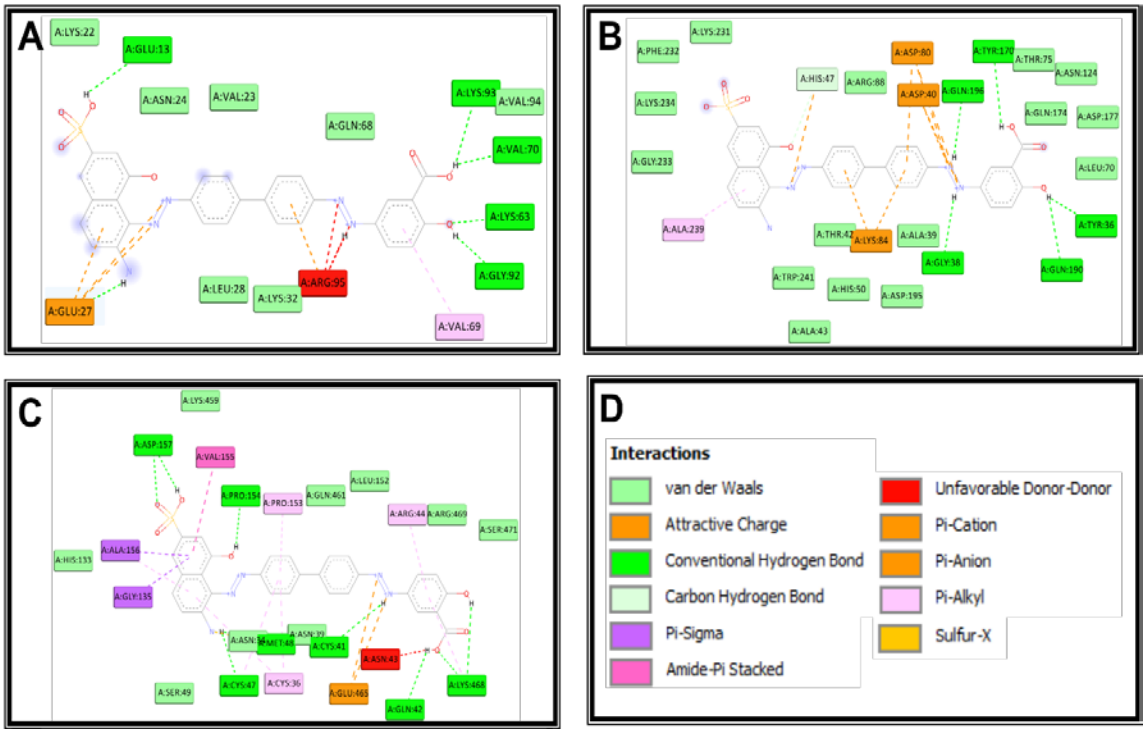

Figure 6. 2D diagrams of the interaction of 4-OH Benzoic acid, which possessed the best binding score for each of human peroxiredoxin 5 (1H2D), TyrRS from S. aureus (1JIJ), and cyclooxygenase 2 (1CX2) macromolecules**.**

4. Conclusion

This study demonstrates that both aqueous and ethanolic extracts of *M. pubescens* exhibit potent antimicrobial, antioxidant, and anti-inflammatory activities, with the ethanolic extract showing superior efficacy due to its richness in polyphenols and flavonoids. Computational modelling confirmed strong binding affinities of its compounds to key biological targets (1HD2, 1JIJ, and 1CX2), supporting their bioactive potential. These findings highlight the relevance of *M. pubescens* as a natural source of therapeutic agents, particularly in addressing antimicrobial resistance, a pressing global health issue. Its demonstrated activity against resistant pathogens, combined with its antioxidant and anti-inflammatory effects, underscores its potential for developing plant-based alternatives or adjuvants to conventional drugs. Future research should focus on in vivo validation, mechanism-of-action studies, toxicity assessments, and clinical trials. The integration of *M. pubescens* into drug discovery pipelines and nutraceutical development could pave the way for innovative strategies to combat antimicrobial resistance and oxidative stress-related disorders.

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Conflict of interests

On behalf of all authors, the corresponding author states that there is no conflict of interest.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent

All the authors read the manuscript and approved it for submission.

Authorship contribution

H. C: Writing – review & editing, Supervision. Writing – original draft, Methodology, Investigation**. M.L. D:** Writing – review & editing**. C. B:** Methodology**. R. B:** Software**. G. Z:** Methodology**. M.A. Y:** Methodology**. C. D:** Methodology**. I. H:** Methodology**. M. S:** Methodology. **M. B:** visualization**. A. B:** validation**. M. M:** Conceptualization, Project administration**.**

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