

Original Research

Beyond Traditional Use: Mechanistic Insights into the Medicinal Potential of *Thymus tiflisiensis* and Modulatory Enhancement Effects of Gallic Acid

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Abstract

Background: *Thymus tiflisiensis* is used in Georgian traditional medicine, but its pharmacological properties remain poorly characterized. This study evaluated the antioxidant, protein denaturation inhibitory, and antibacterial activities of leaf and root extracts and examined the modulatory effects of gallic acid (GA) on these activities. **Methods:** Methanolic extracts of leaves and roots were analyzed for total phenolic and flavonoid contents, rosmarinic acid (RA), ascorbic acid, and glutathione. Antioxidant activity was evaluated using the DPPH assay, protein denaturation inhibitory activity using a bovine serum albumin denaturation assay, and antibacterial activity against *Streptococcus agalactiae* and *Escherichia coli* using broth microdilution. The combined effects of the plant extracts and GA were evaluated across multiple concentration matrices. Molecular docking was used to predict the interactions of RA and GA with the bacterial enzymes MurF and LpxC. **Results:** The methanolic leaf extract exhibited greater antioxidant activity ($IC_{50} = 34.8 \mu\text{g/mL}$) than the root extract ($IC_{50} = 81.9 \mu\text{g/mL}$), accompanied by higher total phenolic (113.6 vs. 56.9 mg GAE/g DW) and flavonoid (63.8 vs. 19.0 mg RE/g DW) contents and higher ASC/DHA and GSH/GSSG ratios. Conversely, the root extract exhibited greater protein denaturation inhibitory activity ($IC_{50} = 51.3$ vs. $159.7 \mu\text{g/mL}$ for the leaf extract) and moderate antibacterial activity against *S. agalactiae* (MIC = 6.0 mg/mL) and contained higher levels of RA. The addition of 5 μM GA enhanced the antioxidant activity of both leaf and root extracts. In protein denaturation assays, 10 μM GA produced a minor enhancement at low extract concentrations, whereas 100 μM GA reduced the inhibitory effects. At 1 mM, GA enhanced the antibacterial activity of both extracts against *S. agalactiae* and that of the root extract against *E. coli*. Molecular docking predicted more favorable interactions of RA with MurF and LpxC than those of GA. **Conclusions:** *T. tiflisiensis* root extracts exhibited greater protein denaturation inhibitory and antibacterial activities, whereas leaf extracts showed greater antioxidant activity. GA modulated these activities in a concentration- and tissue-dependent manner, with enhancement observed at specific concentrations and attenuation at higher concentrations in the protein denaturation assay. Exploratory molecular docking predicted favorable interactions of RA with the bacterial enzymes MurF and LpxC. These findings support further investigation of the bioactive properties of *T. tiflisiensis* and the mechanisms underlying their modulation by GA.

Keywords: *Thymus* plant; gallic acid; antioxidants; anti-bacterial agents; plant extracts; protein denaturation inhibitors

1. Introduction

The genus *Thymus* L. comprises around 350 species of perennial aromatic herbaceous plants with widespread applications in the food, pharmaceutical, and cosmetic industries [1]. Distributed across Europe, North Africa, and Asia, these species possess notable antioxidant, antimicrobial, anti-inflammatory, and anticancer properties, underpinning their historical use in traditional medicine to treat fever and digestive ailments [2]. In Georgia, twelve species are indigenous, including the endemic *Thymus tiflisiensis* Klovov

& Des.-Shost, found in the lower and middle mountain belts of Kakheti, Kartli, and Trialeti [3,4]. Although *Thymus* species are utilised in traditional Georgian medicine for the treatment of pulmonary diseases [5], the biological activities of *T. tiflisiensis* remain unexplored.

While the essential oil composition of the *Thymus* genus is widely documented [6,7,8], the non-volatile polar compounds in methanolic extracts, particularly phenolic acids, flavonoids, and other redox-active metabolites, serve as equally critical drivers of biological functionality [9,10]. Substantial antioxidant activity has been documented for



various polar extracts of related species, including *Thymus vulgaris* L. [6], *Thymus zygis subsp. gracilis*, *Thymus membranaceus*, *Thymus longiflorus* [11], six Croatian *Thymus* species [12]. In these studies, the antioxidant activity correlated with high phenolic and flavonoid content [9], with rosmarinic acid (RA) frequently identified as a major bioactive component [10]. However, because the phytochemical matrix is highly species- and solvent-dependent, systematic evaluation of the non-volatile polar fractions of uncharacterized endemics such as *T. tiflisiensis* is essential to establish their distinct pharmacological baselines.

The antibacterial activity of *Thymus* extracts has been recognised since the 1960s (reviewed in [13]). Methanolic and other polar extracts from the aerial part of *T. vulgaris* inhibited the growth of Gram-positive and Gram-negative bacteria: *Staphylococcus aureus*, *Escherichia coli*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* [14,15]. This efficacy was primarily determined by phenolic monoterpenes, flavonoids [16], and non-volatile polyphenols like rosmarinic acid [13]. These polar compounds disrupt the lipid bilayer and inhibit enzymes [17], collapse the proton gradient, and cause ion leakage [18]. Despite these documented effects in other *Thymus* species, the antibacterial potential of *T. tiflisiensis* remains unexplored.

Beyond antimicrobial properties, *Thymus* extracts demonstrate anti-inflammatory potential. *In vitro* studies using *T. vulgaris* have shown that polar polyphenols and flavonoids prevent protein thermal denaturation [19], an effect associated with the stabilisation of serum proteins. *In vivo*, these compounds reduce inflammation by downregulating pro-inflammatory cytokines (tumour necrosis factor- α (TNF- α), interleukin-1 beta (IL-1 β), and interleukin-6 (IL-6)) and inhibiting the key NF- κ B and MAPK intracellular signalling pathways [20].

Plant extract bioactivity can be modulated by individual phenolic compounds in a concentration- and phytochemical matrix-dependent manner [21,22]. Gallic acid (3,4,5-trihydroxybenzoic acid, GA) is a phenolic acid characterized by a benzene ring with three adjacent hydroxyl groups and a carboxylic acid group, widely distributed in plants [23]. This structure underpins its antioxidant activity via electron donation [24], antibacterial action via membrane disruption, metabolic interference, and biofilm inhibition [25] and protein denaturation inhibitory effects [26]. These multifaceted bioactivities make GA a promising modulator of plant extract functionality.

To address the complete absence of pharmacological data on *T. tiflisiensis*, this study systematically evaluates the antioxidant, protein denaturation inhibitory, and antibacterial efficacy of its leaf and root methanolic extracts. We quantified total phenolic and flavonoid content, rosmarinic acid concentration, and ascorbate and glutathione levels. Furthermore, we investigated the concentration-dependent modulatory effects of GA on extract bioactivity and em-

ployed molecular docking simulations against MurF and LpxC to provide exploratory structural insights into potential mechanisms of action.

2. Materials and Methods

2.1 Plant Material

Thymus tiflisiensis Klokov & Des.-Shost. plants were collected in June 2025, at the beginning of flowering, in the National Botanical Garden of Georgia (Tbilisi, coordinates: 41.68574° N, 44.80217° E). The time of collection followed Georgian traditional medicine and data show that *Thymus* plants exhibit the highest antibacterial activity during the flowering stage [27]. Middle stem leaves and roots were collected, frozen in liquid nitrogen, and stored at -80 °C.

2.2 Drying Processes

Leaves and roots (5 g) were freeze-dried (DW-10N freeze dryer, Drawell, China) at 10 Pa and -55 °C.

2.3 Extraction of Plant Samples

Methanol was previously identified as the most efficient solvent [28]. Leaf and root samples were extracted (1:5 w/v) with 80% methanol [28]. Extracts were rotary evaporated at 50 °C (DW-ORE2000, Drawell, China), and the residue was re-dissolved in 100 mM potassium phosphate buffer, pH 7.5. For antibacterial assays, extracts were filter-sterilised (Millipore filter, 0.22 μ m).

2.4 DPPH Radical Scavenging Activity Assay

DPPH radical scavenging activity was measured at 517 nm [29]. Scavenging effect (%) was calculated as:

Scavenging effect (%) = [(control absorbance - sample absorbance) / (control absorbance)] $\times$ 100.

IC₅₀ values were determined by linear regression. GA was tested at 2–20 μ M; concentrations >7 μ M caused complete decolorization and were excluded.

2.5 Determination of Total Phenolic Content (TPC)

The total phenolic content (TPC) was determined using Folin-Ciocalteu's reagent [30] at 750 nm, quantified from a GA standard curve, and expressed as milligrams of GA equivalents per gram of dry weight (mg GAE/g DW).

2.6 Determination of Total Flavonoid Content (TFC)

The total flavonoid content (TFC) was estimated at 506 nm [31] and quantified using a rutin standard curve, expressed as milligrams of rutin equivalents per gram of dry weight (mg RE/g DW).

2.7 Extraction of Plant Samples for the Determination of Ascorbate and Glutathione Contents

Plant material (1 g) was homogenised in 2 mL 5% (w/v) metaphosphoric acid and 1 mM EDTA at 4 °C, centrifuged (30 min at 15,000 $\times$ g), and the supernatant was collected.

2.8 Determination of Reduced (ASC) and Oxidised (DHA) Ascorbate Content

ASC and DHA were assayed at 525 nm, based on Fe^{3+} reduction to Fe^{2+} by ASC and subsequent Fe^{2+} -bipyridyl complex formation [32]. DHA was reduced to ASC with dithiothreitol (DTT), followed by the addition of *N*-ethylmaleimide, and the total ascorbate was determined. The ASC content was calculated using a standard curve; DHA was determined as the difference between total ascorbate and ASC.

2.9 Determination of Reduced (GSH) and Oxidised (GSSG) Glutathione

Total glutathione and GSSG were measured at 412 nm using the 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB)-glutathione reductase recycling method [33] and 5-thio-2-nitrobenzoate (TNB) formation was monitored for 4 min at 412 nm. GSSG was measured after GSH derivatisation with 2-vinylpyridine. Total glutathione and GSSG were quantified from standard curves; GSH was determined as the difference between total glutathione and GSSG.

2.10 Determination of Rosmarinic Acid

Rosmarinic acid content was determined using a spectrophotometric method based on its complexation reaction with Fe^{2+} ions measured at 572 nm [34].

2.11 Determination of Antibacterial Activity

In the current study, antibacterial activity of the extracts was tested against the following bacterial strains: *Escherichia coli* ATCC 25922 (Gram-negative strain); *Streptococcus agalactiae* ATCC 27956 (Gram-positive strain).

2.11.1 Well Diffusion Method

Overnight cultures of *E. coli* and *S. agalactiae* were spread onto sterile Petri dishes containing LB medium (contents (g/L): tryptone – 10; yeast extract – 5; NaCl – 10; distilled water – 1000 mL; agar 1.5%; pH 7.0) and blood agar medium (contents (g/L): beef heart infusion or tryptose – 10; meat peptone – 10; NaCl – 5; agar – 15; supplemented with 5% (v/v) sterile defibrinated sheep blood; final pH 7.3 $\pm$ 0.2), respectively, to obtain a bacterial lawn. Sterile wells (14 mm diameter) were filled with the studied compound: 100 μL of plant extract, 100 μL of gallic acid (15 mM), or a combination of 50 μL of plant extract + 50 μL of gallic acid (30 mM). Amoxicillin (0.1 mg/mL, 100 μL) and DMSO (10%, 100 μL) served as positive and negative controls, respectively. The plates were incubated at 37 °C for 18–24 h. Antibacterial activity was quantitatively evaluated by measuring the total diameter of the clear inhibition zones.

2.11.2 Broth Liquid Culture Method

MIC and MBC were evaluated using the broth dilution method in a 24-well sterile polystyrene plate [35]. Serial di-

lutions of the leaf and root extracts were prepared in sterile LB broth to obtain the following final concentrations: 0.63; 1.45; 2.9; 5.8; 6.0; 7.5; 8.8; 11.3; 15.6; 17.6; 22.6; 26.4 mg/mL. Gallic acid was added to the corresponding wells to final concentrations of 0.2 mM and 1 mM. Due to the certain level of turbidity that crude extracts produce themselves in the nutrient media, blank wells containing extracts only were used as a baseline control for each investigated concentration. Each well was inoculated with 100 μL of the overnight culture of the studied strain. The bacterial inoculum suspension was standardised to a 0.5 McFarland turbidity standard (approximately 1.5×10^8 CFU/mL). For each strain, a separate plate was used to avoid contamination. DMSO (10%) was used as a negative control; ampicillin (0.1 mg/mL) was used as a positive control. The plates were incubated at 37 °C for 18–24 h. After incubation, bacterial growth was evaluated visually by turbidity and measured spectrophotometrically at $\lambda = 600$ nm. Inhibition was calculated using the following formula:

$$I\% \text{ Inhibition} = \frac{OD_{600} \text{ control} - (OD_{600} \text{ sample} - OD_{600} \text{ extract blank})}{OD_{600} \text{ control}} \times 100$$

To estimate the viability of the cultures after incubation with the extract, 10 μL of the suspension was subcultured on a Petri dish with sterile LB medium for 24 h at 37 °C. The MIC value was defined as the lowest concentration showing growth suppression compared to the control, and MBC as the lowest value showing 99.9% suppression of growth [36]. All experiments were performed in triplicate.

2.12 Evaluation of Protein Denaturation Inhibition Using the Bovine Serum Albumin (BSA) Assay

Protein denaturation inhibitory activity was assessed using the BSA denaturation inhibition assay [37] with modifications for plant extracts. The thermal protocol produces partially denatured BSA [38]. Based on preliminary experiments, GA was tested at concentrations of 10 and 100 μM , as concentrations below 10 μM showed no significant activity and concentrations above 100 μM caused protein coagulation. In the control, the sample solution was replaced with PBS. The percentage of protein denaturation inhibition was determined using the formula:

$$I\% = \frac{(A_{\text{control}} - A_{\text{control blank}}) - (A_{\text{sample}} - A_{\text{sample blank}})}{A_{\text{control}} - A_{\text{control blank}}} \times 100$$

where, A_{control} : absorbance of the control reaction (BSA + PBS, heated).

$A_{\text{control blank}}$: absorbance of the control reagent blank (PBS only, heated).

A_{sample} : absorbance of the test sample reaction (BSA + extract/gallic acid, heated).

$A_{\text{sample blank}}$: absorbance of the sample background blank (extract/gallic acid + PBS, no BSA, heated).

Concentration-dependent sample background blanks ($A_{\text{sample blank}}$) were simultaneously prepared to correct for matrix turbidity. The leaf extract blanks had OD from 0.01 to 0.12 at the highest tested concentration (375 $\mu\text{g/mL}$). The leaf extract plus gallic acid blanks exhibited a baseline optical density range from 0.02 to 0.13 at 660 nm. Root extract blanks were practically transparent, registering a baseline OD from 0.004 to 0.05 at 375 $\mu\text{g/mL}$. The root extract plus gallic acid had OD ranging from 0.004 to 0.06. Reagent and sample blanks were simultaneously heated alongside the assay treatments to maintain uniform physical conditions. The heated control reaction (A_{control}), consisting of BSA alone, yielded a baseline absorbance of 0.85 at 660 nm, while the heated PBS reagent blank ($A_{\text{control blank}}$) remained near 0.

2.13 Calculation of Combined Modulation Profiles

The interaction profiles between GA and the extracts were evaluated across multiple combined concentration matrices spanning fractional effect levels ($F_a = 0.1\text{--}0.9$) using the Chou-Talalay combination index (CI) method [39] via CompuSyn software (Version 1.0; CompuSyn Inc., Paramus, NJ, USA). The CI was calculated as:

$$CI = \frac{D_1}{D_{x1}} + \frac{D_2}{D_{x2}}$$

where D_1 and D_2 are the combined concentrations of extract and GA required to produce a specific F_a level and D_{x1} and D_{x2} are their standalone concentrations causing the identical fractional effect. CI values <1 , $=1$, and >1 were interpreted as concentration-dependent bioactivity enhancement, parallel additivity, and activity suppression, respectively.

2.14 Statistical Analysis

Data are expressed as mean $\pm$ SD from at least three biological replicates (with three technical replicates each). Normality and homogeneity of variances were confirmed (Kolmogorov–Smirnov, Shapiro–Wilk, Levene's; $p > 0.05$). Two-group comparisons used an unpaired t -test; multi-group comparisons used one-way ANOVA with Tukey's HSD post-hoc ($p < 0.05$). Analyses were performed in Excel (Analysis ToolPak).

2.15 Molecular Docking

Three-dimensional conformers of gallic acid and rosmarinic acid were obtained from PubChem (<https://pubchem.ncbi.nlm.nih.gov/> accessed on 26 June 2026) in SDF format and converted to PDB format. Molecular docking was performed using the HDock server [40] in blind dock-

ing mode with MurF from *Streptococcus pneumoniae* (PDB ID: 3ZM5 [41]) and LpxC from *Escherichia coli* (PDB ID: 3P3G [42]). Before docking, water and co-crystallized non-protein molecules were removed. Essential catalytic cofactors present in the MurF and LpxC crystal structures were retained during protein preparation to preserve the native active-site environment. HDock generates dimensionless docking scores, with more negative values indicating more favourable predicted protein–ligand interactions. The top-ranked docking poses were analyzed based on docking score and protein residues within 4.0 Å of the ligand.

3. Results

3.1. Antioxidant Activity of Leaf and Root Extracts With Assessment of Gallic Acid as a Modulator

DPPH radical scavenging activity of leaf and root extracts is used as an indicator of plant antioxidant potential [29]. The leaf extract exhibited stronger DPPH scavenging activity than the root (IC_{50} of 34.8 ± 0.7 vs. 81.9 ± 1.1 $\mu\text{g/mL}$, Fig. 1). The IC_{50} of pure GA was 1.0 ± 0.01 $\mu\text{g/mL}$ (5.8 μM). Addition of 2 μM GA reduced the leaf extract IC_{50} to 26.6 ± 0.03 $\mu\text{g/mL}$ (Fig. 1A), representing a 23.6% reduction compared to extract alone; however, this value remained significantly higher than pure GA ($p < 0.05$). At 5 μM GA, the leaf extract IC_{50} decreased further to 0.8 ± 0.03 $\mu\text{g/mL}$ (97.7% reduction vs untreated, $p < 0.05$), which was significantly below pure GA ($p < 0.05$).

Statistical comparisons for all antioxidant assays were performed using one-way ANOVA with Tukey's HSD post hoc test. Pairwise comparisons were conducted between the following groups: extract alone vs. extract + 2 μM GA vs. extract + 5 μM GA, with GA alone serving as a reference control.

Similarly, addition of 2 μM GA reduced the root extract IC_{50} to 49.3 ± 0.7 $\mu\text{g/mL}$ (Fig. 1B), a 39.8% decrease compared to extract alone ($p < 0.05$), though it remained above pure GA. At 5 μM GA, the root extract IC_{50} dropped to 3.3 ± 0.05 $\mu\text{g/mL}$ (95.9% reduction, $p < 0.05$) but remained higher than pure GA ($p < 0.001$).

Thus, the addition of 5 μM GA resulted in a significant enhancement of the antioxidant capacity for both leaves and roots, driving down the IC_{50} values. At 2 μM GA, a more modest, concentration-dependent enhancement of the radical scavenging profile was observed.

3.2 Total Phenolic (TPC), Rosmarinic Acid (RA), and Flavonoid (TFC) Content

Leaves had higher total phenolic content (113.6 ± 6.3 mg GAE/g DW) than roots (56.9 ± 3.3 mg GAE/g DW), approximately twice as much (Fig. 2A). In contrast, rosmarinic acid was more abundant in roots (17.7 ± 0.3 mg/g DW) than in leaves (12.3 ± 0.2 mg/g DW), a 1.4-fold difference (Fig. 2B). Leaves also had higher TFC (63.8 ± 2.1 mg RE/g DW) than roots (19.0 ± 1.3 mg RE/g DW) (Fig. 2C). The higher TPC and TFC in leaves align with their stronger DPPH activity.

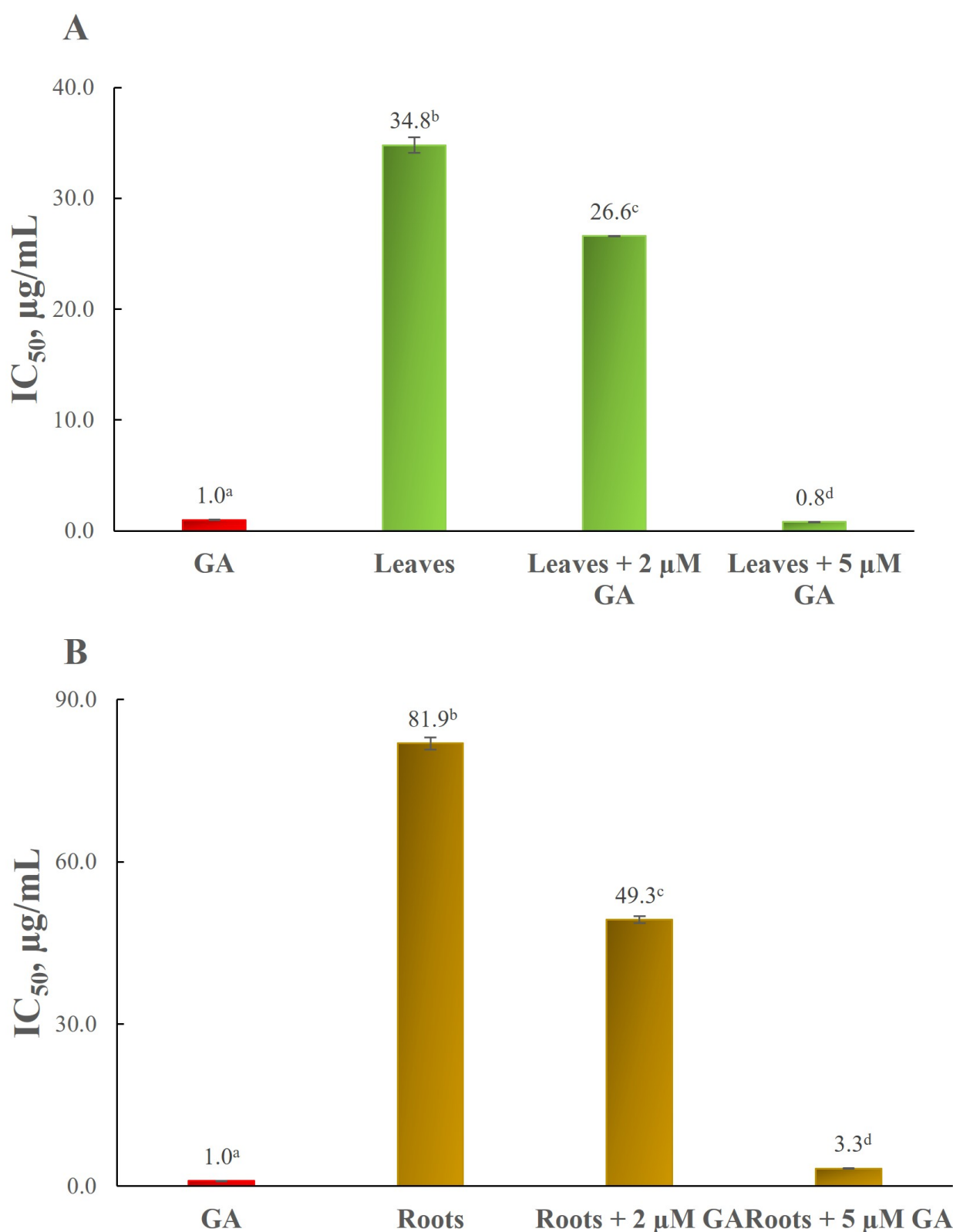


Fig. 1. Antioxidant activity of *T. tifiensis* leaf (A) and root (B) extracts determined by the DPPH method. Extracts were tested alone and with GA (2 μM and 5 μM). Data are mean ± SD (n = 3). IC₅₀ values (μg/mL) are shown as mean ± SD (n = 3). GA alone showed an IC₅₀ of 1.0 ± 0.01 μg/mL (5.8 μM). Different letters above bars indicate significant differences between groups (one-way ANOVA, Tukey's HSD, $p < 0.05$). Comparisons were performed independently for leaf (A) and root (B) panels. GA, gallic acid.

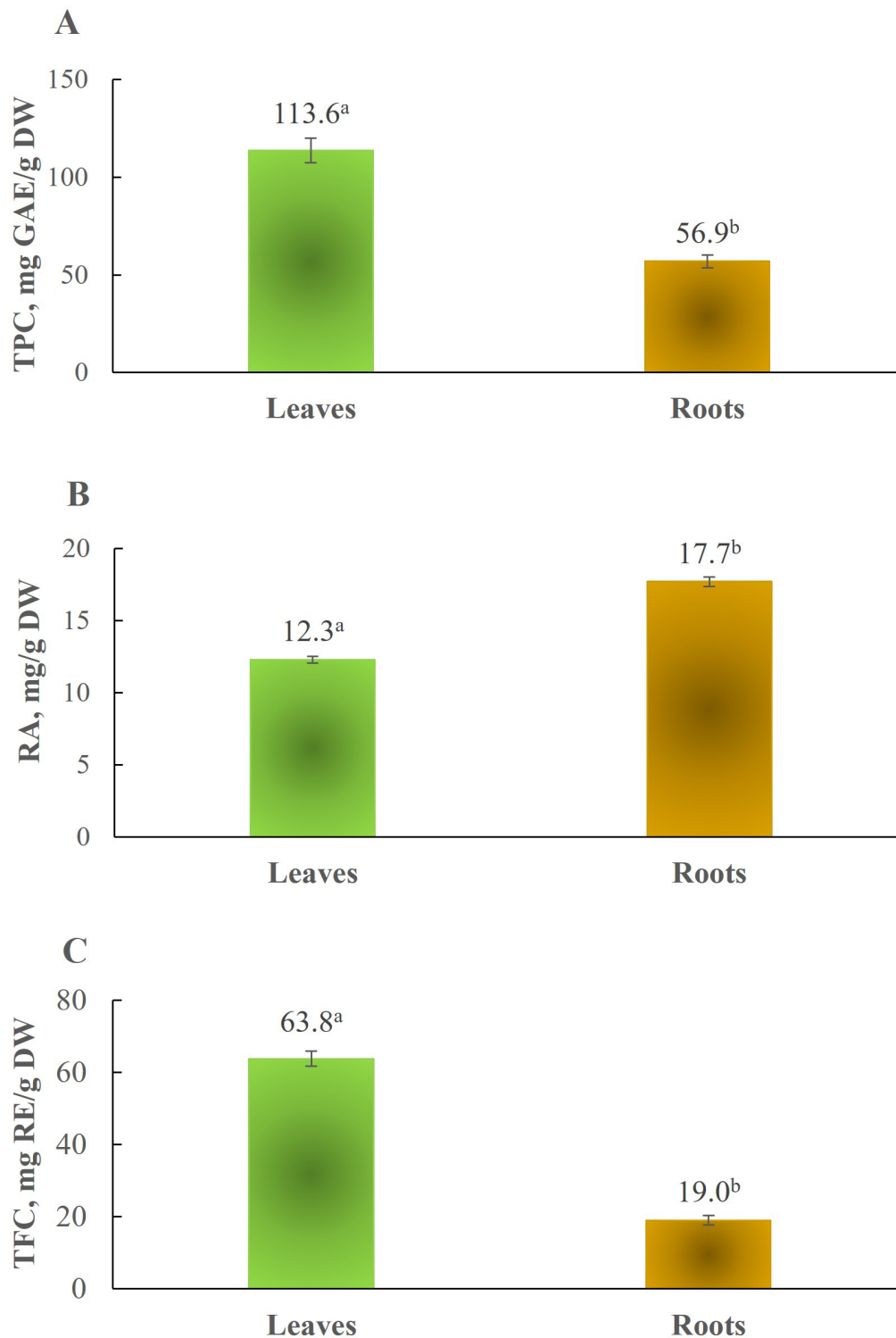


Fig. 2. Phenol (A), rosmarinic acid (RA, B) and flavonoid (C) contents in leaves and roots of *T. tifflesiensis*. Data are mean $\pm$ SD (n = 3). Different letters above bars indicate significant differences between leaves and roots for each parameter (one-way ANOVA, Tukey's HSD, $p < 0.05$). TPC, total phenolic content; RA, rosmarinic acid; TFC, total flavonoid content.

3.3 Ascorbate and Glutathione Content

Leaves had higher levels of ASC (220.6 ± 16.4 vs. 144.5 ± 12.8 mg/100 g DW, $p < 0.001$), DHA (59.8 ± 3.5 vs. 51.0 ± 3.8 mg/100 g DW, $p < 0.01$), as well as a greater

ASC/DHA ratio (3.7 ± 0.3 vs. 2.8 ± 0.2 , $p < 0.001$), compared to roots (Table 1). GSH was also higher in leaves (6.5 ± 0.3 vs. 3.3 ± 0.3 mg/100 g DW, $p < 0.001$), while GSSG was lower (1.9 ± 0.1 vs. 2.3 ± 0.3 mg/100 g DW, $p <$

Table 1. The content of reduced (ASC), oxidised (DHA) ascorbate, ASC/DHA ratio and reduced (GSH), oxidised (GSSG) glutathione, GSH/GSSG ratio in *T. tiffliensis* leaves and roots.

Parameter	Leaves	Roots	Significance
ASC (mg/100 g DW)	220.6 ± 16.4 ^a	144.5 ± 12.8 ^b	$p < 0.001$
DHA (mg/100 g DW)	59.8 ± 3.5 ^a	51.0 ± 3.8 ^b	$p < 0.01$
ASC/DHA	3.7 ± 0.3 ^a	2.8 ± 0.2 ^b	$p < 0.001$
GSH (mg/100 g DW)	6.5 ± 0.3 ^a	3.3 ± 0.3 ^b	$p < 0.001$
GSSG (mg/100 g DW)	1.9 ± 0.1 ^a	2.3 ± 0.3 ^b	$p < 0.05$
GSH/GSSG	3.4 ± 0.3 ^a	1.4 ± 0.2 ^b	$p < 0.001$

Data are mean ± SD (n = 3). Different superscript letters within a row denote significant differences between leaves and roots for each parameter, calculated using an unpaired Student's *t*-test. The *p*-values shown in the final column reflect leaf-to-root comparison.

0.05), resulting in a GSH/GSSG ratio more than twice that of roots. These results indicate that leaves maintain a more reduced redox state than roots (Table 1).

3.4 Correlation Analysis of Bioactive Compounds and Antioxidant Activity

Pearson correlation analysis showed a strong, statistically significant negative linear association between DPPH IC₅₀ values and each individual antioxidant parameter (TPC, TFC, ASC, ASC/DHA ratio, GSH, and GSH/GSSG ratio) across both leaf and root extracts (with *r* values reaching -0.999 ; $R^2 \geq 0.998$). These data indicate a strong statistical association, demonstrating that the observed radical scavenging capacity changes in parallel with the collective presence of these antioxidant pools rather than establishing a direct, isolated causal link to a single dominant constituent.

In contrast, Pearson correlation analysis revealed a strong positive relationship between rosmarinic acid (RA) content and the DPPH IC₅₀ values ($r = 0.988$; $R^2 = 0.976$; $p < 0.05$). Because RA accumulation is significantly higher in roots while antioxidant potency is concentrated in leaves, this positive correlation confirms that rosmarinic acid is not the dominant constituent driving the overall radical scavenging capacity of the plant.

3.5 Protein Denaturation Inhibitory Activity, BSA Binding Properties, and Modulatory Effects of GA

Protein denaturation inhibition is an indirect screening assay commonly used as a preliminary test for potential protein-stabilising activity [43], as preventing denaturation may reduce inflammation [44]. BSA is often used as a model protein due to its similarity to human serum albumin [45]. In this study, ibuprofen served as the reference drug.

For the BSA denaturation assay, statistical comparisons were performed independently at each concentration point. One-way ANOVA with Tukey's HSD post-hoc test was used to compare the three treatment groups (extract alone, extract + 10 µM GA, extract + 100 µM GA)

at each concentration. For comparisons between extracts and ibuprofen, unpaired Student's *t*-tests were performed at each concentration.

The leaf extracts inhibited protein denaturation in a concentration-dependent manner (4.9% at 10 µg/mL to 91.1% at 375 µg/mL; Fig. 3A), with an IC₅₀ of 159.7 ± 12.8 µg/mL. This was twice that of ibuprofen (74.6 ± 6.2 µg/mL; $p < 0.05$), under the tested *in vitro* conditions. However, this comparison does not imply therapeutic equivalence or clinical superiority over ibuprofen. Between 10 and 250 µg/mL, ibuprofen was more effective ($p < 0.05$); at 375 µg/mL, the leaf extract showed greater inhibition (91.06% vs. 86.2%; $p < 0.05$).

Adding 10 µM GA slightly increased inhibition at low leaf extract concentrations (from 4.9% to 8.2% at 10 µM GA), but there was little difference at ≥ 60 µg/mL (Fig. 3B). The IC₅₀ for leaf extract + 10 µM GA (164.6 ± 14.1 µg/mL) was not significantly different from extract alone ($p > 0.05$). In contrast, 100 µM GA reduced inhibition at low-to-mid concentrations, raising IC₅₀ to 203.5 ± 18.5 µg/mL ($p < 0.05$).

Chou-Talalay modelling revealed a concentration-dependent interaction between the extract and GA across multiple effect levels ($F_a = 0.1$ – 0.9). For the 10 µM GA combination, activity enhancement response ($CI < 1$) occurred exclusively at low concentrations ($F_a \leq 0.2$), transitioning to parallel additivity ($CI \approx 1$) at higher doses with an unchanged IC₅₀. Conversely, the 100 µM GA combination exhibited definitive activity suppression ($CI > 1$) at low-to-mid effect levels, significantly increasing the IC₅₀. At 375 µg/mL, all combinations converged back to parallel additivity ($CI \approx 1$).

The root extract also showed concentration-dependent inhibition, ranging from 14.1% at 10 µg/mL to 98.4% at 375 µg/mL (Fig. 4A), with an IC₅₀ of 51.3 ± 4.2 µg/mL. The IC₅₀ was significantly lower than those of the leaf extract (159.7 ± 12.8 µg/mL) and ibuprofen (74.6 ± 6.2 µg/mL; $p < 0.05$) under the tested *in vitro* conditions. Above 40 µg/mL, the root extract showed greater inhibition than ibuprofen in this assay ($p < 0.05$), achieving 98.4% inhibition at the high-

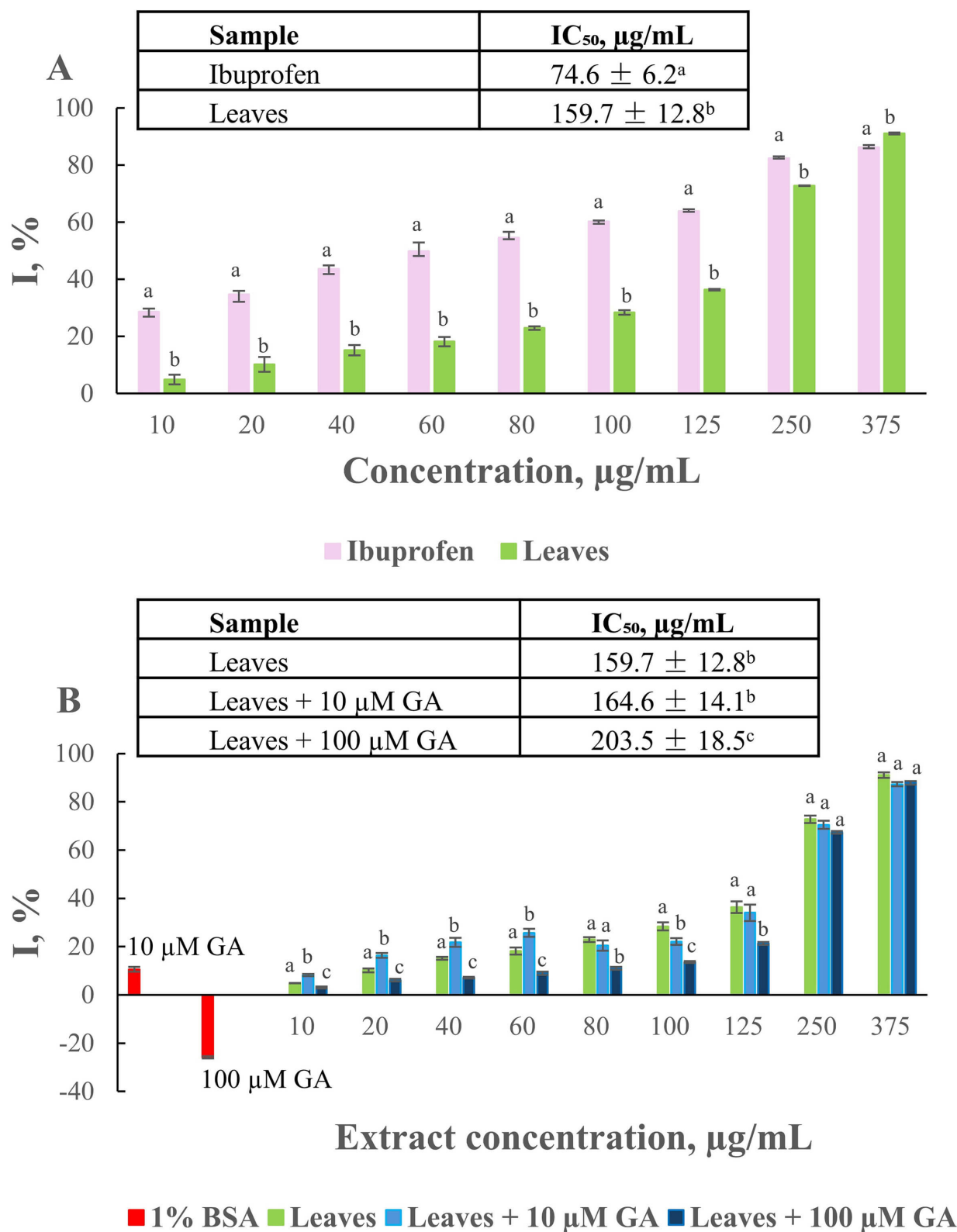


Fig. 3. Percentage inhibition (I, %) of BSA denaturation by ibuprofen and *T. tifiensis* leaf extract alone (A) and in the presence of 10 and 100 µM GA (B). Data are mean ± SD (n = 3). Different letters indicate significant differences ($p < 0.05$). In (A), letters compare leaf extract vs. ibuprofen at each concentration (unpaired t -test). In (B), letters compare treatments at each concentration (one-way ANOVA, Tukey's HSD); leaves alone are designated as "a".

Table 2. Inhibition zones (mm) of *T. tifiensis* leaf and root extracts against *S. agalactiae* and *E. coli* in an agar well diffusion assay.

Bacterial species	Treatment/extract	Inhibition zone (mm)
<i>S. agalactiae</i>	GA (340 µg/well)	NZ ^a
	Leaf (5 mg/well)	31.0 ± 4.2 ^b
	Leaf + GA (5 mg/well + 340 µg/well)	31.2 ± 4.0 ^b
	Root (5 mg/well)	35.0 ± 3.9 ^b
	Root + GA (5 mg/well + 340 µg/ well)	33.1 ± 6.9 ^b
	Amoxicillin, 0.1 mg/mL	51.2 ± 4.0 ^c
<i>E. coli</i>	GA (340 µg/well)	NZ ^a
	Leaf (5 mg/well)	25.1 ± 2.2 ^b
	Leaf + GA (5 mg/well + 340 µg/ well)	26.4 ± 3.2 ^b
	Root (5 mg/well)	23.1 ± 3.1 ^b
	Root + GA (5 mg/well + 340 µg/ well)	25.3 ± 1.2 ^b
	Amoxicillin, 0.1 mg/mL	57.9 ± 3.8 ^c

Data are mean ± SD (n = 3). Different superscripts within each bacterial species section indicate statistically significant differences among treatment groups (one-way ANOVA, Tukey's HSD, $p < 0.05$). NZ = no zone (0 mm).

est concentration. Overall, the root extract was three times more potent than the leaf extract.

Analysis of root extract combinations revealed a highly concentration-dependent modification of activity by GA (Fig. 4B). At low extract concentrations, 10 µM GA provided localised enhancement of the inhibitory effect ($CI < 1$), whereas 100 µM GA actively suppressed the baseline anti-denaturation performance ($CI > 1$), before both converged toward linear additivity ($CI \approx 1$) at concentrations ≥ 60 µg/mL.

3.6 Antibacterial Activity of Plant Extracts

3.6.1 Well Diffusion Method

The well diffusion assay revealed that both leaf and root extracts inhibited the growth of Gram-positive and Gram-negative bacterial strains (Table 2). GA alone (340 µg/well) exhibited no inhibitory effect against *S. agalactiae* or *E. coli*. Statistical comparisons for inhibition zone diameters were performed using one-way ANOVA with Tukey's HSD post-hoc test to compare all treatment groups (GA alone, leaf alone, leaf + GA, root alone, root + GA, amoxicillin) within each bacterial species. For *S. agalactiae*, the leaf extract (5 mg/well) produced an inhibition zone of 31.0 ± 4.2 mm, with no significant enhancement upon co-administration with GA. The root extract produced an inhibition zone of 35.0 ± 3.9 mm, which decreased to 33.1 ± 6.9 mm with GA addition. The differences between leaf and root extracts were not statistically significant ($p > 0.05$). Amoxicillin (0.1 mg/mL) produced an inhibition zone of 51.2 ± 4.0 mm.

Against *E. coli*, the leaf and root extracts produced inhibition zones of 25.1 ± 2.2 mm and 23.1 ± 3.1 mm, respectively, with no significant difference between them or following GA addition ($p > 0.05$). Amoxicillin produced the largest inhibition zone (57.9 ± 3.8 mm). Overall, both

extracts exhibited antibacterial activity against both bacterial species, with no significant differences between the leaf and root extracts or between extracts alone and in combination with GA.

3.6.2 Broth Liquid Culture Method

MIC and MBC values were determined by broth microdilution. The antibacterial activity of leaf and root extracts (1.5 mg/mL) against *S. agalactiae* was evaluated alone and with 0.2 and 1 mM GA (Fig. 5). Amoxicillin (0.1 mg/mL) was used as a positive control (99.9% inhibition of growth). The leaf extract alone inhibited growth by $16.3 \pm 0.6\%$ (MIC = 7.5 mg/mL, Fig. 5A). With 0.2 mM GA, inhibition was $17.0 \pm 1.3\%$ (MIC unchanged). At 1 mM GA, inhibition increased to $25.1 \pm 1.6\%$ ($p < 0.05$; MIC reduced to 5.8 mg/mL). GA alone at 1 mM exhibited minimal activity ($2.6 \pm 0.6\%$).

Statistical comparisons for antibacterial activity were performed using one-way ANOVA with Tukey's HSD post-hoc test to compare treatment groups (extract alone, extract + 0.2 mM GA, extract + 1 mM GA) within each bacterial species and extract type. Amoxicillin served as a positive control and was not included in the statistical comparisons due to its distinct mechanism and substantially higher activity.

The root extract exhibited significantly greater antibacterial activity than the leaf extract (Fig. 5B), achieving $41.2 \pm 1.1\%$ growth inhibition (MIC = 6.0 mg/mL). Addition of 0.2 mM GA had no significant effect, whereas 1 mM GA increased inhibition to $57.4 \pm 3.1\%$ ($p < 0.05$; 39.0% improvement) and reduced the MIC to 2.9 mg/mL. Overall, the root extract was approximately 2.5-fold more active than the leaf. For both extracts, 1 mM GA enhanced activity and lowered the MIC, while 0.2 mM GA had no impact.

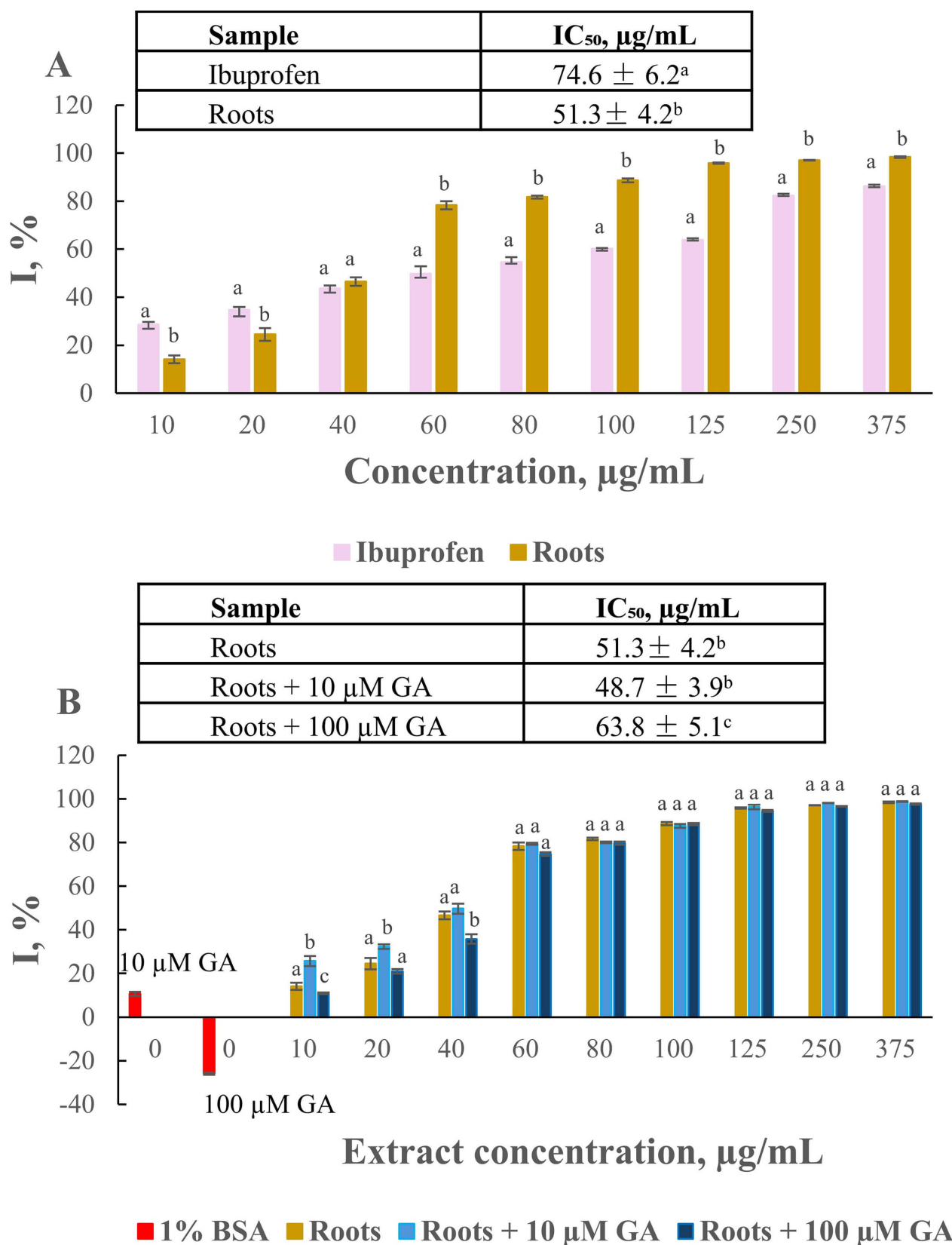


Fig. 4. Percentage inhibition (I, %) of BSA denaturation by ibuprofen and *T. tiflisiensis* root extract alone (A) and in the presence of 10 and 100 µM GA (B). Data are mean ± SD (n = 3). Different letters indicate significant differences ($p < 0.05$). In (A), letters compare root extract vs. ibuprofen at each concentration (unpaired *t*-test). In (B), letters compare treatments at each concentration (one-way ANOVA, Tukey's HSD); roots alone are designated as "a".

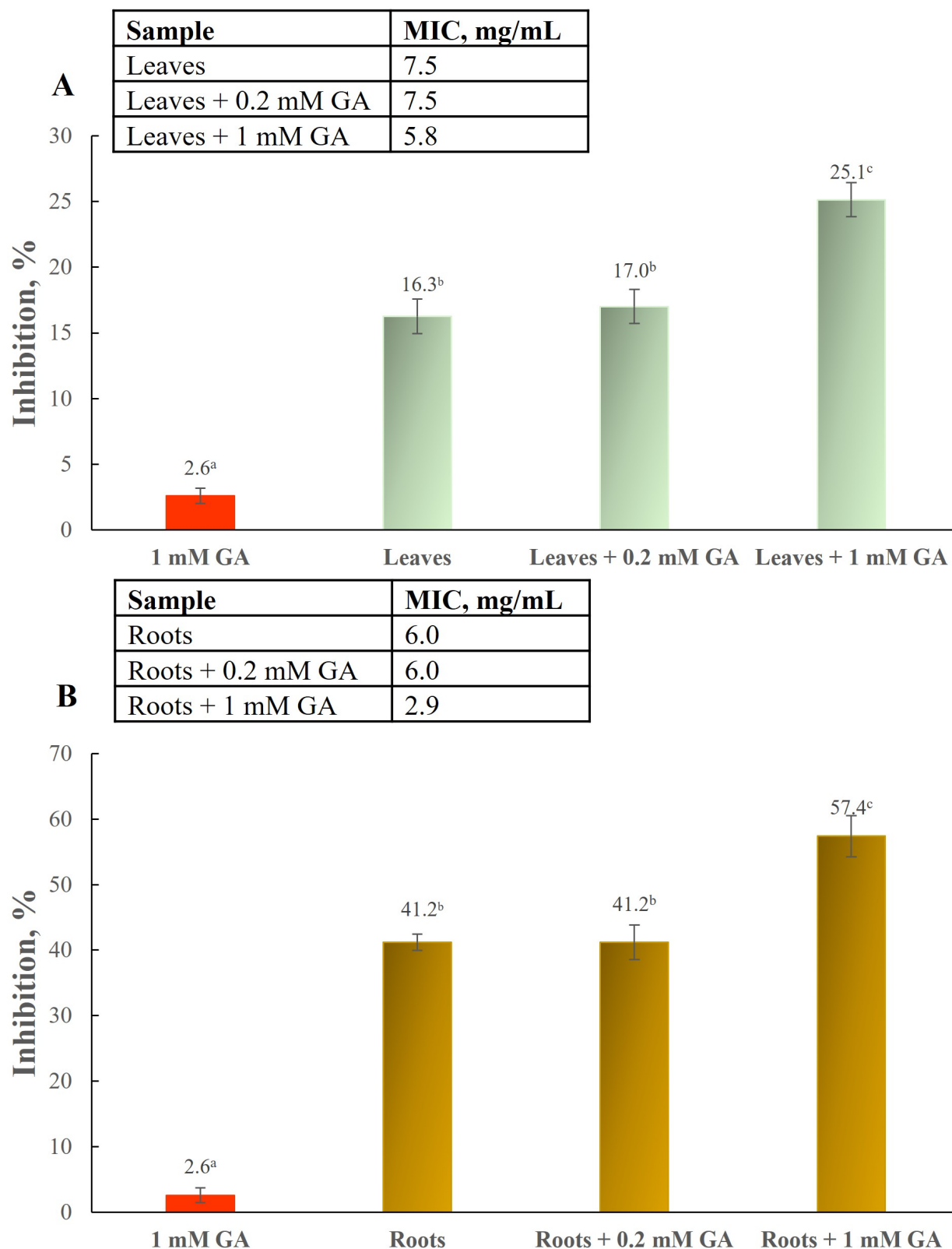


Fig. 5. Suppression of *S. agalactiae* growth by *T. tiflisiensis* leaf (A) and root (B) extracts (1.5 mg/mL) alone and in the presence of 0.2 and 1 mM of GA. Data are mean $\pm$ SD ($n = 3$). Different letters above bars indicate statistically significant differences among treatment groups within each panel (one-way ANOVA, Tukey's HSD, $p < 0.05$).

The experimental data demonstrated that GA augments the antibacterial activity of both extracts against *S. agalactiae* in a concentration-dependent manner. At 1 mM GA, a clear enhancement of growth inhibition was observed, decreasing the leaf MIC from 7.5 to 5.8 mg/mL and the root MIC from 6.0 to 2.9 mg/mL.

Antibacterial activity against *E. coli* was evaluated in parallel (Fig. 6). The leaf extract alone resulted in $30.2 \pm 2.8\%$ inhibition (Fig. 6A), with an MIC of 8.8 mg/mL and $\text{MBC} > 26.4$ mg/mL. Addition of 0.2 mM GA produced $30.5 \pm 2.4\%$ inhibition ($p > 0.05$; MIC unchanged), while 1 mM GA resulted in $32.3 \pm 2.9\%$ inhibition ($p > 0.05$; MIC unchanged). GA alone at 1 mM exhibited minimal activity ($1.0 \pm 0.1\%$).

The root extract yielded $32.5 \pm 2.1\%$ inhibition against *E. coli* (Fig. 6B), with an MIC of 11.3 mg/mL and $\text{MBC} > 26.4$ mg/mL. At 0.2 mM GA, inhibition was $31.0 \pm 3.4\%$ ($p > 0.05$; MIC unchanged). At 1 mM GA, inhibition increased to $38.2 \pm 2.1\%$ ($p < 0.05$), with the MIC reduced to 8.9 mg/mL. While leaf and root extracts showed comparable inhibition at 1.5 mg/mL (30.2% vs. 32.5% , $p > 0.05$), the leaf extract was more potent based on MIC (8.8 vs. 11.3 mg/mL).

Chou-Talalay analysis for *E. coli* revealed distinct extract-GA interactions. At 0.2 mM GA, neither extract exhibited MIC reduction or measurable activity enhancement. At 1 mM GA, the leaf extract interaction remained within the boundaries of parallel additivity ($\text{CI} \approx 1$, MIC 8.8 mg/mL), while the root extract shifted into a clear bioactivity enhancement zone ($\text{CI} < 1$), with the MIC reduced from 11.3 to 8.9 mg/mL. Thus, GA provided a significant modulatory enhancement of antibacterial activity only in the root extract at 1 mM, confirming tissue-specific modulation.

3.7 Molecular Docking

MurF, an intracellular target involved in peptidoglycan biosynthesis in Gram-positive bacteria (*Streptococcus* spp.), and LpxC, an enzyme in lipid A biosynthesis in Gram-negative bacteria (*E. coli*), were chosen for docking studies to investigate possible mechanisms behind the differing antibacterial effects of the root and leaf extracts. The docking analyses of GA and RA (Fig. 7 and Table 3) indicate that RA achieved better binding scores with both MurF and LpxC, forming multiple short-range interactions in common functional regions.

For MurF, RA engaged a cluster of residues: TYR129, GLN132, TYR135, TYR143, HIS147, and ASN326 (Table 3), indicating that it occupies a substrate-recognition zone. GA interacted with only part of this region (ASP32, ARG34, TYR135, and TYR143), suggesting a more limited and less stabilising binding footprint. GA exhibited the least favourable HDOCK scores toward both MurF and LpxC compared with RA, consistent with the lack of inhibition zones in agar diffusion assays against either *S. agalactiae* or *E. coli*.

For LpxC, RA occupied a pocket defined by ASP160, PHE161, PHE192, ASP197, LYS239, and SER263, whereas GA was associated with a distinct set of residues: GLN4, ASN34, ASP90, ASN91, and LYS128. The larger polyphenolic structure of RA therefore appears capable of engaging the protein binding regions more extensively than GA, consistent with its superior docking scores. The antibacterial assay demonstrated that *T. tiflisiensis* extracts were more active against *S. agalactiae* than against *E. coli* (inhibition zones of 31–35 mm and 23–26 mm, Table 2). This experimental trend is supported by the *in silico* data, where the primary root polyphenol (RA) demonstrated more favourable predicted binding scores over GA within both target enzyme models (Table 3). This suggests a predicted structural complementarity between RA from *T. tiflisiensis* and the modelled binding pockets of MurF and LpxC, providing a tentative mechanistic hypothesis that warrants future experimental enzymatic validation. The stronger predicted binding to MurF, together with the greater susceptibility of Gram-positive bacteria due to the absence of an outer membrane, supports the observed higher antibacterial potency against *S. agalactiae*. The stronger activity of the root extract, containing a higher amount of RA, than leaf extracts against *S. agalactiae* is consistent with the more favourable docking scores observed for MurF, suggesting that inhibition of peptidoglycan biosynthesis may contribute to the antibacterial effect of root-derived phytochemicals, in line with the more favourable predicted docking scores observed for the homologous *S. pneumoniae* MurF target.

In contrast, although the leaf extract showed slightly greater activity against *E. coli* than the root extract, the docking scores toward LpxC were less favourable than for MurF. This suggests that inhibition of LpxC alone does not fully explain the antibacterial activity of the plant extract. Other variables, including tissue-specific differences in non-volatile phytochemical composition, outer-membrane permeability constraints, reinforcing interactions among minor matrix constituents, or alternative molecular targets, contribute to the observed activity against Gram-negative bacteria.

4. Discussion

4.1 Antioxidant Activity and Modulatory Enhancement by GA

This study demonstrated for the first time that *T. tiflisiensis* leaf and root extracts have significant antioxidant activity. Leaves displayed stronger antioxidant effects than roots ($\text{IC}_{50} = 34.8$ $\mu\text{g/mL}$ vs. 81.9 $\mu\text{g/mL}$), closely linked to higher phenolic and flavonoid content ($r = -0.999$, $R^2 = 0.998$). These results support the role of phenolics and flavonoids as major plant antioxidants [46]. Despite the higher RA content in roots than leaves, the superior antioxidant defence in leaves may be attributed to higher ASC and

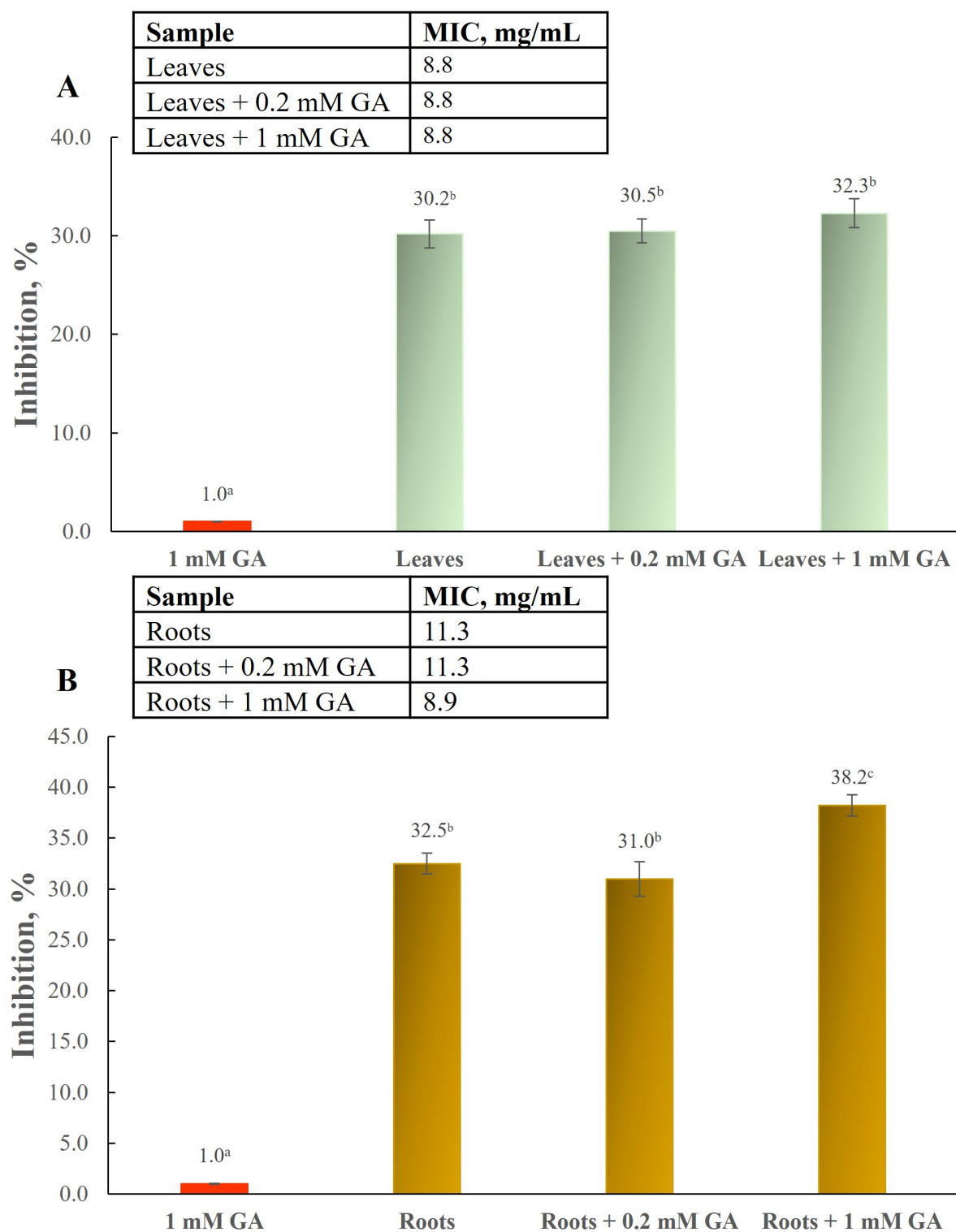
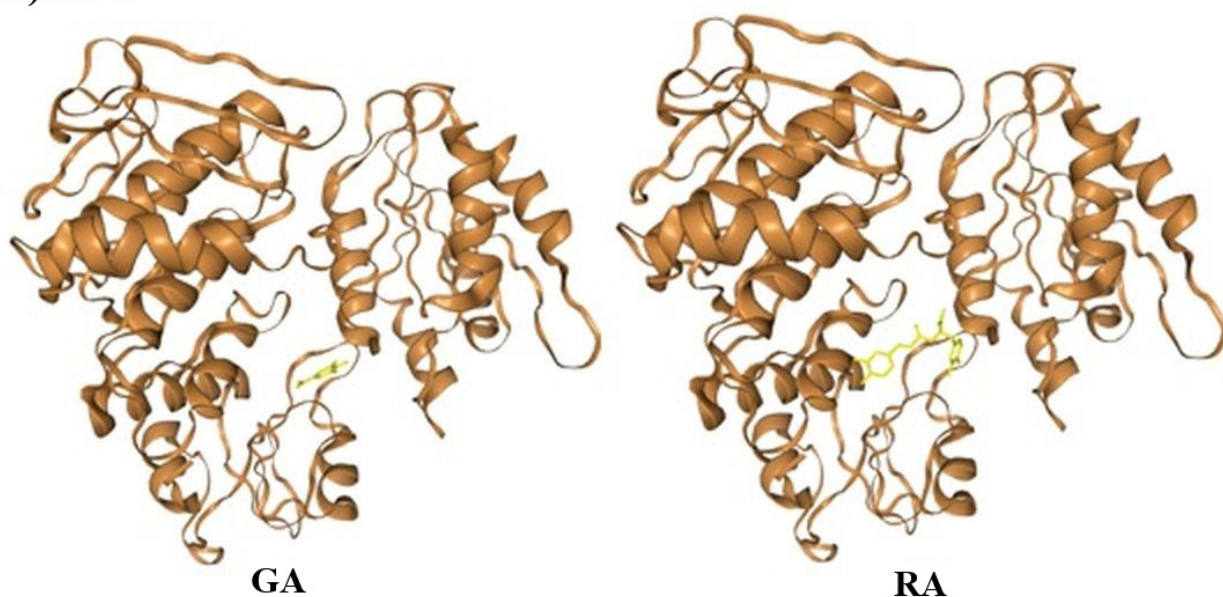


Fig. 6. Suppression of *E. coli* growth by *T. tifiensis* leaf (A) and root (B) extracts (1.5 mg/mL) alone and in the presence of 0.2 and 1 mM of GA. Data are mean $\pm$ SD ($n = 3$). Different letters above bars indicate statistically significant differences among treatment groups within each panel (one-way ANOVA, Tukey's HSD, $p < 0.05$).

GSH levels and greater ASC/DHA and GSH/GSSG ratios, which are key for redox balance and radical neutralisation [47].

Combining both extracts with 5 μ M GA yielded a modulatory enhancement ($CI < 1$) across all tested fractional effect levels ($F_a = 0.1-0.9$), reducing IC_{50} values (0.8 and 3.3 μ g/mL). This bioactivity enhancement, driven by GA's

A) MurF



B) LpxC

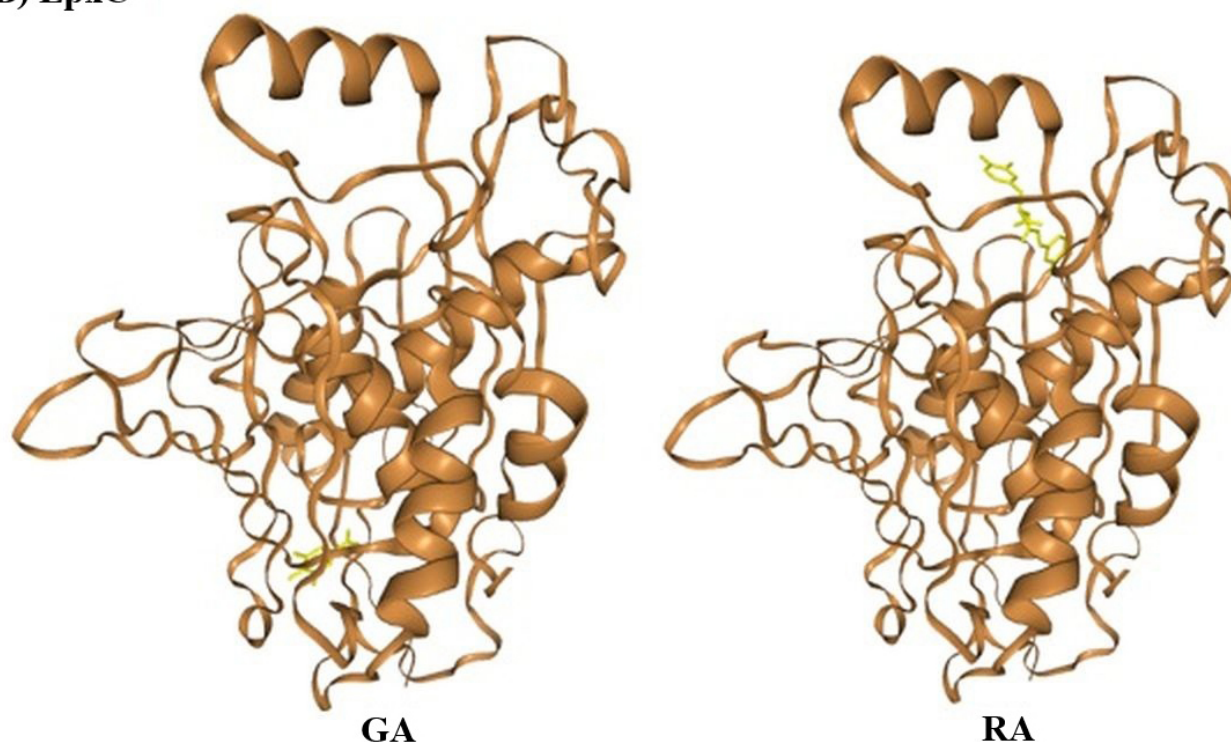


Fig. 7. Three-dimensional views of the top-ranked HDOCK docking poses for gallic acid (GA) and rosmarinic acid (RA) bound to *Streptococcus pneumoniae* MurF (PDB ID: 3ZM5 (A)) and *Escherichia coli* LpxC (PDB ID: 3P3G (B)). Ligands are displayed in yellow to highlight their placement within the predicted binding pockets.

electron-donating properties [48], may allow for lower extract doses; however, this requires confirmation in *in vivo* studies. Similar reinforcing enhancement profiles between RA, caffeic acid, and GA in preventing oxidative DNA

damage have been reported [49]. Conversely, 2 μ M GA shifted toward mostly parallel additive boundaries (leaf: CI = 1.10; root: CI = 0.94), reinforcing the necessity of dose optimisation.

Table 3. Molecular docking results of gallic acid (GA) and rosmarinic acid (RA) against MurF (PDB: 3ZM5) and LpxC (PDB: 3P3G).

Target (PDB)	Ligand	Top 1 Score	Mean Top1–3	SD	Top 1 Interacting Residues (≤ 4.0 Å)
MurF (3ZM5)	GA	–120.14	–115.70	6.66	GLU30 (2.79), PHE31 (3.25), ASP32 (2.93), ARG34 (3.62), LEU35 (3.52), TYR135 (2.78), TYR143 (2.70), ALA327 (3.26), ASN328 (2.42)
	RA	–197.50	–190.15	11.65	ASP32 (2.43), ARG34 (3.20), LEU35 (2.99), TYR129 (2.25), GLN132 (3.10), TYR135 (2.74), TYR143 (3.37), THR144 (3.01), HIS147 (2.35), ARG306 (3.59), TYR325 (3.35), ASN326 (3.02), ALA327 (3.37), ASN328 (2.80)
LpxC (3P3G)	GA	–126.44	–113.45	11.29	GLN4 (2.86), ASN34 (3.28), GLY36 (3.37), ASP90 (2.62), ASN91 (2.95), ALA126 (3.39), LYS127 (3.35), LYS128 (2.69), GLY251 (2.56), HIS252 (3.28), ASN253 (3.65)
	RA	–185.33	–175.60	11.55	ILE159 (2.96), ASP160 (2.64), PHE161 (2.96), PHE192 (2.25), GLY193 (3.69), ASP197 (3.30), LYS239 (3.91), LYS262 (3.14), SER263 (2.11)

The table summarises the top docking score, mean docking score of the three best-ranked poses, standard deviation (SD), and amino acid residues located within 4.0 Å of the top-ranked binding pose.

4.2 Protein Denaturation Inhibitory Activity and Modulation by GA

Concerns about the efficacy and side effects of non-steroidal anti-inflammatory drugs (NSAIDs) have led to interest in plant extracts as safer alternatives [50]. The BSA denaturation assay, an indirect screening model for potential protein-stabilising activity [51], showed that the root extract ($IC_{50} = 51.3$ µg/mL) exhibited greater inhibition than leaf extract ($IC_{50} = 159.7$ µg/mL) and ibuprofen ($IC_{50} = 74.6$ µg/mL) under the tested *in vitro* conditions. However, this comparison does not imply therapeutic equivalence or clinical superiority over ibuprofen, as the BSA assay measures protein stabilisation rather than cellular inflammatory responses. These results suggest that root extracts have potent protein-stabilising properties that warrant further investigation in cellular inflammatory models (e.g., LPS-stimulated macrophages) and *in vivo* models of inflammation [52]. The elevated anti-denaturation activity of root extracts, despite low antioxidant capacity, implies the presence of specific protein-stabilising compounds, such as rosmarinic acid. It is important to note that BSA denaturation inhibition is an indirect screening model for potential protein-stabilising activity. This assay does not capture the full complexity of inflammatory responses, including cytokine signalling, immune cell activation, or *in vivo* pharmacokinetics. The results should therefore be interpreted as preliminary evidence of protein-stabilising activity that warrants further investigation in cellular and animal models.

4.3 Antibacterial Activity and Modulation by GA

The well diffusion assay indicated that both leaf and root extracts exhibit antibacterial activity. Broth microdilution revealed that root extract was more effective against *S. agalactiae* than leaf extract. Given the clinical significance of *S. agalactiae* in neonatal sepsis and rising antibiotic resistance [53,54], *T. tiflisiensis* roots warrant further exploration as a source of anti-streptococcal agents. Both *T. tiflisiensis* extracts showed lower efficacy against Gram-negative *E. coli* than Gram-positive *S. agalactiae*. This reduced susceptibility is typical for polar, non-volatile phenolics due to the restrictive outer lipopolysaccharide membrane barrier of Gram-negative bacteria, which limits the passive influx of hydrophilic constituents into the periplasmic space [55]. These findings for polar, methanolic extracts must be distinguished from volatile, lipophilic monoterpenes such as *T. vulgaris* essential oils, which cause rapid membrane destabilisation [56]. The observed resistance profile of *E. coli* highlights the need for targeted formulations that can bypass the outer membrane without relying on volatile lipophilic mechanisms.

The broth assay also demonstrated that 1 mM GA potently enhanced the activity of both extracts against *S. agalactiae*, likely driven by GA–phenolic/flavonoid interactions [57]. For *E. coli*, activity enhancement at 1 mM GA was observed only with the root extract, indicating preferential root–GA interactions. The lower susceptibility enhancement against *E. coli* is structurally consistent with

active Gram-negative cell resistance mechanisms, such as transmembrane efflux pump activity, which limit intracellular accumulation [58]. Exploratory molecular docking supported these findings: RA showed favourable binding energies to MurF and LpxC and established multiple interactions within their active sites, more extensively than GA. This suggests that RA from *T. tiflisiensis* may interact with MurF and LpxC, providing a mechanistic rationale for the observed antibacterial effects.

4.4 Advisability of Combining GA With *T. tiflisiensis* Extracts

Our study demonstrated that the therapeutic efficacy of combining GA with *T. tiflisiensis* is concentration- and purpose-dependent. For antioxidant use, 5 μM GA is beneficial due to sustained enhancement of activity. Conversely, elevating the modulator to 100 μM GA actively reduced the native extract anti-denaturation performance.

For protein denaturation inhibition in the BSA assay, GA combinations are not recommended based on the current *in vitro* data. While a low dose of 10 μM GA offers a narrow, localised activity enhancement window restricted to early effect levels ($F_a \leq 0.2$), it fails to improve the overall IC_{50} . Furthermore, elevating the modulator to 100 μM GA triggers a distinct activity suppression ($\text{CI} > 1$), actively diminishing the native extract's protein-stabilising capacity by 24–27%. However, these findings are derived from an indirect screening assay (BSA denaturation) and require confirmation in cellular inflammatory models (e.g., LPS-stimulated macrophages) to assess cytokine modulation and NF- κB pathway involvement before any conclusions can be drawn about true anti-inflammatory potential.

The combination of GA with plant extracts is a well-established approach to combat resistance, since it affects membrane permeability, inhibits formation of biofilms, and disrupts efflux pumps [59]. For antibacterial trends, combining 1 mM GA with both extracts provided a clear bioactivity enhancement against *S. agalactiae*, whereas the enhancement response against *E. coli* was strictly limited to root extract; the complete lack of functional enhancement observed at 0.2 mM GA indicates a clear minimum effective concentration threshold.

Overall, GA combinations are recommended only for antioxidant (5 μM GA, roots) and specific antibacterial uses (1 mM GA with both extracts against *S. agalactiae*, and with root extract against *E. coli*). These results underscore the importance of dose optimisation and tissue selection, and caution against indiscriminate GA use as a therapeutic enhancer.

5. Limitations

Several limitations of the current study should be noted. First, the biological activities of *T. tiflisiensis* extracts and their modulation by gallic acid were evaluated exclusively using *in vitro* assays and *in silico* molecular

docking models. Therefore, these preliminary screening findings cannot fully replicate systemic physiological responses, bioavailability, or complex tissue microenvironments *in vivo*, which is a recognised transition boundary for baseline phytomedicinal investigations. Second, our study relied on crude methanolic extracts rather than isolated pure compounds, leaving the exact interactions among minor phytochemical constituents uncharacterized. Comprehensive HPLC-DAD/ESI-MS profiling to identify and quantify individual phenolic compounds was beyond the scope of this preliminary study. While we quantified total phenolics, flavonoids, rosmarinic acid (by spectrophotometry), ascorbate, and glutathione, the specific contributions of individual phenolic constituents to the observed bioactivities remain to be determined. Third, protein denaturation inhibitory activity was assessed solely by BSA denaturation inhibition, an indirect screening assay. Cellular inflammatory models (e.g., LPS-stimulated macrophages) are needed to confirm cytokine modulation (TNF- α , IL-1 β , IL-6) and NF- κB pathway involvement before proposing clinical applications. Fourth, the antibacterial evaluation was restricted to two specific standard reference strains (*S. agalactiae* and *E. coli*); while these are well-established benchmarks for examining plant extract potencies [60], our results may not fully extrapolate to multidrug-resistant clinical isolates or broader bacterial taxonomies. Finally, although molecular docking suggested favourable binding scores for rosmarinic acid with MurF and LpxC, prospective *in vitro* enzymatic inhibition assays are required to definitively validate these specific cellular targets, as computationally predicted footprints do not automatically equate to true enzymatic suppression cascades [61].

6. Conclusions

This is the first study to characterize the phytomedicinal profile of the endemic species *Thymus tiflisiensis*. The methanolic leaf extracts demonstrated a superior baseline antioxidant profile, which correlated strongly with elevated total phenolic and flavonoid accumulation. Conversely, pronounced tissue-specific variation was observed in the roots, which exhibited significantly more potent *in vitro* protein denaturation inhibitory activity and distinct growth inhibition against *S. agalactiae* and *E. coli*. The integration of exogenous GA as a bioactive modulator requires meticulous dose optimization; a 5 μM concentration effectively enhances native antioxidant capacity, whereas higher concentrations (100 μM) actively suppress the preliminary protein denaturation inhibitory performance of the extracts. For targeted antibacterial applications, a 1 mM GA concentration successfully enhances bacterial growth inhibition against *S. agalactiae* for both tissues and selectively enhances root extract performance against *E. coli*, whereas lower modulator doses fail to yield functional improvements. Docking analysis suggested that RA was the top compound for MurF and LpxC targets, with GA partially

overlapping these binding sites. *T. tiflisiensis* extracts were more active against *S. agalactiae*, which is consistent with the exploratory docking studies showing higher affinity of RA for Gram-positive MurF. Collectively, these baseline findings demonstrate that *T. tiflisiensis* is a promising source of tissue-specific bioactive compounds, establishing a definitive foundation for mandatory future downstream cellular validation, cytokine modulation profiling, and *in vivo* efficacy evaluations.

Abbreviations

Apig, Apigenin; BSA, bovine serum albumin; DTT, dithiothreitol; *E. coli*, *Escherichia coli*; DTNB, 5,5'-dithiobis(2-nitrobenzoic acid); GA, gallic acid; Lut, luteolin; NSAIDs, non-steroidal anti-inflammatory drugs; RA, rosmarinic acid; *S. agalactiae*, *Streptococcus agalactiae*; TNB, 5-thio-2-nitrobenzoate; *T. tiflisiensis*, *Thymus tiflisiensis*.

Availability of Data and Materials

The datasets used and analyzed during the current study are available from the corresponding authors upon reasonable request.

Author Contributions

VM, EB, MP, NE and GR designed the research study. VM, EB, and GR performed the research. ZT and CM analyzed the data. NE provided help and advice on the studied plant material, its obtaining, characteristics and distribution range. VM, EB, MP, NE, ZT and GR wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Ethical approval was not required for this study. The research was conducted entirely *in vitro* using plant extracts and bacterial cultures, and did not involve human participants, human material, human data, or animal experiments. This type of research is not subject to review by an ethics committee according to the national laws of Georgia or the Code of Ethics of University Geomedi, which adhere to international guidelines for research on humans. The plant material used in this study, *Thymus tiflisiensis* Klokov & Des.-Shost. (Lamiaceae), was collected in June 2025 at the onset of flowering from the living collections maintained at the National Botanical Garden of Georgia (Tbilisi, Georgia). Plant collection was conducted in accordance with institutional policies of the National Botanical Garden of Georgia, the National Law of Georgia on Environmental Protection (1996), and the Law of Georgia on the Special Protection of Green Plantations and State Forests within

and Adjacent to the City of Tbilisi (2026), governing the research use and conservation of local botanical resources. The species was identified by Dr. Nino Eradze, National Botanical Garden of Georgia (Department of Living Collections), and a voucher specimen has been deposited in the working herbarium of the National Botanical Garden of Georgia, Tbilisi, under the accession number (voucher number NBGG-LC-2025-0196). The voucher specimen is available for verification upon request.

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Conflicts of Interest

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