Original Research Article

Methodological Modifications for Crude *Thymus tiflisiensis* Leaf Extract Screening for *In Vitro* Anti-Inflammatory and Antibacterial Activities

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Abstract

Thymus tiflisiensis Klokov & Des.-Shost. (Lamiaceae) is a medicinal plant traditionally used in the Caucasus region for inflammatory conditions and infectious diseases. However, standard *in vitro* assays often require methodological modifications when applied to crude plant extracts due to interference from coloured phytochemicals, tannins, and other matrix components. This study aimed to: (1) optimize the bovine serum albumin (BSA) denaturation assay for crude plant extracts by determining the optimal buffer pH and denaturation temperature, and (2) compare the broth microdilution (liquid culture) method versus disk diffusion (agar) method for antibacterial screening of plant extracts, and to demonstrate the superiority of liquid culture over agar diffusion for MIC determination. Leaves of *T. tiflisiensis* were extracted by maceration using 80% methanol. For BSA denaturation optimization, buffer pH (6.0-7.4) and denaturation temperature (65 °C, 70 °C, 75 °C) were systematically evaluated using a fixed BSA concentration (1 %). Optimal conditions were identified as pH 6.4 and incubation at 37 °C for 20 minutes, followed by heating at 70 °C for an additional 20 minutes. For antibacterial screening, broth microdilution is unequivocally superior to disk diffusion for crude plant extracts, as it eliminates diffusion-related artifacts and provides quantitative MIC data required for meaningful comparison. These optimized protocols provide a validated framework for ethnopharmacological screening of Caucasian medicinal plants.

Keywords: *Thymus tiflisiensis*; BSA denaturation assay modification; broth microdilution; disk diffusion comparison; methodological optimization

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Introduction

The screening of plant extracts for anti-inflammatory and antibacterial activities is fundamental to ethnopharmacological research. The most common and accessible *in vitro* assays are the bovine serum albumin (BSA) denaturation inhibition assay for anti-inflammatory activity and the disk diffusion (Kirby-Bauer) method for antibacterial screening. However, these standard protocols frequently yield unreliable or irreproducible results when applied to crude plant extracts, representing complex mixtures containing pigments, tannins, polysaccharides, and other matrix components.

An *in vitro* anti-inflammatory assay can be conducted to measure the activity of natural substances before animal testing using various methods. One of these methods is the protein denaturation inhibition method using bovine serum albumin (BSA), which is stable and non-reactive. The BSA denaturation assay is based on the principle that heat-induced protein denaturation mimics the protein unfolding that occurs during inflammatory tissue damage. Compounds that prevent this denaturation are considered potential anti-inflammatory agents [1]. The absorbance of the turbidity caused by protein denaturation at its maximum wavelength is measured using a UV-Vis spectrophotometer and anti-inflammatory activity is expressed as the half-maximal inhibitory concentration (IC50) [2].

Standard protocols of this method typically employ phosphate buffer at pH 6.5-7.0 and denaturation temperatures of 57-70 °C [3], [4]. However, crude plant extracts introduce two major confounders: coloured compounds (chlorophyll, anthocyanins, flavonoids) that absorb at the measurement wavelength (660 nm), artificially elevating absorbance readings [5]; and problems associated with pH. BSA has an isoelectric point (pI) of approximately 4.7 to 5.5 [6]. When assay buffers or acidic crude plant extracts drop too close to the pI, the protein loses electrostatic stability and spontaneously aggregates [7]. This causes falsely high denaturation readings, masking the true anti-denaturing effects of the extracts.

Similarly, the disk diffusion method presents critical limitations for crude plant extracts. Hydrophobic or high-molecular-weight compounds may fail to diffuse through agar, leading to false-negative results [8]. Furthermore, the method provides only qualitative or semi-quantitative data (e.g., zones of inhibition), whereas studies of medicinal plants quite often require quantitative minimum inhibitory concentration (MIC) values to enable direct potency comparisons between extracts, fractions, or isolated compounds [9]. Beyond these well-recognized drawbacks, the disk diffusion technique is also extremely sensitive to several experimental variables that are difficult to standardize when working with complex plant matrices. Factors such as the rate of compound diffusion (influenced by molecular size, agar composition, and temperature), the inoculum density, and the choice of culture medium can significantly alter the resulting inhibition zones. Moreover, pigmented or turbid crude extracts may obscure the visibility of faint inhibition halos, introducing subjective interpretation

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bias. Consequently, reliance on disk diffusion alone for screening medicinal plants risks both false negatives (due to poor diffusion) and false positives (caused by non-specific effects or physical interference), making it an inappropriate method for rigorous antimicrobial evaluation. Therefore, complementary broth-based dilution methods, for example microdilution are strongly recommended, particularly when working with hydrophobic, high-molecular-weight, or coloured phytochemical preparations.

This study addresses these methodological challenges using leaf extracts of *Thymus tiflisiensis* Klokov & Des.-Shost.

This study aimed to optimize and validate methodological approaches for the anti-inflammatory and antibacterial screening of extracts of Caucasian medicinal plants. The specific objectives were:

1. To determine optimal conditions (buffer pH and denaturation temperature) for the BSA denaturation assay when applied to crude plant extracts.
2. To compare the broth microdilution and disk diffusion methods for assessing the antibacterial activity of plant extracts.
3. To develop validated protocols suitable for future ethnopharmacological screening based on the above findings.

Materials and Methods

Plant material and authentication

Thymus tiflisiensis Klokov & Des.-Shost. plants were collected in July 2025 in the National Botanical Garden of Georgia, Tbilisi, Georgia. Five plants were collected, placed in paper bags, and transported to the laboratory within 1 h. Fully expanded leaves were collected from all plants. Samples of fresh plant material were frozen in liquid nitrogen and stored at –80 °C.

Drying process

The leaves (5 g) were freeze-dried using a DW-10N freeze dryer (Drawell, China) in a 500 mL vacuum flask at 10 Pa and a final condenser temperature of –55 °C, until constant weight was achieved, as was determined by measuring the dry weight.

Extraction of plant samples

The extraction efficiencies of different solvents for plant material were tested previously, and methanol was demonstrated to be the most efficient solvent, allowing the highest DPPH scavenging activity to be obtained [10]. *T. tiflisiensis* leaf samples were homogenized in a ratio of 1:5 with 80% methanol, followed by continuous stirring for 24 h at room temperature using an orbital shaker at 270 rpm. The crude extract was clarified by centrifugation at 5000×*g* for 15 minutes (TD6 Benchtop Centrifuge Drawell, Chongqing, China). Leaf extracts obtained using 80% methanol were rotary evaporated at 50 °C using DW-ORE2000 rotary evaporator (Drawell, Chongqing, China), and the residue was re-dissolved in 150 mM potassium-phosphate buffer, pH 7. For antibacterial assays, re-dissolved residues were sterilized through a 0.22 µm sterile filter (Millipore) and then loaded on sterile filter paper disks.

Anti-inflammatory activity

Denaturation of proteins is one of the predominant reasons for inflammatory and arthritic diseases [11]. Therefore, the ability

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of a substance to prevent protein denaturation may also help to prevent the inflammatory conditions [12]. BSA is widely used as a model protein in many areas of research because it holds similar properties to those of human serum albumin [12]. In this study, ibuprofen, a non-steroidal anti-inflammatory drug, was used as a reference drug, preventing protein denaturation.

***In vitro* inhibition of albumin denaturation**

Inhibition of albumin denaturation was performed by following a standard method with slight modifications [13]. A 1 % (w/v) solution of bovine serum albumin (BSA) was prepared in phosphate-buffered saline (PBS) at a pH ranging from 6.0 to 7.4 by stirring at 400 rpm for 20 minutes. Different concentrations of samples (100 µL) or the standard drug (Ibuprofen) were mixed with 2.9 mL of 1% BSA solution. The resulting samples were incubated at 37 °C for 20 minutes, then heated at 70 °C for an additional 20 minutes. Finally, the samples' absorbance was measured at 660 nm in 10 mm path length quartz cuvettes using a DU-8800 RS spectrophotometer (Drawell, Chongqing, China) to assess the reaction outcomes. Given that BSA unfolds upon heating and undergoes substantial denaturation and aggregation at 70 °C, the thermal protocol used in this assay produces a partially denatured protein population. Accordingly, the UV-Vis binding parameters obtained reflect interactions with partially denatured BSA [14].

A control was established using the same procedure; the sample solution was replaced with 50 µL of phosphate-buffered saline (PBS). The percentage of protein denaturation inhibition was determined using the following formula:

$$I\% = \frac{Ac - As}{Ac} \times 100$$

where I% denotes the percentage of inhibition, A_S represents the absorbance value of the sample, and A_c represents the absorbance value of the control.

Antibacterial activity

Determination of antibacterial activity

The Kirby-Bauer diffusion method was employed for antibacterial activity screening [15]. The *Escherichia coli* ATCC 25922 strain was used in the study. The bacteria were grown in LB broth for 16–18 h at 37 °C (10^9–10^{10} CFU/mL). Sterile blank disks with a 6 mm diameter were individually placed on an LB nutrient agar plate covered with 300 µL of the bacterial strain. Sterile disks were impregnated with 20 µL of extract solutions at concentrations of 500, 1000, and 2000 µg/mL. The plates were incubated at 37 °C for 24 h. The antimicrobial activity was determined in triplicate by measuring the diameter of the inhibition zone (IZ, mm). Amoxycillin (5 µg/disk) was used as the positive control. Dimethyl sulfoxide (10%) was used as a negative control.

Determination of minimal inhibitory concentration (MIC)

The antibacterial activity of the plant extracts was determined using sterile test tubes (modified from [16]). Tubes were filled with sterilized LB broth and corresponding concentration of the plant extract, to create a concentration sequence from 0.01 mg/mL to 15 mg/mL. The negative control received no extract; the positive control received amoxicillin (0.1 mg/mL). 5% v/v inoculum

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was added into the tubes. The tubes were incubated for 24 h at 37 °C. Turbidity was assessed after 24 h by measuring optical density at 600 nm using a DU-8800 RS spectrophotometer. The MIC was defined as the lowest concentration with no visible growth. At least three replicates were run for each assay. According to Bussmann et al. [17] , strong antibacterial activity was defined as MIC < 5 mg/mL.

Results

BSA denaturation assay – methodological optimisation

Preliminary screening: Initial experiments (data not shown) identified that coloured compounds in both extracts significantly interfered with absorbance readings at 660 nm, and tannin-like constituents caused protein precipitation even without heating. This necessitated systematic optimisation.

pH optimization

Buffer systems were prepared at four pH values:

6.0 (1X phosphate-buffered saline (PBS))

6.4 (1X phosphate-buffered saline (PBS))

7.0 (1X phosphate-buffered saline (PBS))

7.4 (1X phosphate-buffered saline (PBS))

BSA (1 % w/v) was dissolved separately in each buffer.

Critical: Colour controls (extract + buffer without BSA, heated identically) were prepared for each extract at each pH.

The anti-denaturation activity of *Thymus tiflisiensis* leaf extract was strongly pH-dependent, with maximum inhibition (70.3 ± 1.8%) observed at pH 6.4 (1× PBS) using 0.25 mg/mL extract (Fig. 1). At pH 6.0 and 7.4, inhibition decreased to 43.5 ± 1.9% and 55.9 ± 1.2%, respectively, while pH 7.0 gave 68.9 ± 2.8%. Ibuprofen (0.05 mg/mL) also showed its highest inhibition at pH 6.4 (47.8 ± 0.9%). Importantly, colour interference from the extract was reduced by 42% at pH 6.4 compared to pH 7.4 ($p < 0.01$).

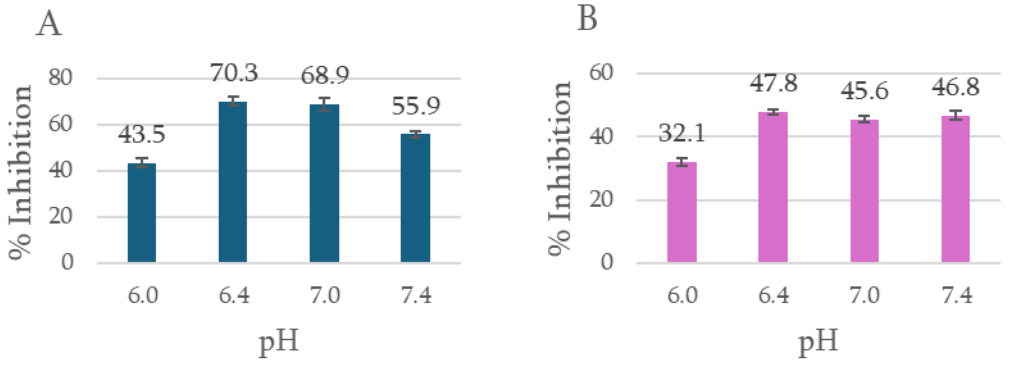

Fig. 1. Effect of pH on the anti-denaturation activity of *Thymus tiflisiensis* leaf extracts (0.25 mg/mL, A) and ibuprofen (0.05 mg/mL, B) in the BSA denaturation assay performed at incubation at 37 °C for 20 minutes followed by incubation at 70 °C for 20 minutes.

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Extract concentration was selected based on preliminary dose-response data. Values represent mean of three independent experiments ± standard deviation (n=3).

Note: Colour controls (extract + buffer without BSA, heated identically) were subtracted for each extract at each pH to correct for extract colour interference.

Temperature Optimization

Using the optimal pH identified (6.4), the denaturation temperature was systematically varied in experiments (Table 1):

65 °C for 20 minutes;

70 °C for 15 minutes;

70 °C for 20 minutes;

75 °C for 15 minutes

The highest anti-denaturation activity for *Thymus tiflisiensis* leaf extract (70.3 ± 1.8%) was achieved at 70 °C with a 20-minute incubation. Lower inhibition was observed at 65 °C (59.4 ± 1.3%), while both 70 °C with 15 minutes incubation (63.7 ± 0.6%) and 75°C with 15 minutes incubation (67.3 ± 2.5%) produced intermediate values, confirming 70 °C for 20 minutes as optimal.

Statistical Analysis

All experiments performed in triplicate on three independent occasions (n=9 technical replicates across 3 biological replicates). Results expressed as mean ± standard deviation. Comparisons between methods used paired *t*-test (disk diffusion vs. broth microdilution results for identical extract-bacteria combinations). The analysis of the obtained data indicated that final incubation at 70 °C for 20 minutes increased assay sensitivity while maintaining thermal stability of extract components.

Table 1. Effect of temperature and exposure time on the anti-denaturation activity of *Thymus tiflisiensis* leaf extracts and ibuprofen in the BSA denaturation assay.

Sample	Temperature (°C)	Incubation Time (minutes)	Concentration (mg/mL)[i]	% Inhibition (Mean ± SD)[ii]
T. tiflisiensis leaf extract	65	20	0.25	59.4 ± 1.3
T. tiflisiensis leaf extract	70	15	0.25	63.7 ± 0.6
T. tiflisiensis leaf extract	70	20	0.25	70.3 ± 1.8
T. tiflisiensis leaf extract	75	15	0.25	67.3 ± 2.5

DOI: 10.56580/GEOMEDI80

Sample	Temperature (°C)	Incubation Time (minutes)	Concentration (mg/mL)[i]	% Inhibition (Mean ± SD)[ii]
Ibuprofen	65	20	0.05	30.2 ± 0.7
Ibuprofen	70	15	0.05	38.1 ± 1.5
Ibuprofen	70	20	0.05	47.8 ± 0.9
Ibuprofen	75	15	0.05	43.1 ± 1.9

[i]Extract concentration (0.25 mg/mL) was selected based on preliminary dose-response data and maintained constant across all temperature conditions.

[ii]Values represent mean of three independent experiments ± standard deviation (n=3). Assays were performed at pH 6.4 (1X PBS).

Note: Colour controls (extract + buffer without BSA, heated identically) were subtracted for each temperature condition to correct for extract colour interference.

Based on optimization results, the following protocol was adopted (Table 2).

Table 2. Final optimized protocol for the determination of anti-denaturation activity of Georgian medicinal plants

Parameter	Standard protocol	Protocol, optimized for plant extracts
Buffer	Phosphate buffer pH 6.8	1xPBS pH6.4
BSA concentration	0.5-1 %	**1%**
Pre-incubation	37 °C, 15-20 minutes	**37 °C, 20 minutes**
Denaturation	65-75 °C, 15-25 minutes	**70 °C, 20 minutes**
Color control	Usually omitted	**Mandatory** (extract + buffer, no BSA)
Wavelength	660 nm	660 nm

DOI: 10.56580/GEOMEDI80

Parameter	Standard protocol	Protocol, optimized for plant extracts
Positive control	Ibuprofen (10-200 μg/mL)	Ibuprofen (10-200 μg/mL)

Complete procedure for the determination of anti-denaturation activity of Georgian medicinal plants

1. Prepare extract dilutions in 150 mM potassium-phosphate buffer, pH=7 (10-1000 μg/mL)
2. For each sample both sample measurement (2900 μL BSA + 100 μL extract) and colour control measurements (2900 μL buffer + 100 μL extract) should be taken.
3. Positive control: ibuprofen (10, 25, 50, 100, 200 μg/mL) should be measured without colour control (negligible colour)
4. Negative control: 2900 μL BSA + 100 μL buffer
5. All samples should be incubated at 37 °C for 20 minutes
6. The incubation should be followed up by incubation at 70 °C for 20 minutes
7. Samples should be cooled to room temperature for 10 minutes
8. Absorbance should be measured at 660 nm
9. Corrected absorbance should be calculated: $OD_{corrected} = OD_{sample} - OD_{color\ control}$
10. Accordingly, % inhibition should be calculated based on corrected absorbance

Complete procedure for the determination of antibacterial activity of Georgian medicinal plants by disk diffusion method and broth microdilution method

Disk Diffusion Method (Kirby-Bauer)

Procedure (CLSI M02 standard):

1. LB plates (150 mm diameter) were inoculated by swabbing with bacterial suspension (0.5 McFarland)
2. Sterile filter paper disks (6 mm diameter, Whatman) were impregnated with 20 μL of leaf extract solutions (concentrations: 500, 1000, 2000 μg/mL in 10% DMSO)
3. Disks were air-dried for 15 minutes to evaporate solvent
4. Disks placed on inoculated agar (max 6 disks per plate)
5. Positive control: amoxicillin (5 μg/disk)
6. Negative control: 10% DMSO disk
7. Incubate at 37 °C for 24 hours
8. Measure inhibition zone diameters (mm) including disk diameter (6 mm)

Interpretation: No zone = no activity; zone ≤8 mm = weak; 9-14 mm = moderate; ≥15 mm = strong (modified from standard criteria for plant extracts)

Broth Microdilution Method

Procedure (CLSI M07 standard):

1. Two-fold serial dilutions of extracts in LB (containing 0.5% DMSO final). Concentration range: 3.9, 7.8, 15.6, 31.25, 62.5, 125, 250, 500 μg/mL
2. Dispense 100 μL of each dilution into 96-well plate

DOI: 10.56580/GEOMEDI80

3. Add 100 µL bacterial suspension (~5×10^5 CFU/mL)
4. Positive controls: amoxicillin (0.1 mg/mL)
5. Growth control: LB + bacteria (no extract)
6. Sterility control: LB + extract (no bacteria)
7. Solvent control: LB + bacteria + 0.5% DMSO
8. Incubate at 37 °C for 24 hours
9. Read MIC as lowest concentration with no visible turbidity (as OD, 600 nm wavelength).
10. MBC determination: subculture 10 µL from clear wells onto LB agar, incubate 24 hours; MBC = lowest concentration with ≥99.9% killing (no colonies)

Table 3 demonstrates results of growth inhibition for 2 methods. MIC for extracts is estimated as 125.0 µg/mL. The comparison of antibacterial testing methods revealed a clear discrepancy between disk diffusion and broth microdilution. Disk diffusion produced small, highly variable inhibition zones for *Thymus tiflisiensis* leaf extract at 2000 µg/disk (12 ± 4.81 mm), with root extract showing similarly inconsistent results (11 ± 3.71 mm). In contrast, broth microdilution demonstrated concentration-dependent activity with excellent reproducibility. Leaf extract achieved 91 ± 4.32% inhibition at 60.5 µg/mL and complete (100%) inhibition at 125.0 µg/mL, establishing the MIC at 125.0 µg/mL for both extracts. Broth microdilution produced low standard deviations (typically <10% of the mean), whereas disk diffusion gave highly variable outcomes, indicating that the latter substantially underestimates the true antibacterial potential of crude plant extracts.

Table 3. Comparison of results of growth inhibition obtained for two methods of determination of antibacterial activity of Georgian medicinal plants

Disk diffusion method		
Extract	**Concentration, µg/mL**	**Inhibition zone, mm (mean value ± standard deviation)**
Leaf	500	7±1.17
	1000	10±4.60
	2000	12±4.81
Root	500	6±1.89
	1000	8±2.79
	2000	11±3.71
Amoxicillin	5 µg/disk	24±4.12
DMSO	10%	0
Broth microdilution method		
Extract	**Concentration, µg/mL**	**% inhibition (mean value ± standard deviation)**

DOI: 10.56580/GEOMEDI80

Leaf	3.9	46±7.02
	7.5	50±5.10
	15.0	59±1.11
	30.0	75±5.31
	60.5	91±4.32
	125.0	100
Root	3.9	37±4.27
	7.5	42±3.23
	15.0	49±5.72
	30.0	68±1.90
	60.5	85±5.18
	125.0	100
Amoxicillin	100	100
DMSO	10%	0

Method Comparison Criteria

Disk diffusion method produced small and highly variable inhibition, while broth microdilution method revealed clear, concentration-dependent antibacterial activity. The two antibacterial testing methods were compared based on the following criteria: quantitative output, requirement of extract diffusion, susceptibility to extract colour interference, susceptibility to extract hydrophobicity, dependence on agar composition, time requirements, and availability of standardized interpretation (Table 4).

Table 4. Method comparison criteria for antibacterial activity

Criterion	**Disk diffusion**	**Broth microdilution**
Quantitative output	No (zone diameter only)	Yes (MIC µg/mL)
Requires extract diffusion	Yes	No
Affected by extract colour	No	Yes (can be corrected)

DOI: 10.56580/GEOMEDI80

Criterion	Disk diffusion	Broth microdilution
Affected by extract hydrophobicity	Yes (major limitation)	No
Affected by agar composition	Yes	N/A
Time requirements	24 h	24-48 h
Standardized interpretation	No	Yes
CLSI guideline	M02	M07

Discussion

Methodological optimizations for crude plant extracts

This study systematically addressed two critical methodological challenges in ethnopharmacological screening: protein denaturation assays in the presence of coloured, tannin-rich extracts, and the choice of antibacterial screening method for complex plant matrices.

BSA denaturation optimization: pH 6.4 and 70 °C

The shift from standard pH 7.4 to pH 6.4 proved crucial for reducing non-specific interference. At higher pH (7.4), phenolic compounds present in *Thymus* leaf extracts (flavonoids, tannic acid derivatives) may undergo autoxidation, potentially forming coloured products that can interfere with absorbance measurements. Additionally, alkaline conditions promote tannin-protein complexation even without heating, is a well-documented phenomenon where hydrogen bonding between phenolic hydroxyl groups and protein amide groups is maximized at pH 7-8 [18],[19]. By lowering the pH to 6.4, the value well below the pKa values of most phenolic compounds (typically 8.5–10.9) [20], the phenolic hydroxyl groups remain protonated and uncharged, minimizing their autoxidation and subsequent colour formation. This was confirmed by a 42% reduction in colour control absorbance compared to pH 7.4."

The selection of 70 °C denaturation for 20 minutes addresses a different issue: thermal stability of plant-derived anti-inflammatory compounds. Many phenolic compounds exhibit optimal bioactivity at slightly higher temperatures due to improved solubility and molecular unfolding that exposes active sites[21]. At 75 °C, however, degradation became more pronounced, which may be explained by thermal degradation of phenolic compounds, caramelization of sugars, or oxidation of thermolabile constituents [22].

Mandatory colour controls: The most important modification which are quite often omitted in published plant extract studies is the inclusion of extract colour controls

DOI: 10.56580/GEOMEDI80

(extract + buffer without BSA, heated identically). This correction should be considered mandatory for any plant extract containing visible pigments (chlorophyll, anthocyanins, flavonoids).

Antibacterial method comparison: liquid culture superior to disk diffusion

The dramatic discrepancy between disk diffusion and broth microdilution results demands explanation. Three mechanisms explain why disk diffusion fails for crude plant extracts:

1. Diffusion limitation in agar: The aqueous agar matrix (approximately 98% water, 2% agarose) effectively excludes hydrophobic compounds. Many antimicrobial plant metabolites including thymol, carvacrol (from *Thymus*) are lipophilic and partition preferentially into the disk or form micelles rather than diffusing radially. The result is a steep concentration gradient immediately adjacent to the disk, with insufficient compound reaching the bacterial lawn to produce a visible zone even when the MIC in liquid culture is modest.

2. High molecular weight constituents: Crude extracts contain polysaccharides, tannins, and proteinaceous material that cannot diffuse through the agar meshwork (pore size approximately 50-100 nm for 1.5% agar). These components are trapped near the disk, failing to contact bacteria even if they possess antimicrobial activity.

3. Quantitative vs. qualitative output: Disk diffusion provides only a threshold measurement (zone diameter ≥X mm = susceptible). For plant extracts, no standardized breakpoints exist. Broth microdilution provides continuous quantitative data (MIC in μg/mL) allowing comparison across studies, determination of bactericidal vs. bacteriostatic activity (MBC/MIC ratio), and calculation of selectivity indices when cytotoxicity data are available.

According to the previously published results, MIC broth dilution method is more reliable as it provides more standardized results. Thus, it is suggested to use disk diffusion method as a qualitative and/or additional method for estimating the antibacterial activity [9], [23].

In conclusion, this study demonstrates that crude plant extracts require significant methodological modifications for reliable *in vitro* screening. For the BSA denaturation assay, optimal conditions were identified as pH 6.4 with denaturation at 70 °C for 20 minutes, plus mandatory colour controls to correct for pigment interference. For antibacterial testing, broth microdilution proved unequivocally superior to disk diffusion, providing quantitative, reproducible MIC data. These optimized protocols provide a validated framework for future ethnopharmacological screening of Caucasian medicinal plants.

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მეთოდოლოგიური მოდიფიკაციები *Thymus tiflisiensis*-ის (თბილისური ბეგქონდარა) ფოთლის უხეში ექსტრაქტის სკრინინგისთვის *in vitro* ანთების საწინააღმდეგო და ანტიბაქტერიული აქტივობების დასადგენად

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რეზიუმე

Thymus tiflisiensis(Lamiaceae-ტუჩოსანთა ოჯახი) სამკურნალო მცენარეა, რომელიც, კავკასიის რეგიონში, ტრადიციულად, გამოიყენება ანთებითი მდგომარეობებისა და ინფექციური დაავადებების სამკურნალოდ. თუმცა, მცენარის უხეში ექსტრაქტების შემთხვევაში, სტანდარტული *in vitro* ანალიზები ხშირად საჭიროებს მეთოდოლოგიურ მოდიფიკაციებს შეფერილი ფიტოქიმიკატების, ტანინებისა და მატრიქსის სხვა კომპონენტების მხრიდან ხელის შეშლის გამო(ინტერფერენცია). მოცემული კვლევის მიზანი იყო: **1.** ხარის შრატის ალბუმინის(BSA) დენატურაციის ანალიზის ოპტიმიზაცია მცენარის უხეში ექსტრაქტებისთვის ბუფერის ოპტიმალური pH-ისა და დენატურაციის ტემპერატურის განსაზღვრის გზით; **2.** ბულიონში მიკროგანზავების(თხევადი კულტურა) მეთოდის შედარება დისკო-დიფუზიურ(აგარი) მეთოდთან მცენარის ექსტრაქტის ანტიბაქტერიული სკრინინგისთვის და თხევადი კულტურის უპირატესობის ჩვენება აგარში დიფუზიასთან შედარებით მინიმალური მაინჰიბირებელი კონცენტრაციის(MIC) დასადგენად. *Thymus tiflisiensis* ექსტრაგირდა მაცერაციის(დალბობა) მეთოდით, 80%-იანი მეთანოლის გამოყენებით. ხარის შრატის ალბუმინის ოპტიმიზაციისთვის, ბუფერის pH(6,0-7,4) და დენატურაციის ტემპერატურა(65°C, 70°C, 75°C) სისტემურად შეფასდა ხარის შრატის ალბუმინის სისტემური კონცენტრაციის(1%) გამოყენებით. ოპტიმალურ პირობებად განისაზღვრა pH=6,4 და ინკუბაცია 37°C-ზე 20წთ-ის განმავლობაში, რასაც მოჰყვა გაცხელება 70°C ტემპერატურაზე დამატებით 20წთ-ის განმავლობაში. ანტიბაქტერიული სკრინინგისთვის, ბულიონში მიკროგანზავების მეთოდი ცალსახად სჯობს დისკო-დიფუზიურ მეთოდს მცენარის უხეში ექსტრაქტების შემთხვევაში, ვინაიდან, იგი გამორიცხავს დიფუზიასთან დაკავშირებულ არტეფაქტებს და იძლევა მინიმალური მაინჰიბირებელი კონცენტრაციის(MIC) რაოდენობრივ მონაცემებს, რაც

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აუცილებელია საფუძვლიანი შედარებისთვის. ეს ოპტიმიზებული პროტოკოლები ვალიდურ ჩარჩოს ქმნის კავკასიური სამკურნალო მცენარეების ეთნოფარმაკოლოგიური სკრინინგისთვის.

საკვანძო სიტყვები: *Thymus tiflisiensis* (თბილისური ბეგქონდარა), BSA-ის (ხარის შრატის ალბუმინი) დენატურაციის ტესტის მოდიფიკაცია, ბულიონში მიკროგანზავების მეთოდი, დისკო-დიფუზიური მეთოდების შედარება, მეთოდოლოგიური ოპტიმიზაცია.

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