

Phytochemical screening and antibacterial activity of the methanolic extract of *Indigofera oblongifolia* leaves naturally grown in Saudi Arabia

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[Triagem fitoquímica e atividade antibacteriana do extrato metanólico das folhas de *Indigofera oblongifolia* cultivadas naturalmente na Arábia Saudita]

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ABSTRACT

The growing prevalence of drug-resistant pathogens necessitates the search for novel antimicrobial agents. This study evaluated the antimicrobial and antibiofilm activities of the methanolic leaf extract of *Indigofera oblongifolia* against selected Gram-positive (*Listeria monocytogenes* ATCC 7644 and *Staphylococcus aureus* ATCC 25923) and Gram-negative bacteria (*Escherichia coli* ATCC 8739 and *Salmonella enterica* ATCC 14028). The methanolic extract was prepared, and its phytochemical composition was analyzed. Qualitative analysis revealed the presence of phenolics, flavonoids, tannins, and saponins, with phenolics being the predominant constituents. Antimicrobial activity was assessed using the agar well-diffusion method, while antibiofilm activity was determined by the microtiter plate assay. The extract exhibited pronounced antibacterial effects against Gram-positive strains, whereas Gram-negative bacteria showed limited susceptibility. Furthermore, the extract significantly inhibited biofilm formation in *L. monocytogenes* and *E. coli*, while a slight enhancement of biofilm production was observed in *S. enterica*. These results indicate that *I. oblongifolia* leaves possess bioactive compounds with potent antimicrobial and antibiofilm properties. Further studies are recommended to isolate and characterize the active constituents and elucidate their mechanisms of action, which may support the potential development of plant-derived alternative therapeutic agents.

Keywords: *Indigofera oblongifolia*; phytochemicals; bacteria; antimicrobial activity; biofilm inhibitory activity

RESUMO

A crescente prevalência de patógenos resistentes a medicamentos exige a busca por novos agentes antimicrobianos. Este estudo avaliou as atividades antimicrobianas e antibiofilme do extrato metanólico das folhas de *Indigofera oblongifolia* contra bactérias Gram-positivas (*Listeria monocytogenes* ATCC 7644 e *Staphylococcus aureus* ATCC 25923) e Gram-negativas selecionadas (*Escherichia coli* ATCC 8739 e *Salmonella enterica* ATCC 14028). O extrato metanólico foi preparado e sua composição fitoquímica foi analisada. A análise qualitativa revelou a presença de fenólicos, flavonóides, taninos e saponinas, sendo os fenólicos os constituintes predominantes. A atividade antimicrobiana foi avaliada usando o método de difusão em poços de ágar, enquanto a atividade antibiofilme foi determinada pelo ensaio em placa de microtitulação. O extrato exibiu efeitos antibacterianos pronunciados contra cepas Gram-positivas, enquanto as bactérias Gram-negativas mostraram suscetibilidade limitada. Além disso, o extrato inibiu significativamente a formação de biofilme em *L. monocytogenes* e *E. coli*, enquanto um ligeiro aumento na produção de biofilme foi observado em *S. enterica*. Esses resultados indicam que as folhas de *I. oblongifolia* possuem compostos bioativos com potentes propriedades antimicrobianas e antibiofilme. Estudos adicionais são recomendados

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para isolar e caracterizar os constituintes ativos e elucidar seus mecanismos de ação, o que pode apoiar o desenvolvimento potencial de agentes terapêuticos alternativos derivados de plantas.

Palavras-chave: *Indigofera oblongifolia*, fitoquímicos, bactérias, atividade antimicrobiana, atividade inibidora de biofilme

INTRODUCTION

Currently, several synthetic antibiotics are widely used to treat infectious diseases in humans and animals. However, the increasing prevalence of antimicrobial resistance has prompted researchers to explore alternative strategies for controlling microbial infections (Natarajan *et al.*, 2010; Singh *et al.*, 2021). Over the past two decades, significant efforts have focused on discovering new antimicrobial drugs, including those derived from traditional ethnomedicine. Plant-based medicines, which have long been integral to healthcare practices worldwide, have garnered attention as potential sources of novel antimicrobial agents (Cowan, 1999; Charindy *et al.*, 1999; Vijayanand and Hemapriya, 2011). The economic and societal impacts of antimicrobial resistance extend beyond clinical settings, affecting healthcare providers, patients, administrators, and the broader public (Singh *et al.*, 2021).

Among medicinal plants, *Indigofera oblongifolia* (Fabaceae), commonly known as "hasr" in Arabic, is traditionally valued for its analgesic and anti-inflammatory effects and is distributed across selected regions of Asia and Africa (Sharif *et al.*, 2005). The leaves of this plant are rich in polyphenols, flavonoids, and organic acids, contributing to its potent antioxidant activity (Dkhil *et al.*, 2019). Experimental studies have demonstrated its antimalarial properties, protecting the spleen from *Plasmodium chabaudi* infection in mice (Dkhil *et al.*, 2015), as well as its protective effects against *Trypanosoma evansi*-induced injury (Dkhil *et al.*, 2019; Dkhil *et al.*, 2020). Additionally, *I. oblongifolia* exhibits antimicrobial (Dahot, 1999), hepatoprotective (Shahjahan *et al.*, 2005), and lipoxigenase inhibitory activities (Sharif *et al.*, 2005), highlighting its potential as a multifunctional therapeutic agent.

Given its documented ethnomedicinal use and diverse biological activities, this study aimed to investigate the phytochemical constituents of *I. oblongifolia* leaves and to evaluate the

antimicrobial and antibiofilm properties of its methanolic extract against selected Gram-positive and Gram-negative bacterial strains. This research seeks to provide a scientific basis for the plant's traditional use and its potential application in combating microbial infections.

ETHICAL ASPECTS

The research was submitted to the Research Ethics Committee (REC) on Animal Use and Care at King Saud University and approved under the number KSU-SE-19-67.

MATERIALS AND METHODS

Collection of plant material. Fresh leaves of *Indigofera oblongifolia* were collected in March 2023 from naturally growing populations in the Jazan Province, located in the southwestern region of Saudi Arabia (16°53'N, 42°33'E; altitude approximately 60 m above sea level). The plants were harvested from semi-arid, sandy soil habitats under full sunlight conditions. The species was identified based on morphological characteristics using standard taxonomic keys and verified against the current nomenclature listed in the World Flora Online database. A qualified taxonomist from the Department of Botany and Microbiology, College of Science, King Saud University, Riyadh, Saudi Arabia, confirmed the identification. A voucher specimen (No. 9028) has been deposited in the King Saud University Herbarium (KSUH), Riyadh, for future reference. The leaves were transported to the laboratory in sterile paper bags, washed with distilled water to remove dust and debris, and shade-dried at ambient temperature (25–28°C) for 10 days. The dried leaves were then ground into fine powder and stored in airtight containers at 4°C until extraction.

Preparation of Plant Extract. The collected leaves of *Indigofera oblongifolia* were first washed thoroughly with sterile, filtered tap water, followed by rinsing with distilled water to remove surface contaminants. The leaves were then air-dried in a clean, shaded environment at a

controlled temperature of 40 °C for 3 hr, as optimized based on Al-Shaebi *et al.* (2018) to prevent degradation of thermolabile compounds. The dried material was ground into a fine, homogeneous powder (particle size <0.5 mm) using a Hummer Grinder (Edison Electric, ED-CG1400, China).

Approximately 100g of powdered leaves were extracted with 1 L of 70% (v/v) analytical-grade methanol (solvent-to-solid ratio 10:1, w/v). The mixture was maintained at 4 °C for 24 hr in the dark to minimize oxidation and photodegradation, with intermittent stirring every 2 hr to enhance solvent penetration and diffusion of metabolites. After incubation, the extract was filtered using Whatman filter paper, and the filtrate was concentrated under reduced pressure at 40°C using a Büchi vacuum evaporator (Switzerland) to remove methanol completely.

The resulting crude extract was further dried under a gentle nitrogen stream to eliminate residual solvent and then reconstituted in sterile distilled water to a final concentration of 100 mg/mL. The extract was stored in amber glass vials at 4 °C until further use. The extraction yield was recorded, and microbial sterility was confirmed by plating a small aliquot on nutrient agar before use in biological assays.

Determination of Total Phenolic Compounds. 100mg of the methanolic extract of the sample was weighed accurately and dissolved in 100mL of triple-distilled water (TDW). 1mL of this solution was transferred to a test tube, then 0.5mL of 2N Folin-Ciocalteu reagent and 1.5mL of 20% Na₂CO₃ solution were added. Ultimately, the volume was made up to 8mL with TDW, followed by vigorous shaking, and finally allowed to stand for 2 hr, after which the absorbance was taken at 765nm. These data were used to estimate the total phenolic content using a standard calibration curve obtained from various diluted concentrations of gallic acid (50, 100, 150, 200, and 250mg/mL).

Determination of Total Flavonoids. The method is based on forming the flavonoids-aluminum complex, which has an absorptivity maximum at 415nm. 100µL of the plant extracts in methanol were mixed with 100µL of 20 % aluminum trichloride in methanol and a drop of acetic acid, and then diluted with methanol to 5mL. The

absorption at 415 nm was read after 40 min. Blank samples were prepared from 100µL of plant extracts and a drop of acetic acid, and then diluted to 5 mL with methanol. The absorption of standard quercetin solution (100µg/mL) in methanol was measured under the same conditions and expressed as milligrams of quercetin equivalents (QUE) per gram of extract.

Estimation of Total Tannin Content. The tannins were determined by the Folin-Ciocalteu method. About 100µL of the sample extract was added to a volumetric flask (10 mL) containing 7.5mL of distilled water and 0.5mL of Folin-Ciocalteu phenol reagent, 1mL of 35% sodium carbonate solution, and diluted to 10 mL with distilled water. The mixture was shaken well and kept at room temperature for 30 min. A set of reference standard solutions of tannic acid (100, 200, 300, 400, and 500µg/mL) was prepared in the same manner as described earlier. Absorbance for test and standard solutions was measured against the blank at 700nm with a UV/Visible spectrophotometer. Tannin content was expressed in terms of mg of tannic acid equivalents/g of sample extract.

Total Saponin Determination. The determination of the total saponin content in the extracts was made according to the vanillin-sulfuric acid colorimetric method. The mixture containing 250µL of vanillin reagent (8% dissolved in methanol), 500µL of extract, and 2.5mL of 72% sulfuric acid was mixed and incubated at 60 °C for 10 min. At the end of the incubation, the absorbance of the solution cooled in an ice bath was read at 544nm. Saponin content was expressed as mg diosgenin equivalent (DE)/g methanolic extract.

Determination of Antioxidant Activity Using the Reducing Power (RP) Method. The reducing power of the plant extracts was determined by the method described by Oyaizu (1986). Aliquots of 1 mL of methanolic extract of each sample, two replicates per sample, were mixed with 2.5 mL of 0.2 mM phosphate buffer solution at pH 6.6 and 2.5 mL of 1% potassium ferrocyanide. The mixture was incubated for 20 min at 50°C in a water bath. Then, 2.5 mL of 10% trichloroacetic acid was added, and the mixture was centrifuged at 3000 rpm for 10 min. After centrifugation, 2.5 mL of the supernatant was mixed with 2.5 mL of distilled water and 0.5 mL

of 0.1% FeCl₃. Absorbance was measured at 700 nm in a UV-30 spectrophotometer. The blank was prepared by substituting the same amount of diluted extract with methanol. The calibration line was established using the following concentrations of Ascorbic acid: 50, 100, 150, and 200 µg/mL. Antioxidant was expressed in terms of mg of ascorbic acid equivalents (AAE)/g methanolic extract.

Antimicrobial Susceptibility Testing, MIC, and MBC Determination. The antimicrobial susceptibility of *Indigofera oblongifolia* leaf extract was evaluated using the agar well-diffusion method according to the guidelines of Clinical & Laboratory Standards Institute (CLSI 2012, 2019) and protocols of Gholizadeh *et al.* (2012) and Balouri *et al.* (2016). Test strains obtained from the American Type Culture Collection (ATCC, USA) included Gram-positive bacteria *Listeria monocytogenes* (ATCC 7644) and *Staphylococcus aureus* (ATCC 25923), and Gram-negative bacteria *Escherichia coli* (ATCC 8739) and *Salmonella enterica* (ATCC 14028).

Bacterial suspensions were standardized to 1×10^5 CFU/mL using optical density verification and spread evenly on Mueller-Hinton Agar (MHA) plates poured to a depth of 4 mm. Wells of 0.6 cm diameter were filled with 100 µL of extract at four concentrations (0, 25, 50, 100 mg/mL) prepared in 1% sterile DMSO. DMSO-only wells served as negative controls. Plates were pre-incubated at 4 °C for 30 min to allow diffusion, then incubated at 37 °C for 24 hr under aerobic conditions with controlled humidity. Zones of inhibition (ZOI) were measured in millimeters using a digital caliper. Positive controls included conventional antibiotics: amikacin (30 µg/mL), amoxicillin (25 µg/mL), ampicillin/sulbactam (20 µg/mL), norfloxacin (10 µg/mL), and ofloxacin (5 µg/mL). All assays were performed in triplicate.

Minimum inhibitory concentrations (MICs) were determined by the broth microdilution method (Gholizadeh *et al.*, 2013; CLSI, 2019). A stock solution of the extract (100 mg/mL in 1% DMSO) was serially diluted two-fold across 10 wells containing 100 µL Mueller-Hinton Broth. Bacterial inocula (1×10^5 CFU/mL) were added to each well except for sterility controls. Solvent blanks and growth controls were included. Plates

were incubated at 37 °C for 24 hr, and bacterial growth was assessed by measuring absorbance at 600 nm using a microplate reader (Multiskan SkyHigh, ThermoFisher Scientific, USA). MIC was defined as the lowest extract concentration showing no visible growth, confirmed by comparison with controls. Statistical significance was evaluated at $p < 0.05$ via one-way ANOVA.

Minimum bactericidal concentrations (MBCs) were determined from wells showing no visible growth in the MIC assay. Aliquots of 50 µL were plated onto MHA and incubated at 37°C for 24 hr. Plates showing no colony formation (detection limit < 10 CFU/mL) were recorded as bactericidal. The MBC was defined as the lowest extract concentration reducing $\geq 99.99\%$ of the initial bacterial population. All MBC assays were performed in triplicate, and results were expressed in mg/mL as mean $\pm$ SD. Positive and negative controls were included to validate assay accuracy, and the MBC/MIC ratio was used to distinguish bactericidal from bacteriostatic activity.

Antibiofilm Formation Quantitative Assay. The antibiofilm potential of the crude extract was evaluated according to the method described by Kang *et al.* (2019) and Hamada *et al.* (2022), with slight modifications to optimize reproducibility and control for solvent and color interference. Briefly, an overnight culture of the test strain was adjusted to a density of 1×10^5 CFU/mL using sterile nutrient broth, corresponding to an optical density of 0.08–0.1 at 600 nm. Aliquots (100 µL) of the standardized microbial suspension were added to sterile, flat-bottom 96-well polystyrene microplates, followed by 100 µL of the crude extract diluted in broth to achieve final concentrations ranging from 0 to 100 mg/mL. Wells containing only bacteria and medium served as negative (untreated) controls, while those with medium alone were used as blanks. Solvent control wells containing the same concentration of methanol used for extract solubilization were included to ensure the solvent did not affect biofilm formation. Each treatment was performed in triplicate, and all assays were repeated three times independently.

The plates were covered with lids to minimize evaporation and incubated statically at 37 °C for 48 h, which corresponds to the optimal period for

mature biofilm development under these conditions. After incubation, non-adherent cells were removed, and the wells were washed twice with sterile phosphate-buffered saline (PBS, pH 7.3) and air-dried at room temperature. The biofilms were fixed with 400 μ L of methanol for 15 min, air-dried, and subsequently stained with 1% (w/v) crystal violet for 10 min. Excess dye was removed by washing with sterile distilled water, and the bound dye was solubilized using absolute ethanol. To avoid pigment interference, absorbance from extract-only blank wells was subtracted from each corresponding sample. The optical density (OD) was measured at 630 nm using a microplate reader (Multiskan SkyHigh, Thermo Fisher Scientific, USA), a wavelength validated for consistent detection of crystal violet-stained biofilm biomass. The percentage inhibition of biofilm formation was calculated using the formula of

$$[(OD_{\text{control}} - OD_{\text{sample}}) / (OD_{\text{control}})] \times 100$$

All results were expressed as mean $\pm$ standard deviation (SD) from three independent experiments. Positive controls using a known antibiofilm agent were included for comparative validation. Additionally, the potential bactericidal effect of the extract on planktonic cells was evaluated in a parallel assay to confirm that reductions in biofilm mass reflected true antibiofilm activity rather than growth inhibition. Representative images of stained wells were captured to visually support the quantitative data.

RESULTS

Phytochemical analysis of the *Indigofera oblongifolia* leaf extract revealed the presence of phenolics, flavonoids, tannins, and saponins as the major bioactive constituents (Table 1). Among these, phenolics were the most abundant, measured at 49.49 ± 8.38 mg gallic acid equivalent (GAE)/g of extract, whereas flavonoids were the least abundant, at 1.15 ± 0.2 mg quercetin equivalent (QE)/g. The antioxidant capacity of the extract, expressed as ascorbic acid equivalents (AAE), was 0.06 ± 0.001 mg AAE/g. These phytochemical compounds are known to contribute to the antimicrobial and antioxidant activities of plant extracts, highlighting their potential biological relevance. The antimicrobial activity of the extract was assessed against Gram-positive and Gram-negative bacteria at different concentrations of

the extract using the agar well-diffusion susceptibility method, in comparison to standard antibiotics. Estimation of the diameter of the zones of inhibition produced by serial dilutions of the extract (ranging from 12.5 to 100 mg/mL) as shown in Figure (1) and Table (2). The extract exhibited potent antimicrobial activity against all Gram-positive bacteria tested (*L. monocytogenes* ATCC 7644 and *S. aureus* ATCC 25923). The concentration of the extract was proportional to the degree of inhibition, particularly at a concentration of 100 mg/mL (Table 2). No antibacterial activity was observed against *S. aureus* at concentrations of 12.5 mg/mL for the extract. Moreover, the antimicrobial activity of the extract was tested against Gram-negative bacteria. At all concentrations of the extract, *S. enterica* ATCC 14028 and *E. coli* ATCC 8739 had no inhibition zone (Figure 1 and Table 2). These results indicate that the extract has an anti-Gram-positive bacterial effect.

The MIC results (Table 3, Figure 2) displayed the investigated extract's promising ability to effectively suppress the growth of the bacterial strains, exhibiting variable levels of potency. Interestingly, the extract showed potent activity. It exerted potent growth inhibition against *S. aureus* ATCC 25923 (MIC = 50 mg/mL) and *L. monocytogenes* ATCC 7644 (MIC = 6.25 mg/mL). Additionally, it showed moderate activity against *S. enterica* ATCC 14028 (MIC = >50 mg/mL). Moreover, the MBC values for the tested extract were higher than the MICs, and considerable variations were observed among Gram-positive bacterial strains. Table (3) presents the recorded values for MBCs (mg/mL), which range from higher than or equal to 50. As shown by the result, since the MBC is equal to or higher than the MIC, we expect the extract to have a bactericidal effect.

The results of the anti-biofilm (ABF) potential of the leaf extract of *I. oblongifolia* against selected bacterial strains are shown in Table (4). Extracts that inhibit more than 50% are considered to have good ABF activity (++), while those with inhibition between 0 and 50% are classified as having poor ABF activity (+). The tested extract demonstrated good inhibitory activity against the bacterial strains of *L. monocytogenes* ATCC 7644 and *E. coli* ATCC 8739 (Table 4). However, extracts increased the formation of biofilm by *S. enterica* ATCC 14028.

Table 1. Result of the qualitative and quantitative phytochemical analysis of the methanolic extract of *Indigofera oblongifolia* leaves

Phytochemical	Flavonoids (mg/g QE/g)	Phenolics (mg/g GAE/g)	Tannins (mg/g TAE/g)	Saponin (mg DE/g)	Total antioxidant (mg AAE/g)
Qualitative	+	++	+	+	+
Quantitative	1.15 ± 0.2	49.49 ± 8.38	1.58 ± 0.23	6.16 ± 0.59	0.06 ± 0.001

-: absent; +: low; ++: moderate; +++: high

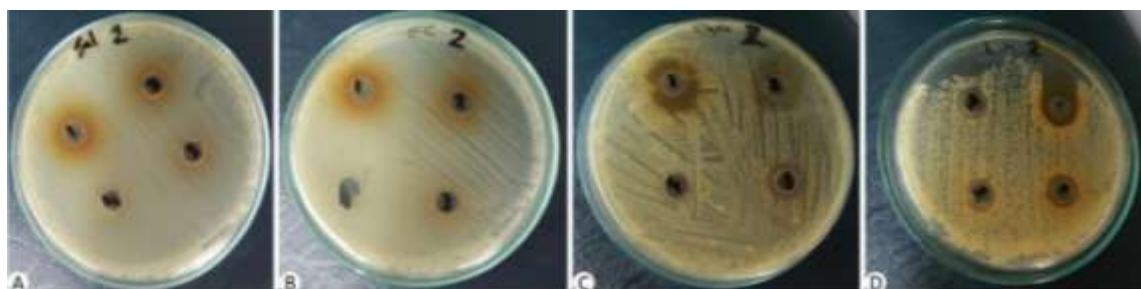


Figure 1. Zone of inhibition of the methanolic extract at different concentrations (1= 100, 2= 50, 3= 25, 4= 12.5 mg/mL) against four bacterial strains. (A) *Salmonella enterica* (ATCC: 14028). (B) *Escherichia coli* (ATCC: 8739). (C) *Staphylococcus aureus* (ATCC: 25923). (D) *Listeria monocytogenes* (ATCC: 7644).

Table 2. Antimicrobial susceptibility test results of the extract

Sample	Extract mg/mL				AK	AX	SAM	NOR	OFX
Concentration	100	50	25	12.5	30	25	20	10	5
<i>S. enterica</i> ATCC 14028	N/D	N/D	N/D	N/D	21	21	10	35	30
<i>E. coli</i> ATCC 8739	N/D	N/D	N/D	N/D	22	N/D	19	30	29
<i>S. aureus</i> ATCC 25923	18	17.67	12.67	N/D	22	21	24	25	27
<i>L. monocytogenes</i> ATCC 7644	20	12.33	10.67	10	7	7	7	8	7

N/D not detected, Results represent the mean of the clear zone of inhibition for three replicates diameter (mm) of each trial; Amikacin (AK 30), protein synthesis inhibitor; Amoxicillin (AX 25) and Ampicillin/sulbactam (SAM 20), cell wall synthesis inhibitors, Norfloxacin (NOR 10) and Ofloxacin (OFX 5), DNA replication inhibitor; Diluted DMSO was used as a negative control

Table 3. Minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of the tested extract

Bacterial reference strains	Concentrations mg/mL			
	MIC	MBC	C	CIP
Gram-negative bacteria				
<i>S. enterica</i> ATCC 14028	>50	N/D	0.25	0.25
<i>E. coli</i> ATCC 8739	N/D	N/D	0.5	0.25
Gram-positive bacteria				
<i>S. aureus</i> ATCC 25923	50	>50	0.25	0.5
<i>L. monocytogenes</i> ATCC 7644	6.25	50	0.5	0.25

P-value * < 0.000001. MIC antibacterial positive controls: Chloramphenicol (C, 1 mg/ml); Ciprofloxacin (CIP, 1 mg/mL).

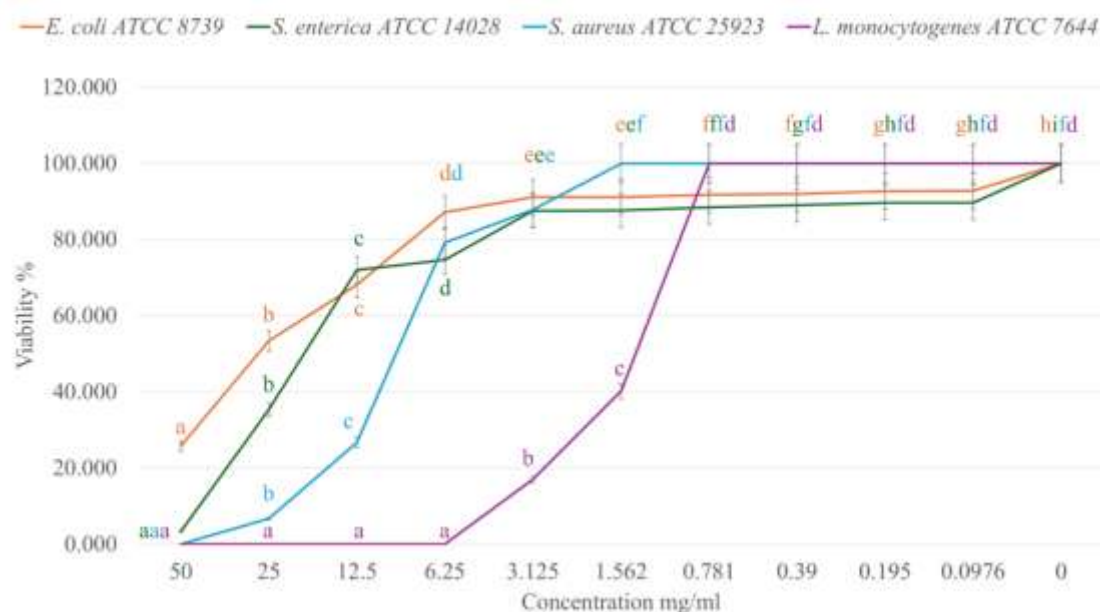


Figure 2. Dose-response effect of the extract on the viability (%) of selected reference strains in the broth dilution assay. Error bars represent standard deviations from the means (n = 3). Means with different letters are significantly different (p < 0.0001) by Duncan's post hoc test

Table 4. Percentage of biofilm formation inhibition of the extract (p < 0.001)

Bacterial reference strains	Concentrations mg/mL										
	50	25	12.5	6.25	3.125	1.562	0.781	0.39	0.195	0.097	0
Gram-negative bacteria											
<i>E. coli</i> ATCC 8739	76.89 _a	33.24 _b	17.36 _c	0 ^d	0 ^d	0 ^d	0 ^d	0 ^d	0 ^d	0 ^d	0 ^d
<i>S. enterica</i> ATCC 14028	0	0	0	0	0	0	0	0	0	0	0
Gram-positive bacteria											
<i>S. aureus</i> ATCC 25923	11.35 ^a	0 ^b	0 ^b	0 ^b	0 ^b	0 ^b	0 ^b	0 ^b	0 ^b	0 ^b	0 ^b
<i>L. monocytogenes</i> ATCC 7644	100 ^a	100 ^a	100 ^a	100 ^a	100 ^a	69.29 ^b	64.7 ^c	61.2 ^d	58.08 ^e	57.95 ^e	0 ^f

Means followed by different letters are significantly different at the (p < 0.0001) level, Duncan's post-hoc test.

DISCUSSION

Bacterial infectious diseases continue to be a major cause of morbidity and mortality worldwide (Abejew et al., 2025). Medicinal plants have long been utilized to prevent and control pathogenic microorganisms due to their rich phytochemical content. In this study, the methanolic leaf extract of *Indigofera oblongifolia* was evaluated for its antibacterial and antibiofilm activities against Gram-positive bacteria (*Listeria monocytogenes* ATCC 7644

and *Staphylococcus aureus* ATCC 25923) and Gram-negative bacteria (*Escherichia coli* ATCC 8739 and *Salmonella enterica* ATCC 14028).

Our results demonstrated that the extract exhibited stronger antimicrobial activity against Gram-positive bacteria, with comparatively lower activity against Gram-negative strains. This observation aligns with previous reports on *Indigofera tinctoria* (Renukadevi and Sultana, 2011) and *I. oblongifolia* (Dkhil et al., 2020), where Gram-positive bacteria were more

susceptible. The reduced susceptibility of Gram-negative bacteria is likely attributable to the presence of an outer phospholipid membrane with lipopolysaccharide components, which acts as a barrier to many antibacterial agents (Silhavy *et al.*, 2010; Nostro *et al.*, 2000). In contrast, Gram-positive bacteria possess a thicker, hydrophilic peptidoglycan layer that is more permeable to bioactive compounds (Trombetta *et al.*, 2005; Kaur and Arora, 2009).

Regarding biofilm formation, the extract effectively inhibited biofilms produced by Gram-positive bacteria, likely by interfering with quorum sensing, disrupting the extracellular polymeric substance (EPS) matrix, and/or directly affecting bacterial cell structures (Donlan and Costerton, 2002; Balaban *et al.*, 2007). Notably, *S. enterica* biofilm formation was slightly enhanced, indicating that the extract's effects may be strain-specific.

Phytochemical analysis revealed the presence of phenolics, flavonoids, tannins, and saponins, with phenolics being the most abundant. These compounds are known to exert antibacterial effects through multiple mechanisms: flavonoids can disrupt energy metabolism, inhibit nucleic acid synthesis, and impair membrane function (Khotimah *et al.*, 2020); saponins can compromise cell membranes, inhibit biofilm and capsule formation, and reduce toxin production (Tatli Cankaya and Somuncuoglu, 2021); tannins can inactivate enzymes, bind transport proteins, and prevent microbial adherence (Mummed *et al.*, 2018). The antimicrobial activity observed in this study is likely due to the combined effects of these bioactive compounds.

Overall, these findings support the potential of *I. oblongifolia* leaf extract as a source of natural antimicrobial and antibiofilm agents, particularly against Gram-positive pathogens. Further studies are warranted to elucidate precise mechanisms of action and to explore their potential applications in developing alternative therapeutics.

CONCLUSION

Overall, these findings support the potential of *I. oblongifolia* leaf extract as a source of natural antimicrobial and antibiofilm agents, particularly against Gram-positive pathogens. Further studies are warranted to elucidate precise mechanisms of

action and to explore their potential applications in developing alternative therapeutics.

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CONTRIBUTORS

E.M. Al-Shaebi, M. Dkhil, R. Abdel-Gaber: study conception, R. Abdel-Gaber, K.A. Abdelmeguid: objective, M. Dkhil, R. Abdel-Gaber, D. Delic: data analysis, M. Dkhil, R. Abdel-Gaber, H.M. Alharbi, S. Al Quraishy: discussion, M. Dkhil, R. Abdel-Gaber, K.A. Abdelmeguid: manuscript writing, and all authors' approval of the version to be published.

DATA AVAILABILITY STATEMENT

The research data are available within the article itself.

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