

Antibacterial Activity of Sequential Organic Extracts from *Gliricidia sepium* Leaves Against *Staphylococcus aureus*[†]

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Abstract

Natural products remain an important source of structurally diverse compounds for the discovery of new antimicrobial agents. *Gliricidia sepium* (Jacq.) Kunth ex Walp. is a medicinal species widely distributed in tropical regions and is recognized as a source of flavonoids, phenolic acids, coumarins, glycosides, and other specialized metabolites with diverse biological activities. Previous phytochemical investigations of this species have reported compounds such as oxytroside, o-coumaric acid, and melilotic acid, highlighting its potential as a source of bioactive natural products. In the present study, the influence of sequential solvent extraction on the recovery of antibacterial metabolites from *G. sepium* leaves was evaluated. Dried leaves were sequentially extracted by maceration with n-hexane, ethyl acetate, and methanol, and the resulting crude extracts were tested against *Staphylococcus aureus* using the agar disk diffusion assay at concentrations of 12.5, 25, 50, 100, and 200 µg/mL. Antibacterial activity varied according to solvent polarity and extract concentration. The methanolic extract exhibited the highest activity, producing an inhibition zone of 1.1 cm at 200 µg/mL, followed by the ethyl acetate extract (0.8 cm), whereas the hexane extract showed only slight activity (0.7 cm). The positive control produced inhibition zones ranging from 2.8 to 3.7 cm. These findings indicate that sequential solvent extraction influences the recovery of antibacterial constituents from *G. sepium* and support further bioactivity-guided fractionation and phytochemical characterization of the most active extracts.

Keywords: *Gliricidia sepium*; *Staphylococcus aureus*; antibacterial activity; natural products; bioactive metabolites

1. Introduction

Antimicrobial resistance is one of the most pressing challenges to global public health, particularly in relation to Gram-positive pathogens such as *Staphylococcus aureus* [1]. This bacterium has a remarkable ability to acquire resistance to different classes of antimicrobial agents, making staphylococcal infections increasingly difficult to treat and control. Reported resistance mechanisms include reduced drug uptake, modification of the pharmacological target, enzymatic inactivation, and active efflux [1].

Medicinal plants are an important source of secondary metabolites with biomedical potential because they contain bioactive compounds such as polyphenols, flavonoids, tannins, and terpenes, among others [2]. Among these species, *Gliricidia sepium* (Jacq.)



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Kunth ex Walp., a member of the Fabaceae family, has attracted scientific interest because of its reported antimicrobial, anti-inflammatory, and antioxidant properties. This tree is traditionally used in tropical regions and has a diverse chemical composition that includes flavonoids, phenolic acids, coumarins, alkaloids, and glycosides [2,3].

However, the antimicrobial activity of plant extracts may vary depending on the extraction method and the polarity of the solvents used, as these factors directly influence the chemical composition and metabolite profile of the final extract.

In this context, the aim of the present study was to evaluate the antibacterial activity of sequential *Gliricidia sepium* extracts obtained with n-hexane, ethyl acetate, and methanol against *Staphylococcus aureus*, in order to compare their inhibitory effects according to solvent polarity and identify the extracts with the greatest potential for subsequent phytochemical studies.

2. Materials and Methods

2.1. Plant Material

Gliricidia sepium leaves were collected at the Universidad Juárez Autónoma de Tabasco, Mexico, in 2026. The collection site was located at 18°04'33.8'' N, 93°10'08.8'' W.

After collection, the plant material was transported to the laboratory for processing. Leaves in good condition were selected, while those showing signs of deterioration or visible contamination were discarded.

2.2. Preparation of Extracts

The collected plant material was dried in an oven at a controlled temperature of 40 °C for 48 h to reduce its moisture content [4].

After drying, the extracts were obtained by successive maceration with solvents of increasing polarity: n-hexane, ethyl acetate, and methanol. The plant material remained in contact with each solvent for 48 h. The extracts were then filtered and concentrated to dryness using a rotary evaporator to remove the solvent and recover the corresponding extract.

2.3. Evaluation of the Extracts

The antibacterial activity of the extracts obtained from *Gliricidia sepium* leaves was determined using the Kirby–Bauer agar disk diffusion method. Mueller–Hinton agar plates were first inoculated with the test microorganism, after which disks impregnated with the different concentrations of each extract were placed on the agar surface.

For each extract, concentrations of 12.5, 25, 50, 100, and 200 µg/mL were prepared. Disks impregnated with the different concentrations were placed on the inoculated medium. Positive and negative controls were also included.

The plates were incubated for 24 h at 37 ± 1 °C. The inhibition zones were then measured to determine the antibacterial activity of the extracts [5].

2.4. Microorganisms

Staphylococcus aureus was used in the assay. The microorganism was inoculated on Mueller–Hinton agar for antibacterial evaluation.

3. Results

The antibacterial activity of *Gliricidia sepium* extracts obtained with n-hexane, ethyl acetate, and methanol was evaluated against *Staphylococcus aureus*. The methanolic extract displayed inhibitory activity from 12.5 µg/mL and reached a maximum inhibition zone of 1.1 cm at 200 µg/mL. The ethyl acetate extract showed activity from 50 µg/mL, with a

maximum inhibition zone of 0.8 cm, whereas the hexane extract showed inhibitory activity only at 200 µg/mL, with an inhibition zone of 0.7 cm (see Table 1).

Table 1. Antibacterial activity of *Gliricidia sepium* extracts against *Staphylococcus aureus*.

Microorganism	HE	EAE	MeOH
<i>Staphylococcus aureus</i>	n/a	n/a	12.5: 0.7 cm
	n/a	n/a	25: 0.7 cm
	n/a	50: 0.7 cm	50: 0.8 cm
	n/a	100: 0.8 cm	100: 0.9 cm
	200: 0.7 cm	200: 0.8 cm	200: 1.1 cm

Positive control: Amikacin. Negative controls: DMSO and H₂O. No activity: n/a.

The controls were used to verify the performance of the assay. The positive control (amikacin, 3 mg/mL) produced inhibition zones of 2.8, 3.6, and 3.7 cm. The negative control (DMSO and H₂O) showed no inhibitory activity, indicating that the solvents did not contribute to the observed inhibition zones.

4. Discussion

Evaluation of the sequential *Gliricidia sepium* leaf extracts against *Staphylococcus aureus* showed differences associated with the polarity of the solvent used. The methanolic extract (MeOH) exhibited the greatest inhibitory activity, with a 1.1 cm inhibition zone at 200 µg/mL, followed by the ethyl acetate extract (EAE), with 0.8 cm, whereas the hexane extract (HE) showed lower activity, with 0.7 cm. This pattern is consistent with the phytochemical profile reported for the species, which includes medium- to high-polarity metabolites such as flavonoids, phenolic acids, tannins, and saponins [2,6]. Methanol and ethyl acetate may favor the extraction of several of these bioactive compounds. Some flavonoids and tannins have shown antibacterial mechanisms involving disruption of the cell membrane, interference with cell-wall synthesis, and inhibition of microbial enzymes [2,3]. In contrast, the lower activity of the hexane extract may be related to the predominantly lipophilic profile of the compounds extracted by this solvent.

These findings can be compared with those reported by Alade et al. [7], who evaluated volatile oils obtained by hydrodistillation from *G. sepium* leaves and stems and found no significant antibacterial activity against *S. aureus* or *Escherichia coli*. The differences between the two studies highlight the influence of the extraction method on the resulting chemical profile. Whereas hydrodistillation favors the recovery of volatile compounds, maceration with methanol and ethyl acetate allows the extraction of more polar constituents, which may contribute to the differences observed in antibacterial activity [6,7].

The present results provide additional evidence of the antibacterial potential of *G. sepium*. Several reports describe the traditional use of its leaves in preparations for skin conditions and wound healing in tropical regions [3,6]. Given the relevance of *S. aureus* in skin and soft-tissue infections, the activity observed here supports further chemical and biological characterization of the most active extracts. Nevertheless, the present findings do not allow the activity to be attributed to specific compounds, nor do they establish therapeutic efficacy on their own.

Although the crude extracts showed antibacterial activity and, particularly for the methanolic extract, a tendency toward larger inhibition zones with increasing concentration, the zones were smaller than those produced by the positive control (amikacin, 2.8–3.7 cm). These results suggest the presence of constituents with antibacterial activity, but their relative potency cannot be established without additional quantitative measurements. Future studies should therefore focus on bioactivity-guided fractionation of the MeOH and EAE extracts and on the isolation and identification of the compounds responsible for

the activity. Determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), together with in vitro cytotoxicity assessment of the extracts and isolated compounds, will also be important [2,6].

5. Conclusions

Sequential extraction of *Gliricidia sepium* leaves showed that antibacterial activity against *Staphylococcus aureus* varied according to the solvent used. The methanolic extract exhibited the greatest activity, followed by the ethyl acetate extract, whereas the hexane extract showed the lowest inhibition. These findings support the selection of the methanolic and ethyl acetate extracts for subsequent bioactivity-guided fractionation and phytochemical characterization. Identification of the active compounds and determination of the MIC and MBC will be necessary to define their antibacterial potential more precisely.

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