Keywords
Gliricidia sepium; oral health promotion; phytochemicals; natural antimicrobials; antioxidant potential; preventive healthcare.

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Received: 10-04-2026
Revised: 17-05-2026
Accepted: 25-05-2026
DOI: 10.1922/ejprd.v34i3s.1418

Antimicrobial and Antioxidant Potential of Gliricidia sepium Leaf Extract for Preventive Oral Health Promotion

ABSTRACT

Preventive oral health promotion increasingly emphasizes safe, sustainable, and plant-derived bioactive agents with potential antimicrobial and antioxidant relevance. Gliricidia sepium is a medicinal plant known to contain diverse phytochemicals that may support future natural product-based preventive health applications. This study evaluated the phytochemical composition, biological activity, environmental safety, and enzyme-modulating potential of ethanolic leaf extract of Gliricidia sepium, with emphasis on its relevance as a natural bioactive resource for preventive oral health promotion. Leaves of G. sepium were collected from Baranoa, Atlántico, Colombia, and extracted using 96% ethanol. Phytochemical screening was performed to identify major secondary metabolites. Biological activity was assessed through standardized bioassays to determine LC_{50} and LC_{90} values, while Artemia salina toxicity testing was used to evaluate preliminary safety. Enzymatic assays involving glutathione S-transferase, α-esterase, β-esterase, and mixed-function oxidases were conducted to examine biochemical activity. The extract contained alkaloids, phenols, flavonoids, saponins, sterols, terpenes, glycosides, and tannins. It demonstrated notable biological activity, with an LC_{50} of 281.15 ppm at 48 h in the Rockefeller strain, and showed low toxicity toward A. salina, with LC_{50} values greater than 1000 ppm. Enzyme analysis indicated measurable modulation of detoxification-related enzymes, including a 61% increase in GST activity. The phytochemical richness, biological activity, favorable safety profile, and enzyme-modulating properties of G. sepium suggest its potential as a sustainable natural bioactive resource for future antimicrobial and antioxidant investigations related to preventive oral health promotion, community health education, and natural product-based healthcare strategies.

INTRODUCTION

Oral diseases continue to be one of the most common non-communicable health problems in the world, affecting billions of people, and contributing to significant social, economic and health care costs. Oral diseases such as dental caries, periodontal diseases, oral mucosal infections and other oral health conditions are major causes of pain and discomfort, impaired nutrition, loss of quality of life and health care costs. Oral diseases continue to disproportionately affect vulnerable populations despite the progress made in preventive dentistry and oral healthcare delivery, with limited access to preventive services, and oral health education, in low and middle-income countries (1). As a result, interest has been sparked in the search for natural products that are sustainable, affordable and biologically active to assist in preventive oral health promotion and complement existing public health measures (2).

The use of natural bioactive compounds that have antimicrobial properties and can reduce oxidative stress and promote general oral health is a growing focus of preventive oral care. The oral cavity supports a variety of microbes and disruption of the microbial balance may be linked to dental caries,

gingivitis, periodontitis and other inflammatory diseases. Moreover, oxidative stress is known to play a role in inflammatory reactions in the oral cavity and in periodontal tissue loss (3). Hence, a significant effort has been made towards identifying plant-based bioactive molecules with antimicrobial, antioxidant, anti-inflammatory, and protective activity that can help in the maintenance of oral health and prevention of oral diseases (4).

The importance of medicinal plants as valuable sources of therapeutic agents from them and their use in community health practices have been appreciated since long time ago and continue to be important all over the world. Many plant extracts have secondary metabolites like phenolic compounds, flavonoids, alkaloids, terpenes, saponins and glycosides that possess potent biological activities. These compounds are reported to have antimicrobial activity against pathogenic microorganisms, antioxidant activity which may help neutralize reactive oxygen species and anti-inflammatory activity that may help protect and heal tissues (5,6). These attributes have prompted growing interest in research on the development of plant-based health products and educational programs to encourage evidence-based use of medicinal plants in health care prevention programmes (7,8).

In addition, the increasing trend towards using natural products that are eco-friendly and biologically active has fuelled research into the biodiversity of plants for the discovery of new health-promoting compounds. With an estimated 11% of the world's plant diversity and over 31,000 registered species, Colombia is known as one of the most biodiverse countries in the world. Thousands of these have been studied for their medicinal, antimicrobial, insecticidal, antioxidant and pharmacological properties, showing the great potential of flora of Colombia as an important source of biologically active compounds that might be used in human health (9, 10). Further research into these plant resources could help not only in the research field, but in the field of community health promotion and education as well in a sustainable manner.

In this list of promising species is Gliricidia sepium, a species of the Fabaceae family which originates in America and grows from Mexico to Colombia. The species is usually found in slightly acid soil, and is distinguished by its average height (10-12 m), imparipinnate compound leaf of 25-30 cm. long and the small flower produced along a smooth twig (11). G. sepium has a traditional use in folk medicine for different diseases such as dermatitis and inflammatory diseases. Previous investigations have shown anti-inflammatory, antispasmodic, diuretic, hypothermic, insecticidal and central nervous system depressant properties, which show the presence of various bioactive compounds (12).

Phytochemical analysis of G. sepium has shown the presence of various types of secondary metabolites such as phenols, flavonoids, alkaloids, terpenes, and saponins, that are well known for their biological activity and therapeutic potential. Besides its traditional use in medicine, previous studies have already shown some biological effects of G. sepium extracts, such as ovicidal and larvicidal activities, which have led to a decrease in egg hatching and larval survival in a concentration-dependent manner (13–17). The results indicated the presence of potent bioactive compounds within the plant, which can interact with biological systems and impact cellular and metabolic processes.

The phytochemical diversity and biological activity of G. sepium suggest that it may have potential in other areas of preventive healthcare research, with previous studies mainly focusing on its ecological and insecticidal uses. Natural compounds that have antimicrobial, antioxidant and enzyme-modulating properties are gaining interest for their potential role in the promotion of oral health, especially in community-based and education-based approaches, which focus on prevention and sustainable healthcare. Such knowledge of medicinal plants' biological properties is thus a significant milestone toward elucidating potential natural resources that may yield future investigations concerning oral health.

Taking this into account, in the present study, it was aimed to characterize the phytochemical composition and biological activity of Gliricidia sepium ethanolic extract (GSEE), as well as evaluate its environmental safety and enzymatic effects. This research's bioactive profile and biological properties offer baseline data for future studies to explore the potential of G. sepium as a natural resource for preventive oral health promotion, community health promotion, and education on sustainable approaches to healthcare using plants.

MATERIALS AND METHODS

Study Design

The study design used was experimental laboratory-based to characterize the phytochemical composition, biological activity, environmental safety and enzyme-modulating activity of the ethanolic leaf extract of Gliricidia sepium. The study was designed as an exploratory examination of bioactive compounds in plants of relevance to the potential use of these compounds in preventive health care and future oral health promotion research.

Plant Material and Extraction

Leaves of Gliricidia sepium were collected in Baranoa, Atlántico, Colombia (10°47′44″N, 74°55′10″W) and verified in the Colombian National Herbarium. Fresh plant material was cleaned, air-dried and processed for extraction. The 50 g of the dried leaves was macerated in 500 mL of 96% ethanol for 72 h. After extraction, the extract was filtered and then concentrated on a Buchi R-201 rotary evaporator. The resulting residue was then dissolved in ethanol to get the ethanolic extract which was used throughout the study (18).

Phytochemical Screening

Qualitative phytochemical screening was carried out for the identification of major classes of secondary metabolites in the extract. The detection of chemical constituents like alkaloids, anthraquinones, coumarins, terpenes, phenols, flavonoids, glycosides and tannins were carried out by the standard phytochemical assays

(19). Each reaction was graded as − (absent), + (mild), ++ (moderate) or +++ (strong). Many of these compounds have been attributed to antimicrobial, antioxidant and health promoting effects in medicinal plant studies and the identification of these bioactive constituents was deemed important.

Biological Activity Assessment

The biological activity of the ethanolic extract was tested with the standardized bioassays as outlined by the World Health Organization (WHO) guidelines (20). The larvae of Aedes aegypti (Rockefeller strain and wild strain) were exposed to extract concentrations of 50, 100, 250, 500 and 1000 ppm. Mortality was noted 24 h and 48 h after exposure. Median lethal concentration (LC_{50}) and ninety-percent lethal concentration (LC_{90}) values were calculated using PROBIT analysis, which was run in R statistical software (version 4.5). The bioassays were used as a model system to evaluate the biological activity of the phytochemical components of the extract.

Environmental Safety Assessment

The preliminary safety evaluation of the extract was carried out by assessing its toxicity on Artemia salina nauplii (Meyer bioassay method) (21). Artemia salina is the most common non-target species for initial toxicity testing of natural products and plant extracts. LC_{50} values > 1000 ppm were considered to represent low toxicity and a good safety profile.

Enzyme Activity Assays

Colorimetric methods were used to determine the effect of the extract on certain detoxification related enzymes. The enzymatic activity assays were those of α-esterases, β-esterases, mixed-function oxidases (MFO) and glutathione S-transferases (GST) according to Lima, (22). The percentage inhibition of the enzyme, relative activity index (RAI) and percentage increase in activity was then determined based on known procedures (23). These analyses were carried out to explore the possibility of biochemical interactions with the extract and detoxification pathways in cells, which would give more insight into the biological activity of the identified phytochemicals.

Data Recording and Statistical Analysis

Qualitative-Descriptive method was used to record and analyze the data. Data analysis was performed using R statistical software package (version 4.5.0) and Microsoft Excel. The analytical procedures such as descriptive statistics, PROBIT analysis and enzyme activity calculations and toxicity estimations were carried out accordingly. The results were then analyzed to describe the phytochemical finger print, biological activity, environmental safety and enzyme-modulating effect of Gliricidia sepium ethanolic leaf extract.

Results and discussion

Phytochemical Composition

The extract was found to contain several secondary metabolites such as alkaloids, phenolic compounds, flavonoids, saponins, terpenes, glycosides and tannins (Table 1). These phytochemicals are well known for biological activities and have been correlated with antimicrobial, antioxidant, anti-inflammatory and protective properties in medicinal plant studies [24] [25].

Oral health aspects of phenolic compounds and flavonoids include antimicrobial activity, anti-inflammatory properties, and tissue homeostasis, whereas the bioactive properties of terpenes, alkaloids and saponins indicate their potential for oral health applications. The presence of these bioactive compounds indicates that Gliricidia sepium could be a viable natural source of compounds that could be studied in the future for use in the promotion of oral health and community based preventive health strategies.

Table 1. *Gliricidia sepium* chemical composition

t	Essay	*A. indica*
Alkaloids	*Dragendorff*	+++
Alkaloids	*Mayer*	+++
Alkaloids	*Warner*	+
Alkaloids	*Hager*	+
Anthraquinones	*Bornträger*	-
Coumarins	*Hidroxamato ferric*	+
Sterols y terpenes	*Libermand Bouchard*	+
Phenols	*Trichloro ferric*	+++
Flavonoids	*Shinoda*	+
Flavonoids	*Pacheco*	+
Cardiotonic glycosides	*Baljet*	++
Leucoantocianinas	*Rosenheim*	+
Saponins	*Spume*	++
Tannins	*Gelatine 1%*	+
Tannins	*Gelatine-Sal 1%-10%*	+

Based on the phytochemical profile exhibited by G. sepium, it is highly rich in metabolites that are biologically active. It is noteworthy that phenols, flavonoids, alkaloids, saponins and terpenes are all present simultaneously, and these compounds have been widely used in research on natural antimicrobial agents, as antioxidants and as healthcare products derived from plants for preventive uses.

Larvicidal activity

The LC_{50} values indicate high larvicidal potency (< 400 ppm) [22] *G. sepium* showed greater activity at 48 h exposure (Table 2).

Table 2. Toxicity results of ethanolic extracts on third-stage *Ae. aegypti* larvae.

Specie	Strain	Tiempo (h)	CL50 (ppm)	IC95%	CL90 (ppm)	IC95%
G. sepium	Rockefeller	24	315,11	(279,78 - 357,53)	469,57	(417,63 - 547,28)
G. sepium	Rockefeller	48	281,15	(255,29 - 311,6)	437,74	(396,16 - 495,22)
G. sepium	Wild	24	363,55	(333,54 - 397,66)	547,62	(501,25 - 610,37)
G. sepium	Wild	48	336,84	(306,27 - 372,08)	538,2	(488,47 - 606,12)

*n=25 per triplicate per concentration

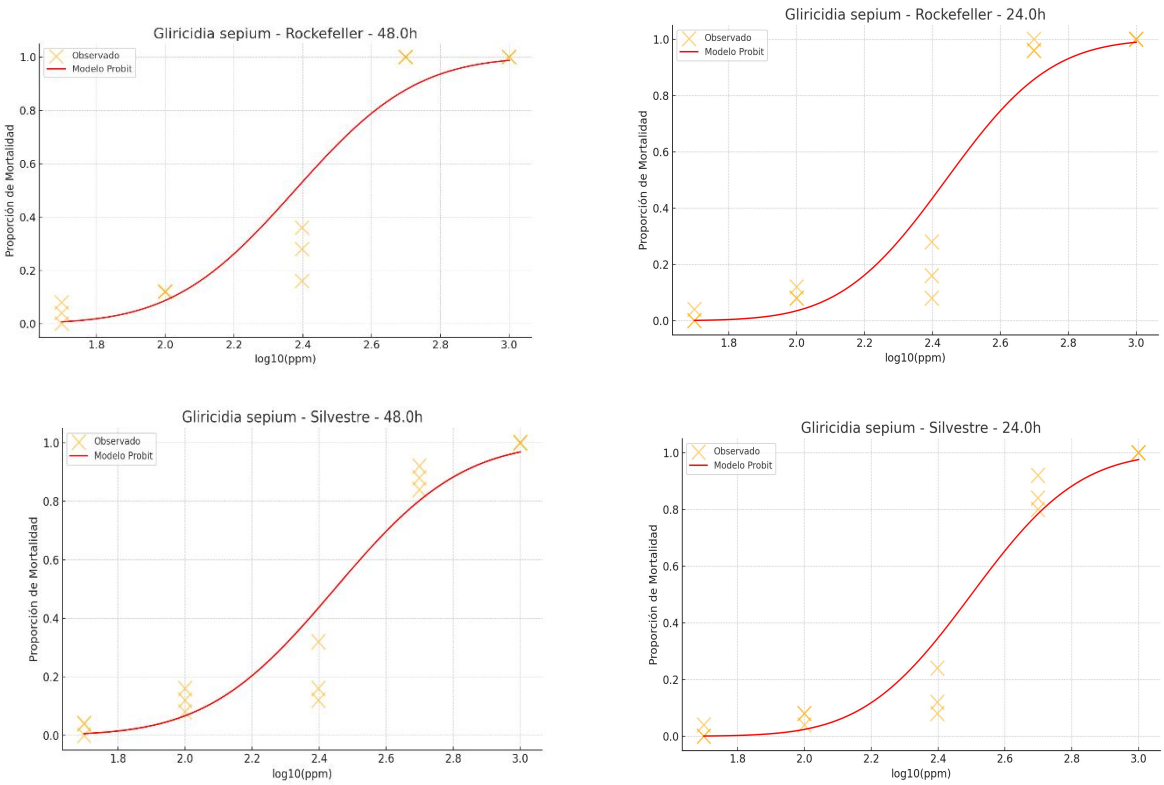

Figure 1: Median lethal concentration, a) Rockefeller strain at 24 hours. b) Rockefeller strain at 48 hours. c) Wild strain at 24 hours. d) Wild strain at 48 hours

Figure 1 demonstrates the biological activity profile of Gliricidia sepium extract across different concentrations and exposure times, confirming the presence of potent bioactive constituents that warrant further investigation for antimicrobial and antioxidant applications.

The species Gliricidia sepium exhibited toxicity levels against Aedes aegypti larvae that classify it within the high-toxicity category according to the toxicity scale adapted by Meyer et al. (24), with LC_{50} values below 400 ppm. The observed biological activity is likely associated with the presence of alkaloids, phenols, saponins, sterols, and terpenes identified during phytochemical screening (Table 3). Similar findings have been reported by Widiyaningrum et al. (14) and Krishnaveni et al. (25).

The current experimental model did not aim to assess oral microorganisms, but the significant biological activity of the extract suggests that it contains potent bioactive compounds. These results offer a preliminary indication for further research on the antimicrobial and antioxidant activity of G. sepium for preventive oral care, especially in the community-oriented health promotion program that uses natural therapeutic resources.

Table 3: Toxicity results of ethanolic extracts in Artemia salina

Species	Time (h)	Cl50 (ppm)	IC_95%	Cl90 (ppm)	IC_95%
G. sepium	24	1141,74	(956,93 - 1468,07)	2073,06	(1682,77 - 2809,79)

*n=25 per triplicate per concentration

The opposite side, Gliricidia sepium extract showed low toxicity for the non-target organism of Artemia salina, similar to the results reported by Sánchez and Neira (26), so it can be considered to be not hazardous for the environment. The selectivity index of 3.62 is an indicator of the favorable safety margin, which makes G. sepium a promising and environmentally friendly agent for controlling the vector A. aegypti. Besides environmental compatibility, the good safety profile, as shown in the Artemia salina assay, could be a key

attribute for further studies of health products derived from plants (Table 4). Preliminary safety assessment is a key step in the process of designing natural preventive healthcare interventions and community health promotion activities.

Table 4. Absorbance recorded in detoxifying enzymes exposed to ethanolic extracts

Extract	Enzyme											
	GST			α-esterase			MFO			β-esterase		
	media	sd	p-value	media	sd	p-value	media	sd	p-value	media	sd	p-value
Rockefeller (susceptible)	0,401	0,051		0,227	0,056		0,348	0,038		0,246	0,009	
Gliricidia sepium	0,644	0,291	<2,2e-16	0,255	0,040	3e-03	0,411	0,023	<2,2e-16	0,248	0,020	0,034

*The results represent statistical comparisons between each group treated with extracts and

Moreover, Gliricidia sepium ethanolic extract showed a potent and selective inhibitory activity on detoxifying enzymes α-esterase, β-esterase, mixed-function oxidase (MFO), and glutathione S-transferase (GST) which would further advocate its use as a larvicidal agent. The results obtained showed that the mean absorbance values were found to be higher in wild strain than in the susceptible strain group. There was also significant difference between the mean data obtained from exposed wild strain and Rockefeller (susceptible) strain of Aedes aegypti larvae by t-test, which showed that the detoxifying enzyme activity differs between these strains (Table 5).

The effect of modulation of enzymatic activity observed further confirms the biological activity of the extract. Phytochemicals that modulate enzymes are a growing focus of interest due to their possible roles in cellular protection, managing oxidative stress and biological responses of interest in preventive healthcare research.

Table 5. Percentage inhibition (%), Relative Activity Index (RAI), and % increase in activity

Extract	Enzyme	Positive Control	Susceptible	Exp	Percent Inhibition (%)	Relative Activity Index (IAR)	% Increase in Activity
Gliricidia sepium	α-esterase	0,598	0,227	0,255	57%	1,12	12%
	β-esterase	0,627	0,246	0,248	60%	1,01	1%
	Glutathione S-transferase (GST)	0,735	0,401	0,644	12%	1,61	61%
	Mixed-function oxidases (MFO)	0,674	0,348	0,411	39%	1,18	18%

To identify the inhibitory potential the calculated inhibition percentage was applied, according to the criteria adapted from Brogdon and McAllister (23) and Lima, (22), where the comparison of the enzymatic activity of larvae exposed to the ethanolic extract of Gliricidia sepium with that of the control group. The level of inhibition was considered as mild (below 40%), moderate (40 to 60%) and strong (above 60%). Moderate inhibition (57%) was observed with α-esterase and moderate inhibition (60%) was obtained with the activity of β-esterase. The glutathione S-transferase (GST) enzyme was exhibited low inhibition (12%) and the mixed-function oxidase (MFO) enzyme was exhibited low inhibition (39%). However, inhibition of all the detoxifying enzymes studied was observed.

The fact that an enzyme inhibition was observed in each system tested suggests that the extract is interacting with key biochemical pathways. These interactions are often investigated during evaluation of the general biological significance of medicinal plants and their bioactive compounds, with the aim of considering them as sources for bioactive agents for health promotion.

The degree of increased or decreased enzymatic activity compared between field strains and the susceptible strain was determined by the Relative Activity Index (RAI). The α-esterase enzyme showed an RAI of 1.12, β-esterase 1.01, GST 1.61, and MFO 1.18. The percentage increase in enzyme activity corresponding to the reference values can also be used as an indicator to estimate the enzymatic inhibition. Specific percentage increases for each of the enzymes were as follows: α-esterase (12%), β-esterase (1%), GST (61%) and MFO (18%) in present study.

After comparing the inhibition percentage, the relative activity index, enzyme activity increase, the results of biological activity and the safety profile of non-target species, it was concluded that the ethanolic extract of Gliricidia sepium had good biological activity and low toxicity to non-target species. Measurable effects were found in the extract on α-esterase, β-esterase, mixed-function oxidases and glutathione S-transferase, suggesting interactions with biochemical pathways involved in detoxification.

The phytochemical composition of G. sepium, along with its excellent safety profile and enzyme modulation activity, underlines its potential as a rich source of natural bioactive compounds. The current study did not aim to directly assess oral pathogens, but the biological traits found indicate that the antimicrobial and antioxidant properties of this study warrant further research for preventive oral health promotion. These data can help to build a body of evidence for the use of plant materials for community health education, sustainable healthcare, and prevention oral health education.

Implications for Oral Health Promotion and Education

Given this increased need for safe, low cost and sustainable health care interventions, there is a renewed interest in medicinal plants as educational and preventative health care resources. Oral health promotion programs are increasingly adding plant-derived bioactive compounds to their strategies, due to their antimicrobial, antioxidant, protective properties. The phytochemical profile revealed in Gliricidia sepium reveals the presence of a number of classes of compounds which are often linked to biological activities of interest for preventive medicine.

In an educational context, evidence based information on medicinal plants could be used as part of community outreach and health literacy and preventative health education. Raising awareness of the plant resources that have been scientifically studied can facilitate informed decision-making and promote sustainable health maintenance practices. Thus, the current results could serve as a basis for future research regarding G. sepium for oral health promotion, education in natural products and community-based preventive healthcare strategies.

Conclusion

Based on the findings of the present study, the bioactive phytochemicals such as sterols, terpenes, alkaloids, phenolic compounds, flavonoids, saponins and glycosides found in Gliricidia sepium leaf extract reveal its significant biological activity. The extract displayed a significant bioactivity with the observed LC_{50} values and it was also found to be less toxic to the non-target organism Artemia salina with low LC_{50} and a good selectivity index of 3.62, which indicates a satisfactory preliminary safety profile. Moreover, the modulation of key detoxification enzymes such as α-esterase, β-esterase, glutathione S-transferase and mixed-function oxidases demonstrate the ability of the extract to interact with important biochemical pathways. The phytochemical profile, biological activity, enzyme-modulating effects, and environmental sustainability highlighted in this study underscore the potential benefits of G. sepium as a valuable natural source of bioactive compounds. The phytochemicals identified in the present investigation are well known to possess antimicrobial, antioxidant and health promoting properties, although the study was not aimed at direct evaluation of the oral microorganisms. The results of this study support further research on the use of G. sepium in preventive oral health promotion and community health education, natural product interventions, and sustainable healthcare strategies to promote oral health and awareness of community health.

Acknowledgments

The authors would like to thank PHD Nelson Grisales from the World Mosquito program for providing with the *Aedes aegypti* Rockefeller strain. Atlantico University and all the populations from the localities investigated for their collaborations. All the technical support from the Cellular Biology Laboratory and the Tropical Medicine laboratory from Atlantico University.

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