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Antioxidant and antimicrobial activities of sustainable *Salvia hispanica* L. seed extracts against gram-positive and gram-negative bacteria

Actividades antioxidantes y antimicrobianas de los extractos sostenibles de semillas de *Salvia hispanica* L. frente a bacterias Gram-positivas y Gram-negativas

Atividades antioxidantes e antimicrobianas dos extratos sustentáveis de sementes de *Salvia hispanica* L. contra bactérias Gram-positivas e Gram-negativas

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Rev. Fac. Agron. (LUZ). 2026, 43(1): e254308
ISSN 2477-9407
DOI: https://doi.org/10.47280/RevFacAgron(LUZ).v43.n1.VIII

Received: 19-11-2025
Accepted: 29-12-2025
Published: 18-01-2026

Food technology

Associate editor: Dra. Gretty R. Ettiene Rojas
University of Zulia, Faculty of Agronomy
Bolivarian Republic of Venezuela.

Keywords:
Salvia hispanica L.
Green extraction
Antioxidant activity
Antimicrobial activity
Food safety
Sustainable bioingredients

Abstract

Salvia hispanica L. (chia) seeds are rich in bioactive compounds with antioxidant and antimicrobial properties, making them valuable natural ingredients for food and agro-industrial applications. Food contamination by Gram-positive and Gram-negative bacteria remains a major challenge for food safety, highlighting the need for sustainable natural preservatives. This study aimed to compare two green extraction methods for recovering bioactive compounds from Algerian dark chia seeds and to evaluate the antioxidant and antimicrobial activities of the resulting extracts against Gram-positive and Gram-negative bacteria. Aqueous Soxhlet extraction and ethanolic maceration were applied. Extracts were analyzed for total phenolics and flavonoids, antioxidant activity (DPPH assay), and antimicrobial activity using the disc diffusion method against nine microbial strains, including Gram-positive and Gram-negative bacteria and *Candida albicans*. The aqueous Soxhlet extract exhibited the highest total phenolic content and strong antioxidant activity (88 % DPPH inhibition at 400 $\mu g.mL^{-1}$). The ethanolic maceration extract was richer in flavonoids and showed moderate, dose-dependent antimicrobial activity. Gram-positive bacteria (*Staphylococcus aureus* and *Listeria monocytogenes*) were most sensitive (up to 12 ± 1 mm), while Gram-negative bacteria were generally more resistant, except *Klebsiella pneumoniae* (15 ± 1 mm at 15 $mg.mL^{-1}$). *Candida albicans* was highly susceptible (14 ± 1 mm). Green-extracted chia seed extracts demonstrate significant antioxidant and moderate antimicrobial activities, supporting their potential use in food preservation, sustainable product development, and eco-friendly agro-industrial applications.

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Resumen

Las semillas de *Salvia hispanica* L. (chia) son ricas en compuestos bioactivos con propiedades antioxidantes y antimicrobianas, lo que las convierte en ingredientes naturales valiosos para aplicaciones alimentarias y agroindustriales. La contaminación de los alimentos por bacterias Gram-positivas y Gram-negativas sigue siendo un desafío importante para la seguridad alimentaria, lo que resalta la necesidad de conservantes naturales sostenibles. Este estudio tuvo como objetivo comparar dos métodos de extracción verde para recuperar compuestos bioactivos de semillas de chia oscuras de Argelia y evaluar las actividades antioxidante y antimicrobiana de los extractos resultantes frente a bacterias Gram-positivas y Gram-negativas. Se aplicaron extracción acuosa Soxhlet y maceración etanólica. Los extractos se analizaron para determinar fenoles totales y flavonoides, actividad antioxidante (ensayo DPPH) y actividad antimicrobiana mediante el método de difusión en disco frente a nueve cepas microbianas, incluidas bacterias Gram-positivas y Gram-negativas y *Candida albicans*. El extracto acuoso Soxhlet mostró el mayor contenido total de fenoles y una fuerte actividad antioxidante (88 % de inhibición DPPH a 400 $\mu g.mL^{-1}$). El extracto de maceración etanólica fue más rico en flavonoides y presentó una actividad antimicrobiana moderada, dependiente de la dosis. Las bacterias Gram-positivas (*Staphylococcus aureus* y *Listeria monocytogenes*) fueron las más sensibles (hasta 12 ± 1 mm), mientras que las bacterias Gram-negativas generalmente fueron más resistentes, excepto *Klebsiella pneumoniae* (15 ± 1 mm a 15 $mg.mL^{-1}$). *Candida albicans* mostró alta susceptibilidad (14 ± 1 mm). Los extractos de semillas de chia obtenidos mediante métodos verdes demuestran actividades antioxidantes significativas y moderadas actividades antimicrobianas, respaldando su potencial uso en la conservación de alimentos, desarrollo de productos sostenibles y aplicaciones agroindustriales ecológicas.

Palabras clave: *Salvia hispanica* L., extracción verde, actividad antioxidante, actividad antimicrobiana, seguridad alimentaria, bioingredientes sostenibles.

Resumo

As sementes de *Salvia hispanica* L. (chia) são ricas em compostos bioativos com propriedades antioxidantes e antimicrobianas, tornando-se ingredientes naturais valiosos para aplicações alimentares e agroindustriais. A contaminação de alimentos por bactérias Gram-positivas e Gram-negativas continua sendo um grande desafio para a segurança alimentar, destacando a necessidade de conservantes naturais sustentáveis. Este estudo teve como objetivo comparar dois métodos de extração verde para recuperar compostos bioativos de sementes escuras de chia da Argélia e avaliar as atividades antioxidante e antimicrobiana dos extratos obtidos frente a bactérias Gram-positivas e Gram-negativas. Foram aplicadas extração aquosa Soxhlet e maceração etanólica. Os extratos foram analisados quanto ao teor total de fenóis e flavonoides, atividade antioxidante (ensaio DPPH) e atividade antimicrobiana pelo método de difusão em disco contra nove cepas microbianas, incluindo bactérias Gram-positivas e Gram-negativas e *Candida albicans*. O extrato aquoso Soxhlet apresentou o maior teor total de fenóis e forte atividade antioxidante (88 % de inibição DPPH a 400 $\mu g.mL^{-1}$). O extrato de maceração etanólica foi mais rico em flavonoides e mostrou atividade antimicrobiana moderada, dependente da dose. As bactérias Gram-positivas (*Staphylococcus aureus* e *Listeria monocytogenes*) foram as mais sensíveis (até 12 ± 1 mm), enquanto as Gram-negativas foram geralmente mais resistentes, exceto *Klebsiella pneumoniae* (15 ± 1 mm a 15 $mg.mL^{-1}$). *Candida albicans* apresentou alta suscetibilidade (14 ± 1 mm). Extratos de sementes de chia obtidos por métodos verdes demonstram atividades antioxidantes significativas e atividade antimicrobiana moderada, apoiando seu potencial uso na conservação de alimentos, desenvolvimento de produtos sustentáveis e aplicações agroindustriais ecológicas.

Palavras-chave: *Salvia hispanica* L., extração verde, atividade antioxidante, atividade antimicrobiana, segurança alimentar, bioingredientes sustentáveis.

Introduction

Salvia hispanica L., commonly known as chia, is an emerging high-value crop with adaptability to diverse climates and potential for sustainable cultivation. In Algeria, it offers opportunities to diversify agriculture and develop functional food and agro-industrial products.

Food contamination by Gram-positive and Gram-negative bacteria remains a major concern for food safety and public health. Natural bioactive compounds from plants provide a promising strategy to inhibit microbial growth, reduce chemical preservatives, and improve food quality.

Chia seeds are rich in omega-3 fatty acids, dietary fibers, proteins, vitamins, minerals, polyphenols, and flavonoids (Rahmoune *et al.,* 2025; Dib *et al.,* 2021), which exhibit strong antioxidant and antimicrobial activities (Tavera-Hernández *et al.,* 2023; Tavares *et al.,* 2018). Green extraction methods, such as aqueous Soxhlet extraction and ethanolic maceration, allow efficient recovery of these bioactive compounds while minimizing chemical waste and environmental impact.

This study aims to evaluate the antioxidant and antibacterial activities of Algerian dark chia seed extracts against Gram-positive and Gram-negative bacteria, and to compare the effects of two green extraction methods on the yield and bioactivity of these extracts. The work highlights the potential of sustainable chia extracts for enhancing food safety and producing eco-friendly bioingredients for agro-industrial applications.

Materials and methods

Plant material

Commercial *Salvia hispanica* L. (black chia) seeds were first carefully sorted and cleaned to eliminate foreign particles and impurities, ensuring sample purity and analytical reliability. Subsequently, 10 g of seeds were gently ground at room temperature to enhance the contact surface with the extraction solvent, thereby improving the efficiency of compound recovery (Silva *et al.,* 2022).

Preparation of extracts

Ethanolic extract (Maceration)

Ten grams of black chia seed powder were placed in 100 mL of ethanol (1:10 w/v) in a 250 mL Erlenmeyer flask and stirred continuously at room temperature for 24 h. The mixture was filtered through Whatman paper, concentrated under reduced pressure using a rotary evaporator (Heidolph Instruments, Germany), and stored at 4 °C until analysis (Motyka *et al.,* 2023).

Aqueous extract (Soxhlet Extraction)

Ten grams of black chia seed powder were sequentially extracted in a Soxhlet apparatus with 100 mL of hexane (65 °C), chloroform (60 °C), ethanol (78 °C), and distilled water (100 °C), each for 6 h. Solvents were removed with a rotary evaporator, and the final aqueous extract was stored at 4 °C (Najafi *et al.,* 2022).

Microorganisms and culture conditions

The tested microorganisms included four Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 49452, *Listeria monocytogenes* ATCC 15313, *Bacillus cereus* ATCC 11778), four Gram-negative bacteria (*Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603, *Salmonella enteritidis* ATCC 2453, *Pseudomonas aeruginosa* ATCC 27853), and one yeast, *Candida albicans* ATCC 10231. Strains were obtained from the Laboratory of Applied Microbiology to Food, Biomedical and Environmental Sciences (LAMAABE), Abou Bekr Belkaïd University, Tlemcen, Algeria. Cultures were grown 18–24 h and adjusted to 0.5 McFarland standard (≈10^8 CFU.mL^{-1}).

Determination of total phenolic and flavonoid contents

Total phenolic content (TPC) was measured using the Folin–Ciocalteu method (Pérez *et al.,* 1990; Singleton and Rossi, 1965). Total flavonoid content (TFC) was determined via the aluminum chloride colorimetric assay (Chang *et al.*, 2002).

Antioxidant activity

Antioxidant activity was assessed using the DPPH assay according to Brand-Williams *et al.* (1995) with minor modifications. Extract stock solutions were prepared in methanol and diluted to final concentrations ranging from 12.5 to 400 µg.mL^{-1}. In 96-well plates, 100 µL of each dilution were mixed with 100 µL of 0.1 mM DPPH solution, incubated for 30 min in the dark, and the absorbance was measured at 517 nm. The percentage of DPPH radical scavenging activity was calculated using the following equation:

$$\% \text{ Inhibition} = \frac{\text{A control} - (\text{A sample} - \text{A blank}}{\text{A control}} \text{ X } 100$$

Antimicrobial activity

Antibacterial and antifungal activities were assessed using the agar well diffusion method. Mueller-Hinton agar plates were inoculated with standardized microbial suspensions (CA-SFM, 2025), and four 6 mm wells per plate were filled with 20 µL of either ethanolic or aqueous extracts at concentrations ranging from 0.5 to 15 mg.mL^{-1}. Gentamicin (10 µg) was used as an positive control, while the corresponding solvents served as negative controls. Experiments were performed in triplicate. The chosen concentration range allowed evaluation of the dose-dependent response of microorganisms and determination of both minimal and maximal antimicrobial effects, in accordance with established agar well diffusion protocols for natural extracts (Gonzalez Pastor *et al.,* 2023; Matuschek *et al.,* 2014). Inhibition zones were classified as non-sensitive (<8 mm), sensitive (9 - 14 mm), very sensitive (15 - 19 mm), and extremely sensitive (>20 mm).

Experimental design and statistical analysis

The study followed a completely randomized experimental design comparing two extraction methods (ethanolic and aqueous) at different concentrations for antioxidant and antimicrobial responses. All experiments were conducted in triplicate, and results are expressed as mean ± standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA) to evaluate differences among extracts and concentrations. Differences were considered statistically significant at $p < 0.05$. Statistical analyses were carried out using Microsoft Excel (Microsoft Corp., USA).

Results and discussion

Total phenolic and flavonoid contents

Table 1 presents the total phenolic content (TPC) and total flavonoid content (TFC) of black chia seed extracts obtained using ethanolic maceration (EEM) and aqueous Soxhlet extraction (AES). TPC is expressed as µg gallic acid equivalents per mg of dry extract (µg GAE.mg^{-1}), and TFC as µg quercetin equivalents per mg of dry extract (µg QE.mg^{-1}).

Table 1. Total phenolic and flavonoid contents of ethanolic and aqueous extracts of *S. hispanica*.

Extracts	Ethanolic maceration extract	Aqueous Soxhlet extract
Total Phenolic (µg GAE.mg^{-1})	60.57 ± 4.60	110.67 ± 5.10
Total Flavonoid (µg QE.mg^{-1}).	20.66 ± 0.50	3.60 ± 0.30

A marked variation in the biochemical composition of *Salvia hispanica* L. seeds was observed depending on the extraction method and solvent. The aqueous Soxhlet extract (AES) exhibited the highest phenolic content (110.67 ± 5.10 µg GAE.mg^{-1}), whereas the ethanolic maceration extract (EEM) showed lower phenolics (60.57 ± 4.60 µg GAE.mg^{-1}) but significantly higher flavonoid content (20.66 ± 0.50 µg QE.mg^{-1}) compared to AES (3.60 ± 0.30 µg QE.mg^{-1}), indicating that solvent polarity strongly affects extraction efficiency (Knez Hrnčič *et al.*, 2019; Capitani *et al.*, 2012). The phenolic content of AES is consistent with previously reported aqueous chia extracts ~105 - 115 µg GAE.mg^{-1} (Capitani *et al.*, 2012), while the elevated flavonoid content in EEM confirms ethanol's efficiency in extracting lipophilic flavonoids (Knez Hrnčič *et al.*, 2019).

The extraction approach was designed to maximize recovery of bioactive compounds while minimizing environmental impact, in line with sustainable agronomy and green processing principles (Cravotto *et al.*, 2025; Clarke *et al.,* 2018). Mild pretreatment preserved sensitive compounds by avoiding harsh reagents and high energy consumption. Both ethanolic maceration and aqueous Soxhlet extraction are low-cost, eco-friendly, and suitable for small- and medium-scale agro-industrial applications. Ethanol, notable for its renewability, biodegradability, and safety, is particularly effective for flavonoid recovery, while sequential Soxhlet extraction with water enhances hydrophilic phenolics and reduces solvent waste (Demirkol *et al.*, 2022; Clarke *et al.,* 2018).

Antioxidant activity

The aqueous Soxhlet extract (AES) demonstrated strong antioxidant activity, reaching 88 % inhibition at 400 µg.mL^{-1}, which exceeds the values reported for similar chia extracts ~75 % (Silva *et al.*, 2022) and confirms the efficiency of this extraction method for polar phenolics. Antioxidant potential, assessed using the DPPH assay, showed dose-dependent radical scavenging for both extracts, with AES markedly outperforming the ethanolic maceration extract (EEM, 25 % inhibition) (Figure 1). The high activity of AES can be attributed to its enrichment in hydrophilic phenolics and mucilages, which act synergistically as radical scavengers and stabilizers (Silva *et al.*, 2022; Ullah *et al.,* 2016; Capitani *et al.*, 2012; Sultana *et al.*, 2009). These results underscore the potential application of AES as a natural antioxidant to improve oxidative stability and extend the shelf life of agro-food products.

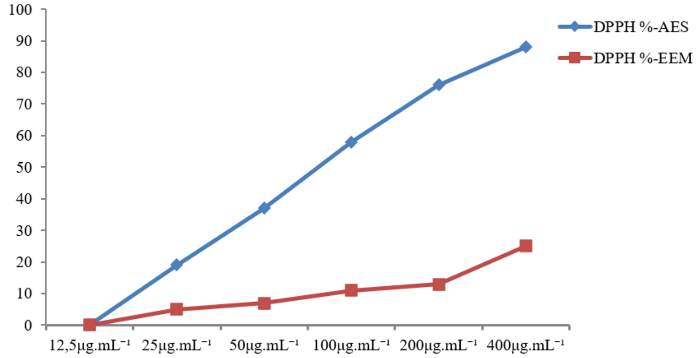

Figure 1. Antioxidant capacity of chia seed extracts using the DPPH Assay.

Antimicrobial activity

Chia seed extracts exhibited dose-dependent antimicrobial activity against nine microbial strains, including Gram-positive and Gram-negative bacteria, as well as *Candida albicans* (figure 2; table 2).

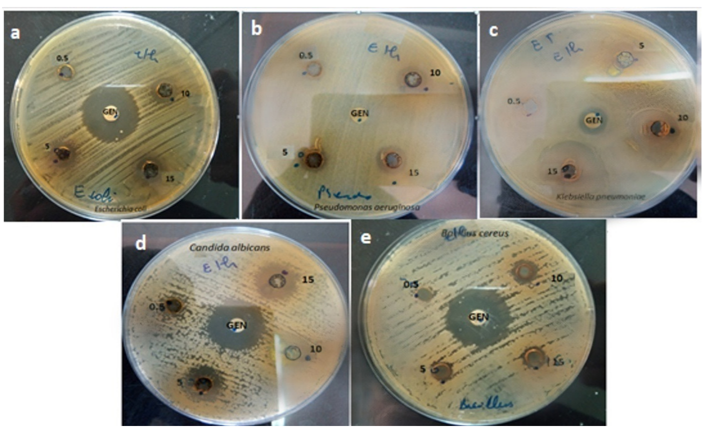

Figure 2. Antimicrobial activity of *Salvia hispanica* L. seed extracts against (a) *Escherichia coli,* (b) *Pseudomonas aeruginosa,* (c) *Klebsiella pneumoniae,* (d) *Candida albicans,* and (e) *Bacillus cereus.*

The ethanolic maceration extract (EEM) demonstrated stronger inhibition, particularly at 10–15 mg.mL-1. *Staphylococcus aureus* (12 ± 1 mm) and *Listeria monocytogenes* (9 ± 1 mm) were highly sensitive, while *Klebsiella pneumoniae* (15 ± 1 mm) showed inhibition comparable to gentamicin (10 µg).

Candida albicans also displayed notable susceptibility (14 ± 1 mm), confirming the antifungal potential of EEM. These effects are consistent with previous studies showing that ethanolic extracts of chia seeds inhibit bacterial and fungal growth due to non-polar metabolites, including methylated flavonoids, terpenoids, and unsaturated fatty acids (Ullah *et al*., 2016; Segura-Campos *et al*., 2014; Topçu *et al*., 2007).

An inverse relationship between antioxidant and antimicrobial activities was observed, reflecting the distinct chemical nature of the bioactive compounds: polar phenolics are primarily responsible for antioxidant effects, whereas non-polar metabolites contribute to antimicrobial action (Kaurinovic and Vastag, 2019; Clarke *et al.,* 2018). These findings confirm the concentration-dependent efficacy of chia extracts, particularly EEM, against *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Candida albicans*, supporting their potential as natural antimicrobial agents, although their activity is generally lower than that of conventional antibiotics.

Solvent polarity strongly influenced both chemical composition and bioactivity: water favored the extraction of polar, antioxidant-rich fractions, while ethanol efficiently recovered lipophilic, antimicrobial compounds (Rahmoune *et al.*, 2024; Knez Hrnčič *et al.*, 2019; Ullah *et al.,* 2016; Capitani *et al.*, 2012). Overall, these results highlight the multifunctionality of *S. hispanica* seeds as a source of bioactive compounds for natural preservatives and sustainable agro-industrial applications, contributing to circular bioeconomy strategies and the valorization of crops in semi-arid regions such as Algeria.

Conclusion

This study demonstrated that the antimicrobial and antioxidant efficacy of *Salvia hispanica* L. seed extracts is strongly influenced by the polarity of the extraction solvent and the sustainability of the extraction process. Aqueous Soxhlet extraction, rich in polar phenolic compounds, provided extracts with remarkable antioxidant capacity, while ethanolic maceration yielded lipophilic bioactives with greater antimicrobial potential, particularly against *Candida albicans*. The complementary bioactivities of these extracts underscore the multifunctionality of chia seeds as a valuable agricultural crop for the development of natural agro-industrial additives and eco-innovative formulations. By using renewable, safe, and biodegradable solvents such as water and ethanol, this work demonstrates sustainable processing approaches that minimize environmental impact and enhance the valorization of agricultural resources.

Table 2. Antimicrobial activities (diameters of inhibition zones in mm) of ethanolic (EEM) and aqueous (AES) extracts of *S. hispanica* against various microbial strains.

Extracts	Control	Aqueous Soxhlet extract (AES, mg.mL^{-1})				Ethanolic maceration extract (EEM, mg.mL^{-1})			
Strains	GEN 10 µg	0.5	5	10	15	0.5	5	10	15
Staphylococcus aureus	22 ± 1	6 ± 1	7 ± 1	10 ± 1	10 ± 1	6 ± 1	8 ± 1	9 ± 1	12 ± 1
Enterococcus faecalis	26 ± 1	6 ± 1	6 ± 1	6 ± 1	7 ± 1	6 ± 1	7 ± 1	7 ± 1	8 ± 1
Listeria monocytogenes	19 ± 1	6 ± 1	7 ± 1	8 ± 1	8 ± 1	7 ± 1	8 ± 1	9 ± 1	9 ± 1
Bacillus cereus	20 ± 1	6 ± 1	7 ± 1	7 ± 1	8 ± 1	6 ± 1	7 ± 1	7 ± 1	9 ± 1
Escherichia coli	22 ± 1	6 ± 1	6 ± 1	7 ± 1	8 ± 1	7 ± 1	8 ± 1	9 ± 1	10 ± 1
Klebsiella pneumoniae	15 ± 1	6 ± 1	7 ± 1	8 ± 1	14 ± 1	7 ± 1	8 ± 1	11 ± 1	15 ± 1
Salmonella enteritidis	24 ± 1	6 ± 1	7 ± 1	8 ± 1	9 ± 1	7 ± 1	8 ± 1	9 ± 1	10 ± 1
Pseudomonas aeruginosa	18 ± 1	6 ± 1	7 ± 1	7 ± 1	8 ± 1	7 ± 1	8 ± 1	8 ± 1	9 ± 1
Candida albicans	21 ± 1	7 ± 1	8 ± 1	8 ± 1	10 ± 1	8 ± 1	9 ± 1	10 ± 1	14 ± 1

Inhibition zone diameter (mm); Values are expressed as mean ± SD.

Overall, the results reinforce the potential of *Salvia hispanica* L. as a sustainable agricultural resource whose bioactive compounds can be efficiently harnessed through environmentally sound extraction technologies, supporting responsible agricultural innovation and contributing to a circular bioeconomy.

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