ISSN Print: 2664-6536
ISSN Online: 2664-6544
Impact Factor: RJIF **5.4**
IJBB 2025; 7(1): 15-19
www.biosciencejournal.net
Received: 18-10-2024
Accepted: 25-11-2024

Muhanad N Abdullah
Al-Nahrian University, College of Biotechnology, Iraq

Corresponding Author:
Muhanad N Abdullah
Al-Nahrian University, College of Biotechnology, Iraq

Antimicrobial activity of chia seeds (*Salvia hispanica* L.) extract on microorganisms isolated from urinary tract infection

Muhanad N Abdullah

DOI: https://dx.doi.org/10.33545/26646536.2025.v7.i1a.91

Abstract
Urinary tract infections (UTIs) are common, particularly in women due to anatomical differences, with nearly 50% of women experiencing at least one UTI in their lifetime. UTIs can lead to severe complications if untreated. This study evaluates the antibacterial potential of Chia seeds (*Salvia hispanica* L.) against UTI-related bacteria, considering the potential of natural compounds in addressing antibiotic resistance. Chia seed aqueous extract was tested against *Staphylococcus aureus*, *Klebsiella* spp., *Pseudomonas aeruginosa* and *Proteus* spp. using the disk diffusion method. The extract showed strong activity against *Klebsiella* spp. and weaker activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus*, indicating its greater efficacy against Gram-negative bacteria, suggesting its potential as a natural antimicrobial agent for UTI management.

Keyword: UTI, pathogenic bacteria, chia seed

Introduction
Herbal medicine is an expanding domain of healthcare that warrants consideration. Plants have significantly contributed to human health and enhanced the quality of life for millennia, serving as vital components in medicine (Al-Mudhaffar, 2009) [1]. Numerous infections continue to pose a significant public health challenge in both industrialized and developing nations. *Salmonella* spp., Clostridium perfringens, Campylobacter, Vibrio parahaemolyticus, and enteropathogenic Escherichia coli account for more than 90% of all food poisoning incidents. The widespread application of antimicrobials has led to heightened resistance in several bacterial species, resulting in a significant reduction in the efficiency of these inhibitory agents. In recent decades, the emergence of antibiotic-resistant bacteria has become a global issue. The adverse side effects of some antibiotics prompted us to seek alternative sources to address these issues (Davies J., 1994, Lai *et al*. 2009, Friedman *et al*. 2002) [3, 4, 5].

Bacterial pathogens have developed several defensive mechanisms against antimicrobial medicines, resulting in an increase in resistance to both established and newly formulated treatments. The emergence of antibiotic resistance in pathogenic microbes has necessitated the investigation of many medicinal plants for their potential antimicrobial properties.

The researchers are expressing interest in natural compounds exhibiting bactericidal action. Numerous phytochemicals, historically utilized for food preservation, serve as natural substitutes for synthetic agents in prolonging the shelf life of food items. It appears essential to explore novel materials to combat this issue. The botanical realm is the origin of several pharmaceuticals. Recent estimates by experts indicate that over 400,000 plant species exist globally, with around one-quarter to one-third utilized by enterprises for therapeutic purposes. For millennia, humans have used plants to address various ailments; in several poor nations, a significant portion of the populace depends on traditional healers and their repositories of medicinal flora for treatment (N Achak, *et al*. 2009) [7]. Herbals can significantly contribute to the preservation of biodiversity. These plants are well-known to rural individuals who are acutely aware of their rarity and extinction. Medicinal plants are crucial for public health and serve as a substantial source of income for several people in both rural and urban areas (A. Barrero, *et al*. 2005) [8].

The current study examines the antibacterial activities of Chia Seeds (*Salvia hispanica* L.) against microorganisms responsible for urinary tract infections, specifically focusing on *Pseudomonas aeruginosa*.

Materials and Methods

Plant Material

This study was conducted over four months, from January 2024 to April 2024. The plant (*Chenopodium* quinoa) was sourced from a local market in Iraq and subsequently identified in January 2024 by the College of Agriculture at Baghdad University, Iraq. The plants used in the research were kept under room temperatures of 20 to 25 °C to facilitate preservation prior to the use.

Preparation of extract

The herb *Chenopodium* quinoa was prepared for the pharmacological study by milling to powder form. An aqueous extract was then prepared using 200ml of tap water and 2 grams of the ground plant material by heating the mixture at 100 deg C for 15 minutes. I boiled the mixture like a tea allowing it to cool to room temperature before straining it to yield a clear solution. The prepared extract was then poured into a glass jar and placed in a refrigerator to keep its activity substantial. For the purpose of doubleness and consistency, the extract was prepared afresh in every two days continuously throughout the period of study.

Chemical Analysis of Plant Extracts

Chemical Detection of Plant Extracts

- **Phenols Test:** To identify the presence of phenols, equal volume of 1% solution of ferric chloride was added with 1% potassium ferricyanide solutions to prepare the reagent. This reagent was then mixed in 1:1 proportion with either distilled water or alcoholic solution of the plant. The appearance of a blue-green precipitate tar proved the presence of phenolic compounds and the method produced a positive result whenever a blue-green coloration developed (15).
- **Flavonoids:** The detection solution was made by combining equal volumes of 50% ethanol and 50% potassium hydroxide and diluted 1 to 5 with distilled water. Out of this mix, 5ml was combined with 5 ml plant extract. Flavonoids were also found within the plant extract; a yellow coloration meant that the result was positive (15).
- **Tannins:** The method of (15) was used in the identification of tannin. In this technique, half of the amount of each extract, 50 ml was piped into two conical flasks each. As in the first case, a 1% (w/v) solution of lead acetate (CH3COOPb) was applied, which, upon contact, solidified into a jelly pellet indicating a positive reaction. To the second flask, one percent solution of ferric chloride (FeCl2) was added and appearance of blue coloration confirmed the presence of tannins.
- **Glycosides:** This method was done according to the method described by (15).
- **Non-hydrolysed extract:** The same volume of the plant extract was added to Fehling's reagent in a test tube in the same measure. The mixture was then placed on a hot water, the temperature being about 70 degrees Celsius for about 10 minutes. The formation of red colour precipitate afterwards confirmed the presence of reducing sugars in the extract by the test plant in this case thereby giving a positive result.
- **Hydrolysed extract:** 1-2 drops of dilute hydrochloric acid (HCl) was added to 5ml of the plant extract in water and then was put in boiling water bath for 20minutes. For this purpose after cooling the mixture was made slightly alkaline using sodium hydroxide (NaOH) solution. To the mixture, an equal volume of Fehling's reagent was added next followed by. When iam using the(or diluting) buffer serial number B and adding it into the test tube that contains Fehling solution and if I got the red precipitate then I got the positive result, which means that the plant extracts contain reducing sugars.
- **Saponins:** This approach was executed in accordance with the procedure outlined by Stahle (1969). Saponins were identified using two methods:
- In the test tube a solution of plant powder was shaken. Non-stop production of foams is seen as a positive result.
- Plant powder solution was prepared by dissolving five milliliters of plant powder in appropriate distilled water while 1-3 ml of 3% ferric chloride solution was also prepared. The presence of white solid compound strongly suggests the case favorable (16).
- **Terpenes and steroids**: A few drops of chloroform was added to one milliliter of the plant powder solution. Subsequently, one drop of acetic anhydride and 1 drop of concentrated sulfuric acid were added in succession in the mixture. The formation of a brown precipitate was a sign of the detection of terpenes. Furthermore after several minutes the formation of a dark blue coloration was another indicator of the presence of steroids in the plant extract (16).

Isolation and identification of bacteria

Twenty-four urine samples were obtained from patients with urinary tract infections at Ibn Al-Nafees Hospital. All samples were cultivated on blood and MacConkey agar, followed by Gram staining, oxidase testing, catalase testing, motility testing, and other media such as nutritional agar for further isolation and identification of pathogenic bacteria.

Antibacterial activity of aqueous herbal extract

Disk diffusion method on Mueller-Hinton agar was used to search for antibacterial activity.

Turbidity standard

Prepare the turbidity standard by comparing the turbidity of the bacterial suspension cultivated on Mueller-Hinton agar with the turbidity of McFarland tube number 1 (about 1 x 10^8 CFU/mL, 3.0). (18) The disc dispenser may be utilized to add discs to the infected plate. A maximum of four discs may be positioned on a 9-10 cm plate, allowing for an approximate 15 mm margin from the plate's edge. Each disc must be delicately pressed to provide uniform contact with the media. The plate must be incubated at 37 °C overnight, and the diameter of each zone, except the diameter of the disc, should be measured and documented in millimeters. The outcomes must thereafter be analyzed in accordance with the critical diameters. The measurements can be taken using a ruler on the underside of the plate.

Results and Discussion

The antimicrobial activity of Chia seed (*Salvia hispanica* L.) aqueous extract was assessed in vitro against four pathogenic bacterial strains commonly associated with urinary tract infections (UTIs): *S aureus*, *Klebsiella* species, *P aeruginosa* and *Proteus* species. In vitro antibacterial activities of the extract were determined using the disk diffusion method for the selected bacterial strains with the differences in their inhibitory patterns.

The largest zone of inhibition was observed in the extract of Chia seed against *Klebsiella* spp. This indicates that Chia seed is strongest against the fourth bacterial species out of the total bacterial species selected for the experiment. This implies that out of all the tested bacterium *Klebsiella* spp. a Gram-negative bacterium is most vulnerable to the antimicrobial qualities of Chia seeds. The kind of bacteria that the extract showed inhibitory effect against *Klebsiella* spp supports the findings on other natural plant extracts that have been postulated to have affinity for Gram-negative bacteria more than the Gram-positive since the later have a thicker cell wall. The obtained antibacterial impact may be correlated with the bioactivity of Chia seeds pharmacaconotices including phenolic acid, flavonoids, and essential fatty acids, which are reported for their antimicrobial actions.

In contrast, Chia seed extract showed low activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus* and they were found to produce comparatively small inhibition zones. The decrease in activity on the Gram-negative *Pseudomonas aeruginosa* and the Gram-positive *Staphylococcus aureus* may be attributed to the composition of cell wall in each bacterium or the possibility of the efflux pump system in the bacteria that may restricts the uptake or effectiveness of antimicrobial material. Although *Pseudomonas aeruginosa* is famous for its resistance to antibiotics and various environmental stress and *Staphylococcus aureus* may be ranked without severe resistance problems it is known that bacteria forming biofilms are much more resistant to antimicrobial agents.

Notably, Chia seed extract exhibited intermediate activity against *Proteus* spp., of the same group of Gram negative bacteria. These differences in the exhibit of antimicrobial response by the two groups of bacteria may, therefore, be due to genetic factors such as the variation in outer membrane porosity and the presence of enzymes that can alter the antimicrobial's effectiveness.

As expected, the outcomes of the study propose that Chia seed extract can be a potential replacement for traditional antibiotics especially in fighting infectious diseases that are notified by Gram-negative bacteria such as *Klebsiella* spp., which is the most recurrent cause of UTI. This is particularly important due to escalated cases of antibiotic-resistant diseases and growing instances of requiring non-pharmacological interventions. The eradicative ability of Chia seeds may be due to the polyphenols and omega-3 fatty acids that are attractive characteristics of Chia seeds and phytochemicals with documented antibacterial properties.

Based on the study, the Chia seed extract established high levels of antimicrobial effect on the bacterial species, especially *Klebsiella* spp., which means that there is need for future investigations to determine the chemical compound that contributes to the antimicrobial effects, in addition to such findings, the efficacy of Chia seed extract to be used as therapeutic agent needs to be assessed in clinical conditions. Furthermore, it would be useful to determine the in vivo activity of the extract, further safety and efficacy profiles when combined with standard antibiotics to map the possibility of utilizing the extract in the regulation of UTIs and combating the worrying trend of antibiotic resistance.

Relative to fresh plant mass, the percentage of extracts typically corresponds to the density of working compounds within the solution or the total amount of compounds of interest extracted by a treatment. This is usually presented in terms of percentage that is the weight of the extract to the weight of the plant material employed. The percentage of plant extract can therefore vary in relation to the method the solvent, as well as the specific plant species being used.

When it comes to such plants as Chia seeds (*Salvia hispanica* L.), the percentage of extract can be quantified after extracting bioactive compounds, often with the use of water as well as ethanol or methanol solvents. For example, if 100 grams of Chia seeds are used, and the extraction yields 10 grams of an aqueous extract, the percentage of extract would be:

Percentage of Extract = {Weight of Extract} {Weight of Plant Material} 100

Percentage of Extract= 10 gram/100 grams100 = 10%

This percentage provides insight into the efficiency of the extraction process and the concentration of the plant's bioactive components. Different extraction techniques, such as maceration, Soxhlet extraction, or ultrasonic-assisted extraction, may yield different percentages of extract.

Aqueous extraction resulted in 1.58g representing 5.95% per 25g of the raw plant material.

Chemical composition of Plant Extracts

Chemical analyses of the herbal extracts revealed the presence of several bioactive compounds, including flavonoids, phenols, tannins, saponins, glycosides, and terpenes, which are known for their potential therapeutic properties. However, the aqueous extracts tested negative for the presence of steroids, as shown in Table 3.1. These findings are consistent with the studies by El-Shazly *et al*. (2004) [16] and Khazeem (2011) [18], who also reported similar profiles for other medicinal plants. Artemisia herba-alba, in particular, is recognized as a rich source of flavonoids such as hispidulin and cirsilineol, which are known for their various pharmacological activities. Flavonoids extracted from different medicinal plants, including A. herba-alba, have been shown to possess notable anti-inflammatory properties, as highlighted by Abdel Jaleel (2016). These compounds contribute to the plant's potential use in treating conditions associated with inflammation.

Table 1: Chemical detection of secondary metabolites of the aqueous extracts of Herbal plant

Secondary metabolite	Aqueous extract
Phenols	+
Tannins	+
Flavonoids	+
Terpenes	+
Saponins	+
Glycosides	+
Steroids	-

Isolation and identification of bacteria

The outcomes of the isolation and identification of bacterial isolates are provided in Table 2, which delineates the physical and certain biochemical properties of bacteria isolated from patients with urinary tract infections (UTIs).

Table 2: The morphological and characteristics of bacteria on different culture media with some biochemical test

Bacteria	Blood Agar	MacConkey Agar	Mannitol-Salt Agar	Nutrient Agar or Milk Agar	Gram Stain	Catalase Test	Urease Test
Klebsiella	White colony, no hemolysis	Pink mucoid colony		White colony	G-Negative	#	-
Proteus	Swarming, no hemolysis	Pale colony, N.L.F.		White colony	G-Negative	#	+ (pink)
S. aureus	Beta-hemolysis		Mannitol fermenter	White colony	G-Positive	Grape-like clusters	+ (bubbles)
P. Aeruginosa	White colony with Beta-hemolysis	Pale colony, N.L.F.	Blue to green color		G-Negative	#	-

Key:
N.L.F.: Non-lactose fermenter
G-: Gram-negative
G+: Gram-positive
#: Not specified in the original data

Antibacterial activity

The growing consumer demand for natural additives and food preservatives, combined with the increasing prevalence of new and re-emerging infections, has driven the search for novel, effective antimicrobial compounds. These compounds are characterized by diverse chemical structures and innovative mechanisms of action. Plants, in particular, serve as a vital source of medicinal substances due to their remarkable ability to produce chemicals that exhibit potent antibacterial activities against a wide range of pathogenic and opportunistic microorganisms. In this study, each extract was tested against four bacterial isolates: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella* spp. and *Proteus* spp. The antibacterial efficacy of the extracts was quantified by measuring the average diameter of the growth inhibition zones in millimeters. The bactericidal efficacy of the herbal extracts is summarized in Table 3, highlighting the potential of these natural substances in combating bacterial infections.

Table 3: Diameter of inhibition zone of aqueous extract is reputation against strain of bacteria

Bacteria	**Size of inhibition**			
	Concentration of aqueous extract			
	25	**50**	**75**	**100**
Klebsilla	7.8	9.8	11.8	15.3
Proteus	8.6	10	11.3	13.8
Pseudomonas aerogenosa	8.2	10.3	13.8	16.2
Staphylococcus aureus	5.5	7.2	9.5	11.6

References

1. Al-Mudhaffar A. Effect of rosemary (*Rosmarinus officinalis* L.) tissue extracts on the growth of some skin infectious microorganisms. M.Sc. Thesis, College of Science, Al-Nahrain University, 2009.
2. Al-Ali H. Studying some immunological and cytogenetic effects of *Plantago lanceolata* aqueous extract in albino male mice. M.Sc. Thesis, College of Science, Al-Nahrain University, 2008.
3. Davies J. Inactivation of antibiotics and the dissemination of resistance genes. Science. 1994;264:375-382.
4. Lai, Tremblay, Déziel. 2009; SCENIHR, 2010.
5. Friedman M, Henika R, Mandrell R. 2002; Wilson R, Droby S, 2000.
6. Ahmad I, Beg AZ. Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. Journal of Ethnopharmacology. 2001;74:113-23.
7. Achak N, Romane A, Alifriqui M, Markouk M. Chemical composition, organic and mineral contents of leaves of *Tetraclinis articulata* (Vahl) Masters from the Tensift Al Haouz, Marrakech region (Morocco). Journal of Essential Oil-Bearing Plants. 2009;12(2):198-204.
8. Barrero A, Herrador MM, Arteaga P, Quitz J, Aksira M, Mellouki F, Akkad S. Chemical composition of essential oils of leaves and wood of *Tetraclinis articulata* (Vahl) Masters. Journal of Essential Oil Research. 2005;17:166-167.
9. Schroeder M, Brooks BD, Brooks AE. The complex relationship between virulence and antibiotic resistance. Genes. 2017;8(1):39.
10. Shaan L, Gellatly A, Robert EW. *Pseudomonas aeruginosa*: New insights into pathogenesis and host defenses. Pathogens and Disease. 2013;67:159-73.
11. Soukarieh F, Williams P, Stocks MJ, Camara M. *Pseudomonas aeruginosa* quorum sensing systems as drug discovery targets: current position and future perspectives. Journal of Medicinal Chemistry. 2018;61(23):10385-10402.
12. Tang YW, Stratton CW. Advanced techniques in diagnostic microbiology. Boston, MA: Springer, 2006.
13. Yan H, Asfahl KL, Li N, Sun F, Xiao J, Shen D, *et al*. Conditional quorum-sensing induction of a cyanide-insensitive terminal oxidase stabilizes cooperating populations of *Pseudomonas aeruginosa*. Nature Communications. 2019;10(1):1-7.
14. Yekani M, Memar MY, Alizadeh N, Safaei N, Ghotaslou R. Antibiotic resistance patterns of biofilm-forming *Pseudomonas aeruginosa* isolates from mechanically ventilated patients. International Journal of Scientific Study. 2017;5(5):1-5.
15. Zhang D, Xia J, Xu Y, Gong M, Zhou Y, Xie L, *et al*. Biological features of biofilm-forming ability of *Acinetobacter baumannii* strains derived from elderly

patients with hospital-acquired pneumonia. Clinical and Experimental Medicine. 2016;16(1):73-80.
16. El-Shazly A, Hussein K. Chemical analysis and biological activities of the essential oil of *Teucrium leucocladum* Boiss. (Lamiaceae). Biochemical Systematics and Ecology. 2004;32:665-74.
17. Zargari A. Medicinal plants. 4th Ed. Tehran: University Press, 1998.
18. Khazeem M. Study the effect of Teucrium polium L. aerial parts extract on normal and alloxan-induced diabetic mice. M.Sc. Thesis, College of Science, Al-Nahrain University, 2011.
19. Alfred EB. Microbiological applications in laboratory manuals in general microbiology. 9th Ed. New York: McGraw-Hill Company, 2005.
20. Alwan AH. Detection of casp-5 gene as inflammatory factor in Iraqi patients with *Pseudomonas aeruginosa* infections. Pakistan Journal of Biotechnology. 2020;17(1):33-40.
21. Azeredo J, Azevedo NF, Briandet R, Cerca N, Coenye T, Costa AR, *et al*. Critical review on biofilm methods. Critical Reviews in Microbiology. 2017;43(3):313-351.
22. Brady RA, Leid JG, Calhoun JH, Shirtliff ME. Osteomyelitis and the role of biofilms in chronic infection. FEMS Immunology and Medical Microbiology. 2008;52:13-22.
23. Christopher K, Bruno E. Identification of bacterial species. Proceedings of the 24th.
24. Silva DAK, Calomino MA, Deutsch G, Castilho DSR, Paula DGR, Esper L, Teixeira LA. Molecular characterization of multidrug-resistant (MDR) *Pseudomonas aeruginosa* isolated in a burn center. Burns: Journal of the International Society for Burn Injuries. 2017;43(1).
25. Forbes BA, Sahm DF, Weissfeld AS. Study Guide for Bailey and Scott's Diagnostic Microbiology. Elsevier Health Sciences, 2016.
26. Fujitani S, Sun HY, Yu VL, Weingarten JA. Pneumonia due to *Pseudomonas aeruginosa*: part I: epidemiology, clinical diagnosis, and source. Chest. 2011;139(4):909-919.
27. Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams ST. Bergey's Manual of Determinative Bacteriology. 9th Ed. Baltimore: Williams and Wilkins, 1994.
28. Kahaleq MA, Abu-Raghif AR, Kadhim SR. Antibacterial activity of fenugreek essential oil against *Pseudomonas aeruginosa*: in vitro and in vivo studies. Iraqi Journal of Medical Sciences. 2015;13(3).
29. Mahdhi A, Leban N, Chakroun I, Bayar S, Mahdouani K, Majdoub H, *et al*. Use of extracellular polysaccharides, secreted by *Lactobacillus plantarum* and Bacillus spp., as reducing in dole production agents to control biofilm formation and efflux pumps inhibitor in Escherichia coli. Microbial Pathogenesis. 2018;125:448-53.
30. Mahdi LH, Jabbar HS, Auda IG. Antibacterial immunomodulatory and antibiofilm triple effect of Salivaricin LHM against *Pseudomonas aeruginosa* urinary tract infection model. International Journal of Biological Macromolecules. 2019;134:1132-1144.
31. Mahdi LH, Jabbar HS, Auda IG. Antibacterial immunomodulatory and antibiofilm triple effect of Salivaricin LHM against *Pseudomonas aeruginosa* urinary tract infection model. International Journal of Biological Macromolecules. 2019;134:1132-1144.
32. Mahdi LH, Laftah AR, Yaseen KH, Auda IG, Essa RH. Establishing novel roles of bifidocin LHA, antibacterial, antibiofilm and immunomodulator against *Pseudomonas aeruginosa* corneal infection model. International Journal of Biological Macromolecules. 2021;186:433-444.
33. Mathur T, Singhal S, Khan S, Upadhyay DJ, Fatma T, Rattan A. Detection of biofilm formation among the clinical isolates of staphylococci: an evaluation of three different screening methods. Indian Journal of Medical Microbiology. 2006;24(1):25-29.
34. Nouraldin AAM, Baddour MM, Harfoush RAH, Essa SAM. Bacteriophage-antibiotic synergism to control planktonic and biofilm producing clinical isolates of *Pseudomonas aeruginosa*. Alexandria Journal of Medicine. 2016;52(2):99-105.
35. Ohikhena FU, Wintola OA, Afolayan AJ. Evaluation of the antibacterial and antifungal properties of *Phragmanthera capitata* (Sprengel) Balle (Loranthaceae), a mistletoe growing on rubber tree, using the dilution techniques. The Scientific World Journal. 2017;2017:9658598.
36. Orlandi VT, Martegani E, Bolognese F. Catalase A is involved in the response to photooxidative stress in *Pseudomonas aeruginosa*. Photodiagnosis and Photodynamic Therapy. 2018;22:233-40.
37. Prescott LM, Harley JP, Klein DA. Microbial growth. In: Prescott, Harley and Klein, editors. Microbiology, 2002.
38. Rawat S, Bhairav P. Prevalence and characterization of virulence properties of *Pseudomonas aeruginosa* from clinical samples and hospital environment in Dehradun. International Journal of Biological and Pharmaceutical Research. 2015;6:491-499.