DOI: https://doi.org/10.21323/2618-9771-2026-9-2-192-198

Received 06.02.2026
Accepted in revised 27.04.2026
Accepted for publication 30.04.2026

Available online at https://www.fsjour.com/jour
Original scientific article
Open access

ANTIMICROBIAL EVALUATION OF THE HYDRO-SOLUBLE EXTRACT FROM *OPUNTIA FICUS-INDICA* SEEDS AGAINST BACTERIAL AND FUNGAL PATHOGENS

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KEYWORDS:
Opuntia ficus-indica, hydro-soluble extract, antibacterial activity, antifungal activity, pathogenic bacteria

ABSTRACT

The increasing emergence of antimicrobial resistance requires the development of alternative natural bioactive compounds. This research describes the evaluation of the antimicrobial activity of hydro-soluble extracts from *Opuntia ficus-indica* seeds. Seeds of *Opuntia ficus-indica* were collected from the Bordj Bou Arreridj region (Algeria). Hydro-soluble extracts were prepared and tested against selected bacterial and fungal strains using the disk diffusion method. Different concentrations were applied, and inhibition zones were measured to assess the antimicrobial activity. The extract exhibited low antibacterial activity, with inhibition observed only at 110 mg/mL against specific bacterial strains. In contrast, the significant antifungal activity was recorded, with clear inhibition zones against several fungi and yeasts. The results indicate a selective antimicrobial effect, suggesting that the bioactive compounds present in the extract are more active against fungal microorganisms than bacteria. Hydro-soluble extracts of *Opuntia ficus-indica* seeds constitute a potential natural source of antifungal agents with limited antibacterial effects. These findings support their potential application in antifungal formulations and encourage further studies to identify active compounds and elucidate their mechanisms of action.

FUNDING: This work was funded by the Algerian Ministry of Higher Education and Scientific Research (PRFU project grant N° D00L01UN340120190001).

ACKNOWLEDGEMENTS: Authors are grateful to Dr. Mekhoukh Nasreddine who helped us to carry out this work.

Поступила 06.02.2026
Поступила после рецензирования 27.04.2026
Принята в печать 30.04.2026

https://www.fsjour.com/jour
Научная статья
Открытый доступ

ОЦЕНКА АНТИМИКРОБНЫХ СВОЙСТВ ВОДОРАСТВОРИМОГО ЭКСТРАКТА ИЗ СЕМЯН ОПУНЦИИ ИНДИЙСКОЙ ПРОТИВ БАКТЕРИАЛЬНЫХ И ГРИБКОВЫХ ПАТОГЕНОВ

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КЛЮЧЕВЫЕ СЛОВА:
Опунция индийская, водорастворимый экстракт, антибактериальная активность, противогрибковая активность, патогенные бактерии

АННОТАЦИЯ

Увеличение проблемы возникновения устойчивости к противомикробным препаратам требует разработки альтернативных натуральных биоактивных соединений. В данной работе описывается определение антимикробной активности водорастворимых экстрактов из семян опунции индийской. Семена опунции индийской были собраны в регионе Бордж-Бу-Арреридж (Алжир). Были приготовлены водорастворимые экстракты и тестированы против отдельных штаммов бактерий и грибов, используя метод диффузии в агар. Были применены различные концентрации и измеряли зоны ингибирования для оценки антимикробной активности. Экстракт проявил низкую антибактериальную активность против определенных штаммов бактерий с ингибированием только при концентрации 110 мг/мл. Напротив, была зарегистрирована существенная противогрибковая активность против некоторых грибов и дрожжей с четкими зонами ингибирования. Результаты указывают на избирательный антимикробный эффект, что говорит о том, что биоактивные соединения, присутствующие в экстракте более активны против грибковых микроорганизмов, по сравнению с бактериями. Водорастворимые экстракты семян опунции индийской представляют собой потенциальный натуральный источник противогрибковых средств с ограниченным антибактериальным эффектом. Эти результаты поддерживают их потенциальное применение в рецептурах противогрибковых препаратов и стимулируют дальнейшие исследования для идентификации активных соединений и выяснения их механизмов действия.

FOR CITATION: **Sakhraoui, A., Touati, N., Hihat, S., Madagh, B., Blanco, F.** (2026). Antimicrobial evaluation of the hydro-soluble extract from *Opuntia ficus-indica* seeds against bacterial and fungal pathogens. *Food Systems,* 9(2), 192–198. https://doi.org/10.21323/2618-9771-2026-9-2-192-198

ДЛЯ ЦИТИРОВАНИЯ: **Сахрауи, А., Туати, Н., Хихат, С., Мадаг, Б., Бландо, Ф.** (2026). Оценка антимикробных свойств водорастворимого экстракта из семян опунции индийской против бактериальных и грибковых патогенов. *Пищевые системы*, 9(2), 192–198. https://doi.org/10.21323/2618-9771-2026-9-2-192-198

ФИНАНСИРОВАНИЕ: Данная работа была финансирована Министерством высшего образования и научных исследований Алжира (грант на выполнение проекта PRFU № 00L01UN340120190001).

БЛАГОДАРНОСТИ: Авторы благодарны Доктору Мехуху Насреддину за помощь в выполнении данной работы.

1. Introduction

In recent years, the exploration of natural products for their antimicrobial properties has gained considerable attention in the fields of medicine, agriculture, and food preservation [1]. With the increasing concern over antibiotic resistance and the demand for safe and effective antimicrobial agents [2], researchers have turned to nature's vast arsenal of bioactive compounds. Alexandre et al. [3] highlighted the potential of natural products in crop protection and food preservation, emphasizing their role as eco-friendly alternatives to synthetic antimicrobials and their promising future applications. In addition, Abdel Fattahet al. [4] investigated the nutritive value and chemical composition of *Opuntia ficus-indica* seeds, demonstrating their richness in bioactive compounds that may contribute to the antimicrobial activity.

Opuntia ficus-indica, commonly known as prickly pear, has emerged as a promising natural resource due to its rich phytochemical composition and long-standing traditional uses [1]. Arba [5] emphasized the importance of plant-derived compounds as sustainable alternatives in food preservation and crop protection, highlighting their antimicrobial potential. Furthermore, Sakhraoui et al. [6] state that "it is a good source of bioactive compounds and may protect against human diseases. Moreover, cactus fruit juice positively affects the body's redox balance, and decreases oxidative lipid damage". In addition, Koubaa et al. [7] demonstrated that prickly pear seeds are rich in phenolic compounds exhibiting significant antioxidant and antibacterial activities.

Despite these findings, the antimicrobial potential of hydro-soluble extracts from *Opuntia ficus-indica* seeds remains insufficiently explored, particularly against both bacterial and fungal pathogens. This research describes the evaluation of the antimicrobial activity of hydro-soluble extracts derived from *Opuntia ficus-indica* seeds against selected pathogenic microorganisms.

Opuntia ficus-indica, a member of the Cactaceae family, is native to arid and semi-arid regions of the world [8]. Beyond its nutritional value, *Opuntia ficus-indica* is also recognized for the therapeutic potential of its different plant parts, including seeds and cladodes. Santos-Díaz et al. [9] and Zaghbib et al. [10] reported that *Opuntia* species contain bioactive compounds such as polyphenols, flavonoids, and betalains, which are associated with antioxidant and anti-inflammatory effects and may contribute to the prevention of chronic diseases. Similarly, El-Hawary et al. [11] identified volatile constituents in cladodes, fruit peel, and pulp with demonstrated antimicrobial activity against several pathogenic microorganisms. The seeds of *Opuntia ficus-indica* have garnered significant scientific interest due to their rich content of bioactive constituents. Silva et al. [12] reported that the seeds contain high levels of phenolic compounds and flavonoids, which contribute to strong antioxidant activity [13], as well as betalains known for their protective effects against oxidative stress and inflammation. In addition, in [14], authors highlighted the presence of essential fatty acids, particularly linoleic acid, which plays a key role in nutritional and health-promoting properties. Senouci-Bereksi et al. [15] furthermore characterized various *Opuntia ficus-indica* products and confirmed the presence of diverse phenolic compounds, flavonoids, and lipid fractions in the seeds, emphasizing their contribution to antioxidant capacity and potential antimicrobial activity. These compounds are well-documented for their various health-promoting properties, including antioxidant, anti-inflammatory, and anticancer activities [16,17]. Among these benefits, the antimicrobial potential of *Opuntia ficus-indica* extracts has emerged as a particularly noteworthy aspect. Historically, traditional medicine has recognized the antimicrobial efficacy of certain plant-derived substances, and advances in modern scientific methodologies have provided robust frameworks to validate and investigate these attributes further [18]. The global challenge posed by antibiotic resistance has intensified the search for natural antimicrobial agents, encouraging the exploration of plant-based alternatives to conventional therapies. Slimen et al. [19] provided a comprehensive review of the antimicrobial properties of *Opuntia ficus-indica,* highlighting the presence of bioactive compounds such as phenolics, flavonoids, and alkaloids that exhibit inhibitory effects against a wide range of pathogenic microorganisms. In addition, Koop et al. [20] discussed natural additives, particularly plant-derived pigments. Iftikhar et al. [21] emphasized that compounds such as betalains not only serve as natural colorants but also possess antioxidant and antimicrobial properties, making them valuable in food preservation. Furthermore, Castro et al. [22] demonstrated that extracts from yellow prickly pear peels are rich in bioactive compounds and exhibit significant antimicrobial activity against various microorganisms, supporting their potential application as natural preservatives.

This study focuses on the hydro-soluble extract obtained from *Opuntia ficus-indica* seeds and aims to evaluate its antimicrobial potential against a range of pathogenic bacteria and fungi. Plant-derived extracts have increasingly attracted scientific attention as alternative sources of bioactive compounds, particularly in response to the growing concerns regarding antimicrobial resistance and the excessive use of synthetic chemicals. In this context, gaining a deeper understanding of the antimicrobial properties of *Opuntia ficus-indica* seed extracts may provide valuable insights for several practical applications. These include the development of new natural antimicrobial agents, the improvement of food preservation strategies, and the promotion of more sustainable and environmentally friendly agricultural practices. Moreover, the valorization of plant by-products such as cactus seeds could represent an important step toward the sustainable use of natural resources.

The current study aims to methodically examine the antibacterial and antifungal properties of the hydro-soluble extract made from *Opuntia ficus-indica* seeds. The study evaluates the effect of this extract on different pathogenic microorganisms in order to determine its potential effectiveness as a natural antimicrobial source. Particular attention is also given to the influence of different concentration levels on microbial growth, allowing a more detailed assessment of the bioactivity of the extract. In addition, an attempt is made to discuss the possible mechanisms that may contribute to the observed antimicrobial effects, based on the interaction between the bioactive compounds present in the extract and microbial cells. Overall, the results obtained from this work may contribute to a better understanding of the antimicrobial potential of cactus seed extracts and may support future research aimed at developing natural, safe, and sustainable solutions for controlling microbial contamination in various fields, including food safety, agriculture, and public health.

2. Objects and methods

2.1. Sample preparation

The fruits were harvested in Hasnaoua, department of Bordj Bou Arreridj (Latitude: 36°15′44'81″S, Longitude: 4°79′13'85″W) towards the end of August 2020. The fruits were stripped of their thorns, washed and then peeled to recover the pulp, which is sprinkled with several small seeds that are stacked fairly evenly. The cohesion between the seeds is ensured by the mucilage and the fibers contained in the pulp. The fruits were then kneaded with an electric mixer SEB HT618 (SEB, France). The seeds from the pulp were cleaned and separated using water and sieve with increasingly reduced pores by rinsing abundantly with running water in order to be sure to eliminate all the mucilage. After that, the seeds were arranged in single layer on a plate and exposed to sunlight (with daily temperature of 30 °C) for about one day and then ground in an electrical grinder Type 108 N (SEB, France) to obtained powder, which was passed through standard 0.02 Um sieve and stored in airtight box until use.

2.2. Microwave-assisted extraction

A domestic microwave oven (CMW-A2602, Condor, Algeria) with cavity dimensions of 22.5 cm × 37.5 cm × 38.6 cm and 2450 kHz was used. The oven contained a digital control system for irradiation time and microwave power (the microwave was linearly adjustable from 100 to 1000W). The latter was modified in order to condensate the vapors generated during extraction into the sample. Different amounts of seed powder were placed in round bottom flasks of 250 mL (medium neck of 45 mm) containing water/ 80 % acetone). The extraction was carried out under 750 W and after 200 s. The sample extracts were separated by centrifugation using a centrifuge Universal 320 (Hettich, Germany) at 3000 rmp (SIGMA 3–30 ks) for 15 min. At the end, they were stored at 4 °C until use. A volume of 20 mL of a sample was passed through a rotary evaporator (model: R-210, type: rotary evaporator, Büchi, Switzerland) to evaporate the solvent. After 1 hour, the recovered sample was dried in an oven (Memmert, Germany) at 40 °C. After 24 hours the dry seed extract was obtained.

2.3. Antimicrobial activity

2.3.1. Antibacterial activity

The antibacterial potential of the *Opuntia ficus-indica* seed extracts was evaluated *in vitro* using the disc diffusion method. Sterile Whatman filter paper discs (6 mm in diameter) were used as the delivery vehicle for the extracts. Each disc was impregnated with a specific concentration of the *Opuntia ficus-indica* seed extract and subsequently placed onto the surface of Mueller-Hinton agar plates, which had been previously inoculated with the selected pathogenic bacterial strains. After incubation, the antibacterial activity was determined by measuring the diameter of the zones of inhibition (in mm) formed around each disc. These zones represent the area where bacterial growth was prevented by the diffusion of the extract [23]. The bacterial strains selected for this study are pathogenic bacteria (Table 1). These strains were provided to us by the microbiology and phytopathology laboratory at Mohamed El Bachir El Ibrahimi University (BBA). They were maintained by subculturing on nutrient agar medium (type: standard nutrient agar, Oxoid, UK) conducive to their growth in the dark for 24 hours at 37 °C.

Table 1. **Bacterial strains used and their pathogenic powers**

Таблица 1. **Использованные штаммы бактерий и их патогенность**

Strains	GRAM	Pathogenic power
Bacillus cereus	+	food poisoning due to food contamination (vegetable origin such as rice, spices)
Enterococcus faecalis	+	— Chronic intestinal inflammation — Bladder and prostate infections
Proteus mirabilis	–	— Urinary tract infections — Cysts and acute pyelonephritis
Salmonella typhimirium	–	— Typhoid fever — Gastroenteritis
Pseudomonas aeruginosa	–	— Septicemia, infant meningitis, surgical wound infections, gastroenteritis — Abdominal pain and bloody diarrhea
Escherichia coli	–	— Septicemia, infant meningitis, surgical wound infections, gastroenteritis — Abdominal pain and bloody diarrhea
Staphylococcus aureus	+	— Hospital-acquired infections — Abscesses, wound infections, septicemia, pneumonia, food poisoning — Potentially fatal infections in humans
Micrococcus luteus	+	— Bacteremia — Infections associated with ventricular shunts — Cases of abscesses and pneumonia

2.3.2. Antifungal activity

The antifungal activity of the dried extract was determined against five fungi: *Phytophthora infestans, Aspergillus parasiticus, Penicillium* sp., *Trichoderma* sp., *Fusarium* sp., and two yeasts: *Candida albicans, Candida glabrata.* These microorganisms were obtained from laboratory culture collections of the microbiology unit (Mohamed El Bachir El Ibrahimi University) and maintained under standard conditions prior to use. The selection of these strains was based on their relevance as common plant and food-contaminating fungi, as well as opportunistic human pathogens, and according to previous studies reported in the literature evaluating antimicrobial activity of plant extracts. This choice allows for a comprehensive assessment of the antifungal potential of the extract against a diverse range of microorganisms with agricultural, food, and clinical importance. The tested strains were used as model organisms to determine whether the extract exhibits inhibitory activity and to evaluate its spectrum of action.

2.4. Preparation of culture media and Opuntia ficus-indica (OFI) seed extract dilutions

Distilled water, test tubes used for the preparation of bacterial solutions, and Whatman filter paper disks (6 mm in diameter) wrapped in aluminum foil were sterilized in an autoclave Tuttnauer 2540 EL (Tuttnauer, Netherlands) at 121 °C/15 min.

The Mueller Hinton Agar medium was used to study the antibacterial activity because it is the most commonly used medium for antimicrobial susceptibility testing. Sabouraud agar and PDA medium were used for the isolation and maintenance of yeasts and fungi, as well as studying their sensitivity to the extracts.

The *OFI* seed extracts were dissolved. A stock solution of the acetone extract (110 mg/mL) was prepared and further diluted using a serial two-fold dilution method (Table 2). Dimethyl sulfoxide (DMSO) was used as the diluent to maintain a constant total volume of 400 uL for all concentrations (C1-C8). Pure DMSO (without extract) served as the negative control (C0) to ensure that the solvent did not interfere with bacterial growth. The solutions were prepared with agitation and refrigerated until use (Table 2).

Table 2. **Different concentrations of the acetone extract and corresponding DMSO (mg/ml)**

Таблица 2. **Различные концентрации ацетонового экстракта и соответствующий ДМСО (мг/мл)**

C	C0	C1	C2	C3	C4	C5	C6	C7	C8
ES	0	110	55	27.5	13.75	6.87	3.43	1.71	0.85
VOL	0	400	200	100	50	25	12.5	6.247	3.123
DMSO	400	0	200	300	350	375	387.5	393.753	396.877

Control (**C0**), extract concentrations (**C1-C8**), extract (**ES**), extract volume (**VOL**), and dimethyl sulfoxide (**DMSO**).

2.5. Evaluation of the antimicrobial potential

The antimicrobial potential of *Opuntia ficus-indica seed* extracts was evaluated using the disk diffusion method (Kirby-Bauer protocol) as recommended by the American Society for Microbiology (ASM) [24].

2.5.1. Standardization and preparation of the inoculum

After thawing the preserved microbial strains and allowing them to reach room temperature, well-isolated colonies were picked from young pre-cultures using a sterile platinum loop. The colonies were suspended in sterile saline solution (0.85 % NaCl) and thoroughly homogenized using a Vortex-Genie 2 (Scientific Industries, Inc., Bohemia, NY, USA). The turbidity of the inoculum was standardized to the 0.5 McFarland standard by visual comparison and confirmed spectrophotometrically. Specifically, the optical density (OD) was measured at 625 nm using a UV-1800 spectrophotometer (Shimadzu, Japan). The suspension was adjusted until an OD value between 0.08 and 0.10 was reached, corresponding to approximately $1–2 \times 10^8$ CFU/mL for bacteria and $1–5 \times 10^6$ CFU/mL for yeasts and fungi.

2.5.2. Inoculation and disk application

Petri dishes containing Mueller-Hinton (MH) agar for bacteria, Potato Dextrose Agar (PDA) for fungi [25], or Sabouraud medium for yeasts were aseptically inoculated. A sterile swab was soaked in the standardized microbial suspension and streaked over the entire agar surface in a tight zigzag pattern. To ensure a confluent lawn of growth, this procedure was repeated three times, rotating the plate 60° each time, and finalized by swabbing the periphery of the medium [24]. Subsequently, a two-fold serial dilution was performed in sterile microtubes to obtain eight concentrations ranging from 110 mg/mL (C1) to 0.85 mg/mL (C8). Standardized 6 mm sterile filter paper disks were immersed in each concentration until complete saturation and placed onto the inoculated agar surface using sterile forceps.

2.5.3. Controls and incubation

A disk impregnated with pure DMSO (C0) was included in each assay as a negative control. In our study, DMSO exhibited no inhibitory effect against the tested strains, confirming the neutrality of the solvent. The plates were then incubated in a regulated incubator at 37 °C for 24 hours (bacteria) and at 25–28 °C for 48–72 hours (fungi and yeasts).

2.5.4. Interpretation

The interpretation was done by measuring the diameters of the inhibition zones in millimeters (mm) around the disks using a caliper. The results were expressed as the diameter of the inhibition zone and could be represented by symbols based on the sensitivity of the strains to the extracts.

The bacteria were classified into the following categories [23]:

- Non-sensitive (–) or resistant: diameter < 8 mm.
- Sensitive (+): diameter between 9 and 14 mm.
- Highly sensitive (++): diameter between 15 and 19 mm.
- Extremely sensitive (+++): diameter > 20 mm.

2.6. Statistical analysis

The results ($n = 3$) were subjected to a two-factor analysis of variance. Mean values were compared using Fisher's test ($p < 0.05$). All statistical analyses were carried out using Infostat®.

3. Results and discussion

3.1. Antimicrobial activity

The antibacterial and antifungal activities of the *OFI* seed extracts were estimated in terms of the diameter of the inhibition zone around the disks containing the extracts at various concentrations against pathogenic bacteria, molds and yeasts.

3.1.1 Antibacterial activity of hydro-soluble extract of OFI seeds

The following table (Table 3) presents the values in mm of the inhibition zones achieved with the studied strains.

Table 3. **Diameter of inhibition zones (in mm) of bacteria for the hydro-soluble extract of prickly pear seeds in the antimicrobial test**

Таблица 3. **Диаметр зон ингибирования (мм) бактерий для водорастворимого экстракта опунции индийской в анализе антимикробной активности**

Acetonic extract concentration	Gram	Diameters of inhibition zones							
		C1	C2	C3	C4	C5	C6	C7	C8
Bacillus cereus	+	–	–	–	–	–	–	–	–
Enterococcus faecalis	+	15[a]	–	–	–	–	–	–	–
Micrococcus luteus	+	–	–	–	–	–	–	–	–
Pseudomonas aeruginosa	–	15[a]	14[b]	12[c]	–	–	–	–	–
Escherichia coli	–	14[a]	–	–	–	–	–	–	–
Proteus mirabilis	–	15[a]	14[b]	–	–	–	–	–	–
Salmonella typhimurium	–	–	–	–	–	–	–	–	–

The same letters in the same column refer to means that are not statistically different.

The results presented in Table 3 show the antibacterial activity of the hydro-soluble extract of *Opuntia ficus-indica* seeds against several bacterial strains. After 24 h of incubation at 37 °C, the extract did not exhibit any antibacterial activity against *Bacillus cereus*, *Micrococcus luteus*, or *Salmonella typhimurium* at any of the tested concentrations. These results indicate that the hydro-soluble extract of *Opuntia ficus-indica* seeds may have limited effectiveness against certain bacterial species.

However, the antibacterial activity was observed against some bacterial strains at higher concentrations. At a concentration of 110 mg/mL, the extract produced inhibition zones against *Escherichia coli* (14 mm), *Enterococcus faecalis* (15 mm), *Pseudomonas aeruginosa* (15 mm), and *Proteus mirabilis* (15 mm). In addition, *Pseudomonas aeruginosa* and *Proteus mirabilis* showed inhibition zones at lower concentrations as well, although the diameters decreased progressively with decreasing concentrations. These findings suggest that the antibacterial activity of the *OFI* seed extract is concentration-dependent, with stronger inhibition observed at higher extract concentrations [23].

These findings differ from those reported by Castro et al. [22], who found that prickly pear peels extracts had the antimicrobial activity against *Pseudomonas aeruginosa, Escherichia coli*, and *Micrococcus luteus*. Similarly, Temagoult et al. [26] also reported an inhibitory effect against *E. coli*. The differences observed between studies may be explained by several factors, including the plant part used, extraction solvent, extraction method, and the concentration of bioactive compounds present in the extract. According to Ciocan and Bara [27], the chemical structure of bioactive compounds, their type, and their concentration around microbial cells can influence their ability to penetrate the microbial membrane, which may lead to variations in microbial sensitivity to plant extracts.

Furthermore, Bandigari et al. [28] reported that the ethanolic extract of prickly pear exhibited the antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Lactobacillus* spp. at a concentration of 40 mg/mL. In contrast, in the present study the antibacterial activity was observed only at a higher concentration (110 mg/mL). This difference may be attributed to variations in the extraction solvent and method. While Bandigari et al. [28] used an ethanolic extract, the present study employed an acetonic extract, which may differ in the composition and concentration of bioactive compounds extracted from the plant material.

The antibacterial activity observed in this study may be associated with the presence of phenolic compounds, which are known for their antimicrobial properties [6]. Phenolic compounds can inhibit microbial growth by disrupting cell membranes, altering cell permeability, interfering with enzyme activity, and causing leakage of cellular components. However, the limited activity observed against some bacterial strains suggests that the effectiveness of the hydro-soluble *OFI* seed extract may depend on the susceptibility of the bacterial species as well as the concentration and composition of the bioactive compounds present in the extract.

The differences in the antibacterial activity observed among the tested bacterial strains may also be related to the structural differences between Gram-positive and Gram-negative bacteria [29]. The observed variations in susceptibility likely stem from the fundamental architectural differences in the bacterial cell walls [29]. While the thick peptidoglycan layer of Gram-positive strains generally allows for easier diffusion of plant metabolites, the complex outer membrane of Gram-negative bacteria — rich in lipopolysaccharides — serves as a robust permeability barrier. Interestingly, the total resistance exhibited by *Bacillus cereus* and *Micrococcus luteus* in this study suggests that Gram-status is not the sole determinant of sensitivity. Intrinsic resistance mechanisms, such as efflux pumps or specific enzymatic defenses, may further modulate the effectiveness of the *Opuntia ficus-indica* extract regardless of cell wall thickness. These findings highlight that the antibacterial efficacy of *Opuntia ficus-indica* seed extracts is not solely determined by Gram classification but also by the specific physiological and structural characteristics of each bacterial strain [19].

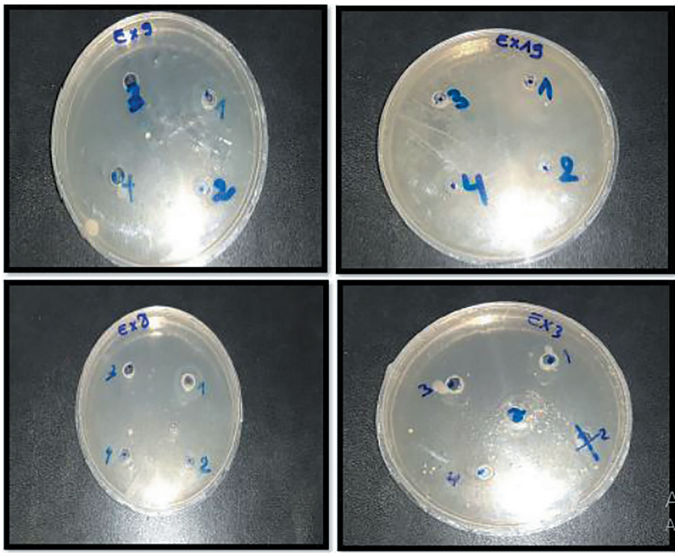

Figure 1. **Inhibition zones of the hydro-soluble extract for some bacteria (*Escherichia coli, Micrococcus luteus, Pseudomonas aeruginosa, Enterococcus faecalis*)**

Рисунок 1. **Зоны ингибирования роста некоторых бактерий (*Escherichia coli, Micrococcus luteus, Pseudomonas aeruginosa, Enterococcus faecalis*) под действием водорастворимого экстракта**

Figure 1 presents diameters of inhibition zones (in mm) of bacteria for the hydro-soluble extract of prickly pear seeds in the antimicrobial test.

According to the results presented in Figure 1, concentrations C2 (55 mg/mL; inhibition diameter = 14 mm) and C3 (27.5 mg/mL; inhibition diameter = 12 mm) exhibited the antibacterial activity against *Pseudomonas aeruginosa*. In addition, concentration C2 (diameter = 14 mm) showed inhibitory activity against *Proteus mirabilis*. These results suggest that the antibacterial activity of the hydro-soluble extract is concentration-dependent, with higher concentrations producing more pronounced inhibitory effects.

These findings are consistent with those reported by Aruwa et al. [30], who observed the antimicrobial activity against *Pseudomonas aeruginosa* and *Proteus mirabilis* using prickly pear fruit and peel extracts at different concentrations. The similarities between the results may indicate that various parts of the *Opuntia ficus-indica* plant contain bioactive compounds with antimicrobial potential. Therefore, prickly pear seed extracts may represent a promising natural source of antimicrobial agents, although their effectiveness appears to depend on the concentration and the bacterial strain tested.

However, our results differ from those reported by Özcan and Al Juhaimi [31], who investigated prickly pear seed extracts and found no antibacterial activity against *Escherichia coli, Pseudomonas aeruginosa*, and *Micrococcus luteus*. Such discrepancies between studies may be explained by differences in extraction methods, solvent polarity, plant origin, or environmental conditions affecting plant metabolism. According to Ramírez-Moreno et al. [32], variations in extraction procedures can significantly influence the chemical composition and biological activity of plant extracts.

Previous studies have demonstrated that the antimicrobial properties of plant extracts are largely attributed to secondary metabolites such as flavonoids, phenolic acids, and polyphenols [33]. These bioactive compounds can exert antibacterial effects through several mechanisms, including disruption of bacterial cell membranes, alteration of membrane permeability, inhibition of essential enzymes, and interference with nucleic acid synthesis. As reported by Iftikhar et al. [21], phenolic compounds can penetrate bacterial cell membranes and interact with intracellular targets, ultimately leading to bacterial growth inhibition or cell death.

According to the classification scale for antimicrobial activity, bacterial strains are considered resistant when the diameter of the inhibition zone is less than 10 mm [34]. Based on this criterion, the inhibition zones observed in the present study (12–15 mm) indicate that some bacterial strains were moderately sensitive to the hydro-soluble extract of *OFI* seeds. The antimicrobial activity observed may not be attributed to a single compound but rather to the synergistic interaction of several bioactive constituents present in the extract. Essawi et al. [35] reported that such synergistic effects among phytochemicals may enhance the overall antibacterial activity of plant extracts.

Furthermore, differences in bacterial susceptibility may also be associated with the structural characteristics of Gram-positive and Gram-negative bacteria. The antibacterial efficacy of the *Opuntia ficus-indica* seed extract was evident across both Gram-positive and Gram-negative classifications. Specifically, the extract successfully inhibited the growth of *Enterococcus faecalis*, *Escherichia coli*, and *Pseudomonas aeruginosa*. This suggests that the bioactive constituents within the hydro-soluble fraction possess a versatile mechanism of action capable of penetrating diverse cellular envelopes. Such broad-spectrum activity is significant, as it indicates that the functional compounds of the extract are not strictly limited by the structural barriers typically associated with different bacterial taxa [36].

Overall, these findings highlight the potential antimicrobial properties of *Opuntia ficus-indica* seed extracts and support their possible application as natural antibacterial agents.

However, their relatively limited antibacterial activity and variability depending on concentration indicate the need for further optimization and comprehensive investigation.

3.1.2 Antifungal activity of the hydro-soluble extract

The *in vitro* antifungal activity of various extracts from prickly pear seeds was investigated using the diffusion method. The table below (Table 4) presents the values in mm of the inhibition zones achieved with the studied strains.

Table 4. **Diameter of inhibition zones (mm) of the hydro-soluble extracts from prickly pear seeds against yeasts and fungi**

Таблица 4. **Диаметр зон ингибирования (мм) для водорастворимого экстракта опунции индийской против дрожжей и грибов**

Acetonic extract concentration	Diameters of inhibition zones							
	C1	C2	C3	C4	C5	C6	C7	C8
Candida albicans	Very sensitive							
Candida glabrata	15ᵃ	–	–	–	–	–	–	–
Phytophthora infestans	9ᵃ	9ᵃ	9ᵃ	–	–	–	–	–
Aspergillus parasiticus	16ᵃ	–	–	–	–	–	–	–
Penicillium sp.	–	–	–	–	–	–	–	–
Trichoderma sp.	12ᵃ	10ᵇ	10ᵇ	10ᵇ	9ᵇ	9ᵇ	8ᶜ	
Fusarium sp.	9ᵃ	9ᵃ	–	–	–	–	–	–

The same letters in the same column refer to means that are not statistically different.

Table 4 presents the antifungal activity of the hydro-soluble extract of *Opuntia ficus-indica* seeds against several fungal and yeast strains. The results indicate that the extract exhibited inhibitory activity against most of the tested microorganisms, except for *Penicillium* sp., which showed no sensitivity at any of the tested concentrations. The observed inhibition zones were 9 mm for *Phytophthora infestans*, 16 mm for *Aspergillus parasiticus*, 12 mm for *Trichoderma* sp., and 9 mm for *Fusarium* sp. These results indicate that the extract possesses moderate antifungal activity against certain filamentous fungi.

According to the classification scale proposed by Mutaiet al. [37], fungal strains are considered sensitive to antimicrobial agents when the inhibition zone diameter ranges between 9 and 19 mm. Based on this criterion, the fungal strains tested in this study can be considered sensitive to the hydro-soluble extract of *Opuntia ficus-indica* seeds, particularly *Aspergillus parasiticus*, which showed the largest inhibition zone (16 mm). The antifungal activity observed may be attributed to the presence of bioactive phytochemicals capable of inhibiting fungal growth.

Regarding yeasts, the extract demonstrated strong inhibitory activity against both tested strains. *Candida albicans* was particularly sensitive to the extract, with an inhibition diameter exceeding 20 mm, while *Candida glabrata* exhibited an inhibition zone of 15 mm at the highest concentration tested. These findings suggest that the hydro-soluble extract of *OFI* seeds may possess promising antifungal properties against pathogenic yeasts. The inhibition zones observed also indicate that the antifungal effect may be concentration-dependent, with higher concentrations of the extract resulting in stronger inhibitory activity.

The antifungal activity observed in this study may be associated with the presence of secondary metabolites such as flavonoids, phenolic acids, and polyphenols in the plant extract. These compounds are known to possess strong antimicrobial properties and may exert their effects through different mechanisms. According to Elkady et al. [38], phenolic compounds can penetrate fungal cell membranes and interact with essential intracellular components such as enzymes, proteins, and nucleic acids. This interaction may disrupt important cellular processes, leading to the inhibition of fungal growth or cell death.

Furthermore, differences in sensitivity among fungal species may be related to variations in their cellular structure and metabolic characteristics. Fungal cell walls are mainly composed of chitin, glucans, and other polysaccharides, which may influence the permeability of antifungal compounds. Some fungal species possess more effective defense mechanisms, such as detoxification enzymes or efflux pumps, which may reduce their susceptibility to plant-derived antimicrobial compounds. This may explain the lack of activity observed against *Penicillium* sp. in the present study.

In addition, the antifungal activity of plant extracts is often attributed to the synergistic effects of multiple bioactive compounds [39], rather than a single active constituent. The interaction between different phenolic compounds, flavonoids, and other secondary metabolites may enhance the overall antimicrobial activity of the extract [40]. Such synergistic interactions can increase the ability of plant extracts to disrupt fungal cell structures and inhibit microbial growth [41].

Overall, the results obtained in this study highlight the potential antifungal properties of the hydro-soluble extract derived from *Opuntia ficus-indica* seeds against several pathogenic fungi and yeasts. The observed inhibitory effects suggest that compounds naturally present in prickly pear seeds may contribute to limiting the growth of certain microorganisms of medical and agricultural importance. These findings reinforce the growing interest in plant-based resources as possible alternatives to synthetic antifungal agents, particularly in the context of increasing microbial resistance and environmental concerns related to chemical preservatives. In addition, the valorization of cactus seed by-products could represent a sustainable approach for the development of natural antimicrobial substances with potential applications in food preservation, agriculture, and public health.

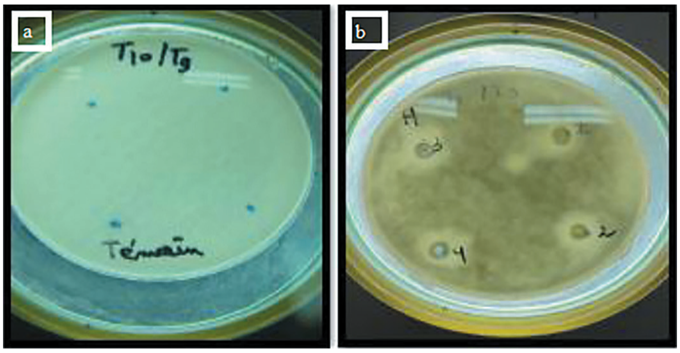

Figure 2. **Effect of water-soluble extract on *Trichoderma* sp.: (a) control; (b) inhibition zone**

Рисунок 2. **Влияние водорастворимого экстракта на *Trichoderma* sp.: (a) контроль; (b) зона ингибирования**

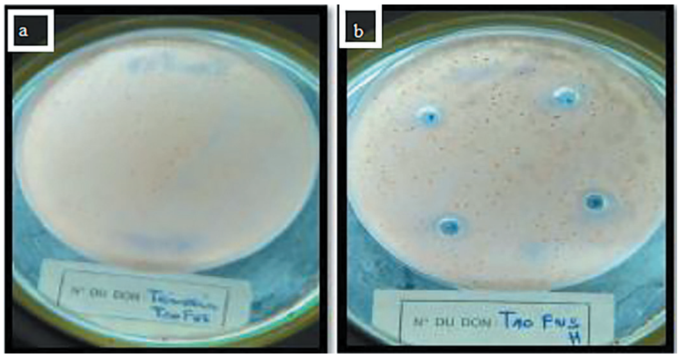

Figure 3. **Effect of water-soluble extract on *Fusarium* sp.: (a) control; (b) inhibition zone**

Рисунок 3. **Влияние водорастворимого экстракта на *Fusarium* sp.: (a) контроль; (b) зона ингибирования**

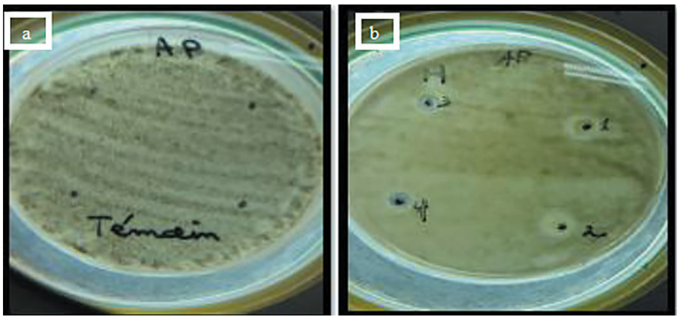

Figure 4. **Effect of water-soluble extract on *Aspergillus parasiticus*: (a) control; (b) inhibition zone**

Рисунок 4. **Влияние водорастворимого экстракта на *Aspergillus parasiticus*: (a) контроль; (b) зона ингибирования**

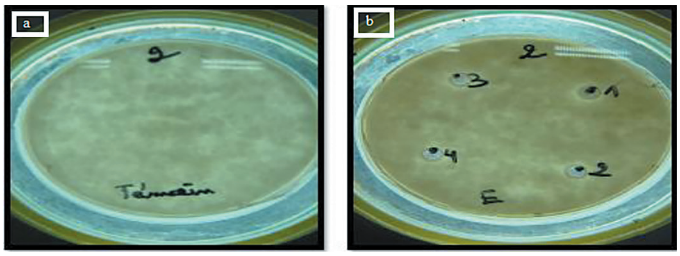

Figure 5. **Effect of water-soluble extract on *Phytophthora infestans*: (a) control; (b) inhibition zone**

Рисунок 5. **Влияние водорастворимого экстракта на *Phytophthora infestans*: (a) контроль; (b) зона ингибирования**

Figures 2 to 5 present the diameters of inhibition zones (mm) produced by the hydro-soluble extracts of *Opuntia ficus-indica* seeds against the tested yeasts and fungi. These figures illustrate the antifungal activity observed during the experiments and allow a clearer comparison of the sensitivity of the different microbial strains. Overall, the results indicate a variable response depending on the microorganism and the concentration of the extract. Some strains exhibited pronounced inhibition zones, reflecting a higher sensitivity to the extract, while others showed reduced or no inhibition, suggesting a lower susceptibility. In particular, yeasts such as *Candida albicans* and *Candida glabrata* demonstrated noticeable sensitivity, whereas certain filamentous fungi displayed more resistance. Furthermore, an increase in extract concentration generally resulted in larger inhibition zones, indicating a dose-dependent effect. These observations highlight the selective antifungal potential of the extract and suggest that its bioactive compounds may act more effectively against specific fungal groups.

4. Conclusion

The hydro-soluble extract obtained from the seeds of *Opuntia ficus-indica* demonstrated noticeable antimicrobial activity against several tested microorganisms, including certain bacterial and fungal species. The results indicate that the inhibitory effect of the extract tends to increase with concentration, suggesting a clear dose-dependent relationship between the amount of extract applied and the level of microbial growth suppression observed during the experiments. Such behavior may be associated with the presence of various bioactive compounds naturally occurring in prickly pear seeds, which are known to contain phenolic substances and other metabolites with potential antimicrobial properties. These findings highlight the interest in *Opuntia ficus-indica* as a valuable natural resource for the exploration of new antibacterial and antifungal substances. In recent years, plant-derived compounds have attracted increasing attention as researchers seek safer and more sustainable alternatives to synthetic antimicrobial agents. In this context, the prickly pear cactus, which is widely distributed in arid and semi-arid regions, represents an accessible and promising source of biologically active molecules. Moreover, the growing global concern related to antibiotic resistance has intensified the search for new antimicrobial strategies capable of limiting the spread of resistant pathogens. Investigating natural products such as extracts obtained from *Opuntia ficus-indica* seeds may therefore contribute to the development of innovative and environmentally friendly antimicrobial solutions. Further studies focusing on the identification of the active constituents and the evaluation of their biological mechanisms would provide deeper insight into the therapeutic and technological potential of this plant resource.

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