*https://doi.org/10.31925/farmacia.2026.1.18* **ORIGINAL ARTICLE**

# EVALUATION OF THE ANTIBACTERIAL POTENTIAL OF AQUEOUS EXTRACTS OF *SCHINUS MOLLE* L. SEEDS

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*Manuscript received: November 2025*

**Abstract**

This study explores the phytochemical composition and antimicrobial properties of *Schinus molle* L. seeds collected from Mascara region in Algeria. Aqueous extracts were prepared using two extraction methods: infusion and maceration. The organoleptic characteristics were evaluated, and chemical composition was determined through phytochemical screening and spectrophotometric quantification. The antibacterial activity was tested against *Lactobacillus bulgaricu*s, *Escherichia coli*, *Salmonella typhi*, *Enterobacter* sp., *Klebsiella* sp., and *Staphylococcus epidermidis*. Seeds of *S. molle* were found to be rich in phytochemicals, while extraction yield and phenolic content were differing between macerated and infused extracts. Total polyphenol content ranged from 4.79 to 3.08, HPLC identification reflected notable molecular variability. In addition, infused extract showed significant antibacterial activity against *E. coli* and Enterobacter, supporting ethnobotanical claims of *S. molle*'s therapeutic potential in treating certain infections. Overall, these results underscore the value of *S. molle* L. seeds as a promising source of natural bioactive compounds for medicinal and food applications.

**Rezumat**

Studiul explorează compoziția fitochimică și proprietățile antimicrobiene ale semințelor de *Schinus molle* L. colectate din regiunea Mascara din Algeria. Extractele apoase au fost preparate folosind două metode de extracție: infuzie și macerare. Caracteristicile organoleptice au fost evaluate, iar compoziția chimică a fost determinată prin screening fitochimic și cuantificare spectrofotometrică. Activitatea antibacteriană a fost testată pe *Lactobacillus bulgaricus*, *Escherichia coli*, *Salmonella typhi*, *Enterobacter* sp., *Klebsiella* sp. și *Staphylococcus epidermidis*. Semințele de *S. molle* s-au dovedit a fi bogate în compuși fitochimici, iar randamentul extracției și conținutul fenolic au fost diferite între extractele macerate și cele infuzate. Conținutul total de polifenoli a variat între 4,79 și 3,08. În plus, extractul infuzat a prezentat o activitate antibacteriană semnificativă asupra *E. coli* și *Enterobacter*, susținând afirmațiile etnobotanice privind potențialul terapeutic al *S. molle* în tratarea anumitor infecții. În ansamblu, aceste rezultate subliniază valoarea semințelor de *S. molle* L. ca sursă promițătoare de compuși bioactivi naturali pentru aplicații medicinale și alimentare.

**Keywords**: *Schinus molle L.*, extraction methods, organoleptic properties, phytochemical analysis, antibacterial activity

## Introduction

Medicinal plants are recognized as valuable reservoirs of bioactive compounds, particularly phenolics and flavonoids, which possess antioxidant, antimicrobial, and other health-promoting properties (1, 2). *Schinus molle* L., commonly known as false pepper in Algeria, is a flowering plant belonging to the *Anacardiaceae* family, native to South America and now widely distributed in subtropical and Mediterranean regions, including Algeria (3, 4).

Traditionally, different parts of the plant have been employed in folk medicine to treat rheumatism, gastrointestinal disorders, respiratory ailments, toothache, and menstrual disorder, wound healing, and various infections (5, 4, 6-7). Its leaves and seeds are also used for their spicy essential oils, which exhibit diuretic, stomachic, tonic, and microbial properties (8). Additionally, recent studies have demonstrated its antioxidant, antiparasitic, and antifungal activities (9, 10-11).

Despite its wide ethnopharmacological applications, studies focusing on the seeds of *S. molle* L. remain scarce, particularly concerning their nutritional value and antibacterial properties. The toxicological effect of this plant has been previously documented (12, 13). Several studies have indicated that *S. molle* L. leaf and fruit extracts are non-toxic for human use (14), while others have demonstrated phytotoxic effects on certain animals and insect-repellent properties (15-17).

Furthermore, the efficiency and stability of bioactive compounds largely depend on the extraction technique employed (18, 19). Many conventional methods require elevated temperatures or prolonged

solvent exposure. The solvents vary in polarity, volatility, and toxicity; these parameters directly affect the yield and quality of the recovered compounds. An optimized extraction strategy is therefore essential to maximize the recovery of target molecules while maintaining their structural integrity and biological activity (19, 20).

However, the solvent selected must be compatible with downstream applications, particularly in the perfume, cosmetic, pharmaceutical, food, nutraceutical, biofuel, and fine chemical industries, where biocompatibility and safety are critical.

The extraction process is largely related to the extraction solvent selected. The petroleum-derived solvents remain efficient for non-polar compounds but raise safety and sustainability concerns, while aqueous-based solvents are more suitable for polar substances such as polyphenols and flavonoids, and they offer significant advantages in terms of safety, non-toxicity, and sustainability, making them a promising alternative for green extraction approaches (21).

Therefore, the present study highlights the phyto-chemical composition and antibacterial activity of *S. molle* seeds from the Mascara region of Algeria. Two aqueous extraction methods; infusion and maceration were compared in order to assess their effects on extract yield, phytochemical profile, and antibacterial potential against selected bacterial strains.

## Materials and Methods

*Plant material*

Mature seeds of *S. molle* L. were collected in March, 2023 from Bouhanifia region in Mascara, Algeria. The selected seeds were dried at 50°C in an oven for 24 hrs, the moisture content was (32.12%). The seeds were then grounded to a fine powder and stored at 4°C for further use.

*Aqueous extracts protocol*

Two aqueous extraction techniques were tested: infusion and maceration.

Infusion method: 10g of powder were combined with 100ml of boiling distilled water for 30min with frequent agitation.

Maceration method: 10g of powder were immersed in 100ml of cold distilled water at room temperature for 24hrs under continuous agitation.

The extracts were filtered, concentrated under vacuum at 40°C for 24hrs, and stored at 4 °C until further analysis. All experiments were performed in triplicate to minimize the resulting errors.

The extraction yield of each extracts was calculated using equation 1:

$$(\%) = \frac{W}{M} * 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where: Y %: extraction yield; W: weight of dry extract; M: Initial weight of seed powder (g)

*Organoleptic tests*

The aqueous extracts of *S. molle* L. seeds were subjected to sensory evaluation based on appearance, colour, odour, and taste. The sensory panel comprised 25 participants' adults.

*Phytochemical Screening tests*

The extracts were filtered and verified through several phytochemical tests to detect tannins, flavonoids, alkaloids, steroids, saponins, cardiac glycosides, mucilage, anthraquinones, starch, anthracenosides, and amino acids (22).

*Determination of total phenolic content (TPC)*

Total polyphenols in the aqueous extracts were determined using Purssian blue method14. Gallic acid (25 - 500 mg/l) was used as a standard, and the absorbance was measured at 725 nm. Results were expressed as mg of gallic acid equivalent per gram of extract (mg GAE/g) (23).

*Phytochemical analysis of polyphenol by HPLC*

Polyphenolic constituents of the aqueous extract were analysed using an Agilent 1260 Infinity HPLC system equipped with a quaternary pump. Separation was performed on a Zorbax Eclipse Plus C18 column (4.6 mm i.d., 100 mm length) maintained at 30 °C. A linear gradient of water containing 0.2% H2PO4 (v/v, HPLC grade), methanol, and acetonitrile was applied as the mobile phase. The injection volume was 20 µL, and detection was performed using a VWD detector at 284 nm. Individual compounds were identified by comparing their retention times (RTs) with authentic standards, including vanillin (VA), syringic acid, caffeine, vanillic acid, ferulic acid, ellagic acid, benzoic acid, salicylic acid, and cinnamic acid (24).

*Determination of antimicrobial effects*

*Bacterial strains*

Six bacterial strains were tested, including $Gram^+$ (*Staphylococcus epidermidi, Lactobacillus bulgaricus*) and $Gram^-$ (*Enterobacter* sp, *Escherichia coli, Salmonella typhi,* and Klebsiella sp), obtained from the Laboratory of Microbiological Studies University of Mascara.

*Antibacterial activity tests*

The antibacterial activity of infused and macerated extracts was evaluated using both qualitative (agar diffusion) and a quantitative method (solid medium dilution) method.

Purified cultures were grown on nonselective medium from 18 to 24 hours to adapt to each bacterial strain. Bacterial suspension was adjusted to 0.5 McFarland standard in sterile physiological water ($\approx 10^7$–$10^8$ CFU/mL) and confirmed spectrophotometrically at 620 nm. Inoculum were spread on Mueller Hinton agar plates. Disks (6 mm) were loaded with 20µl of each aqueous extract at different concentrations of 100. 50 to 25 mg/ml. Disks are then carefully deposited on the agar surface. Disks containing DMSO served as negative controls. Petri plates were left for 30min for the pre-

diffusion of the extracts and then incubated at 37°C for 24 hours. Antibacterial activity was expressed as the diameter of the inhibition zone (mm) (25).

Minimum inhibitory concentration (MIC) was determined by microdilution in 96-well plates. 1ml of the bacterial suspension was added to tubes containing increasing concentrations of each extract (3–200 mg/mL, prepared by double dilution). Tubes were incubated for 18–24 hours. MIC was defined as the lowest concentration with no visible bacterial growth. MIC determination was performed by observing bacterial growth in each tube (26).

*Statistical analysis*

All statistical analyses were performed using XLSTAT. p-values ≤ 0.05 were considered statistically significant.

## Results and Discussion

*Extraction yield*

The extraction yield differed between both infusion and maceration methods, confirming that extraction conditions significantly influence the recovery of bioactive compound (27). The macerated extract showed a higher (15.97) compared to the infused extract (11.97%). Extraction efficiency is attributed to numerous parameters such as duration, temperature, pressure, and solvent polarity.

*Organoleptic tests*

Organoleptic evaluation is important for analysing the sensory profile of plant material. The organoleptic properties of the colour, texture, odour, and taste of both liquid and dry aqueous extracts are presented in (Table I).

**Table I**

Extraction yield (%) and organoleptic characteristics of aqueous extracts of *S. molle* L. seeds. The sensory evaluation includes colour, texture, odour, and taste for both infusion and maceration methods

| Organoleptic parameters | Infusion | | Maceration | |
|---|---|---|---|---|
| | Liquid aqueous Extract | Dry aqueous Extract | Liquid aqueous Extract | Dry aqueous Extract |
| **Yield** | / | **11.9 ± 1.23** | / | **15.97 ± 2.58** |
| **Aspect** | Liquid | Shiny and viscous | Viscous | Shiny and viscous |
| **Colour** | Light honey like | Light brown | Dark honey like | Dark brown |
| **Odour** | Pepper like | Pepper like | Pepper like | Pepper like |
| **Taste** | Pleasant pepper and lemon taste | Pleasant pepper and lemon taste | Pleasant pepper and lemon taste | Pleasant pepper and lemon taste |

Results showed variations in colour and texture between the infused and macerated extracts, while the odour and taste remained consistent in both extracts. These variations are related to the differences in phytochemical composition of each extract.

*Phytochemical screening*

Preliminary qualitative tests revealed significant amounts of secondary metabolites, including alkaloids, phenolics, flavonoids, saponins, and tannins in aqueous extracts. Jojic et al. (28) has revealed that many monoterpenes of *Juniperi galbulus* aqueous extract were highly bioactive.

Flavonoids and saponins were more abundant in infused extracts, whereas mucilage was exclusively detected in the macerated extract. Cardiac glycosides, coumarins, anthraquinones, and sterols were absent in both extracts (Table II).

*Total Phenolic Content (TPC)*

Polyphenol quantification showed a significant variation in TPC between the two extraction methods. The amount of TPC ranged from 3.08 GAE/g in the infused extract to 4.79 mg GAE/g, in the macerated extract (Figure 1). These compounds are affected by many factors such as geographical origin, drying method, and extraction technique (29, 30).

**Table II**

Phytochemical screening of aqueous extracts of *S. molle* L. seeds.

| Tests \ Aqueous extract | | GI | GM |
|---|---|---|---|
| **Flavonoids** | | +++ | + |
| **Tannins** | | +++ | +++ |
| **Alkaloids** | | +++ | +++ |
| **Sterols and steroids** | | - | - |
| **Saponosids** | **Test 1** | +++ | + |
| | **Test 2** | +++ | ++ |
| **Cardiac glycosides** | | - | - |
| **Starch** | | - | - |
| **Mucilages** | | - | +++ |
| **Coumarins** | | - | |
| **Anthraquinones** | | - | |

Qualitative detection of secondary metabolites: tannins, flavonoids, alkaloids, saponins, mucilage, anthraquinones, steroids, and others. GI (seeds infusion), GM (seeds maceration), Negative (–), weak (+), moderate (++), high (+++)

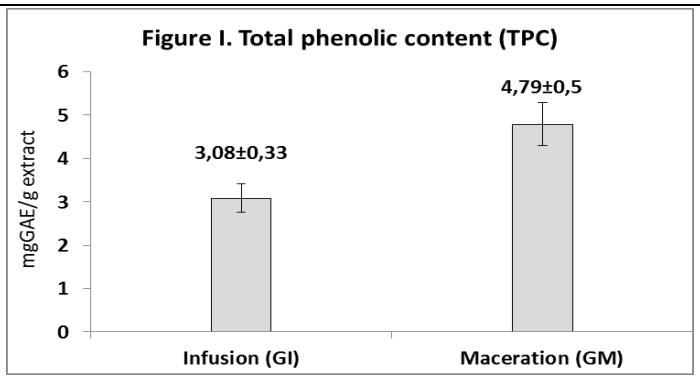


**Figure 1.**

Total phenolic content (TPC) of aqueous extracts of *S. molle* L. seeds. Comparison between macerated (GM) and infused (GI) extracts. Results are expressed as mg Gallic acid equivalent per gram of extract (mg GAE/g). Values represent mean ± SD (n = 3)

*HPLC analysis*

High-Performance Liquid Chromatography (HPLC) quantified 16 phenolic compounds in both aqueous extracts (Table III). A notable variation was detected between the two extracts. Among the targeted compounds, Epicatechin (5.973ppm), Quercetin (5.567ppm), and Catechin (4.395ppm) were higher amount in macerated extract. Infused extract had higher level of Gallic acid (4.919ppm) and Quercetin (4.995ppm). Additionaly, Chlorogenic acid and Syringic acid were detected only in the macerated extract.

**Table III**

HPLC quantification of phenolic compounds in aqueous extracts of *S. molle* L. seeds (ppm/mg/L) in macerated (GM) and infused (GI) extracts

| | Phenolic bile | Correlation (r2) | λ (mm) | RT (min) | GM (ppm) | GI (ppm) |
|---|---|---|---|---|---|---|
| **1** | **3-HydroyBenzoic Acid** | 0.99928 | 287 | 22.545 | N.D. | N.D. |
| **2** | **Benzoic Acid** | 0.99994 | 278 | 17.647 | **0.509** | **0.139** |
| **3** | **Benzoic Acid** | 0.99986 | 278 | 47.629 | N.D. | N.D. |
| **4** | **Catechin Hydrate** | 0.99906 | 278 | 11.499 | **4.395** | N.D. |
| **5** | **Chlorogenic Acid** | 0.9997 | 330 | 16.239 | **0.931** | N.D. |
| **6** | **Coffeic Acid** | 0.99892 | 330 | 21.476 | N.D. | N.D. |
| **7** | **Epicatechin** | 0.99879 | 278 | 20.169 | **5.973** | N.D. |
| **8** | **Gallic Acid** | 0.99966 | 278 | 5.912 | **3.025** | **4.919** |
| **9** | **Hesperidin** | 0.99705 | 287 | 65.989 | N.D. | N.D. |
| **10** | **P-Coumaric** | 0.99982 | 330 | 33.597 | N.D. | N.D. |
| **11** | **Quercetin** | 0.99962 | 254 | 76.313 | **5.567** | **4.995** |
| **12** | **Rosmarinic Acid** | 0.99907 | 330 | 70.655 | N.D. | N.D. |
| **13** | **Sinnapic Acid** | 0.99925 | 330 | 37.264 | N.D. | N.D. |
| **14** | **Syringic Acid** | 0.99839 | 278 | 22.628 | **0.925** | N.D. |
| **15** | **t-Cinnamic Acid** | 0.99998 | 278 | 75.207 | N.D. | N.D. |
| **16** | **t-Ferrulic Acid** | 0.99993 | 330 | 37.202 | **0.573** | N.D. |

These results indicate that maceration is the most effective for extracting phenolic compounds from seeds. These results are consistent with previous reports indicating that extraction temperature and contact time with solvent influence the solubility and stability of phenolic compounds (2, 4, 27, 32, 33-34). High temperature during infusion may increase phenolic oxidation, reducing extract yield, while maceration at room temperature over longer period enhances solubility and preserves thermolabile compounds (35, 36-37).

*Antibacterial activity*

The results revealed that both aqueous extracts exhibited antibacterial activity, varying according to the bacterial strain (Table IV). Infused and macerated extracts showed the highest inhibition zones against *E. coli* and *Enterobacter* sp. It was inhibited by all concentrations of infused extract, while the macerated extract showed inhibitory effects only at the higher concentrations. The other bacteria tested were resistant to both extracts.

The dilution method (MIC) confirmed that *E. coli* was sensitive to both extract. However, infused extract showed stronger activity with MIC and MBC of 25 and 50mg/ml, respectively, for *Enterococcus* sp., MIC and MBC were higher (50 and 100 mg/mL), respectively, with infused extract, and 200 mg/ml for the macerated extract (Table V). The other bacteria strains were resistant to both extracts. These results align with earlier reports on the antimicrobial

potential of *S. molle* seeds and leaves (9, 13, 37-38). However, recent studies by Tadjeddine (37) and Malca-García (39) had confirmed antibacterial activity of methanolic extracts of *S. molle* seeds, particularly against *Staphylococcus aureus* and *Bacillus subtilis*.

**Table IV**

Antibacterial activity of aqueous extracts of *S. molle* L. seeds determined by diameter of inhibition zones (mm) against six bacterial strains. DMSO was used as negative control

| **Extracts** | **Infused extract** | | | | | **Macerated extract** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **[C] (mg/mL)** | **200** | **100** | **50** | **25** | **T** | **200** | **100** | **50** | **25** | **T** |
| ***Staphylococcus epidermis*** | 6.00 | 6.00 | 6.00 | 6.00 | 6.00 | 6.00 | 6.00 | 6.00 | 6.00 | 6.00 |
| ***Lactobacillus bulgaricus*** | 6.00 | 6.00 | 6.00 | 6.00 | 6.00 | 6.00 | 6.00 | 6.00 | 6.00 | 6.00 |
| ***E. coli*** | **20 ± 0.5** | **17 ± 0.3** | **12 ± 0.8** | **10 ± 0.5** | 6.00 | **17 ± 0.66** | **15 ± 0.22** | **8 ± 2.1** | 6.00 | 6.00 |
| ***Salmonella thyphi*** | 6.00 | 6.00 | 6.00 | 6.00 | 6.00 | 6.00 | 6.00 | 6.00 | 6.00 | 6.00 |
| ***Enterobacter* sp** | **15 ± 0.5** | **14 ± 0.65** | **12 ± 0.92** | 6.00 | 6.00 | **10 ± 1.2** | 6.00 | 6.00 | 6.00 | 6.00 |
| ***Klebseilla* sp** | 6.00 | 6.00 | 6.00 | 6.00 | 6.00 | 6.00 | 6.00 | 6.00 | 6.00 | 6.00 |

**Table V**

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of aqueous extracts of *S. molle* L. seeds (mg/mL) against six bacterial strains.

| **Aqueous extracts** | **Infused (GI)** | | **Macerated (GM)** | |
|---|---|---|---|---|
| **Bacterial strains** (mg/mL) | **MIC** | **MBC** | **MIC** | **MBC** |
| ***Staphylococcus epidermidis*** | > 200 | > 200 | > 200 | > 200 |
| ***Lactobacillus bulgaricus*** | > 200 | > 200 | > 200 | > 200 |
| ***Escherichia coli*** | **25** | **50** | **100** | 200 |
| ***Salmonella thyphi*** | > 200 | > 200 | > 200 | > 200 |
| ***Enterobacter* sp** | **50** | **100** | 200 | > 200 |
| ***Klebseilla* sp** | > 200 | > 200 | > 200 | > 200 |

MIC: lowest concentration preventing visible growth; MBC: lowest concentration causing bacterial death.

Variations in the antibacterial activity of seeds extracts can be primarily attributed to differences in their phytochemical profiles, such as Gallic acid, Chlorogenic acid, Syringic acid, Ferulic acid, Catechin, Epicatechin, and Quercetin, which are well-documented for antibacterial potential acting through multiple mechanisms, including disruption of bacterial cell walls, inhibition of enzymatic pathways, and interference with nucleic acid synthesis (12, 13). Other secondary metabolites detected in the extracts, such as flavonoids, tannins, alkaloids and saponins, also contribute significantly to bacterial growth inhibition**.**

Results demonstrate that macerated extract contained higher levels of mucilage, whereas flavonoid and saponin contents were more abundant in the infused extract**.** Saponins, in particular, may exert a stronger effect on Gram-positive bacteria due to their ability to alter membrane ultrastructure. Wallace (38) suggested that saponin may affect the ultra-structure of Gram-positive bacteria more than Gram negative ones.

The infused extract exhibited the strongest antibacterial effects, especially against *E. coli* and *Enterobacter* spp., likely due to its higher Gallic acid concentration. This compound is recognized for its potent antimicrobial effects, including membrane destabilization and metabolic inhibition (40).

Quercetin is also abundant in both extracts and is a major contributor to the observed antibacterial activity. It is known to inhibit both Gram-positive and Gram-negative bacteria through multiple mechanisms such as membrane damage and change of membrane permeability, impaired nucleic acid and protein synthesis, reduced expression of virulence factors, disruption of mitochondrial function, and inhibition of biofilm formation (41, 42, 43).

The greater inhibitory activity of the infused extract against *E. coli,* (up to 20 mm) and Enterobacter sp. (up to 15 mm), suggests that extraction temperature and solvent accessibility significantly influence the recovery of active metabolites. In contrast, the macerated extract exhibited moderate activity, with inhibition zones becoming evident only at higher concentrations. Such variability is likely linked not only to differences in extraction efficiency but also to the intrinsic metabolic characteristics of each bacterial strain.

Additionally, the hydrophobic nature of several bioactive molecules present in the extracts may further facilitates their integration into bacterial membranes and ultimately increasing permeability and leading to cell structural destabilization, as reported by Kwun (44). Synergistic interactions among these phytochemicals often result in greater antibacterial efficacy than individual compounds alone, highlighting the importance of qualitative composition over total phenolic content.

Taken together, these results indicate that the antibacterial properties of *S. molle* extracts depend on both the composition and concentration of

specific bioactive molecules, particularly Gallic acid and Quercetin, as well as the synergistic interplay among various phytochemicals. Therefore, optimizing extraction techniques is therefore crucial to maximize recovery of bioactive molecules responsible for antibacterial activity and therapeutic potential.

## Conclusions

The comparative evaluation of *S. molle* L. seed aqueous extracts, obtained by maceration and infusion, is highlighted not only the total phenolic content but, crucially, by the specific qualitative composition of phenolic compounds and their potential synergistic interactions. While maceration yields higher and more phenolic profile diversity, infusion can concentrate key bioactive molecules such as Gallic acid, resulting in significant antibacterial activity against certain pathogens, such as *E. coli* and *Enterobacter* species. These data highlight the importance of extraction methods in optimizing the therapeutic potential of plant-derived compounds and supports the ethno-pharmacological relevance of *S. molle* L. seeds as natural source of antibacterial agents, warranting further investigation of more extraction methods with other solvent, into the isolation of active constituents, their mechanisms of action, and the development of effective natural therapeutics.

## Acknowledgement

The authors gratefully acknowledge the support provided by the members of the of Mascara laboratory members. We also thank the editor and reviewers for their constructive comments and suggestions.

## Funding

Not applicable.

## Availability of data and materials

The data generated in the present study are included in the figures and/or tables of this article.

## Ethics

Not applicable

## AI use disclosure

During the preparation of this manuscript, the author(s) used DeepL for translation from Arabic to English.

## Conflict of interest

The authors declare no conflict of interest.

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