

In vitro Antibacterial Activity of two plants extracts against bacterial strains

Atividade Antibacteriana In Vitro de Extratos de Duas Plantas contra Cepas Bacterianas

Actividad antibacteriana in vitro de dos extractos de plantas contra cepas bacterianas

DOI: 10.34188/bjaerv7n3-093

Submetido: 01/05/2024

Aprovado: 30/06/2024

Boutarfa Soumia

Assistant Professo at the Department of molecular and cellular biology, Faculty of Natural and Life Sciences, University of Abbes Laghrour Khenchela
Laboratory of Biotechnology, Water, Environment and Health, Faculty of Natural and Life Sciences, University of Abbes Laghrour Khenchela
Algeria
E-mail: soumia.boutarfa@univ-khenchela.dz

Mayouf Nozha

Assistant Professo at the Department of molecular and cellular biology, Faculty of Natural and Life Sciences, University of Abbes Laghrour Khenchela,
Laboratory of Biotechnology, Water, Environment and Health, Faculty of Natural and Life Sciences, University of Abbes Laghrour Khenchela,
Algeria
E-mail: E-mail: nozha.mayouf@univ-khenchela.dz

Belabed Nassira

Ms at Department of molecular and cellular biology, Faculty of Natural and Life Sciences, University of Abbes Laghrour Khenchela
E-mail: nassira.belabed@univ-khenchela.dz

Belaabed Zakiya

Ms at Department of molecular and cellular biology, Faculty of Natural and Life Sciences, University of Abbes Laghrour Khenchela
E-mail: zakiya.belaabed@univ-khenchela.dz

Merzougui Rajaa

Ms at Department of molecular and cellular biology, Faculty of Natural and Life Sciences, University of Abbes Laghrour Khenchela,
E-mail: E-mail: rajaa.merzougui @univ-khenchela.dz

Zedira Dounia

Ms at Department of molecular and cellular biology, Faculty of Natural and Life Sciences, University of Abbes Laghrour Khenchela
E-mail: dounia.zedira@univ-khenchela.dz

Baara Dounia

Ms at Department of molecular and cellular biology, Faculty of Natural and Life Sciences, University of Abbes Laghrour Khenchela
E-mail: dounia.baara@univ-khenchela.dz

ABSTRACT

Plantas medicinais constituem uma fonte potencial de moléculas bioativas que podem fornecer uma nova fonte de agentes antimicrobianos. Neste estudo, relatamos as potentes atividades antimicrobianas de *Salvadora persica* e *Juglans regia* L. Os extratos das plantas foram preparados utilizando seis solventes orgânicos diferentes: clorofórmio, éter de petróleo, acetona, acetato de etila, etanol e metanol. Os extratos de plantas foram avaliados quanto à sua atividade antibacteriana contra bactérias Gram-positivas e Gram-negativas selecionadas (*Bacillus cereus*; *Staphylococcus aureus*; e Gram-negativas: *Escherichia coli*, *Klebsiella pneumoniae*; *Pseudomonas aeruginosa*), utilizando a técnica de difusão em ágar em quatro concentrações diferentes de cada extrato: 100, 50, 25 e 15 mg/ml. Os resultados mostraram que os extratos de *Juglans regia* apresentaram propriedades antibacterianas contra todas as cepas bacterianas testadas e parecem ser mais eficientes do que os extratos de *Salvadora persica*. Os estudos antimicrobianos mostraram níveis variados de atividade, com o extrato etanólico de *J. regia* apresentando a maior eficiência entre todos os extratos das duas plantas estudadas contra *Staphylococcus aureus* (diâmetro médio da zona de inibição = 22 mm). Nosso estudo conclui que a atividade antibacteriana geral do extrato bruto da *J. regia* forneceu evidências de que futuros agentes antibacterianos poderiam ser isolados desta planta.

Keywords: antibacterial activity, *Juglans regia*, medicinal plants, *Salvadora persica*,

RESUMO

Plantas medicinais constituem uma fonte potencial de moléculas bioativas que podem fornecer uma nova fonte de agentes antimicrobianos. Neste estudo, relatamos as potentes atividades antimicrobianas de *Salvadora persica* e *Juglans regia* L. Os extratos das plantas foram preparados utilizando seis solventes orgânicos diferentes: clorofórmio, éter de petróleo, acetona, acetato de etila, etanol e metanol. Os extratos de plantas foram avaliados quanto à sua atividade antibacteriana contra bactérias Gram-positivas e Gram-negativas selecionadas (*Bacillus cereus*; *Staphylococcus aureus*; e Gram-negativas: *Escherichia coli*, *Klebsiella pneumoniae*; *Pseudomonas aeruginosa*), utilizando a técnica de difusão em ágar em quatro concentrações diferentes de cada extrato: 100, 50, 25 e 15 mg/ml. Os resultados mostraram que os extratos de *Juglans regia* apresentaram propriedades antibacterianas contra todas as cepas bacterianas testadas e parecem ser mais eficientes do que os extratos de *Salvadora persica*. Os estudos antimicrobianos mostraram níveis variados de atividade, com o extrato etanólico de *J. regia* apresentando a maior eficiência entre todos os extratos das duas plantas estudadas contra *Staphylococcus aureus* (diâmetro médio da zona de inibição = 22 mm). Nosso estudo conclui que a atividade antibacteriana geral do extrato bruto da *J. regia* forneceu evidências de que futuros agentes antibacterianos poderiam ser isolados desta planta.

Palavras-chave: atividade antibacteriana, *Juglans regia*, plantas medicinais, *Salvadora persica*

RESUMEN

Las plantas medicinales constituyen una fuente potencial de moléculas bioactivas que pueden proporcionar una nueva fuente de agentes antimicrobianos. En este estudio, informamos sobre las potentes actividades antimicrobianas de *Salvadora persica* y *Juglans regia* L. Los extractos de las plantas se prepararon utilizando seis solventes orgánicos diferentes: cloroformo, éter de petróleo, acetona, acetato de etilo, etanol y metanol. Los extractos de plantas fueron evaluados en cuanto a su actividad antibacteriana contra bacterias Gram-positivas y Gram-negativas seleccionadas (*Bacillus cereus*; *Staphylococcus aureus* y Gram-negativas: *Escherichia coli*, *Klebsiella pneumoniae* y *Pseudomonas aeruginosa*), utilizando la técnica de difusión en agar en cuatro concentraciones diferentes de cada extracto: 100, 50, 25 y 15 mg/ml. Los resultados mostraron que los extractos de *Juglans regia* presentaron propiedades antibacterianas contra todas las cepas bacterianas probadas y parecen ser más eficientes que los extractos de *Salvadora persica*. Los estudios antimicrobianos mostraron niveles variados de actividad, con el extracto etanólico de *J. regia* presentando la mayor eficiencia entre todos los extractos de las dos plantas estudiadas contra *Staphylococcus aureus* (diámetro medio de la zona de inhibición = 22 mm). Nuestro estudio concluye que la actividad antibacteriana general del extracto bruto de *J. regia* proporcionó evidencias de que futuros agentes antibacterianos podrían ser aislados de esta planta.

Palabras clave: actividad antibacteriana, *Juglans regia*, plantas medicinales, *Salvadora persica*

1 INTRODUCTION

In developing countries, medicinal plants have been utilized as alternative remedies for health issues for an extended period of time. Several plant extracts and essential oils derived from plants showed biological activity (Noumi *et al.*, 2010; Tasneem *et al.*, 2019). Herbal therapies have the potential to serve as a superior substitute for existing synthetic treatments due to their lower cost and few adverse effects, and they are believed to be relatively safe (Tao *et al.*, 2015; Ashraf *et al.*, 2022).

In recent years, there has been a growing inclination toward natural therapies in developed and developing countries (Agra & Fortuna, 2019; Altemimi *et al.*, 2023). The global prevalence of infectious diseases and antimicrobial resistance pose a significant public health threat. ((Aumeeruddy *et al.*, 2018 ; Pinto Filho Segundo *et al.*, 2023). Antibiotics disrupt gastrointestinal flora, reducing beneficial microorganisms and pathogens and increasing the risk of infections. Therefore, the search for alternative natural antimicrobials is a priority (Aumeeruddy *et al.*, 2018 ; Clébis *et al.*, 2021). Several herbal extracts provide significant efficacy against pathogens and can be utilized for therapeutic purposes (Al-Ayed *et al.*, 2016).

The toothbrush tree, *Salvadora persica* (miswak), Arabic for tooth-cleaning stick belonging to the *Salvadoraceae* family, is one of the most important among 182 species of plants being used as chewing sticks. It has been widely used and scientifically proven to prevent tooth decay even when used without other tooth-cleaning methods (Noumi *et al.*, 2010; Al-Ayed *et al.*, 2016). Miswak possesses significant antibacterial and antifungal properties that can effectively combat periodontal microorganisms, including *Lactobacillus acidophilus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans* (Naseem *et al.*, 2014).

The genus *Juglans* (family *Juglandaceae*) comprises several species found worldwide (Vieira *et al.*, 2019). The walnut tree (*Juglans regia*) is the most ubiquitous tree nut globally and holds significant commercial value. The plant is a renowned botanical species utilized in traditional medicine (Ellafi *et al.*, 2023). Practically, each part of the plant possesses a conventional therapeutic application. The root and stem bark contain anthelmintic, astringent, bactericide, diuretic, digestive, laxative, stimulant, detergent, and insecticidal characteristics (Ebrahimi *et al.*, 2018; Zurek *et al.*, 2022). The dried stem bark *Juglans regia* L. bark possesses anti-inflammatory, blood-purifying, anticancer, depurative, diuretic, and laxative properties. *Juglans regia* bark extract has broad-spectrum antibacterial action against oral microorganisms and can be utilized for tooth cleaning (Zakavi *et al.*, 2013; Bakht *et al.*, 2017).

Considerable attention has been devoted to investigating the suppressive impact of *Salvadora persica* and *juglans regia* on oral microorganisms. However, limited data is available

regarding the antibacterial properties of *Salvadora persica* against other types of pathogens. Therefore, this study was conducted to evaluate the antibacterial effect of both plant extracts (the bark of *Juglans regia* and the stems of *Salvadora persica*) against different reference bacterial species.

2 THEORETICAL FRAMEWORK

2.1 COLLECTION OF PLANT MATERIAL

In the current study, stems of *Salvadora persica* and bark of *Juglans regia* were acquired from local markets in Khenchela City, Algeria, in April 2023. Identification was conducted by a taxonomist at the Department of Agronomy, Khenchela University, Algeria. Voucher specimens were deposited under SAP-1-23 and JUR-2-23 at the pedagogic laboratory, University of Khenchela, Algeria. Plant material was subjected to air drying in the laboratory at ambient temperature. The stems of *Salvadora persica* and the bark of *Juglans regia* underwent air drying at room temperature for ten days. Dried plant samples were sectioned into discs and ground into a fine powder using a laboratory grinder. The powder was stored at four °C in the refrigerator for subsequent processing.

2.2 PREPARATION EXTRACTS

The stems of *Salvadora persica* and the bark of *Juglans regia* were air-dried at room temperature for ten days, chopped into discs, and ground into a fine powder using a coffee grinder. The preparation of the extracts involved combining 20 g of *Salvadora persica* and *Juglans regia* powder with 200 ml of the chosen solvents (95% methanol, 95% Ethanol, Chloroform, petroleum ether, Acetate and Acetone). The solution was agitated for 30 minutes and subsequently allowed to rest for 24 hours. The solution was filtered using Whatman number 1 filter paper, and the resulting solution was subsequently evaporated in a vacuum evaporator at 40 °C. The samples were stored in aseptic containers at four °C until further use (Mau et al., 2001). Dried extracts of *S. persica* and *J. regia* were prepared at various concentrations: 100, 50, 25, and 15 mg/ml, utilizing dimethyl sulfoxide (DMSO) (Abhary and Al-Hazmi, 2015).

2.3 SELECTED PATHOGENIC MICROORGANISMS' SPECIES

In this study, 5 reference bacterial strains including *Bacillus cereus* (ATCC 11778), *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC25923), *Pseudomonas aeruginosa* (ATCC 27853), *Klebsilla pneumonia* (ATCC 4352) were used.

2.4 ANTIBACTERIAL ACTIVITIES

To ensure the accuracy and reliability of the data, each antimicrobial assessment of the extracts was conducted at three different times. Suspensions of fresh cultures of tested bacterial species grown on nutrient agar were prepared using normal saline and compared to McFarland density. The suspensions served as inoculums to evaluate the impact of crude extracts via the agar diffusion method on Mueller Hinton agar plates (Olila et al., 2001). Sterile discs (6 mm in diameter) infused with 20 µl of each extract concentration were positioned on the surfaces of Mueller Hinton agar. Subsequently, the Petri dishes were incubated at 37 °C for 24 hours. The activity is assessed by quantifying the zones of inhibition surrounding the discs; the experiment was conducted thrice (Karou et al., 2005). Sterile DMSO served as a negative control. Commercial antibiotic sensitivity testing discs were utilized for comparison: gentamicin 10 µg. The sizes of the inhibition zones were measured and documented as the average diameter (in millimeters) of the complete growth inhibition (Lino & Deogracious, 2006; Noumi et al., 2011). An extract is deemed efficient when the inhibition zone surrounding the disc exceeds 6 mm in diameter and exhibits no bacterial growth within this area (Amiour et al., 2014).

3 RESULTS AND DISCUSSIONS

The present study was conducted to investigate the antimicrobial activity of *S. persica* and *J. regia* extracts against some microorganisms. Table 1 provides the antimicrobial results obtained using the disc diffusion method. Extracts showed a varying degree of antimicrobial activity against the tested microbial. Overall, *Juglans regia* extracts exhibit highly potent antibacterial action on a variety of microorganisms compared to *Salvadora persica* extracts. Our research supports the findings of Noumi et al. (2011), which show that *Juglans regia* was more efficient against Gram (+) and Gram (-) bacteria than the extract of *Salvadora persica*.

Hence, the methanol, ethanol, acetone and acetate extracts of *J. regia* were significantly active against all tested bacteria in all four concentrations used, and *S. aureus* species were the most sensitive with an inhibition zone of 22 mm with ethanol extract at concentration of 100 mg/ml. The chloroform and petroleum ether extract showed less effect, *Staphylococcus aureus* was the most sensitive with both extracts at the concentration of 50 mg/ml, where the inhibition zones were 15 and 14 mm respectively.

The chloroform extract from the bark of *Juglans regia* has an inhibition diameter of 15mm and 12mm against *Staphylococcus aureus* and *Bacillus cereus* at high concentration, respectively, and an inhibitory activity with a diameter of 10mm against strains of *Klebsiella pneumoniae*, and *Escherichia coli*, at the lowest concentration, and *Pseudomonas aeruginosa* at both concentrations.

Similar results were reported by Ashraf et al. (2022) against *Staphylococcus aureus*, and *Escherichia coli*. Petroleum ether extracts also exhibits inhibitory activity against *Bacillus cereus* (at lowest concentration), and *Klebsiella pneumoniae* with a diameter of 12 mm at high concentration, while a zone of 11 mm at high concentration and 10 mm at lowest concentration, respectively, was seen against *Escherichia coli* and *Pseudomonas aeruginosa*. Even so, *Staphylococcus aureus* has the largest inhibition diameter (14 mm) at high concentration, making it the most sensitive. These findings are supported by the findings of Aissi and Boudjelal (2014), which discovered that *Staphylococcus aureus* is more susceptible to the ether of petroleum extract of *Juglans regia*, with an inhibition zone of 18.2 mm. Even for other bacteria, there is an antibacterial effect. The results obtained from the chloroform extract used for *Juglans regia* were effective against the same strain used in this study.

Table 1: Inhibition diameter (mm) of the sided extracts against bacterial strains tested. Source: Elaborated by the authors (2024).

Extract	Strain	<i>Salvadora persica</i>				<i>Juglens regia</i>			
		100 mg/ml	50 mg/ml	25 mg/ml	15 mg/ml	100 mg/ml	50 mg/ml	25 mg/ml	15 mg/ml
MEC	B1	12	12± 0.0	11± 0.0	11± 0.0	18± 00	17± 0.0	14± 0.0	13± 00
	B2	10± 0.0	09± 0.0	08± 0.0	09± 00	19± 00	19± 0.0	15± 0.0	14± 0.0
	B3	12± 0.0	11± 0.0	11± 0.0	10± 0.0	16± 00	15± 0.0	15± 0.0	15± 00
	B4	8± 0.0	8± 0.0	13± 0.0	13± 0.0	17± 0.0	16± 0.0	15± 0.0	14± 0.0
	B5	9± 00	8± 0.0	9± 0.0	8± 00	20± 00	19± 0.0	10± 0.0	09± 00
EEC	B1	12 ± 00	11± 0.0	11± 00	11± 0.0	15± 00	13± 0.0	14± 00	14± 00
	B2	10± 0.0	08± 0.0	08± 0.0	09± 00	18± 00	18± 00	18± 00	17± 0.0
	B3	12± 0.0	11± 0.0	10± 0.0	10± 0.0	12± 00	12± 0.0	13± 00	12± 0.0
	B4	9± 0.0	9± 00	12± 0.0	13± 0.0	22± 00	15± 0.0	12± 0.0	14± 0.0
	B5	8± 00	8± 00	9± 0.0	9± 00	18± 0.0	15± 0.0	13± 0.0	11± 0.0
ChEC	B1	00	00	08± 0.0	10± 0.0	00	00	9± 00	10± 0.0
	B2	00	00	15± 0.0	12± 0.0	00	00	10± 0.0	10± 0.0
	B3	00	00	09± 0.0	10± 0.0	00	00	12± 0.0	10± 0.0
	B4	00	00	09± 0.0	10± 0.0	00	00	15± 0.0	11± 0.0
	B5	00	00	12± 00	11± 0.0	00	00	9± 0.0	10± 0.0
EthWE	B1	00	00	10± 0.0	9± 0.0	00	00	11± 0.0	10± 0.0
	B2	00	00	10± 0.0	10± 0.0	00	00	10± 0.0	9 ± 0.0
	B3	00	00	9± 0.0	10± 0.0	00	00	10± 0.0	12± 0.0
	B4	00	00	9± 00	9± 0.0	00	00	14± 0.0	12± 0.0
	B5	00	00	9± 0.0	11± 0.0	00	00	12± 0.0	11± 0.0
AcetEC	B1	11± 0.0	14± 0.0	14± 0.0	13± 0.0	17± 00	15± 0.0	14± 00	13± 0.0
	B2	11± 00	10± 0.0	11± 00	11± 0.0	19± 0.0	18± 00	17± 0.0	10± 0.0
	B3	10± 0.0	12± 00	12± 0.0	13± 0.0	16± 00	11± 0.0	14± 0.0	12± 0.0
	B4	10± 00	12± 0.0	12± 0.0	13± 0.0	20± 0.0	17± 0.0	16± 0.0	13± 0.0
	B5	11± 0.0	12± 00	14± 00	8± 0.0	11± 0.0	13± 0.0	15± 0.0	7± 0.0
AWEC	B1	08± 0.0	10± 0.0	12± 0.0	12± 0.0	18± 0.0	15± 00	13± 0.0	14± 0.0
	B2	13± 00	12± 0.0	11± 0.0	11± 0.0	19± 0.0	18± 0.0	17± 0.0	14± 0.0
	B3	12± 0.0	13± 00	14± 0.0	13± 0.0	19± 0.0	16± 0.0	15± 0.0	14± 00
	B4	16± 00	13± 0.0	12± 0.0	13± 0.0	16± 0.0	18± 0.0	13± 0.0	14± 0.0
	B5	10± 0.0	12± 0.0	11± 0.0	11± 0.0	20± 0.0	19± 0.0	12± 0.0	16± 0.0

B1 *Escherichia coli*, B2 *Pseudomonas aeruginosa*, B3 *Bacillus cereus*, B4 *Staphylococcus aureus*, B5 *Klebsiella pneumoniae*, Methanolic extract (MEC), Ethanolic extract (EEC), Chloroform extract (ChWE), petroleum ether extract (EthEC), Acetate extract (AcetEC), Acetone extract (AWEC).

The observations made on the effect of the different extracts on the growth of bacterial and fungal strains demonstrate that all the extracts of *Juglans regia* have antimicrobial activity against all the strains tested. A number of substances commonly found in plants, such as polyphenols, flavonoids, tannins, and alkaloids, are responsible for their antibacterial properties (Nguyen, 1983).

Extract of Tunisian *Juglans regia* L. bark using acetone, ethyl acetate and methanol as solvents (in vitro) against *S. aureus* AT CC 259 23, *P. aeruginosa* AT CC 278 53, *P. aeruginosa*. All three solvent extracts inhibited all tested bacteria, displaying comparable activity in the range (ZOI=7- 10mm) (Noumi et al., 2011). The green shell extracts of *J. regia* possess a good antibacterial activity on the species *E. coli*, *B. subtilis*, *P. aeruginosa*, and *S. aureus* (Sharma et al., 2013). The antibacterial activity also depends on the method of extraction, the type of solvent, and the mixing ratio (Sandu-Bălan Tăbăcariu et al., 2024). An ethanolic extract with a concentration of 5 mg/mL showed a high antibacterial potential for *S. aureus*. A concentration of 10 mg/mL was effective against *E. coli*. At a concentration of 5 mg/mL, the inhibition rates of *S. aureus*, and *E. coli* bacteria were 85.31–90.26% (Oliveira et al., 2008).

Additional research has reported the influence of varying concentrations of the same extract and the choice of solvent used for extraction on the antibacterial properties particularly against Gram-positive bacteria, including *S. aureus*, and *B. cereus* (Pereira et al., 2007 ; Rather et al., 2012). Hence, ethanolic septum extracts of *J.regia*, according to the study of Genovese et al. (2020), exhibited a stronger antibacterial effect on Gram-positive bacteria as (*S. aureus*) than on Gram-negative strains (*E. coli*, *K. pneumoniae*, *P. aeruginosa*), (Genovese et al., 2020).

The extracts of *S persica*, a potential source of antimicrobial agents, demonstrated activity against *S. aureus*. The diameter of the inhibition zone ranged between 12 and 16 mm with Acetone extract, indicating a significant antimicrobial effect. Moreover, an inhibition zone of 13 mm against *S. aureus* was recorded with methanol, ethanol, and Acetate extracts (Table 1). *Pseudomonas aeruginosa* was sensitive to *S persica*'s chloroform and acetone extracts with an inhibition zone of 15 and 13, respectively. Furthermore, acetate and acetone extracts were relatively more potent against the tested bacteria than the other extracts.

Meanwhile, petroleum ether extract of *Salvadora persica* has an activity with a 9- 10 mm perspective against all strains. The highest inhibition zone was seen against the *Pseudomonas aeruginosa* strain with 16 mm of chloroform extract, though the four other strains have activity between 10 and 12 mm. Our results are in agreement with those of Rahmoun *et al.* (2014).

However, the Petroleum ether extract, a component of *S. persica*, exhibited antibacterial activity against *P. aeruginosa*, showcasing its potential as a source of novel antimicrobial agents. The diameter of the inhibition zone was between 07 and 09 mm, indicating a significant

antimicrobial effect. However, there was an absence of inhibitory power for the extract of petroleum ether from *S. persica* on *S. aureus*.

On the other hand, the methanol extract of *S. persica* stems, a potential source of novel antimicrobial agents, showed less inhibitory activity against *S. aureus* (13.6 mm) and *Ps. aeruginosa* resisted all methanol extract concentrations (From 200 to 12.5 mg /ml) (AL-Bayati et al., 2010). Other studies showed that the methanolic stem of *Salvadora persica* extract did not exhibit efficacy against *P. aeruginosa*, in contrast to the aqueous extract (Al-Bayati & Sulaiman, 2008).

The observed variations among these studies suggest that each solvent extracts distinct antibacterial components from *S. persica*, depending on their polarity. The antibacterial properties of *S. persica* can be linked to its wide range of chemical components (Al-Bayati & Sulaiman, 2008). Ethanol extract effectively inhibited the tested microorganisms *E. coli*, *S. aureus*, and *P. aeruginosa*. The miswak extracts showed comparable or slightly stronger activity compared to some toothpastes (Mohammed, 2013)

Other studies were by our results, such as AlAayed et al., (2016) study in which the methanolic (95%), and methanol extract (ZOI=7.4-13.6 mm) were more effective against *E. coli*, *S. aureus*, *P. aeruginosa*, and *K. pneumoniae*. Likewise, the ethanolic extract showed mild activity against *E. coli*, *S. aureus*, and *K. pneumoniae* in Sellami et al., (2013) work. Another study showed that the ethanol and methanol extract effectively inhibited the tested microorganisms *S. aureus* and *K. pneumoniae* (Hesham & Alrumman, 2014). Hence, combined extract of *S. persica* stem and bark using petroleum ether, acetone, methanol as solvents, all solvent-extracts (tested at 200 mg/ml) inhibited *S. aureus* whereas the methanol extract was more effective than the other extracts (Kumar et al., 2016).

Abdallah and AlHarbi (2015) reported that ethanolic extracts of *S. persica* stem (in vitro) against *K. pneumoniae*, *E. coli*, *P. aeruginosa* and, and *B. cereus* inhibited all tested pathogens. The aqueous extract showed more activity than the ethanolic extract against *E. coli*, *P. aeruginosa*, *B. cereus*, and *S. aureus* but was less effective against *S. enterica*. The antibacterial activity of the extracts was comparable to or slightly better than penicillin G (10 units/disc). Likewise, Extract of *S. persica* dry stems using acetone, ethyl acetate, and methanol as solvents (in vitro) Bacteria: *S. aureus* AT CC 25923, *P. aeruginosa* ATCC 27853, *P. aeruginosa*. All three solvent extracts inhibited all tested bacteria, displaying comparable activity in the range (ZOI=7- 10mm) (Noumi et al., 2011). Ethanolic extract of *S. persica* roots (in vitro) *E. coli*, *B. cereus*, The extract showed antibacterial activity against all tested microorganisms (ZOI in the range 27.5-32.8mm) (Ant honey and Timothy, 2015). In addition, it has been shown that *P. aeruginosa* is resistant to ethanolic (80%) extract (Rath et al., 2012).

The antimicrobial activity varies among extracts, attributed to the selective action of the solvent extractor on specific microorganisms (Nguyen, 1983). The efficacy of an extract is contingent upon its concentration, the source plant, and the specific strain evaluated (Klervi, 2005). The antimicrobial efficacy of *J. regia* and *S. persica* is fundamentally determined by the plant-derived antimicrobial compounds contained in each extract. According to their polarity, the extracted compounds differ, suggesting that modifications to the extraction protocol and solvents employed may enable the detection of the antimicrobial activities of *J. regia* and *S. persica* (Nguyen, 1983).

4 CONCLUSION

The search for novel antimicrobial compounds is frequently discussed in academic publications and remains a primary topic of current scientific research since an increasing number of microbial strains are developing resistance to antibiotics. This study investigated the antibacterial activity of different extracts of *S. persica* and *J. regia*, which are widely used in traditional medicine in our country, especially to ensure oral hygiene. These in vitro studies represent the initial phase of the search for new natural antibacterial agents. Hence, the limitation of this study is that the susceptibility assays assess in vitro antimicrobial activity outside the patient. The in vitro efficacy of an antibacterial agent cannot guarantee favorable treatment outcomes. therefore these results remain preliminary. An in vivo investigation in order to identify the compounds that provide *Salvadora persica* and *Juglans regia* with its antibacterial properties, further phytochemical research will be required.

REFERENCES

- Abdallah, E.M., Al-Harbi, K.A. (2015). Phytochemical screening and antibacterial activity of crude aqueous and ethanol extracts of *Salvadora persica* L. stem (Miswak) from Saudi Arabia. *The Journal of Phytopharmacology* 4 (5), 243-247.
- Abhary, M., & Al-Hazmi, A.-A. (2016). Antibacterial activity of Miswak (*Salvadora persica* L.) extracts on oral hygiene. *J. Taibah Univ. Sci*, 10, 513–520. <https://doi.org/10.1016/j.jtusci.2015.09.007>.
- Agra, A. C., & Fortuna, J. L. (2019). Atividade antimicrobiana do óleo fixo de *Allagoptera caudescens* (Mart.) Kuntze sobre bactérias patogênicas / Antimicrobial activity of fixed oil of *Allagoptera caudescens* (Mart.) Kuntze on pathogenic bacteria. *Brazilian Journal of Animal and Environmental Research*, 2(3), 1120–1129. <https://ojs.brazilianjournals.com.br/ojs/index.php/BJAER/article/view/2147>.
- Al-Ayed, M. S., Asaad, A. M., Qureshi, M. A., Attia, H. G., & AlMarrani, A. H. (2016). Antibacterial Activity of *Salvadora persica* L. (Miswak) Extracts against Multidrug Resistant Bacterial Clinical Isolates. *Evidence-based complementary and alternative medicine : eCAM*, 2016, 7083964. <https://doi.org/10.1155/2016/7083964>
- AL-Bayati Firas, A., & Sulaiman Khudir, D. (2008) "In Vitro Antimicrobial Activity of *Salvadora persica* L. Extracts Against Some Isolated Oral Pathogens in Iraq," *Turkish Journal of Biology*, 32(1), 9. Available at: <https://journals.tubitak.gov.tr/biology/vol32/iss1/9>
- Alkhawajah, A.M. (1997). Studies on the antimicrobial activity of *Juglans regia*. *Am. J. Chin. Med*, 25, 175–180.
- Altemimi, A. B., Al-Haliem, S. M., Alkanan, Z. T., Mohammed, M. J., Hesarinejad, M. A., Najm, M. A. A., Bouymajane, A., Cacciola, F., & Abedelmaksoud, T. G. (2023). Exploring the phenolic profile, antibacterial, and antioxidant properties of walnut leaves (*Juglans regia* L.). *Food science & nutrition*, 11(11), 6845–6853. <https://doi.org/10.1002/fsn3.3554>
- Anthoney, S.T, Timothy, L.T. (2015). Phytochemical and antibacterial evaluation of ethanolic extract of *Salvadora persica* root extract against selected microorganisms. *International Journal of Bioassays* 4(12), 4658-4666.
- Ashraf, Z., Beg, A.E., Baig, M.T., Nudrat, F., Tehmina, S., & Iqbal, S. (2022). Phytochemical and Physicochemical Analysis of the In vitro Antimicrobial and Antifungal Activities of *Juglans regia* L. Bark (Walnut Bark). *J. Hunan Univ. Nat. Sci*, (49), 1-10. <https://doi.org/10.55463/issn.1674-2974.49.5.1>.
- Aumeeruddy, M. Z., Zengin, G., & Mahomoodally, M. F. (2018). A review of the traditional and modern uses of *Salvadora persica* L. (Miswak): Toothbrush tree of Prophet Muhammad. *Journal of ethnopharmacology*, 213, 409–444. <https://doi.org/10.1016/j.jep.2017.11.030>
- Bakht, J., Khan, S., Shafi, M., & Iqbal, A. (2017). Fractionation of crude extracts from controlled dried and commercially available stem bark of *Juglans regia* and their antimicrobial effects. *Pakistan journal of pharmaceutical sciences*, 30(5), 1581–1588. <http://www.pjps.pk/wpcontent/uploads/pdfs/30/5/Paper-9.pd>.
- Clébis, V. H., Proni, E., Sarimento, J. J. P., Takayama, R. K., & Nakazato, G. (2021). Atividade antibacteriana de micropartículas de prata encapsuladas com méis de *Apis mellifera* e *Scaptotrigona bipunctata* / Antibacterial activity of silver microparticles encapsulated with honeys from *Apis*

mellifera and Scaptotrigona bipunctata. *Brazilian Journal of Animal and Environmental Research*, 4(1), 933–948. <https://doi.org/10.34188/bjaerv4n1-076>.

Ebrahimi, S., Jamei, R., Nojoomi, F., & Zamanian Z. (2018). Persian walnut composition and its importance in human health. *International Journal of Enteric Pathogens*, 6: 3-9. <https://doi.org/10.15171/ijep.2018.02>

Ellafi, A., Farhat, R., Snoussi, M., Noumi, E., Anouar, E.H., Ben Ali, R., & Ben Younes, S. (2023). Phytochemical profiling, antimicrobial, antibiofilm, insecticidal, and anti-leishmanial properties of aqueous extract from *Juglans regia* L. root bark: *In vitro* and *in silico* approaches. *Int J Food Prop*, 26(1), 1079–1097. <https://doi.org/10.1080/10942912.2023.2200561>.

Genovese, C., Cambria, M. T., D'angeli, F., Addamo, A. P., Malfa, G. A., Siracusa, L., Pulvirenti, L., Anfuso, C. D., Lupo, G., & Salmeri, M. (2020). The double effect of walnut septum extract (*Juglans regia* L.) counteracts A172 glioblastoma cell survival and bacterial growth. *International journal of oncology*, 57(5), 1129–1144. <https://doi.org/10.3892/ijo.2020.5130>

Hesham, A.E., Alrumman, S.A., 2014. In vitro antibacterial activity of different *Salvadora persica* miswak extracts against isolated and genetically identified oral cavity pathogens. *Journal of Biotechnology and Biomaterials* 3(5), 75. <http://dx.doi.org/10.4172/2155-952X.S1.027>

Klervi, L.L. (2005). Connaissance chimiotaxonomique du genre *Turbinaria* et etude des composés de défense de différents espèces de Sargassacées des Iles Salmon (Pacifique sud).p 210.

Koné, K. P. F. O., Soro .Y., & Siaka .S. (2017). Détermination des paramètres influençant le rendement d'extraction hydro-alcoolique des métabolites secondaires de *Alchorneacordifolia*(Euphorbiaceae) et *Tridaxprocumbens*linn(Asteraceae). *Journal de la Société Ouest-Africaine de Chimie*, (44), p 15–22. In french

Kumar, S., Navneet, Gautam, S.S., Kumar, V. (2016). Preliminary phytochemical screening and antimicrobial activity of *Salvadora persica* Linn. extracts against oral pathogens. *Fungal Genomics & Biology* 6(1), 131. doi:10.4172/2165-8056.1000131

Lino, A., & Deogracious, O. (2006). The in-vitro antibacterial activity of *Annona senegalensis*, *Securidacca longipendiculata* and *Steganotaenia araliacea* - Ugandan medicinal plants. *African health sciences*, 6(1), 31–35. <https://doi.org/10.5555/afhs.2006.6.1.31>

Mau, J. L., Chao, G. R., & Wu, K. T. (2001). Antioxidant properties of methanolic extracts from several ear mushrooms. *Journal of agricultural and food chemistry*, 49(11), 5461–5467. <https://doi.org/10.1021/jf010637h>

Mohamed, H.M.H., Mansour, H.A. (2012). Incorporating essential oils of marjoram and rosemary in the formulation of beef patties manufactured with mechanically deboned poultry meat to improve the lipid stability and sensory attributes. *LWT-Food Science and Technology* 45(1), 79-87.

Naeini, A., Naderi, N.J., Shokri, H. (2014). Analysis and in vitro anti-*Candida* antifungal activity of *Cuminum cyminum* and *Salvadora persica* herbs extracts against pathogenic *Candida* strains. *Journal de Mycologie Médicale* 24(1), 13-18.

Naseem, S., Hashmi, K., Fasih, F., Sharafat, S., & Khanani, R. (2014). In vitro evaluation of antimicrobial effect of miswak against common oral pathogens. *Pakistan journal of medical sciences*, 30(2), 398–403. <https://doi.org/10.12669/pjms.302.4284>

Nguyen, D.M. (1983). Des plantes médicinales à propriétés antibactériennes; *Médecine Traditionnelle Chinoise*, (100), P 303-312. In french

- Noumi, E., Snoussi, M., Hajlaoui, H., Valentin, E., & Bakhrouf, A. (2010). Antifungal properties of *Salvadora persica* and *Juglans regia* L. extracts against oral *Candida* strains. *European journal of clinical microbiology & infectious diseases : official publication of the European Society of Clinical Microbiology*, 29(1), 81–88. <https://doi.org/10.1007/s10096-009-0824-3>
- Noumi, E., Snoussi, M., Trabelsi, N., Hajlaoui, H., Ksouri, R., Valentin, E., Bakhrouf, A. (2011). Antibacterial, anticandidal and antioxidant activities of *Salvadora persica* and *Juglans regia* L. extracts. *Journal of Medicinal Plants Research* 5(17), 4138-4146.
- Olila, D., Olwa-Odyek, & Opuda-Asibo, J. (2001). Antibacterial and antifungal activities of extracts of *Zanthoxylum chalybeum* and *Warburgia ugandensis*, Ugandan medicinal plants. *African health sciences*, 1(2), 66–72.
- Oliveira, I., Sousa, A., Ferreira, I. C., Bento, A., Estevinho, L., & Pereira, J. A. (2008). Total phenols, antioxidant potential and antimicrobial activity of walnut (*Juglans regia* L.) green husks. *Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association*, 46(7), 2326–2331. <https://doi.org/10.1016/j.fct.2008.03.017>
- Pereira, J. A., Oliveira, I., Sousa, A., Valentão, P., Andrade, P. B., Ferreira, I. C., Ferreres, F., Bento, A., Seabra, R., & Estevinho, L. (2007). Walnut (*Juglans regia* L.) leaves: phenolic compounds, antibacterial activity and antioxidant potential of different cultivars. *Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association*, 45(11), 2287–2295. <https://doi.org/10.1016/j.fct.2007.06.004>
- Pinto Filho Segundo, W. O., Lima, R. M., de Oliveira, L. A., Cortez, A. C. A., de Souza, Érica S., & de Souza, J. V. B. (2023). Avaliação da atividade antimicrobiana de *Clonastachys rosea* WLB18 frente a cepas de *Escherichia coli* ATCC 25922 e *Escherichia coli* ESBL: Antimicrobial activity of *Clonastachys rosea* WLB18 against strains of *Escherichia coli* ATCC 25922 and *Escherichia coli* ESBL. *Brazilian Journal of Animal and Environmental Research*, 6(3), 2928–2933. <https://doi.org/10.34188/bjaerv6n3-076>
- Pirzada, A.J., Maka, G.A., Shah, S.I.S., & Mughal, S. (2009). Anti-fungal activity of different solvent extracts of medicinal plants *Capparis decidua* edgew and *Salvadora persica* linn. against different parasitic fungi. *Pak J Agric Agric Eng Vet Sci*, 25. 26-34.
- Rath, S., Dubey, D., Sahu, M.C., Debata, N.K., Padhy, R.N. (2012). Surveillance of multidrug resistance of 6 uropathogens in a teaching hospital and in vitro control by 25 ethnomedicinal plants used by an aborigine of India. *Asian Pacific Journal of Tropical Biomedicine* 2(2), S818- S829.
- Rather, M. A., Dar, B. A., Dar, M. Y., Wani, B. A., Shah, W. A., Bhat, B. A., Ganai, B. A., Bhat, K. A., Anand, R., & Qurishi, M. A. (2012). Chemical composition, antioxidant and antibacterial activities of the leaf essential oil of *Juglans regia* L. and its constituents. *Phytomedicine : international journal of phytotherapy and phytopharmacology*, 19(13), 1185–1190. <https://doi.org/10.1016/j.phymed.2012.07.018>
- Sandu-Bălan Tăbăcariu, A., Ifrim, I. L., Patriciu, O. I., Ștefănescu, I. A., & Fînar, A. L. (2024). Walnut By-Products and Elderberry Extracts-Sustainable Alternatives for Human and Plant Health. *Molecules (Basel, Switzerland)*, 29(2), 498. <https://doi.org/10.3390/molecules29020498>
- Sellami, M., Ghariani, B., Louati, H., Miled, N., Gargouri, Y. (2013). Biological activities of extracts of different spices and plants. *International Journal of Current Engineering and Technology* 3(3), 1051-1060

- Sharma, P., Ravikumar, G., Kalaiselvi, M., Gomathi, D., & Uma, C. (2013). *In vitro* antibacterial and free radical scavenging activity of green hull of *Juglans regia*. *Journal of pharmaceutical analysis*, 3(4), 298–302. <https://doi.org/10.1016/j.jpha.2013.01.006>
- Tao, Z., Shi, A., & Zhao, J. (2015). Epidemiological Perspectives of Diabetes. *Cell biochemistry and biophysics*, 73(1), 181–185. <https://doi.org/10.1007/s12013-015-0598-4>
- Tasneem, S., Liu, B., Li, B., Choudhary, M. I., & Wang, W. (2019). Molecular pharmacology of inflammation: Medicinal plants as anti-inflammatory agents. *Pharmacological research*, 139, 126–140. <https://doi.org/10.1016/j.phrs.2018.11.001>
- Upadhyay, V., Kambhoja, S., & Harsha, L.K. (2010). Antibacterial activity and preliminary phytochemical analysis of stem bark extract of *Juglans regia* linn. *Pharmacologyonline* 3, 274–279.
- Upadhyay, V., Kambhoja, S., & Harshaleena, A. (2010 a). Antifungal activity and preliminary phytochemical analysis of stem bark extracts of *Juglans regia* linn. *Int. J. Pharm. Biol. Sci. Arch*, 1, 442–447.
- Vieira, V., Pereira, C., Pires, T.C., Calhelha, R.C., Alves, M.J., Ferreira, O., Barros, L., & Ferreira, I.C. (2019). Phenolic profile, antioxidant and antibacterial properties of *Juglans regia* L. (walnut) leaves from the Northeast of Portugal Industrial Crops and Products, 134 . 347-355. <https://doi.org/10.1016/j.indcrop.2019.04.020>
- Zakavi, F., Golpasand Hagh, L., Daraeighadikolaei, A., Farajzadeh Sheikh, A., Daraeighadikolaei, A., & Leilavi Shooshtari, Z. (2013). Antibacterial Effect of *Juglans Regia* Bark against Oral Pathologic Bacteria. *International journal of dentistry*, 2013, 854765. <https://doi.org/10.1155/2013/854765>
- Żurek, N., Pawłowska, A., Pycia, K., Grabek-Lejko, D., & Kapusta, I. T. (2022). Phenolic Profile and Antioxidant, Antibacterial, and Antiproliferative Activity of *Juglans regia* L. Male Flowers. *Molecules (Basel, Switzerland)*, 27(9), 2762. <https://doi.org/10.3390/molecules27092762>