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In vitro antimicrobial activity of *Salvadora persica* and *Juglans regia* extracts against microbial strains from oral cavity

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A B S T R A C T

Several plants were used in traditional medicine; *Juglans regia* (Souak) and *Salvadora persica* (Miswak) are largely used for the oral hygiene in Arabic countries. Four extracts prepared with organic solvents of increasing polarity; petroleum ether, chloroform, ethyl acetate, and methanol were used to test the antibacterial and antifungal activities of these plants with three different concentrations of each extract; 5, 10 and 15mg/ml. The results showed that *Juglans regia* seems to be more efficient against the isolated strains than *Salvadora persica*. Moreover the ethyl acetate extract of *J. regia* showed a high degree of antimicrobial activity.

Forty bacterial isolates were identified from the oral cavity of healthy individuals of different age categories (from 10 to 44 years) and only seven isolates were selected and characterized as *Staphylococcus saprophyticus* (B1), *Proteus vulgaris* (B2), *Pseudomonas aeruginosa* (B3), *Staphylococcus aureus* (B4), *Serratia marcescens* (B5), *Streptococcus salivarius* (B6), *Streptococcus mutans* (B7) and a yeast species identified as *Candida albicans* (Y). The highest efficiency of all extracts of the two plants achieved with *Streptococcus mutans* (mean of diameters of inhibition zones MDIZs = 10.29mm) followed by *Streptococcus salivarius* (MDIZs = 9.75mm), *Candida albicans* (MDIZs = 6.45mm), *Proteus vulgaris* (MDIZs = 5.75mm), *Staphylococcus aureus* (MDIZs = 5.62mm), *Serratia marcescens* (MDIZs = 5.58mm), *Pseudomonas aeruginosa* (MDIZs = 4.87mm) *and Staphylococcus saprophyticus* (MDIZs = 3.08mm).

1. Introduction

Oral infections and dental caries are a major health problem concerns among people worldwide, while hygiene practices continue to be the best prevention measures against oral infections and gum disease (Aboul-Enein, 2014). Different tools and chemicals exist to maintain and preserve dental and oral health, such as tooth brushes, mouth wash and tooth paste (Bairwa et al., 2012).

The resistance of bacteria to different antibiotics has forced scientists to look for new antimicrobial substances from various sources, such as medicinal plants (Morad Asaad et al., 2013). Medicinal plants are now part of complementary medicine in the world, because of their health benefits. Various plant extracts have great potential against infectious agents and can be used for therapeutic purposes; they are a natural source of antibacterial agents.

Various medicinal plants like, *Azadirachta indica*, *Marmelos aegles*, *Salvadora persica, Acacia Arabica* and *Juglans regia* and others have been used as tooth sticks by people of different cultures (Ahmad and Rajagopal, 2014).

One of these traditional plants, *Juglans regia* being walnut leaves, parts of this plant are important in traditional medicines; the stem is used as anti-helminthic and antidiarrheic agents, Furthermore the bark of the dried stem is also traditionally used in some countries as a toothbrush (Cosmulescu et al., 2014), it is used mostly by women as a dye for coloring the lips for cosmetic purposes (Alkhawajah, 1997).

Many studies *in vitro* have shown the biological activities of extracts of *Juglans regia*; regarding their anti-inflammatory (Hosseinzadeh et al., 2011), anti-proliferative (Santos et al., 2013; Salimi et al., 2014). On the other hand, *J. regia* extract showed broad-spectrum antimicrobial activity. It inhibits the growth of several pathogenic microorganisms (Zakavi et al., 2013).

In addition, *Salvadora persica*, known as Miswak (Arak in Arabic) is a shrub with a soft white wood, belonging to the *Salvadoraceae* family (El-Desouky et al., 2018). This traditional chewing stick growing wild with a very wide geographical distribution (Rahmani and Radvar, 2005).

S. persica can survive in extreme conditions and is capable of tolerating very dry environments and highly saline soils (Aumeeruddy et al., 2018).

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Abbreviations

ADH	Arginine dihydrolase test
API	Analytical profile index
C	Degree Celsius
CNS	Coagulase-negative staphylococci
DMSO	Dimethyl sulfoxide
E. coli	*Escherchia coli*
LPS	Lipopolysaccharids
MDIZs	Mean of diameters of inhibition zones
mg/ml	Milligram per milliliter
ml	Milliliter
mm	Millimeter
NC	Negative control
ND	Not determined
ODC	Ornithine decarboxylase test
PC	Positive control
R	Rod
S	Sphere
SDA	Sabouraud dextrose agar
SEM	Standard error of the mean
VP	Voges-Proskauer test

In Algeria, The traditional medicinal use of *Salvadora persica* as an antimicrobial stick tooth brush for oral hygiene and to treat gum inflammation is considered the most popular for his simplicity, availability, low cost and his traditional and/or religious value (Chelli--Chentouf et al., 2012).

Previous clinical studies and trials have demonstrated the richness of *S. persica* Miswak for the minerals and phytochemical components related to dental care, suggesting that miswak is a favorable oral health tool (Aboul-Enein, 2014; Abdulbaqi et al., 2016). Several clinical studies have reported that the miswak has a positive effect on gingivitis and plaque removal (Sofrata et al., 2011).

The aim of the current study was to assess the *in vitro* antibacterial activity of some marketed *Salvadora persica* and *Juglans regia* extracts against microbial strains from oral cavity. Also, to find the difference between plants extracts performed by different polarity solvents; petroleum ether, chloroform, ethyl acetate, and methanol according to Snyder's polarity index.

2. Materials and methods

2.1. Plant material and extraction

The roots of *Salvadora persica* and the bark of *Juglans regia* were purchased from local markets at Khenchela city (Algeria) in March 2019 and were identified by a taxonomist and a voucher specimen was deposited at the College of Agronomy, University of Khenchela, Algeria.

The bark of *Juglans regia* and the stalks of *Salvadora persica* were dried in a 55 °C oven for three days and then sliced into discs then ground into a fine powder using a coffee grinder.

The extraction method (liquid/solid) that we adopted is the successive maceration by four organic solvents of increasing polarity; petroleum ether (CH_3-$(CH_2)_n$-CH_3), chloroform ($CHCl_3$), ethyl acetate ($C_4H_8O_2$), and methanol (CH_3OH) (Cowan, 1999).

The extraction was done by maceration of 40 g of fine powder in 200ml of solvent (petroleum ether, chloroform, ethyl acetate and methanol). The mixture was agitated for 30 min at room temperature (Mau et al., 2001), and then maintained at rest for 24 h. The resulting extract was filtered by Whatman filter paper No.2 for 2 times to remove coarse plant particles; the solvent completely removed using a rotary evaporator.

All *S. persica* and *J. regia* extracts were suspended in dimethyl sulfoxide (DMSO) to prepare different concentrations.

2.2. Screening and identification of the oral microflora

Oral cavity samples were analyzed for aerobic bacterial and fungal content by cultures on a series of selective media and non-selective (Nutritive agar medium, Blood agar, Hektoen medium, Chapman medium, Sabouraud Dextrose Agar; SDA), incubated at 37 °C for 24h, 48h and up to72 h according to the investigated strains and with the addition of 5% CO_2 in the atmosphere for streptococci.

The bacterial isolates were identified by standard bacteriological procedures. Each representative colony was characterized by its macroscopic morphology, Gram stain, and several conventional approaches, including production of catalase and oxidase. Further identification of bacteria was achieved using commercials kits (API Staph, API20E and API Strep, bioMérieux®, France). The tests were conducted twice for each sample.

2.3. In vitro antimicrobial activities of S. persica and J. regia

All tests for antimicrobial evaluation of the extracts were conducted at three different times to ensure accuracy and reproducibility of the results.

The extracts were dissolved in DMSO to prepare three different concentrations of each extract; 5, 10 and 15mg/ml.

Antimicrobial activity was determined using the disc agar diffusion method described by (Finegold and Martin, 1982). Bacterial cultures of different strains were prepared in trypticase soya broth, standardized to match McFarland N° 0.5 barium inoculate Mueller Hinton agar plates. Discs impregnated with 20 μl of different extracts were placed on the surface of these plates. All plates were prepared and incubated at 37 °C for 24h for bacteria, 48h for yeast (Karou et al., 2005). Diameters of the zones of inhibition were measured and recorded as the mean diameter (mm) of complete growth inhibition. Commercial antibiotic sensitivity testing discs were also used for comparison (Noumi et al., 2011); 20mg of gentamicin was used as a positive control for bacteria, while 10mg of amphotericin B was used for fungi. DMSO served as a negative control.

2.4. Statistical analysis

The statistical analysis of samples was performed by calculating the average of the three replicates for each event and the three repeats of each experiment with the standard deviation.

All data are analyzed using IBM® SPSS® Statistics, Version 20 (IBM Corp., Armonk, NY, USA). One-way analysis of variance was used to compare the mean values of the outcome variable. The significance level was set at $p < 0.05$.

3. Results and discussion

3.1. Isolation and identification of oral microflora

A total of 40 isolates were obtained, 7 of which were in pure form (Table 1) that were selected and characterized based on their Gram reaction characteristics, morphological features and biochemical properties as: *Staphylococcus saprophyticus, Proteus vulgaris, Pseudomonas aeruginosa, Staphylococcus aureus, Serratia marcescens, Streptococcus mutans* and *Streptococcus salivarius*, and a yeast species identified as *Candida albicans.*

The human oral cavity contains a considerable number of bacterial species (estimated at over 500) (Dewhirst et al., 2010), these differences were probably due to several factors that can influence the microbial ecology of oral cavity. *Staphylococcus aureus* is the most common species of this genus in the oral flora (Ohara-Nemoto et al., 2008), which is occasionally isolated from the oral biofilm (Sixou et al., 2007).

Table 1
Phenotype characteristics bacteria isolated from oral cavity.

	Isolates						
Tests	B1	B2	B3	B4	B5	B6	B7
Shape	S	R	R	S	R	S	S
Gram stain	+	–	–	+	–	+	+
Oxidase	–	–	+	–	–	–	–
Catalase	+	+	+	+	+	–	–
Coagulase	–	ND	ND	+	ND	–	+
D-Xylose	–	+	–	–	+	–	–
Mannitol	–	–	+	+	+	+	+
Hemolysis	–	ND	ND	+	ND	–	+
Esculine	ND	+	–	ND	–	+	+
Sucrose	+	+	+	+	+	+	+
Glucose		+	+		+	+	+
VP	–	–	–	+	+	+	+
Lactose	+	–	–	+	+	+	+
Urea	+	+	+	–	–	–	–
ADH	–	–	+	+	–	–	–
Arabinose	–	–	–	–	–	–	–
Trehalose	+	+	+	+	–	–	+
ODC	–	–	–	–	+	ND	ND
King A	ND	ND	+	ND	ND	ND	ND
King B	ND	ND	+	ND	ND	ND	ND
Identification	*Staphylococcus saprophyticus*	*Proteus vulgaris*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Serratia marcescens*	*Streptococcus salivarius*	*Streptococcus mutans*

S: *sphere,* R: *rod,* +: *positive reaction,* −: *negative reaction,* ND: *not determined,* B1: *Staphylococcus saprophyticus,* B2: *Proteus vulgaris,* B3: *Pseudomonas aeruginosa,* B4: *Staphylococcus aureus,* B5: *Serratia marcescens,* B6: *Streptococcus salivarius,* B7: *Streptococcus mutans.*

S. saprophyticus was isolated by (Chelli-Chentouf et al., 2012) from the oral cavity of children with dental caries. Although this genus is known for its pathogenic power in the oral cavity; *Staphylococcus aureus* and coagulase-negative staphylococci (CNS) can cause a number of oral infections (eg. staphylococcal mucositis, angular cheilitis, parotitis) (McCormack et al., 2015), Staphylococci are recognized as major components of the oral flora (Singer, 1952); it is well isolated from the oral flora of healthy individuals (Jackson et al., 2000).

Streptococci were also present in this study; two species have been identified: *Streptococcus mutans* and *Streptococcus salivarius.* The pH in the adherent community is lowered, which selects for the predominance of these acid-loving bacteria.

The genus *Streptococcus* belongs to the commensal flora of the mucosal membranes of the upper respiratory tract (Burton et al., 2006). Several studies previously found that streptococci dominate in advanced caries (Munson et al., 2004; Aas et al., 2008; Chelli-Chentouf et al., 2012), these species develop and form a microbial biofilm as dental plaque (Lai and Li, 2011)**.** *Streptococcus mutans*, a Gram positive facultative anaerobic bacterium, is generally known as a major pathogen of dental caries, it facilitates the destruction of the tooth surface by the fermentation of carbohydrates into lactic acid (Peters et al., 2012).

In addition, this species is a possible causative agent of bacteremia and infective endocarditis (Nakano and Ooshima, 2009)**.**

In contrast, *Streptococcus salivarius* is a prominent member of the oral microbiota. Kaci et al. (2014) reported that *Streptococcus salivarius* colonizes the human oral cavity a few hours after birth and remains there as a predominant commensal flora. This species also inhabits the stomach and jejunum. In addition, *S. salivarius* is a primary colonizer of teeth, acting as a substrate for the attachment of other oral microbes (Nicolas and Lavoie, 2011).

Furthermore, it is interesting to note that this investigation showed a crucial biodiversity with enteric rods (*Proteus vulgaris* and *Serratia marcescens). Proteus vulgaris* is reported as a common inhabitant both in the oral cavity (Drzewiecka, 2016)**.** Moreover (Friedlander, 1975), reported that *Proteus vulgaris* is one of the causative agents of osteomyelitis of the mandible (an inflammation of the lower jaw).

Serratia marcescens is a member of the genus *Serratia*, which is a part of the family *Enterobacteriaceae*. It is widely distributed in nature and considered a causative agent of nosocomial pathogen (Sartor et al., 2000).

The study of (Barbosa et al., 2006) suggest that *Serratia marcescens* isolates from the mouth are not just contaminants from the environment, but that the oral cavity may act as a favorable ecosystem for this strains.

Only one strain of *Pseudomonoas aeruginosa* was isolated in this study, this strain Gram negative, catalase and oxidase positive was isolated from saliva of a man aged 40 years. According to (Caldas et al., 2015)**,** *P.aeruginosa* is an oral species, it can colonize dental plaque, saliva and oral mucosa, and it is an opportunistic pathogen in nosocomial infections.

Several studies showed that the oral cavity is an important reservoir of potentially pathogenic bacteria such as *Pseudomonoas aeruginosa* (Amaral et al., 2009). In addition (Noumi et al., 2011), isolated *P. aeruginosa* from the oral cavity of a patient with prosthetic stomatitis; which is a chronic inflammatory condition of the oral mucosa in contact with a removable prosthesis, it is often asymptomatic. The results of (Noumi et al., 2011) are in agreement with our findings in which *P. aeruginosa* was isolated from an elderly man who wears a dental prosthesis.

Our findings revealed a high prevalence of yeast (*Candida albicans*) in dental plaque and saliva. The results were similar to the study results of (Khurana et al., 2015) who found that the *C. albicans* was isolated from saliva of 88% of the subjects studied. It was reported that *Candida albicans* inhabit the human oral cavity (Chelli-Chentouf et al., 2012).

The acidogenic and heterofermentative characteristics allow the *Candida albicans* strain to play a significant role in the progression of dental caries (Do Rego et al., 2003)**.** Also (Raja et al., 2010), confirm that *Candida albicans* was isolated from carious lesions in children. Moreover (Lai and Li, 2011), reported that *C. albicans* can adhere to dentinal collagen, and can produce collagenolytic enzymes as well as acids that are able to destroy the organic structure of dentine.

Furthermore, this species has the ability to survive at low pH and can colonize the oral cavity with *Streptococcus mutans*; this co-aggregation exacerbates caries development (Peters et al., 2012)**.**

3.2. In vitro antimicrobial activities of Juglans regia

The results of the antimicrobial activity of different extracts of *Juglans regia* are given in Table 2.

Extracts showed a varying degree of antimicrobial activity against

Table 2
Antimicrobial activity profile of different extracts of *Juglans regia.* (Expressed as diameter of inhibition zone in mm).

		Inhibition zone (mm)						
		Extract's concentration (mg/ml)						
	Strain	NC	PC	05	10	15	SEM	*P value*
E 1	Y	0.000	16.267^{a}	10.173^{b}	12.017^{ab}	13.103^{ab}	0.043	0.001
	B1	0.000	18.567	00.010	00.167	00.167	0.059	0.248
	B2	0.000	23.033^{a}	00.007^{b}	00.013^{b}	08.997^{ab}	0.002	0.063
	B3	0.000	20.000^{a}	00.010^{b}	00.010^{b}	09.513^{ab}	0.072	0.009
	B4	0.000	20.333^{a}	00.003^{b}	10.183^{ab}	11.163^{ab}	0.058	0.005
	B5	0.000	20.167^{a}	00.010^{b}	07.200^{b}	08.163^{ab}	0.057	0.008
	B6	0.000	22.067^{a}	09.233^{b}	09.073^{b}	15.000^{ab}	0.040	0.001
	B7	0.000	24.333^{a}	09.133^{b}	10.200^{ab}	17.003^{ab}	0.044	0.000
E 2	Y	0.000	16.267^{a}	08.070^{b}	10.100^{ab}	16.847^{a}	0.043	0.000
	B1	0.000	18.567^{a}	00.173^{b}	00.100^{b}	11.173^{ab}	0.060	0.066
	B2	0.000	23.033^{a}	00.173^{b}	09.373^{b}	14.173^{ab}	0.061	0.009
	B3	0.000	20.000^{a}	07.203^{b}	07.203^{b}	08.980^{b}	0.054	0.061
	B4	0.000	20.333^{a}	00.173^{b}	11.020^{ab}	13.173^{ab}	0.058	0.082
	B5	0.000	20.167^{a}	00.010^{b}	11.973^{ab}	13.007^{ab}	0.010	0.063
	B6	0.000	22.067^{a}	11.300^{ab}	13.003^{ab}	16.400^{ab}	0.058	0.001
	B7	0.000	24.333^{a}	10.203^{b}	15.173^{ab}	15.850^{ab}	0.067	0.001
E 3	Y	0.000	16.267^{a}	10.203^{b}	12.000^{ab}	11.990^{b}	0.038	0.001
	B1	0.000	18.567^{a}	00.173^{b}	08.070^{b}	12.043^{ab}	0.043	0.055
	B2	0.000	23.033^{a}	00.013^{b}	00.167^{b}	10.980^{b}	0.042	0.059
	B3	0.000	20.000^{a}	00.010^{b}	00.010^{b}	14.240^{ab}	0.036	0.051
	B4	0.000	20.333^{a}	10.343^{b}	15.000^{b}	18.003^{ab}	0.022	0.000
	B5	0.000	20.167^{a}	00.167^{b}	17.200^{ab}	19.500^{a}	0.088	0.001
	B6	0.000	22.067^{a}	09.100^{b}	13.000^{b}	14.847^{ab}	0.040	0.009
	B7	0.000	24.333^{a}	09.010^{b}	12.030^{b}	13.080^{b}	0.020	0.009
E 4	Y	0.000	16.267^{a}	08.043^{b}	09.010^{b}	10.273^{b}	0.066	0.022
	B1	0.000	18.567^{a}	08.100^{b}	08.010^{b}	08.393^{b}	0.100	0.034
	B2	0.000	23.033^{a}	00.173^{b}	00.000^{b}	08.980^{b}	0.087	0.055
	B3	0.000	20.000^{a}	00.073^{b}	08.163^{b}	08.820^{b}	0.065	0.051
	B4	0.000	23.033^{a}	00.000^{b}	09.003^{b}	08.980^{b}	0.004	0.051
	B5	0.000	20.167^{a}	00.000^{b}	10.033^{b}	11.660^{b}	0.084	0.052
	B6	0.000	22.067^{a}	15.210^{b}	17.077^{ab}	19.010^{a}	0.041	0.000

E1: Petroleum ether extract, E2: Chloroform extract, E3: Ethyl acetate extract, E4: Methanolic extract. Y: *Candida albicans,* B1: *Staphylococcus saprophyticus,* B2: *Proteus vulgaris,* B3: *Pseudomonas aeruginosa,* B4: *Staphylococcus aureus,* B5: *Serratia marcescens,* B6: *Streptococcus salivarius,* B7: *Streptococcus mutans.* NC: Negative control, PC: Positive control. $^{a,b,\ ab}$Values within columns with unlike superscripts differ *($P < 0.05$).*

the microbial species isolated from the oral cavity. The 15mg/ml of all the extracts of *J. regia* (petroleum ether, chloroform, ethyl acetate, and methanol) had a significant effective ($P < 0.05$) on all strains (Table 2). This plant had an inhibitory effect on the growth of bacterial strains and *Candida albicans*. The highest inhibition was seen against *Serratia marcescens and Streptococcus salivarius* ($P = 0.000$) (Table 2).

Overall, the four extracts of *J. regia* have a stronger antibacterial activity against Gram positive (11–19mm) than Gram negative (7.2–8.9mm) bacteria. Leclerc et al. (1983) explained this difference by the chemical composition of the bacterial cell wall, especially in the presence of a cell wall impermeable to lipophilic solutes in Gram negative bacteria, these bacteria having a layer of lipopolysaccharids (LPS) which form a barrier to diffusion and making Gram negative bacteria less likely to be susceptible to antimicrobial extracts (Nostro et al., 2000). Another study that disagrees with our study indicated that Gram-negative bacteria were more resistant to some plant extracts (Vlietinck et al., 1995). In contrast, Gram positive bacteria have only an outer of peptidoglycan which is not a strong barrier of permeability (Scherrer and Gerhardt, 1971).

Juglans regia extracts, especially methanolic extract, have a significant antimicrobial effect against *Streptococcus salivarius* and *Staphylococcus aureus* ($P < 0.05$) (Fig. 1); our findings are in accordance with other studies; according to (Zakavi et al., 2013), the methanolic extract of *J. regia* exerts an antimicrobial effect against *Streptococcus sanguis, S. salivarius* and *Staphylococcus aureus* with significant difference in contrast to control.

Enterobacteriaceae were also susceptible to the different extracts (Table 2), but their effectiveness varied between them. At the concentration of 15mg/ml of ethyl acetate extract, *Serratia marcescens* was the most sensitive one (19mm), following by *Proteus vulgaris* in the presence of 15mg/ml of chloroform extract (14mm).

(Pereira et al., 2007) affirm that the *Juglans regia* extracts were screened for their antimicrobial properties against different *Enterobacteriaceae* (*E. coli, Proteus vulgaris, Klebsiella pneumonia* and *Serratia marcescens*). Another study showed that *J. regia* could be used as an easily available source of natural compounds to inhibit the growth of different Gram negative bacteria (Sharafati-Chaleshtori et al., 2011).

The results concerning the antibacterial effect of *J. regia* extracts against *Pseudomonas aeruginosa*, a major opportunistic pathogen, also presented in Table 2. Regarding the results, the Acétate d'éthyle extract showed a significant higher antibacterial activity against *P. aeruginosa* (14mm). Our results are similar to those of (Dolatabadi et al., 2018). They showed that *J. regia* extracts had inhibitory effects on biofilm formation by *P. aeruginosa* and triggering the antibiotics activities. However, the study of (Noumi et al., 2011) using walnut extracts against clinical isolates of *P. aeruginosa*, revealed positive results; the authors showed that Gram positive bacteria (*P. aeruginosa, E. coli* and *Klebsiella pneumoniae*) were sensible for some walnut extracts.

Santos et al. (2013) affirm that antimicrobial effects of *J. regia* against pathogens come from its chemical composition. *J. regia* bark contains ketones like regiolone, juglone, phenolic compounds, flavonoid and sterol. *Juglans regia* is rich in flavonoids and naphtoquinones that are responsible for the antibacterial activity of its derived extracts (Quave et al., 2008).

Furthermore, several studies showed that the antibacterial effect of plants may be due to various chemical compositions of extracts such as tannins, terpenoids, alkaloids and saponins (Tomás-Barberán et al.; Al-bayati & Sulaiman, 2008).

The extracts of *Juglans regia* were active against fungal strains, of which diameter of inhibition zone was between 10 and 13mm with

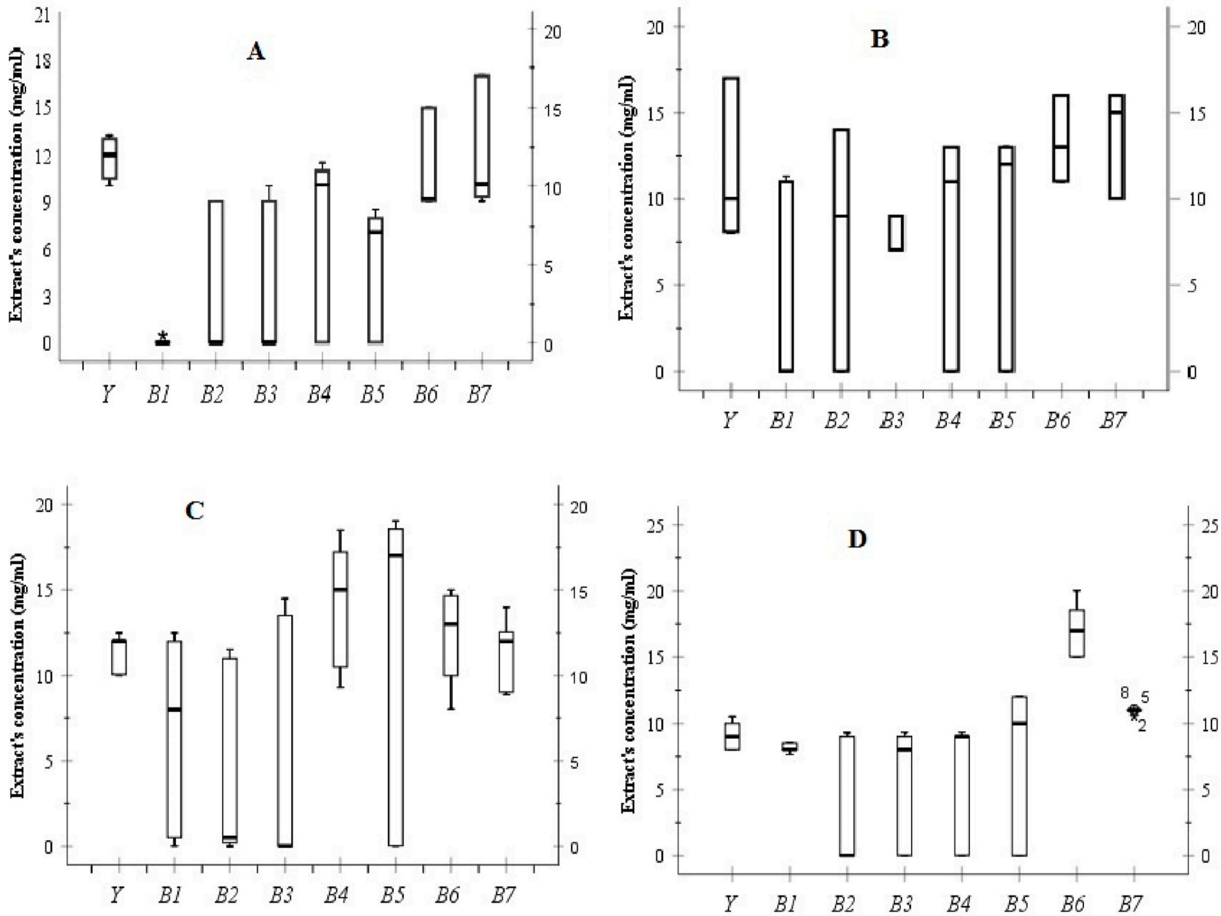

Fig. 1. Antimicrobial effect of different extracts' concentrations of *Juglans regia* on some strains isolated from the oral cavity.
A: Petroleum ether extract, B: Chloroform extract, C: Ethyl acetate extract, D: Methanolic extract. Y: *Candida albicans*, B1: *Staphylococcus saprophyticus,* B2: *Proteus vulgaris,* B3: *Pseudomonas aeruginosa,* B4: *Staphylococcus aureus,* B5: *Serratia marcescens,* B6: *Streptococcus salivarius,* B7: *Streptococcus mutans*.

petroleum ether extract, between 8 and 17mm with chloroform extract, between 10 and 12mm with ethyl acetate extract and between 08 and 10mm with methanolic extract (Table 2).

The present findings corroborate a previous investigation reporting a significant reduction in the carriage rate of *Candida albicans* after using *Juglans regia* mouthwash (Sytykiewicz et al., 2015). Also (Oliveira et al., 2007) affirm that the extract of *Juglans regia* inhibits *in vitro* the majority of oral fungal strains especially *Candida albicans*.

However, researchers investigated that almost all parts of the plant had important antifungal activity against different fungal strains tested with highest activity against *Candida albicans* (Muzaffer and Paul, 2018).

Overall, the data of this study clearly indicated that all extracts of *Juglans regia* bark significantly inhibited the growth of the tested oral bacteria and yeast.

3.3. *In vitro antimicrobial activities of Salvadora persica*

Miswak has been used since ancient times for the hygiene of the mouth (Dutta and Shaikh, 2012), as it plays a role in the protection from pathogens that enter through the mouth (Khalil, 2006). Those sticks provide clean teeth (Farooqi and Srivastava, 1967), and they make the enamel stronger (Akhtar et al., 2011). The results of the antimicrobial activity of different extracts of *Salvadora persica* are given in Table 3.

The results showed that Petroleum ether extract had the greatest effect on the majority of the tested bacteria in all three concentrations used, where as the methanolic extract showed less effect, especially on *Staphylococcus saprophyticus*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Ethyl acetate extract gave the least zone of inhibition among the four solvents in this study (Table 3).

The Petroleum ether extract of *Salvadora persica* was active against the enterobacteria strains, but its effectiveness varied between them. At the concentration of 15mg/ml, *Proteus vulgaris* was the most sensitive one (26mm), following by *Serratia marcescens* (10mm) (Fig. 2). The weakest activity of the same plant extract was demonstrated against *Pseudomonas aeruginosa* (07mm).

The high values of the inhibition zones of *Proteus vulgaris* and *Serratia marcescens* confirm the results of previous reports showing the effect of *Salvadora persica* extracts on Gram-negative bacteria, especially *E.coli* and *P. vulgaris* (Al-Judaibi, 2020). Also (Muzaffer and Paul, 2018), showed the highest mean zone of inhibition against *Proteus vulgaris* (43.12 $\pm$ 1.8mm). In addition, the acidic pH of *S. persica* can disturb the growth of neutrophile flora (Chelli-Chentouf et al., 2012). According to (Guiraud, 1998), the species of the family *Enterobacteriaceae* grow easily in a neutral medium, while an acidic pH make their growth difficult.

A low antibacterial activity was obtained against *Staphylococcus saprophyticus* and *Staphylococcus aureus* with inhibition zones' value of 9mm and 11, respectively (Table 3), at the concentration of 15mg/ml of ethyl acetate extract.

These results disagree with previous reports (AbdElRahman et al., 2002; Chelli-Chentouf et al., 2012; Abhary and Al-Hazmi, 2016; Salem et al., 2018); showed that *S. persica* have a strong antibacterial activity against *Staphylococcus* species. Other studies showed that the extracts of *Salvadora persica* showed inhibition zones ranged from 10.5mm to 31.5mm against *Staphylococcus aureus* (Ahmed et al., 2010). This disagreement may be due to different types of bacteria strains, isolation

Table 3
Antimicrobial activity profile of different extracts of *Salvadora persica* (Expressed as diameter of inhibition zone in mm).

		Inhibition zone (mm)						
		Extract's concentration (mg/ml)						
	Strain	NC	PC	05	10	15	SEM	*P value*
E1	Y	0.000	16.267	00.011	00.101	00.112	0.066	0.312
	B1	0.000	18.567^{a}	00.023^{b}	00.081^{b}	08.018^{ab}	0.066	0.056
	B2	0.000	23.033^{ab}	10.012^{b}	16.111^{ab}	26.023^{a}	0.080	0.000
	B3	0.000	20.000^{a}	00.001^{b}	07.014^{b}	09.003^{ab}	0.062	0.055
	B4	0.000	20.333	00.002	00.123	00.008	0.055	0.142
	B5	0.000	20.167^{a}	00.012^{b}	09.998^{ab}	10.023^{ab}	0.041	0.034
	B6	0.000	22.067^{a}	08.036^{b}	07.956^{b}	07.988^{b}	0.042	0.052
	B7	0.000	24.333^{a}	11.014^{b}	11.032^{b}	12.013^{ab}	0.057	0.022
E2	Y	0.000	16.267^{a}	00.007	00.034	08.023	0.047	0.062
	B1	0.000	18.567^{a}	00.012^{b}	00.214^{b}	07.354^{ab}	0.056	0.059
	B2	0.000	23.033^{a}	07.032^{b}	08.011^{b}	09.033^{b}	0.064	0.050
	B3	0.000	20.000^{a}	09.233^{b}	10.065^{ab}	09.998^{ab}	0.040	0.022
	B4	0.000	20.333^{a}	00.217^{b}	00.001^{b}	08.000^{b}	0.041	0.058
	B5	0.000	20.167^{a}	00.032^{b}	00.111^{b}	07.333^{b}	0.043	0.056
	B6	0.000	22.067^{a}	00.0211	00.366	00.000	0.015	0.412
	B7	0.000	24.333^{a}	06.025^{b}	07.012^{b}	10.321^{ab}	0.044	0.053
E3	Y	0.000	16.267^{a}	00.022^{b}	07.032^{b}	09.221^{ab}	0.036	0.036
	B1	0.000	18.567^{a}	00.001^{b}	00.011^{b}	09.023^{ab}	0.040	0.055
	B2	0.000	23.033^{a}	00.003^{b}	00.103^{b}	10.002^{ab}	0.018	0.058
	B3	0.000	20.000^{a}	00.012^{b}	00.321^{b}	09.011^{ab}	0.065	0.054
	B4	0.000	20.333^{a}	00.001^{b}	10.023^{ab}	11.002^{ab}	0.016	0.026
	B5	0.000	20.167^{a}	00.123	00.002	00.002	0.040	0.112
	B6	0.000	22.067^{a}	00.362^{b}	07.012^{b}	10.153^{ab}	0.016	0.050
	B7	0.000	24.333^{a}	08.003^{b}	08.254^{b}	07.988^{b}	0.001	0.051
E4	Y	0.000	16.267^{a}	00.023	00.213	00.111	0.001	0.243
	B1	0.000	18.567^{a}	00.111	00.023	00.014	0.009	0.457
	B2	0.000	23.033^{a}	00.147	00.123	00.258	0.058	0.325
	B3	0.000	20.000^{a}	00.025	00.036	00.001	0.042	0.365
	B4	0.000	23.033^{a}	00.000	00.022	00.223	0.040	0.475
	B5	0.000	20.167^{a}	00.123^{b}	00.002^{b}	09.452^{ab}	0.051	0.061
	B6	0.000	22.067^{a}	10.001^{b}	09.999^{b}	12.013^{ab}	0.047	0.023
	B7	0.000	24.333^{a}	07.012^{b}	07.222^{b}	08.256^{b}	0.044	0.052

E1: Petroleum ether extract, E2: Chloroform extract, E3: Ethyl acetate extract, E4: Methanolic extract. Y: *Candida albicans*, B1: *Staphylococcus saprophyticus*, B2: *Proteus vulgaris*, B3: Pseudomonas aeruginosa, B4: Staphylococcus aureus, B5: *Serratia marcescens*, B6: *Streptococcus salivarius*, B7: *Streptococcus mutans*. NC: Negative control, PC: Positive control. $^{a,b,\ ab}$Values within columns with unlike superscripts differ *(P < 0.05)*.

area and different methods used.

Based on the findings of the present study, petroleum ether extract, chloroform extract and ethyl acetate extract of *S. persica* exhibited antibacterial activity against *P. aeruginosa*, which diameter of inhibition zone was between 07 and 09mm, whereas methanolic extract had no effect against *P. aeruginosa* at any of the concentrations used, as shown in Fig. 2. Abhary and Hazmi's results which agree with our findings, recommended that the solubility of antimicrobial agents in different polarity solvents had different effects on the growth of the bacterial flora, they affirm that *P. aeruginosa* was more susceptible to the water extract than the methanol or hexane extracts (Abhary and Al-Hazmi, 2016). In addition (Mammad et al., 2015), affirm that the methanol extract of *S. persica* showed less inhibition against *P. aeruginosa.*

Streptococcus mutans and S. salivarius were selected in this study, among all *Streptococci* isolated, as they are most commonly associated with dental caries (Aas et al., 2008; Chava et al., 2012; Balto et al., 2017).

The results showed that petroleum ether extract had the greatest effect on *Streptococcus* species tested in all three concentrations used, whereas the ethyl acetate extract showed less inhibition, especially on *Streptococcus salivarius* (Table 3). *Streptococcus mutans* showed less susceptibility to methanol extract than the other extracts and *S. salivarius* was not affected by the chloroform extract at any of the concentrations used, as shown in Fig. 2.

The antimicrobial activity observed during present study was in agreement with the findings of earlier investigations where *Salvadora persica* exhibited significant antimicrobial activity against streptococci. Chelli-Chentouf et al. (2012) affirm that the miswak had an inhibitory effect on the growth of *Streptococcus mutans* and *Staphylococcus* species. Other studies reported that the *S. persica* extracts showed good antimicrobial effects on *Streptococcus mutans* and *S. sanguis* (Almas, 2001). Additionally, other authors investigated the antimicrobial effects of *S. persica* and its extract on *Streptococcus mutans* and *Lactobacillus*, and it was found a significant decrease in *Streptococcus mutans* count (Almas and Al-Zeid, 2004; Husain and Khan, 2015) suggested that this effect may be due to the interaction with bacteria, which prevents their attachment on the tooth surface.

The results of the antifungal activity of different extracts of *Salvadora persica* against *Candida albicans* are given in Table 3.

With the exception of the ethyl acetate extract, all other extracts failed to show any notable antifungal activity against yeast strains (Fig. 2). The results showed that ethyl acetate extract had a significant effect on *C. albicans* ($P < 0.05$) (Only the higher concentrations showed an inhibitory zone of 7mm and 9mm) (Table 3). This observation disagrees with a previous report of (Noumi et al., 2017) as these authors showed that ethyl acetate extract of *S. persica* had a poor performance as an antifungal agent. Less than optimal antifungal activities of *S. persica* have also been reported in a number of studies (Al Sadhan and Almas, 1999; Paliwal et al., 2007). Also (Almas, 2001), was seen that even the alcoholic extracts of *Salvadora persica* had no inhibitory effect on *C. albicans*.

On the other hand, several investigators have demonstrated that the different extracts of *S. persica* perform well as an antifungal agent (Chelli-Chentouf et al., 2012; Balto et al., 2017). In addition (Balto et al., 2013), affirm that ethyl acetate extract of *S. persica* was able to induce significant inhibition of *Candida albicans* ($P < 0.002$).

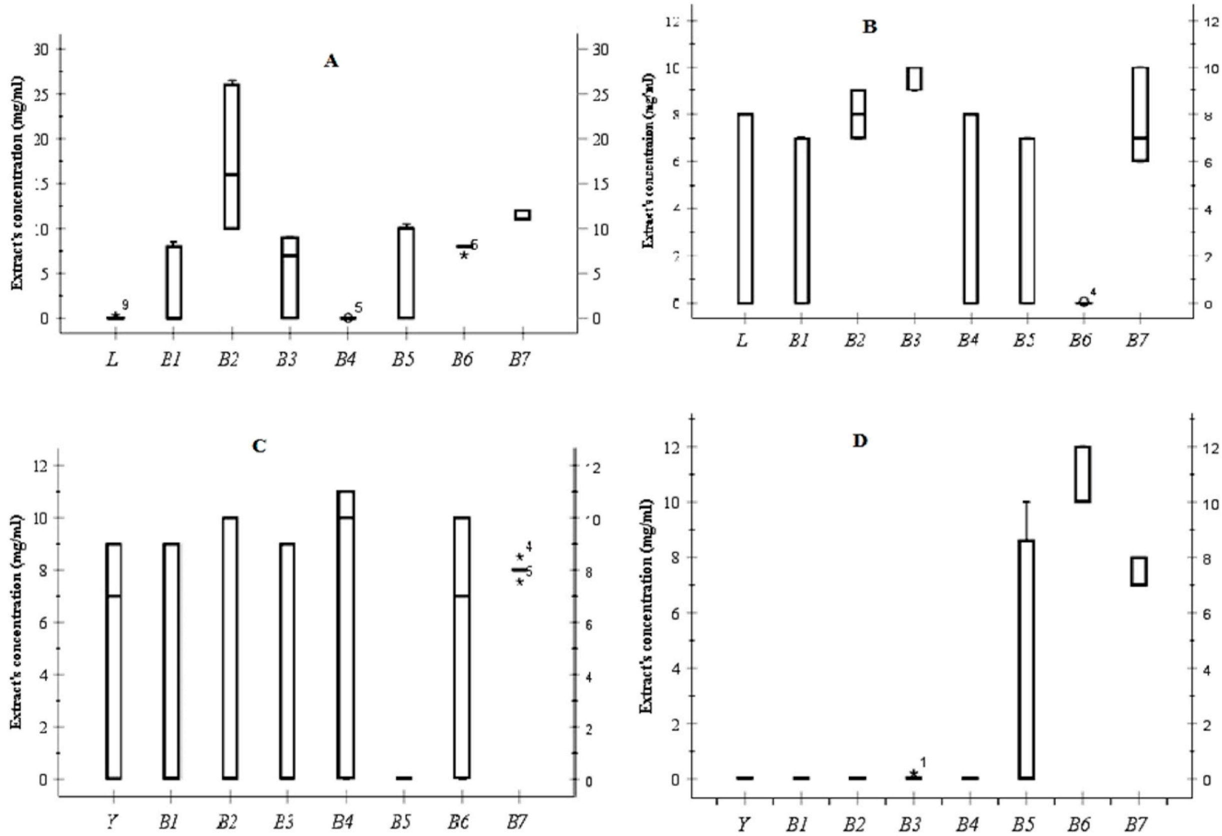

Fig. 2. Antimicrobial effect of different extracts' concentrations of *Salvadora persica* on some strains isolated from the oral cavity.
A: Petroleum ether extract, B: Chloroform extract, C: Ethyl acetate extract, D: Methanolic extract. Y: *Candida albicans*, B1: *Staphylococcus saprophyticus,* B2: *Proteus vulgaris,* B3: *Pseudomonas aeruginosa,* B4: *Staphylococcus aureus,* B5: *Serratia marcescens,* B6: *Streptococcus salivarius,* B7: *Streptococcus mutans*.

4. Conclusion

In the present study, we were able to isolate and identify several oral bacterial and fungal strains which belonging to streptococci, staphylococci, enterococci, *Pseudomonas* and *Candida*. This work was tested the effect of different extracts of *Juglans regia* and *Salvadora persica* the isolated strains.

It was demonstrated that walnut bark (largely used in our country to prevent oral hygiene) seems to be more efficient against several bacterial and fungal strains comparatively to *S. persica*. These activities may be correlated to the high tannins and phenolic content in the *Juglans regia* extracts.

Moreover, according to the results shown in the present work, the ethyl acetate extract from *Juglans regia* presented strong antibacterial activity against the isolated strains. Interestingly, *Streptococcus mutans* was the most sensitive bacteria to all extracts of the two plants tested.

Further phytochemical research is also needed to identify the substances responsible for the antibacterial and antifungal effects of *Salvadora persica* and *Juglans regia*.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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