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Phenolic Compou nds and Antimicrobial Activity of *Ziziphus jujuba* Mill. Fruit from Tlemcen (Algeria)

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Abstract

In this research, the antimicrobial function of the aqueous and organic essences obtained from *Ziziphus jujuba* pulp fruits was investigated against strains of bacteria and fungi. The RP-HPLC-PDA analysis was observed in the hydro-acetone (EA) and ethyl acetate extracts ($Acet_1$, $Acet_2$) in the presence of gallic acid, quercetin, and rutin. Most extracts present a slight antimicrobial activity with an inhibition zone not exceeding 6.5 mm diameter, except for *S. aureus* which was the most sensitive strain. The Minimum Inhibitory Concentration (MIC) values ranged between 450 and 110 mg/mL, *E. coli* and *S. aureus* are the most sensitive strains for all extracts. No particular antifungal activity of extracts was observed against *C. albicans* strain. The antimicrobial activity of *Z. jujuba* pulp fruits has not been reported before, and the present study has revealed that the phenolic compounds identified in jujube fruit extracts cannot provide an important antimicrobial effect to jujube pulp fruits.

Keywords: *Ziziphus jujuba Mill.*, RP-HPLC-PDA analysis, Antimicrobial activity, *E. coli*

Introduction

Infectious diseases are among the main causes of morbidity and mortality affecting the world, (Ahmad & Beg, 2001; Daneshmand *et al.*, 2013a*;* Alzaid *et al.*, 2020). Drug-resistant bacteria and fungi have entangled the therapy of infectious illnesses. Faced with an increasing antibiotic-resistant, the discovery of new antimicrobial compounds is a major concern for several types of research (Taramian *et al.*, 2019; Usman, *et al.*, 2019). Antimicrobial herbal compounds are considered as the potential medical resources (Daneshmand *et al.*, 2013b). *Zizyphus jujuba* Mill. (Rhamnaceae family) commonly known as jujube or Chinese red date has several medicinal properties, it is used as foods and remedies for insomnia, fever, diarrhea, wounds, and ulcers (Abd-alrahman *et al.*, 2013). In previous phytochemical studies of jujube fruits, the results have highlighted a lot of their biological impacts, including the anticancer, anti-inflammatory, sedative, immunostimulating, antioxidant, antimicrobial and hepatoprotective; these effects are mainly related to the richness of this species in various constituents, including Alkaloids (Zizyphine C, D, and E) flavonoids (quercetin, catechin, rutin), terpenoids (Betulinic acid), amino acids (proline, aspartic acid, and glutamic acid), lipids (oleanolic acid, betulonic acid, oleanonic acid, palmitic acid, oleic acid) sugars (glucose, galactose, and sucrose) and polysaccharides (Jiang *et al.*, 2007; San & Yildirim, 2010; Daneshmand *et al.*, 2013a; Elaloui *et al.*, 2014; Elaloui *et al.*, 2015; Damiano *et al.*, 2017; Adjdir *et al.*, 2019b). In Algeria different parts of *Z. jujube* are widely used in traditional medicine, however as far as we know, no reports exist on antimicrobial activity of fruits. Hence, the present study has been established to evaluate the antibacterial and antifungal activity of dried pulp fruits extracts of *Z. jujuba* harvested from Tlemcen in the North of Algeria.

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Materials and Methods

Plant Material

Fruits of *Z. jujuba* were collected in September during the fruit maturation period from Tlemcen city in western Algeria (Latitude: 34° 53′ 24″ Nord, 1° 19′ 12″ Ouest; Altitude: 715m). Botanical identification of the plant was authenticated in the lab of Ecology and Ecosystems Management, Department of Biology, University of Tlemcen, Algeria. In the laboratory, fruits were cleaned and dried at room temperature in the shadow.

Preparation of Plant Extracts

Extracts of *Z. jujuba* Mill. were obtained by maceration at room temperature: 10 g of dried fruits in 300 ml of solvent: distilled

water for the aqueous extract (Aq); acetone–water and methanol-water mixtures (70/30 v/v) to prepare the extracts EA and EM extracts, respectively. The aqueous (Aq) and acetone-water (EA) extracts underwent fractionation by liquid-liquid extraction using ethyl acetate and n-butanol. The fractions recovered from the acetone-water extract were $Acet_2$ and $n\text{-}but_2$; those of the aqueous extract were $Acet_1$ and $n\text{-}but_1$, respectively.

RP-HPLC-PDA Analysis of Phenolic Acids and Flavonoids

Separation and identification of phenolic compounds of the hydro-acetone extract EA of *Z. jujube* and the ethyl acetate fractions $Acet_1$ and $Acet_2$ were carried out by RP-HPLC-PDA technique (Adjdir *et al.*, 2019a; El-Haci *et al.*, 2020). Using a binate pump transmission method and an Eclipse ODS Hypersil C18 column (150 mm × 4.6 µm), RP-HPLC-PDA investigation of phenolic compositions was carried out on a Perkin Elmar Flexar system. The portable phase included resolvent A- Acetic acid (2%) and B-Acetonitrile. The gradient elution system was: 5 min with 10 % of B; 25 min with 90 % of B and 15 min of linear gradient from 90 % to 100% of B, then, 15 min have included equilibration. The outflow extent was 1 ml/min. The chromatograms were controlled at 280 nm. The compositions' diagnosis and maximum transfers were performed according to their confinement durations as well as correlating with criteria applied.

Assessment of the Antibacterial Function

The Clinical and Laboratory Standards Institute (CLSI) recommended Two approaches that were applied; the disk dispersion and the microdilution approach. Only extracts that have shown important results by the disk-method have been evaluated for their activity by the microdilution method. In this study, five various American Type Culture Collection (ATCC) reference pathogenic bacteria and three fungal strains were utilized. Gram-negative types: *Escherichia coli* ATCC 25933, *Acinetobacter baumanni* ATCC19606, Gram-positive species: *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, *Bacillus cereus* ATCC 11778, and fungal strains *Candida albicans* ATCC 10231, 26790 and IP 444.

The Disk Diffusion Method

Every essence was liquefied in dimethyl sulfoxide (DMSO) in order to obtain 1000 mg/mL ultimate condensation. Gentamicin (Gent 10µg/mL) along with amphotéricin B (AmB 10µgmg/mL) served as the positive control antibacterial and antifungal. The dimness of the suspensions was regulated at 0.5 McFarland criteria corresponding to 108 CFU/mL. The Petri dishes injected with the suspension regulated at Mueller-Hinton agar by an ear stick. After dehydrating, the antiseptic filter paper disk (6 mm diameter) was subsequently saturated with 10 µL of every essence. Petri dishes were nurtured at 37 °C, for 18-20 hours. To investigate the sterile or antifungal property, the area of development prevention encompassing the disks was measured.

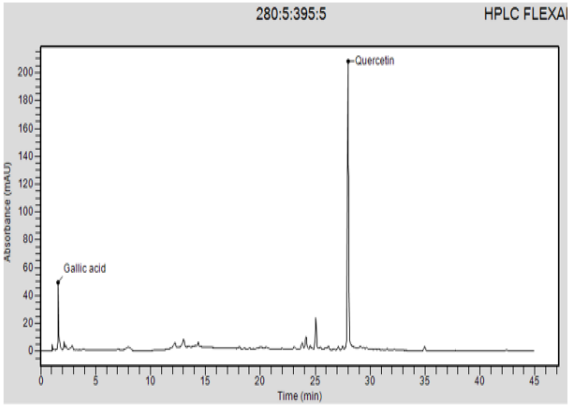
Specification of Minimum Inhibitory Concentration (MIC)

The inoculums got ready and the entire bacterial suspensions were regulated at 0.5 McFarland standard dimness. Sequential subtilizations in Muller Hinton broth were prepared to achieve an ultimate condensation of 5.104CFU /ml by well. In the mentioned experiment, sterile 96 well microplates were utilized. Then, the essences were transmitted to each microplate well, with concentrations ranged from 110 to 450 mg/ml, and the microplates have been nurtured at 35±2 ºC, for 16 to 20 hours. The development of the microorganisms in the microdilution wells, as diagnosed by the unaided eye is completely inhibited by the lowest condensation of the essences called the MIC.

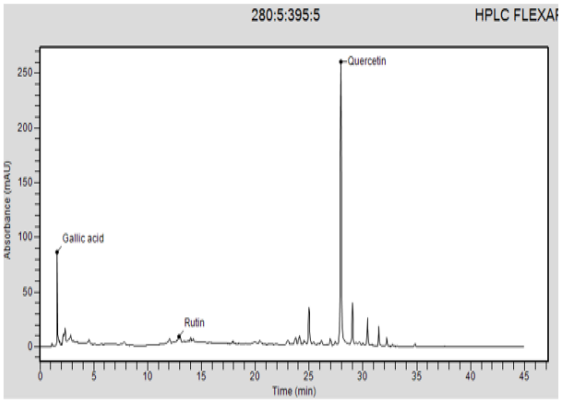
Results and Discussion

Recognition of Phenolic Compounds

Chromatographic evaluation by RP-HPLC-PDA indicated in the crude hydro-acetone extract EA and in the ethyl acetate fractions $Acet_1$ and $Acet_2$ of *Z. jujuba* fruits in the presence of gallic acid and quercetin, rutin was only discovered in Acet2 **(Figure 1)**. In a previous study, these extracts revealed a high content in polyphenols, between 50.96 and 94.70 mg gallic acid equivalent per g extract, and in flavonoids from 75.73 to 427.33 mg catechin equivalent per g extract (Adjdir *et al.*, 2019b). In addition, the total phenolic value of fruits methanolic essences from fifteen selected jujube genotypes of Turkey varies between 42 and 40 mg gallic acid equivalent/g DW (Kamiloglu *et al.*, 2009). According to the literature, Gao *et al.* (2012) have determined a high total phenolic content, in *Z. jujube* harvested in china, ranged from 275.6 to 541.8 mg of GAE/100 g FW and a dozen phenolic compositions: gallic acid, cinnamic acid, chlorogenic acid, caffeic acid, ferulic acid, ellagic acid, catechin, epicatechin, rutin, and quercetin. Elaloui *et al.* (2015) have also shown in *Z. jujube* pulp from Tunisia phenolic compounds especially rutin and chlorogenic acid.



a)



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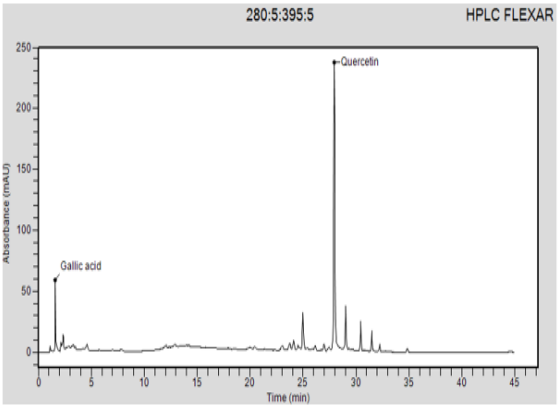

c)

Figure 1. High-performance Liquid Chromatography Profile of Z. Jujube Fruit Extracts (at 280 nm) a): EA; b): Acet2 and c): Acet1

Antibacterial and Antifungal Activity

The growth inhibition zones of the tested extracts, measured by disk diffusion method, have shown that most extracts present a slight antimicrobial activity with an inhibition zone not exceeding 6.5 mm diameter **(Table 1)**, except for *S. aureus* which was sensitive to EA (8mm), $Acet_2$ (7mm), $n\text{-}but_1$ (9mm) and $n\text{-}but_2$ (9mm). The minimum inhibitory concentration results **(Table 2)** show high MIC values ranged between 450 and 110 mg/mL. *E. coli* and *S. aureus* are the most sensitive strains for all extracts. No particular antifungal activity was observed against *C. albicans* strains **(Table 3)**. On the contrary, several studies have demonstrated the antimicrobial effect of the extracts fruits and other parts from Z. jujube. The ethanolic extract of the fruit is an inhibitor of pediatric infectious strains, *S. aureus* (MIC=2.26mg/mL), *C. albicans* (MIC=2.35mg/mL), and *A. fumigatus* (2.86mg/mL), respectively (Daneshmand *et al.*, 2013b). Snakin-Z, a new cationic peptide (31 amino acids) derived from *Z. jujuba* fruits has broad-spectrum antimicrobial activity against bacteria, *S. aureus* (MIC=28.8µg/mL), *E. coli* (MIC=13.6 µg/mL), and fungi *C. albicans* (MIC=8.23µg/mL) (Daneshmand *et al.*, 2013a). The hydro-ethanolic extract of seeds inhibits *S. aureus* (41.25µg/mL; 10mm), *E. coli* (52.5µg/mL; 17.5mm), and *K. pneumonia* (42.5µg/mL; 17mm) (Abd-alrahman *et al.*, 2013). Moreover, alphitolic acid a triterpenoid identified from leaves is a significant antibiofilm against *S. mutans*, a causative agent of human dental caries (Damiano *et al.*, 2017). In the literature, several studies have proved the inhibitory effect of phenolic compounds and flavonoids on microbial strains; it has been reported that Gram-positive bacteria are hypersensitive to herb essences than Gram-negative due to hydrophobic lipopolysaccharide in the outer membrane that protects various factors (Ferreira *et al.*, 2012; Mezni *et al.*, 2015).

Table 1. Mean Inhibition Zone Diameter (mm) of *Ziziphus jujuba* Mill. Fruit Extracts on Tested Bacteria by Disk Diffusion Method

	Extracts (1000mg/mL)							**Control (10µg/ disk)**
	Aq	EM	EA	$Acet_1$	$n\text{-}but_1$	$Acet_2$	$n\text{-}but_2$	Gent
***Staphylococcus aureus* ATCC 25923**	6,5 ± 0,17	6,5 ± 0,2	8 ± 0,8	6,5 ±0,6	9 ±1,0	7 ±0,33	9 ±2,5	22
***Bacillus cereus* ATCC 11778**	6,5 ± 0,17	-	-	-	-	-	6,5 ±0,00	18
***Bacillus subtilis* ATCC 6633**	6,5 ±2,5	-	-	-	-	6,5 ± 0,19	6,5 ±0,6	17
***Escherichia coli* ATCC 25933**	6,5 ± 0,17	6,5 ± 0,19	6,5 ± 0,00	6,5 ±0,00	6,5 ± 0,25	-	6,5 ±0,17	18
***Acinetobacter baumanii* ATCC 19606**	6,5 ± 0,25	-	-	-	6,5 ± 0,00	-	-	17

(-): No activity; ND: no determined,

Table 2. The MIC Values (mg/mL) of *Z. jujuba* Mill. Fruit Extracts against Bacteria

	Extracts							Control
	Aq	EM	EA	$Acet_1$	$n\text{-}but_1$	$Acet_2$	$n\text{-}but_2$	Gent
Staphylococcus aureus ATCC 25923	450	110	110	230	110	230	110	0,19
Bacillus cereus ATCC 11778	230	-	-	-	-	-	230	0,19
Bacillus subtilis ATCC 6633	230	-	-	-	-	110	230	5,20
Escherichia coli ATCC 25933	450	110	110	110	110	-	110	0,32
Acinetobacter baumanii ATCC 19606	230	-	-	-	110	-	-	0,65

(-): No activity

Table 3. Mean Inhibition Zone Diameter (mm) of *Z. jujuba* Mill. Fruit Extracts on *C. albicans* Strain by Disk Diffusion Method

	Extracts (1000mg/mL)							Control (10µg/disk)
	Aq	EM	EA	$Acet_1$	$n\text{-}but_1$	$Acet_2$	$n\text{-}but_2$	AmB
Candida albicans ATCC10231	-	-	-	-	-	-	-	32
Candida albicans IP 444	-	-	-	-	-	-	-	30
Candida albicans ATCC 26790	-	-	-	-	-	-	-	30

(-): No activity

Conclusion

In this study, the antimicrobial activity of *Z. jujuba* fruits pulp from Tlemcen is the first report, these extracts have previously shown *in vitro* an important antioxidant activity related to their higher content on phenolic compounds (Adjdir *et al.*, 2019b). Quercetin, gallic acid, and rutin identified from the pulp fruits have no contribution to the antimicrobial activity of jujube. However, the synergetic effect of these molecules with other phytoconstituents could be primarily responsible for other biological activities of jujube such as the antioxidant effect and the nutritional potential.

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Conflict of Interest: None

Financial Support: None

Ethics Statement: None

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