Antimicrobial activity of fruits extracts of the wild jujube "*Ziziphus Lotus* (L.) Desf.

Rsaissi.N [(1)], EL KAMILI[(1)], B. Bencharki [(1)], L. Hillali[(1)] & M. Bouhache [(2)]

Abstract— In Morocco, Wild jujube "*Ziziphus Lotus* (L.) Desf." is a very common fruit shrub in arid and semi-arid region. Fruits of this species are traditionally used for treatment of many diseases. The objective of this study is to evaluate in vitro the biological activity of the extracts of the fruits of this shrub, extracted successively by maceration with different organic solvents of increasing polarity (ether, dichloromethane and methanol), on four Gram negative and four Gram positive bacteria species and four species of filamentous fungi. All extracts showed an activity on different studied bacterial species. At the concentration of 4000 µg/disk, the etheric and methanolic extracts were the most active by inducing growth inhibition diameters between 11 and 20 mm of *Bacillus subtilis*, *Bacillus cereus*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Salmonella Typhi*, *Escherichia coli*, *Enterococcus faecalis and Pseudomonas aeruginosa*. At the concentration of 20 mg/ml, these extracts showed an interesting activity on the four fungi species: *Fusarium culmorum*, *Aspegillus ochraceus*, *Penicillium italicum*, *Rhizomucor sp*. The inhibition rates ranged from 31 to 85% and 17 to 76% at the second and the fifth day of incubation, respectively. Based on chemical analyses, the fruits of wild jujube contain phenols, flavonoids and tannins, which explain their high antimicrobial activity. Indeed, a strong correlation was noted between the concentrations of these components in the fruits extracts and their antimicrobial activity. These results confirm some uses of wild jujube in traditional medicine.

Keywords— *Ziziphus lotus* (L.) Desf, antimicrobial activity, phenolic acids, flavonoids, tannins.

1 INTRODUCTION

In Morocco, the wild jujube (*Ziziphus lotus (L.) Desf*) commonly called "Sedra" is a species found in many habitats in arid and semi-arid regions. The fruits are drupes welded rings. The endocarp is edible sweet mucilage commonly called "Nbag". They are marketed for human consumption and for their medicinal properties antipyretic, tonic, healing and antiviral [1] and known by its content of biologically active material [2] [3].

The objective of the present study is to evaluate in vitro the activity of extracts from *Ziziphus lotus (L.) Desf* fruits on the growth of some bacteria involved in diseases and poisoning of humans and on some fungi responsible for toxicosis of livestock and to identify the active molecules of the extract by chemical analysis.

2 MATERIALS AND METHODS:

2.1 PREPARATION OF PLANT MATERIAL

The plant material used consisteted of the wild jujube fruit "*Ziziphus lotus (L) Desf*." harvested from the area of El Brouj at Chaouia region, Morocco. After separating the cores by means of a copper mortar. The edible portion (pulp) was ground by using an electric grinder with a sieve with a mesh size of 0.80 mm (20 mesh). The resulting particles were then dried in an oven at 40 ° C for one day. After drying, this homogenate has been transformed into a fine powder by using an electric mixer.

2.2 PREPARATION OF ORGANIC EXTRACTS

The extraction was carried out by successive depletion of homogenates with solvents of increasing polarity according to Diallo et *al.* [4]'s method: petroleum ether followed by dichloromethane and ended by methanol. We used 50 g of fine pulp powder and 400 ml of each solvent. Each maceration was performed under mechanical stirring for 24 hours. The extracts were filtered using Whatman paper and concentrated under Vacuum with Rotary Evaporator to 40 °C. At the end, these extracts were stored in a refrigerator at 4 ° C until use.

2.3 PHYTOCHIMICAL SCREENING

2.3.1 Qualitative screening

-Phenolics

For the detection of phenolics, we added 1 ml of Na2Co3 (20%) and 1 ml of Folin Ciocalteu to 3 ml of the extract. The appearance of a blue color indicates the presence of phenolics.

-Flavonoids

For the detection of flavonoids, we added 1 ml of NaOH (10%) to 3 ml of the extract. The appearance of a yellow color indicates the presence of flavonoids [5].

-Tannins:

For the detection of tannins, we added 2 ml of FeCl3 to 5 ml of the extract. The appearance of a dark blue color indicates the

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presence of tannins [6].

2.3.2 Quantitative screening

2.3.2.1 Determination of total phenolics

The total phenolics were quantified by using reagent Folin-Ciocalteu colorimetric method [7] [8]. A quantity of 0.5 g of each organic extract was dissolved in 10 ml of methanol. After stirring, 1 ml of these solutions was added to 1 ml of Folin Ciocalteu diluted 10 times. After 4 min, 8 ml of distilled water was added and 1 ml of a solution of sodium carbonate Na2CO3 (7.5%). The mixture was vortexed. After incubation for 2 hours at room temperature, the absorbance was measured at 760 nm. The total phenolics content was deduced from the established calibration curve based on Gallic acid (0-200 g /ml) and were expressed in micrograms of Gallic acid equivalent per mg of dry matter (GAE µg /mg).

2.3.2. 2 Determination of flavonoids

The flavonoids in the extracts from different parts of *Zizyphus lotus* were estimated and quantified by the AlCl3 method [9] [10].A quantity of 1 ml of each extract diluted 10 times in methanol was added to 1 ml of AlCl3 (2% in methanol). After incubation for 10 min at room temperature, the absorbance of the mixture was read at 430 nm. The concentrations of flavonoids were deduced from the established calibration curve based on quercitrin (0-100µg/ml) and were expressed as micrograms of quercetin equivalent per milligram of dry matter (µg QE/mg).

2.3.2.3 Determination of tannins

The dosage of condensed tannins in the extracts of *Zizyphus lotus* was performed according to the method of Heimler et *al*. [11]. To 400 µl of each sample extract or standard a 3 ml of a solution of vanillin (4% in methanol) and a 1.5 ml of concentrated hydrochloric acid were added. The mixture was incubated for 15 min and the absorbance read at 500 nm. The concentrations of condensed tannins were deduced from the established calibration curve based on catechin (0-300µg/ml) and were expressed in micrograms of catechin equivalent per milligram of dry matter (CE µg/mg).

2.4. ANTIBACTERIAL TESTS

2.4.1 Bacterial Species:

Bacterial species used are:

Gram-: *Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853, Klebsiella pneumoniae. Salmonella typhi.*

Gram +: *Staphylococcus aureus, Enterococcus faecalis, Bacillus subtilis* and *Bacillus cereus*.

The first two species gram - were obtained from Department of Microbiology and Parasitology health of the Faculty of Pharmacy, Barcelona, Spain. Other species are obtained from the Pasteur Institute of Casablanca, Morocco.

2.4.2 Determination of antibacterial susceptibiliy:

The antibacterial activity of different extracts of *Ziziphus lotus* fruits was tested by using antibiogram method. The Muller Hinton agar medium was inoculated by spreading the bacterial inoculums on the media. Commercial discs (6 mm in diameter), sterilized at 120 ° C for 15 min, were impregnated separately with different extracts previously dissolved in DMSO (1g/ml) and were deposed on the medium. Four rates of each extract were used, 1000, 2000, 3000 and 4000 µg/disk. Two negative checks containing DMSO and solvent used in the extraction and one positive containing 30 µg of Amoxilin were provided. Antibiograms were repeated three times. The evaluation of the activity was assessed on the basis of diameters of inhibition and according to the following scale:

Ø = 0: no activity Ø ≤ 10mm: low activity
10 <Ø ≤ 12mm: significant activity;
12 <Ø ≤ 15mm: moderately significant;
15 <Ø ≤ 20mm: very significant activity;
20mm <Ø: highly significant activity.

2.5 ANTIFUNGALS TEST

2.5.1 Species of fungi

We used four species of fungi including:

- *Fusarium culmorum*: a cosmopolitan fungus. It is pathogenic to cereals and responsible for root rot. This fungus produces rubofusarine. Its extracts are toxic to rabbits [12];
- *Aspegillus ochraceus*: very widespread. It is common on decaying vegetation and isolated moldy grain. This fungus is responsible for fatal poisoning of livestock. It elaborates various toxins among which the phenillic acid and ochracine A [12];
- *Penicillium italicum*: agent of the green-blue rot of citrus [12]. Also isolated from soil and decaying plants;
- *Rhizomucor sp*: very spread in soil, cereal grain stored and its derivatives. It is pathogenic for animals and cause toxicosis with abortive effects [12].

These species were isolated from samples of stored cereal grain by using the culture medium marketed under Czapek Dox Agar. They have been regularly maintained by subculture on the nutrient medium PDA (Potato Dextrose Agar) in the Agri-Food and Health Laboratory of University Hassan I. Settat. Morocco.

2.5.2 Evaluation of antifungal activity:

The evaluation of the antifungal activity of the extracts of jujube fruit was determined by the technique of poisoned medium. Thus, we added 1 g of each extract to 50 ml of culture medium nutrient PDA, which is molten at a temperature of 50 ° C, to get a concentration of 20 mg/ml. After gentle agitation, the mixture was passed on sterilized petri dishes (40 mm diameter). A negative check without extract and a positive check containing the fungicide difenoconazole at rate of 30 µg /ml were used. After solidification of the medium, seeding was done by depositing fragments of 6 mm in diameter, collected aseptically from the periphery of the mycelia mats of each fungal culture of 7 days. The incubation was performed in an oven at a temperature of 25 ° C. Each test was repeated three

times.
The assessment was done at the second and fifth day of incubation, by measuring the diameter of growth of each deposit of four tested species of fungi.
The growth inhibition rate (ICT) was calculated using the formula of Abbot [13]:
ICT = (DT-D0) / DT x 100
DT: diameter of fungal fragments of negative check (mm)
D0: diameter fungal fragments in the presence of the extract or antibiotic.
The evaluation of the activity was based on the growth inhibition according to the following scale [14]:
• Between 75 and 100%: very active. The fungal species is very succeptible.
• Between 50 and 75%: active. The fungal species is succeptible.
• Between 25 and 50%: moderately active. The fungal species is limited.
• Between 0 and 25%: little or no assets. The fungal species is very succeptible or resistant.

2.6. STATISTICAL ANALYSIS

The obtained results were subjected to analysis of variance. The comparison of means was made with the Tukey test (HSD) at 5% probability. The statistical software used is the Statistix. Version 9.0.

3. RESULTS AND DISCUSSION

3.1 CHARACTERIZATION AND YIELD OF EXTRACTS:

The characteristics and yield of each jujube fruit extracts are shown in Table 1.
Table 1. Characteristics and yield of jujube fruit extracts

Extracts	Aspects and color	*Output extraction (%)	Yield pulp/ fruit (g/Kg)	Yield final Extracts/Fruit (g/kg)
Etheric	Pasty,dark green	3,80	500	19,00
Dichloro-methanic	Oily,military green	1,60	500	8,00
Methanolic	Slimy,dark brown	8,30	500	41,50

* (Mass of extract / mass pulp macerated) X 100

Similar yields (3.33, 1.19 and 6.40%) were obtained by Diallo et *al.* [4] of leaves extracts of related species *Ziziphus mauritiana* L. using the same extraction method. So for 1kg of fruit could be extracted in total 68.5 g of extracts: 19; 8 and 41.5 g, respectively of etheric, dichlomethanic and methanol extracts.

3.2 RESULTS OF PHYTOCHEMICAL ANALYSES

3.2.1 Qualitative Analysis

Photochemical analyses of various extracts of jujube fruit pulp showed the presence of phenol acids, flavonoids and tannins (Table 2). The most abundant are composed mainly phenol acids and tannins.

Table 2: Results of photochemical characterization of extracts of jujube fruit

Extraicts	Phenolic acids	Flavonoids	Tannins
Ethéric	++++	++	+
Méthanolic	+++	++	++++
Dichlorométhanic	+	+	+

+: coloring little abundant, + +: medium coloring;
+ + abundant coloring; + + + + very abundant coloring.

3.2.2 Quantitative Analysis

The concentrations of Total phenols, flavonoids and condensed tannins derived from calibration curves are shown in Table 3. These results show that the fruits of the wild jujube "*Z.lotus*" contain about 82.62, 46.21 and 336.24 mg /kg, respectively, total phenols, flavonoids and condensed tannins.
The ethereal extract is rich in phenolic acids (1,31 µg EGA/mg dm) and moderately rich in flavonoids (0,72 µg EQE/mg dm), while the methanol extract is rich in condensed tannins (7,53 µg ECT / mg dm) and phenolic acids (1.26 µg EGA/mg dm) and moderately rich in flavonoids (0.73 µg EQE / mg ms).The dichloromethane extract contains small amounts of these compounds, or 0.68 µg EAG / mg dm, 0.28 µg EQE /mg dm and 0.76 µg ECT/mg dm, respectively, of phenolic acids, flavonoids and condensed tannins. These results are different than those of Djemai Zoughlache et *al.* [15]. They have found the concentrations of total phenols in the range of 2.34, 1.99 and 5 EAG µg /mg dm, flavonoids in the range of 0 , 64, 0.71 and 0.83 µg EQA/mg dm and tannins in the order of 0, 0 and 4.54 ECT µg/mg dm, respectively, for the etheric, dichlorome

Table 3: Results of phytochemical quantitative analysis of jujube fruit extracts

Extracts	Extracts concentrations of pulp phenolic compounds and tannin			Yield Extracts/Fruit (g/Kg)	Content in mg / kg of fruit		
	Total Phenols (µgEGA/mg dry matter)	Flavonoids (µg EQE/mg dry matter)	Tannins (µgECT/mg dry matter)		Total phenols EGA	Flavonoids EQE	Tannins ECT
Etheric	1,31	0,72	0,93	19,00	24,89	13,68	17,67
Dichlorométhanic	0,68	0,28	0,76	8,00	5,44	2,24	6,08
Methanolic	1,26	0,73	7,53	41,50	52,29	30,29	312,49
Tatal	3,25	1,73	9,22	68,50	82.62	46,21	336,24

EGA: Equivalent of Gallic Acid; EQE: Equivalent of Quercetin, ECT: Equivalent of catechin, dm: dry matter

of polyphenolic compounds and tannins may be related not only to the variety, but also to influences of the extraction methods and conditions, the stage of maturity and fruits harvest, storage conditions after harvest , the biogenetic and environmental factors, the dosage of reagents and type of spectrophotometer used [16] [17] [18].

3. ANTIBACTERIAL ACTIVITY:

Table 4 shows the diameters of the inhibition of bacterial growth obtained for the pulp extracts of jujube (*Z. lotus*) fruit and for rates of 1000 to 4000 µg /disc. The zones of inhibition were measured in mm.

Table 4: Evaluation of the antibacterial activity (diameters of the inhibition) of different extracts of fruit (pulp) of jujube "*Ziziphus lotus L. Desf.*)"

Bacterial species	Extract quantity												
	1000 µg			2000 µg			3000 µg			4000 µg			30 µg
	Etheric	Dichloro-méthanic	Methanolic	Etheric	Dichloro-méthanic	Methanolic	Etheric	Dichloro-méthanic	Methanolic	Etheric	Dichloro-méthanic	Methanolic	Amoxillin
Bacillus subtilis	14,33 a	10,33 a	13,00 ab	16,00 a	15,00 a	16,00 a	17,00 a	16,00 a	17,67 a	20,00 a	18,00 a	20,00 a	20,67 bc
Bacillis cereus	13,33 b	9,67 a	11,67 a	15,00 b	11,67 bc	14,67 a	17.00 a	13,67 b	16,00 ab	18,33 b	14,33 b	17,67 b	20,67 bc
Escherichia coli	11.67 c	8,00 b	10,33 abc	14.67 al	11,00 bc	13,00 b	15.67 a	13,00 b	14,67 b	18,00 b	15,00 b	16,67 bc	26,00 a
Klebsiella pneumoniae	11,00 cd	8,00 b	10,33 abc	14,00 b	10,67 c	12,00 b	15.67 a	11.67 c	15,00 b	17,67 b	13,67 bc	16,00 c	22,67 b
Salmonella typhi	10.67 d	8,00 b	8,00 bcd	12.33 c	9,00 d	9,00 c	13.67 b	11.67 c	12,33 c	16,00 c	12,67 c	13,67 d	20,67 bc
Staphylococcus aureus	10.33 d	6,67 bc	6,67 cd	11,67 c	8,00 e	9.67 c	14.00 b	8.67 d	10,67 cd	15,67 c	10,00 d	13,00 d	20,00 c
Enterococcus faecalis	8.33 e	6,67 bc	7,00 d	10,33 d	8,00 e	8,00 d	12.67 bc	9,00 d	9,33 d	14,00 d	10,67 d	11,00 e	16,00 d
Pseudomonas aeruginosa.	7,67 e	6,00 c	6,33 cd	9.67 d	6,67 f	8,00d	11.33 c	7,00 e	9,00 d	12,00 e	7,33 e	11,00 e	0,00 e
Standard error	0,27	0,41	1,12	0,37	0,24	0,45	0,38	0,33	0,47	0,46	0,41	0,34	0,68

Means followed by the same letter are not significantly different according to Tukey HSD (P = 5%).

These results show that the diameters of growth inhibition zones vary depending on the type and quantity of extracted and species tested of bacteria. Moreover, these extracts have antibacterial activity even at a low dose (1000 µg). The 4000 ug/disk etheric extract showed a very significant activity (15 <Ø ≤ 20mm) of *Bacillus subtilis, Bacillus cereus, Staphylococcus aurens, Klebsiella pneumoniae, Escherichia coli* and *Salmonella typhi,* moderately significant (12 <Ø ≤ 15mm) for *Enterococcus faecalis* and significant (Ø = 12 mm) for *Peudomonas aerogignosa*. The Tukey HSD (p=5%) allowed to classify these bacteria according to their succeptibility to the extract as follows: *B.subtilis*>*B.cereus*=*E.coli*=*K.pneumonia*>*S.typhi*=*S.aurens*>*E.Faecalis* >*P.aerogignosa*. Of more or less similar results were observed for the methanol extract with a very important activity against the first four bacteria, moderately important activity against *S.typhi* and *S. aurens* and significant activity (Ø = 11 mm) against *E. faecalis* and *P.aerogignosa*. The ranking of the susceptibility of these bacteria to the methanol extract according to the Tukey HSD test is as follows: *B.subtilis*> *B.cereus* = *E.coli* ≥*K. pneumoniae*>*S. thyphi*= *S.aurens*> *E. Faecalis*= *P. aerogignosa*. In contrast, the dichloromethanic extract has the lowest antibacterial activity, compared to other extracts , with a very significant activity against B. subtilis to moderately significant against *B. cereus, E. coli and K. pneumoniae*, whereas for other bacteria that activity was low. The ranking of the susceptibility of these bacteria according to the Tukey HSD test is as follows:*B.subtilis*>*B.cereus*=*E.coli*≥*K.pneumoniae*≥*S.typhi*>*S.aurens*=*E .Faecalis*>*P.aerogignosa*.

These results are more or less similar to those of Naili et *al.* [19] who reported a highly significant activity (20mm <Ø) against *B. sibtilus* and a very significant activity (15 <Ø ≤ 20mm) against *S. aurens* for the methanol extract of leaves of the same species of jujube at concentrations of 12.5 to 25 micrograms/ml. As for *E. coli, P. aerogignosa* and *S.typhi* these authors noted a low activity (7 ≤ Ø ≤ 10mm) for an extract concentration of 1000 µg /disc.

From Figure 1 and statistical analysis we can conclude that compared to the antibiotic Amoxilin, the etheric extracts of jujube fruit pulp has the highest antibacterial activity, showing growth inhibition zones high on most of the bacteria, followed by the methanol extract and finally by dichlorometanic extract. Also, the etheric extract had significant activity against *P. aeruginosa*, and these bacteria showed a very high resistance against the antibiotic Amoxicillin.These results partially corroborated with those of Djemai Zoughlache et *al*. [15], which showed that the ether extract of *Zizyphus lotus* seems to have the most potent inhibitory effect on four species of bacteria namely *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae and Pseudomonas aeruginosa.*This activity could be due to the synergic effect of other polar molecules in the etheric extract. However, hypothesis could be confirmed by

the work of Nasif [20] on the seeds of *Zizyphus spina christi* and showed that fatty acids have a strong antibacterial activity.

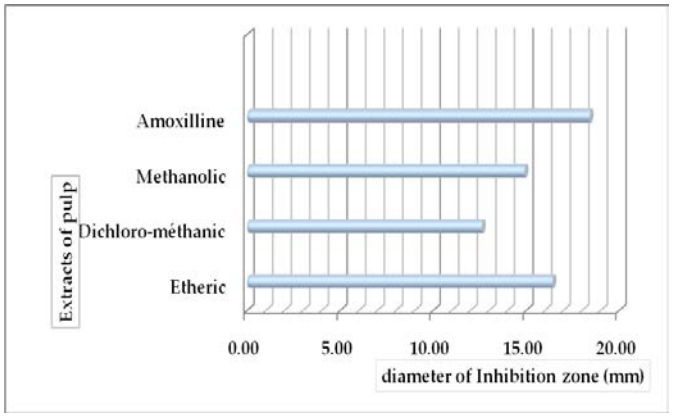

Fig. 1 Antibacterial activity of extracts of jujube fruit pulp (*Z.lotus L.(Desf.*)

With the exception of *E. faecalis* (Gram+) and *P. aeruginosa* (Gram-), all other tested bacterial species were more susceptible to various extracts, which can be attributed to the existence of a mechanism of resistance of genetic origin. In effect, *E. faecalis* has been reported resistant to many antibiotics commonly used [22] [23] [24]. Similarly, *Pseudomonas aeruginosa* is naturally susceptible to a limited number of antibiotics and emerging multi-resistant strains, even tolerant, thus, an anxiety subjet of hospital settings is noted [25]. Almost all strains of this bacterial species produce an inducible beta-lactamase (the enzyme), wide spectrum, AmpC capable of hydrolyzing the amoxilin, ampicillin, cephalosporins, first or second generation, whose céfotaxine and ceftriaxon [26]. The very low permiability of the outer membrane of the bacille pycyanique (10 to 100 times lower than that of *Escherichia coli*) promotes the activity of this enzyme by slowing the penetration of betalactamines in the interior of the bacteria [27]. Also, *P. aeruginosa* possesses a dozen different active efflux systems, with at least two are involved in resistance to antiseptics and antibiotics [28].

A strong linear correlation (r=0.99) between the average diameter of inhibition of all tested bacteria and the extracts of phenolics (Fig. 2) and flavonoids (Fig. 3) was noted. This explains the interesting activity of different extracts which we obtained against the bacterial species. This is consistent with the findings of Ofokansi et *al*. [29] for plant extracts *B. pinnatum,* rich in phenolic compounds, which showed a very interesting efficacy in the treatment of typhoid fever and other bacterial infections, including those caused by *Staphylococcus aureus, Esterichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Klebsiella aerogenes, Klebsiella pneunoniae* and *Salmonella typhi*. In addition, Mbaveng et *al*. [30] showed in their study the antibacterial effect of isolated *Dorstenia barteri* flavonoids.
The mechanism of toxicity of polyphenols against microorganisms is very complex. It is done either by deprivation of metal ions such as iron needed for microbial growth, or by non-specific interactions such as hydrogen bridge facility with cell wall proteins or microbial extracellular enzymes and promoting their inhibition [31] [32] [33] [34].Also, tannins present in high concentrations in the methanolic extract of the fruits of jujube have an interesting activity against different tested bacterial species, without neglecting the synergitic effect of other molecules.These molecules have been reported as a bacteriostatic or bactericidal against several bacterial species such as: *Alealigenes faecalis, Enterobacter aerogenes, Escherichia coli, K pneumoniae, Proteus vulgaris, Pseud fluorescens, Salmonella enteritidis, S paratyphi, Salmonella typhi, Staphylococcus aureus, Strept faecalis pyogenes Strept* and *Yersinia enterocolitica, Bacteroides fragilis, Clostridium clostridiiforme, C perfringens, C paraputrficum, Enterobacter cloacae, Enteroccus aurens* and *Corynebacterium diphtheriae* [35] [36] [37].

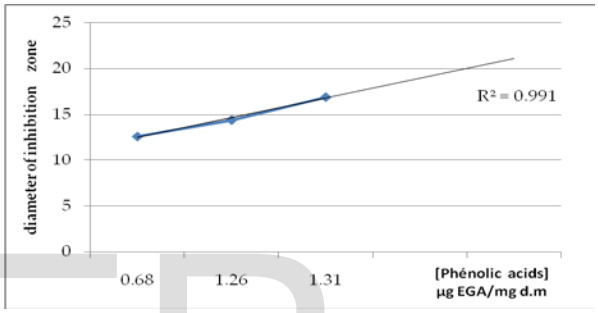

Fig. 2: Correlation between the average inhibition diameter of all tested bacteria and content of the phenolics extracts

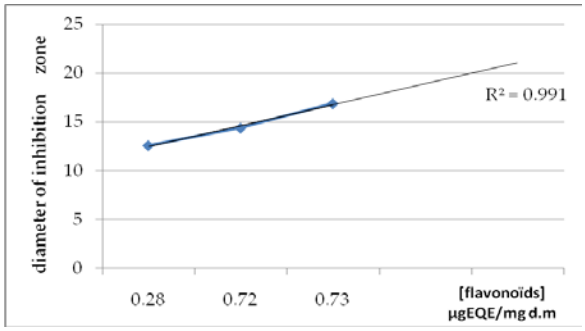

Fig. 3: Correlation between the average inhibition diameter of all tested bacteria and content of flavonoids extracts

McSweeney et *al*. [38] reported that condensed tannins have an inhibitory effect on the growth of the microbial flora of the rumen bacterial species such as *Streptococcus bovis, Butyvibrio fibrosolvens, Fibrobacter succinogenes, Prevotella ruminicola* and *Ruminobacter amylophilis*.

3.4 Antifungal Activity

Table 5 shows the obtained diameters of inhibition of mycelial growth of four fungal species due to fruit pulp of jujube (*Z. lotus*) extracts.

Table 5: Antifungal activity of extracts of jujube fruit "*Ziziphus lotus L. Desf.*)"

	Penicillium Italicum		*Fusarum culmorum*		*Aspergilus ochracerus*		*Rizomucor sp*	
Extracts	2 nd day	5th day	2 nd day	5th day	2 nd day	5th day	2 nd day	5th day
Etheric	60% c	41% b	70% b	42% c	43% b	18%	55% c	40% c
Dichloromethanic	56% d	33% c	67% c	33% d	31% d	17%	55% c	33% d
Methanolic	67% b	50% bc	85% a	76% a	41% c	18%	60% b	45% b
Difenocazole	82% a	70% a	64% d	50% b	75% a	58%	77% a	66% a

Means followed by the same letter are not significantly different according to Tukey HSD (P = 5%).

From these results, it appears that the four extracts possess an activity on four tested species of fungi. The inhibition rates ranging from 31 to 85% for the second day and 17 to 76% for the fifth day of incubation. On the 2nd day, the ehteric extract was active against *Fusarum culmorum* (70%), *Penicilum italicum* (60%) and *Rhizopus sp* (55%) and moderately active on *Aspergillus ochracerus* (43%). Also, the dichlormethanic extract was active but with less degree against *Fusarum culmorum* (67%), *Penicilum italicum* (56%) and *Rhizopus sp* (55%) and moderately active against *Aspergillus ochracerus* (31%). However, the methanolic extract was very active against *Fusarum culmorum* (85%), active against *Penicilum italicum* (67%) and *Rhizopus sp* (60%) and moderately active against *Aspergillus ochracerus* (41%). For the 5th day of incubation, the antifungal activity of all exracts has decreased significantly, except in the case of *Fusarum culmorum* facing the methanol extract which remained very active (76%) against the fungus. Thus, with the exception of *Aspergillus ochracerus* which showed some resistance (inhibition rate of 17-18%), the remaining three species of fungi showed an average effect (33-50%).

From Figure 4 we conclude that *Fusarum culmorum* is the most susceptible species to different extracts of jujube fruit (pulp). Indeed, its susceptibility to these exraits exceeded that obtained for the fungicide (difénocazole) used in the treatment of cereal seed.

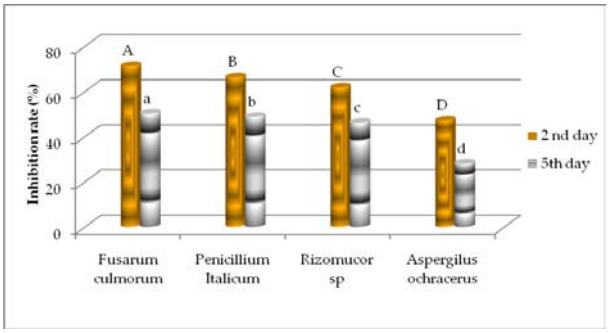

Fig. 4: Susceptibility of different fungi to extracts of jujube fruit "*Ziziphus lotus (Desf.) L.*"

Tuky test (HSD), allowed to calassify the susceptibility to different extracts as follows: *F. culmorum*> *P.talicum*> *Rhizopus sp*> *A. ochracerus*.

While the most active extract after fungicide (difenocazole) is the methanol extract, followed by ethiric extract and ended by dichloromethanic extract (Fig. 5). Indeed, methanol is reported to be effective in the solvent extraction of polyphenols [39], while dichloromethane is effective in extracting terpenoids [31] that is why we are witnessing a large difference between the effects of these solvents.

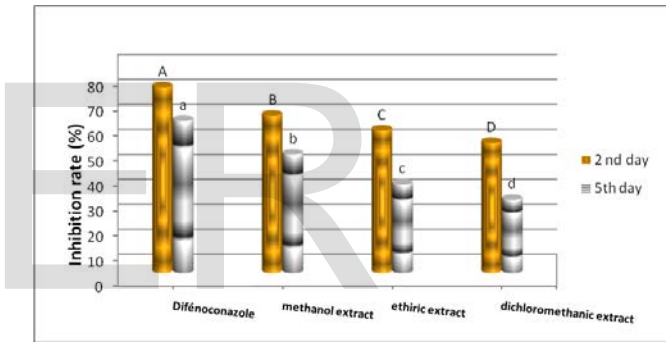

Fig. 5: Antifungal activity of extracts of jujube fruit "*Ziziphus lotus (Desf.) L.*"

The study of correlation between the content of different extracts and antifungal effect allowed to point out a strong correlation between growth inhibition of tested fungi and the concentration of flavonoids (Fig. 6) on one hand and the concentration of tannins (Fig. 7) on the other.

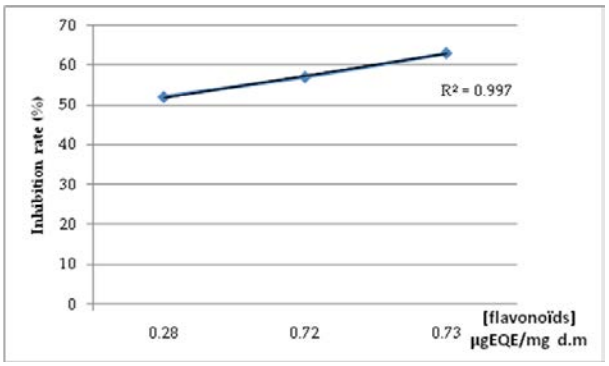

Fig. 6: Correlation between inhibition of the growth of tested fungi and concentration of flavonoids

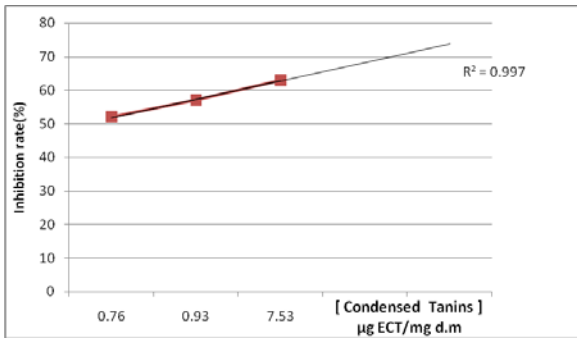

Fig. 7: Correlation between inhibition of growth of tested fungi and concentration of tannins

Several studies on the host-parasite interaction during fungal infections, have allowed us to evidence the toxicity of phenolic substances for many pathogenic fungi by blocking the activity of lytic enzymes involved in the infection [40]. Some of these substances are induced by the penetration of the parasite in the host tissues even before the infection. Among these substances are found phenolics cinnamic acid or benzoic , flavonoids or iso flavonoids and their derivatives as has been reported by many authors [41] [42] [43] [44] [45]. Filamentous fungi such as *Coniphora olivacea, Gloeophyllum trabeum, Collectotrichum graminicola, Penicillium, Aspergillus Niger, Botrytis cinerea, Chaetomium Cupreum, Coriolus versicolor, Crinepellis pernicious, Fomes annosus Merulius Lacrymans, Poria monticola, Trichaderma viride* and *Trametes hirsuta* are inhibited by tannins of different preparations [37]. Baba et *al*. [46] reported that *Pleoopsis duberosa* and *Terminalia avicernoides* contained very high levels of tannins which may be responsible for the antifungal activity of the extracts of these plants.These chemicals are secondary metabolites present in different parts of the plant (roots, leaves, bark, fruit...etc.) and involved in the defense mechanisms. They protect the plant against attack by pathogenic microorganisms (fungi and bacteria), viruses and insects [47] [48].

4 Conclusion

The results obtained in our present study provide a scientific basis for confirming certain uses of jujube fruit in traditional medicine to meet the needs of primary care human health. The presence of active compounds with antimicrobial activity, such as phenols, flavonoids and tannins in the fruit has a natural source of bioactive alternatives to synthetic fungicides and bactericides poorly biodegradable and harmful to the environnement.

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