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IN VITRO ANTIFUNGAL ACTIVITY OF THE EXTRACTS OF *Punica granatum* L OBTAINED BY REFLUX METHOD AGAINST *Fusarium oxysoprum albedenis* IN SOUTH WEST OF ALGERIA

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Abstract. *Punica granatum* (Pg), commonly known as pomegranate is a member of the monogeneric family, Punicaceae. Pg and its chemical components possess various pharmacological and toxicological properties including antioxidant, anti-inflammatory, antifungal and against vegetable and palms diseases. Bayoud is the most dangerous disease, causing significant losses of date palms in Algeria whose the responsible fungi is called *Fusarium oxysporum f.sp.albednis.* This study focused on the evaluation of the *in vitro* antifungal activity of a *Punica granatum* L fruit extracts obtained by the reflux method, considered as a hot method using heating with the following solvents: distilled water, methanol, ethyl acetate, and chloroform; on ten selected strains of *Fusarium oxysporum f.sp. albedinis*. The evaluation of antifusarial activity of *Punica granatum* L. was carried out by using the dilution and the direct contact method to determine the minimum inhibitory concentration (MIC) and the minimum fungicidal concentration (MFC). The results of the extraction yield show that distilled water and methanol are the two best solvents which give a good yield compared to chloroform and ethyl acetate, 42% for methanol and 54% for distilled water, but the low yield is 0.5% for chloroform and between 1.15 for ethyl acetate. The antifungal activity results of these extracts on the solid and liquid medium, the PDA and the PDB, respectively, show that they have good inhibitory activity against all the tested fungi. According to the results obtained in the solid medium, the extracts tested have an inhibitory activity against *Foa*, with different MIC values ranging from 1.2 mg/ml to 4.2 mg/ml. The result of growing on liquid medium, shows that with each increase in the concentration of the extracts in the culture medium, there is a decrease in the fungal biomass, and there is a fungicide activity for all extracts used.

Keywords: *Punica granatum L, Fusarium oxysporum f.sp. albedinis, antifusarial activity, date palm, Bayoud.*

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Abbreviations

CHLF: Chloroform
DNA: Deoxyribonucleic acid
DW: Distilled water.
EA: Ethyl acetate;
EDTA: Ethylene diamine tetra-acetic acido
Et OH: Ethanol
FOA: Fusarium oxposum fsp albedenis
MFC: Minimum fungicidal concentration
MIC: Minimum inhibitory concentration
Me OH: Methanol
PCR: Polymeras chain reaction.
PDA: Potato dextros agar.
PDB: Potato dextrose broth.
PG: Pomegranate
PGF: Pomegranate flowers.
RNA: Ribonucleic acid

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1. Introduction

Punica granatum L. (pomegranate) is a well-known member of Punicaceae family, which comprises two species, *Punica granatum* (indigenous to Mediterranean regions and Iran) and Punica protopunica(endogenous to Socotra islands). It is widely cultivated throughout Central Asia, the Himalayas, Middle East, American Southwest, and Mediterranean area and is believed to originate from Iran and Afghanistan (Shaygannia et *al*., 2016; Eghbali et *al*., 2021).

Punica granatum L, is an ancient, mystical, unique fruit borne on a small, long-living tree . In addition to its ancient historical uses, pomegranate is used in several systems of medicine for a variety of ailments. The synergistic action of the pomegranate constituents appears to be superior to that of single constituents. In the past decade, numerous studies on the antioxidant, anticarcinogenic, and anti-inflammatory properties of pomegranate constituents have been published, focusing on treatment and prevention of cancer, cardiovascular disease, diabetes, dental conditions, erectile dysfunction, bacterial infections and antibiotic resistance, and ultraviolet radiation-induced skin damage. Other potential applications include infant brain ischemia, male infertility, Alzheimer's disease, arthritis, and obesity (Jurenka, 2008).

For centuries, the peels, leaves, flowers and fruits of *Punica granatum* L. (Punicaceae), known as pomegranate, have been used to treat many diseases (Ross *et al*., 2001). Some studies with extracts of *P. granatum* and compounds have shown high antioxidant (Aviram et *al*., 2004, 2008; Reddy et *al*., 2007), anti-inflammatory (Kitchen et *al*., 2004), anticarcinogenic (Adhami & Mukhtar, 2006; Kim et *al*., 2002; Malik et *al*., 2005; Malik & Mukhtar, 2006) and antimicrobial activity (Duraipandiyn et *al*., 2006; Höfling et *al*., 2010; Pereira et *al*., 2006; Voravuthikunchai *et al*., 2004). Among the many compounds present in the *P. granatum* fruit, there are alkaloids, polyphenolics, ellagic acid and gallic acid. The peel of the fruit contains alkaloids and, approximately 20% of tannins, including punicalagin, granatins A and B, gallagyldilacton, casuarinin, pedunculagin, tellimagrandin I and corilagin, which have antibiotic action (Fetrow & Avila, 2000). Compounds such as granatins A and B, punicalagin and punicalin were isolated from the pericarp, and are the main compounds responsible for the antimicrobial activity (Catão et *al.*, 2006).

The objectives of the present study were to find other alternatives to fight the date palm disease, or Bayoud, which is caused by a most dangerous fungus called "*Fusarium oxysporum f.sp.albednis"*causing significant losses of date palms in Algeria, That's the reason why we have studied the antifusarial activity of *Punica granatum* L.bark extracts obtained by the reflux method, using the following solvents: water, methanol, ethyl acetate and chloroform; against ten (10) strains of *Fusarium oxysporum* f. sp. *albedinis* (FOA) causing palme date fusariosis.

2. Materials and Methods

Plant Material

The selected samples of fruits of *Punica granatum* L. were collected from the Bechar oasis, southwest of Algeria; and from an area in Beni Ounif known as "El ksar," north of Bechar, during January and February 2021. This collected vegetal material was dried for 15 days in the dark and ground by an electric grinder machine to obtain a fine powder and less at ambient laboratory temperature (20 °C–28 °C).

Preparation of plant extracts

Pomegranate fruits at the maturity stage were manually peeled washed and air-dried prior to extraction with solvents of different polarities (Al-Zoreky, 2009). Briefly, 10 g of finely powdered peels were extracted with 100 ml of each of the following solvents, water, methanol, chloroform and ethyl acetate using a reflux assembly for 2 hours , each mixture was then left, in the dark, at room temperatures for 1 h prior to filtration (Whatman No. 1), then concentrated by the rotavapor (Vaghasiya & Chanda, 2007; Laoufi *et al*., 2014). All the samples extracted by using the above-mentioned techniques were performed in triplicate. All extracts were bottled properly and stored in the fridge at 4°C (Madan & Singh, 2017).

Determination of extraction yield

The extraction yield is expressed, for each solvent, by the ratio between the mass of the extracted material and the mass of the plant material used.

$$Y(\%) = \frac{M}{M_0} x100$$

where:
Y: extraction yield;
M: weight of the extract after evaporation solvent;
M_0: dry weight of the sample

In vitro Antifungal Activity

Fungal Strains

The antifungal activity was evaluated by both direct contact and dilution methods on ten selected strains of fungi, *Fusarium oxysporum* f. sp. *Albedinis* FOA (1), FOA (2), FOA (3), FOA (4), FOA (5), FOA (6), FOA (7), FOA (8), FOA (9) FOA (10), that were isolated from date palms from the Beni-ounif area infected by the fusariosis disease. Fungal spores were prepared by growing mold on potato dextrose agar (PDA) at 25 °C for 7 days, and spores were suspended in sterile 1% tween-80. Spore count was performed by using a hemocytometer and adjusted to obtain10^5–10^6spores/ml with potato dextrose broth (PDB) (Makhloufi *et al*., 2011, Terfaya *et al*., 2017).

Fungal DNA Isolation, Purification and Quantification

The total DNA of dried mycelium of each strain was extracted and purified as described by Kumar et al. (2012). In the Universal method: a single protocol universally used for plants, algae, blood, bacteria, and fungi will be more demanding than those suited specifically to a particular biological material. The extraction buffer used in this case included 100 mM Tris-HCl, pH 8.0, 25 mM EDTA, 1.5 M NaCl, 2% CTAB, β-mercaptoethanol, and PVP (in case of the plant sample). The method utilizes the classical protocol for homogenization by liquid nitrogen, incubation in a water bath at 65°C, and deproteinization was performed by chloroform–isoamyl alcohol, followed by ethanol precipitation and washing. As an alternative to the above, other universal methods termed as the "nuclei method" and "protoplast method" may be successfully used to prepare high quality, megabase-sized DNA.

The RNA was eliminated by adding proteinase K (RNase; 10 mg/ml). The DNA was dissolved in 200µl TE buffer(10 mM Tris-HCl, pH 6.0, 1 mM EDTA, pH 8.0), quantified and diluted to an approximate concentration of 5 ng/µl for PCR reactions (Zhang *et al*., 2012).

PCR Amplification of Fungal DNA

The primers used were preselected among several primers because they permitted to reveal polymorphism on small DNA samples used for preliminary trials. The PCR techniques were optimized according to primers. Reaction was achieved by 1 cycle consist of 4 min of denaturation at 95 °C followed by 30 cycles consisting of 1 min at 94 °C,1 min at 36 °C and 1 min 30 s at 72 °C. One cycle for 15 min at 72 °C was conducted at the end. PCR reactions were performed in a total volume of 25µl, containing 25 ng genomic DNA, 10X Taq buffer, 10 mM dNTP, 25 mM MgCl2, 1-unitTaq DNA polymerase (Promega) and 10 mM primer.For FOA-specific amplification, PCR primers included two primer pairs; FOA1 (CAGTTTATTAGAAATGCCGCC) coupled with BIO3 (GGCGATCTTGATTG-TATTGTGGTG) and FOA28 (ATCCCCGTAAAGCCCTGAAGC) coupled with TL3 (GGTC GTCCGCAGAGTATACCGGC) (Fernandez *et al.*, 1998).

FOA-specific PCR reactions were performed according to Fernandez et *al*. (1998) as follows: 1 cycle for 4 min at 95 °C followed by 30 cycles for 30 sat 92°C,30 sat 60° Cand30sat 72 °C for the FOA1-BIO3 primer pair; and 30 cycles for30 sat 92 °C, 30 sat 62°C and 45s at 72°C for the FOA28 TL3 primer pair. Thereafter, a cycle of 15 min at 72°C was conducted. PCR reactions were performed in a total volume of 20 µl, containing 10–100 ng genomic DNA,10 X Taq buffer,0.2 mM of dNTP, 1.5 mM MgCl2, 1unit Taq DNA polymerase (Promega) and 1µM primer, as previously described by Fernandez et al. (1998). The PCR reactions were incubated in a TC 3000 Thermocycler (Progene, Techne England). All amplification products were separated in stained agarose gels(1.8% w/v; 15 × 10 cm, W × L) with ethidium bromide in TAE buffer electrophoresed at 100 V for 1 h 30 min (Terfaya *et al.*, 2017). The DNA weight marker used was λ (lambda) digested by enzymes Hind III and EcoR1. At the end of electrophoresis, the gels were visualized by UV illumination and photographed using a Bioprint System 3000WL X-PRESS computer assisted machine (software BIO-1D).

Dilution method (MIC)

The minimum inhibitory concentration (MIC), defined as the lowest concentration of the treatment that inhibits visible fungal growth, was determined with a microdilution method. Antifungal tests were performed according to the method reported by Hassikou et al. (2002), with the antifungal activity evaluated via the dilution and the direct contact method. The direct contact method on solid medium was performed in Petri dishes containing PDA, a culture media and extract, at different concentrations (0.2, 0.4, 0.6, 0.8, 1 mg/ml) which were inoculated with fungus suspension obtained from pure culture, and incubated at 25 °C for 7 days. The diameter of the fungus colony was obtained by calculating the average of two perpendicular diameters compared with the witness petri dishes. However, the direct contact method on liquid medium or the minimum fungicide concentration (MFC) is defined as the lowest concentration that kills 99.9% of the final cell concentration. To determine it, the method described by Canton et al. (2003) was used. Concentrations strictly above the MIC will be used. It was performed in sterile vials where the fungal inoculum was taken and added to 50 ml of PDBac medium, and then precise volumes of the extract were taken according to the given concentrations. As a witness, sterile vials are prepared using PDBac culture medium and fungal inoculum only. At the end, incubation is done at 25°C for 14 days. After determining the MIC, aliquots of the wells with concentrations corresponding to the MIC as well as with higher concentrations were used to inoculate PDA plates for the

determination of the minimum fungicidal concentration (MFC). It was defined as the lowest treatment concentration required killing a pathogen and corresponded to an invisible growth of the subculture. Three replicates of each assay were assessed.

3. Results

Extraction yield

The extraction yield of *Punica granatum* L with different solvents is shown in table 1.

Table 1. Extraction yield of *Punica granatum* L with different solvents

	Reflux method			
Solvents	MOH	CHLF	EA	DW
Extraction yield (%)	42	0.50	1.15	54

MOH: Methanol; CHLF: Chloroform; EA: Ethyl acetate; DW: Distilled water.

Antifungal Activity

Analysis of the Population of F. o. f. sp. Albedinis by the Specific PCR Technique

The amplification of genomic DNA by a specific primer couple to Foa, TL3-FOA28, revealed a specific band which has a size of 400 bp (Fig. 1). However, this band has also been amplified in the case of six strains of *Fusarium oxysporum*, of which one was isolated from the Beni-ounif palm grove. For amplification a specific band of size 200 bp using BIO3-FOA1 coupled primer revealed a specific band. The assembly of the two types of bands revealed by two primer couples (TL3-FOA28 and BIO3-FOA1) only gave 30% of reliability. The dendrogram based on recombined results obtained by two primer couples permitted to distinguish two big distinct groups (data not shown): the groups 1 and 2 are composed of a mixture of FOA strains from different origins and *Fusarium oxysporum* strains (Sedra & Zhar, 2010).

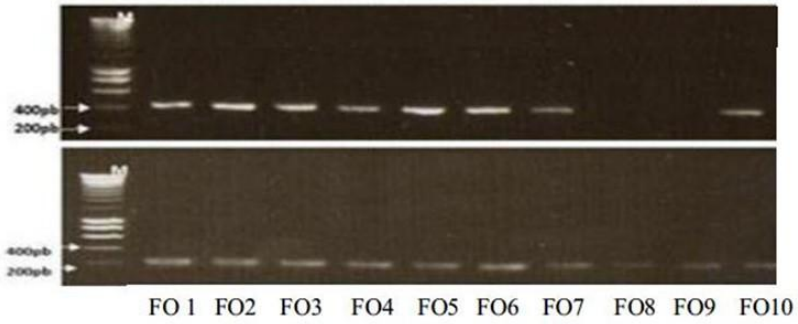

Fig. 1 Agarose gels of DNA PCR products from isolates FOA

Dilution Method (MIC)

The inhibition growth zones measured by the dilution method (MIC) are presented in tables 2, 3, 4 and 5. The antifungal activity of studied extracts against ten strains of *Fusarium oxysporum f. sp. Albedinis* showed that *Punica granatum* L extracts have great antifungal activity against all the investigated strains. These results revealed that the fungal growth was inhibited completely. Nevertheless, S6 was the most

sensitive strain with a minimal inhibition concentration value of 1.2, 1.6, 2.8 and 3 mg/ml for methanol, aqueous, ethyl acetate, and chloroform extracts, respectively (Fig. 2, 3, 4, 5).

Table 2. MIC of *Punica granatum* L. methanol extracts (mg/ml) on fungal strains

Strains	Wit	0.6	0.8	1.2	1.4	1.6	1.8	2	2.2	2.4	2.6	2.8
S1	+	+	+	+	+	+	+	MIC	-	-	-	-
S2	+	+	+	+	+	+	MIC	-	-	-	-	-
S3	+	+	+	+	MIC	-	-	-	-	-	-	-
S4	+	+	+	+	+	+	+	+	MIC	-	-	-
S5	+	+	+	+	+	MIC	-	-	-	-	-	-
S6	+	+	+	MIC	-	-	-	-	-	-	-	-
S7	+	+	+	+	+	+	+	+	+	MIC	-	-
S8	+	+	+	+	+	+	+	MIC	-	-	-	-
S9	+	+	+	+	+	+	+	+	MIC	-	-	-
S10	+	+	+	+	+	+	+	+	+	+	MIC	-

(-): Inhibition (+): Growth ; Wit: Witness

Table 3. MIC of *Punica granatum* L aqueous extracts (mg/ml) on fungal strains

Strains	Wit	0.6	0.8	1.2	1.4	1.6	1.8	2	2.2	2.4	2.6	2.8	3
S1	+	+	+	+	+	+	+	+	MIC	-	-	-	-
S2	+	+	+	+	+	+	+	+	+	MIC	-	-	-
S3	+	+	+	+	+	+	+	+	MIC	-	-	-	-
S4	+	+	+	+	+	+	+	+	+	MIC	-	-	-
S5	+	+	+	+	+	+	+	MIC	-	-	-	-	-
S6	+	+	+	+	+	MIC	-	-	-	-	-	-	-
S7	+	+	+	+	+	+	+	+	+	+	MIC	-	-
S8	+	+	+	+	+	+	MIC	-	-	-	-	-	-
S9	+	+	+	+	+	+	+	MIC	-	-	-	-	-
S10	+	+	+	+	+	+	+	+	+	+	+	MIC	-

(-): Inhibition (+): Growth ; Wit: Witness

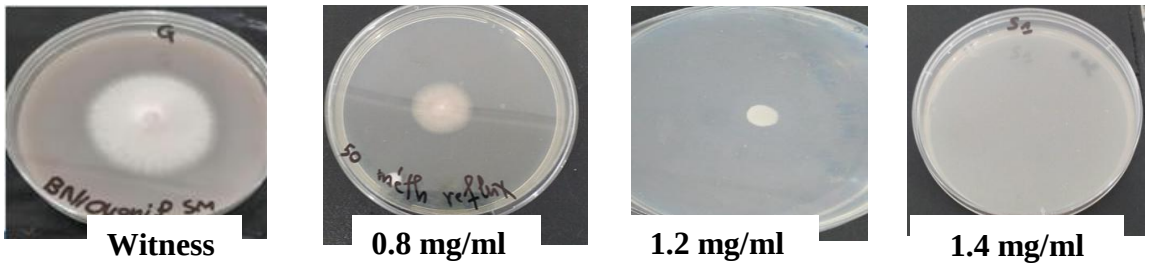

Fig. 2. Effect of Punica granatum L methanol extracts against S6

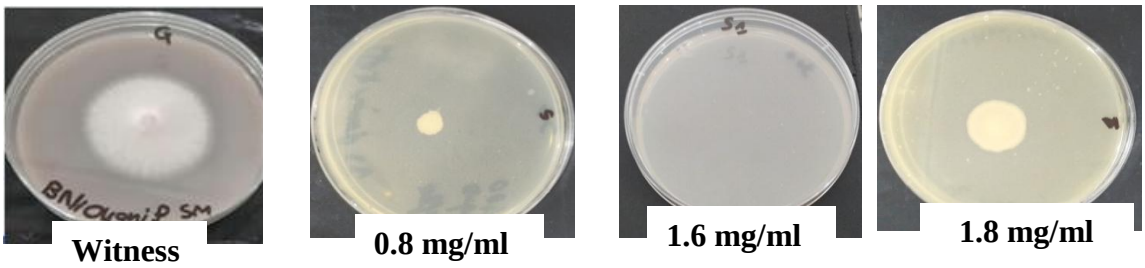

Fig. 3 Effect of *Punica granatum* L aqueous extracts with various concentrations against S6 of FOA

Table 4. MIC of *Punica granatum* L ethyl acetate extracts (mg/ml) on fungal strains

Strains	Wit	2	2.2	2.4	2.6	2.8	3	3.2	3.4	3.6	3.8	4	3
S1	+	+	+	+	+	+	+	MIC	-	-	-	-	-
S2	+	+	+	+	+	+	+	+	+	+	MIC	-	-
S3	+	+	+	+	+	+	+	+	+	MIC	-	-	-
S4	+	+	+	+	+	+	+	+	MIC	-	-	-	-
S5	+	+	+	+	+	+	+	+	+	MIC	-	-	-
S6	+	+	+	+	+	MIC	-	-	-	-	-	-	-
S7	+	+	+	+	+	+	+	+	MIC	-	-	-	-
S8	+	+	+	+	+	+	+	MIC	-	-	-	-	-
S9	+	+	+	+	+	+	+	+	+	+	+	MIC	-
S10	+	+	+	+	+	+	+	+	+	+	MIC	-	-

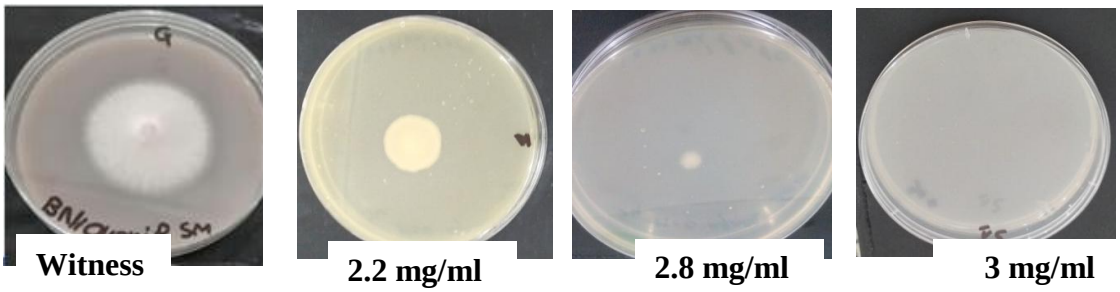

Fig. 4 Effect of *Punica granatum* L ethyl acetate extracts with various concentrations against S6 of FOA

Table 5. MIC of *Punica granatum* L chloroform extracts (mg/ml) on fungal strains

Strains	Wit	2	2.2	2.4	2.6	2.8	3	3.2	3.4	3.6	3.8	4	4.2	4.4
S1	+	+	+	+	+	+	+	+	MIC	-	-	-	-	-
S2	+	+	+	+	+	+	+	+	+	MIC	-	-	-	-
S3	+	+	+	+	+	+	+	+	+	+	MIC	-	-	-
S4	+	+	+	+	+	+	+	+	+	+	MIC	-	-	-
S5	+	+	+	+	+	+	+	+	MIC	-	-	-	-	-
S6	+	+	+	+	+	+	MIC	-	-	-	-	-	-	-
S7	+	+	+	+	+	+	+	MIC	-	-	-	-	-	-
S8	+	+	+	+	+	+	+	+	+	+	+	MIC	-	-
S9	+	+	+	+	+	+	+	+	+	+	+	+	MIC	-
S10	+	+	+	+	+	+	+	+	+	MIC	-	-	-	-

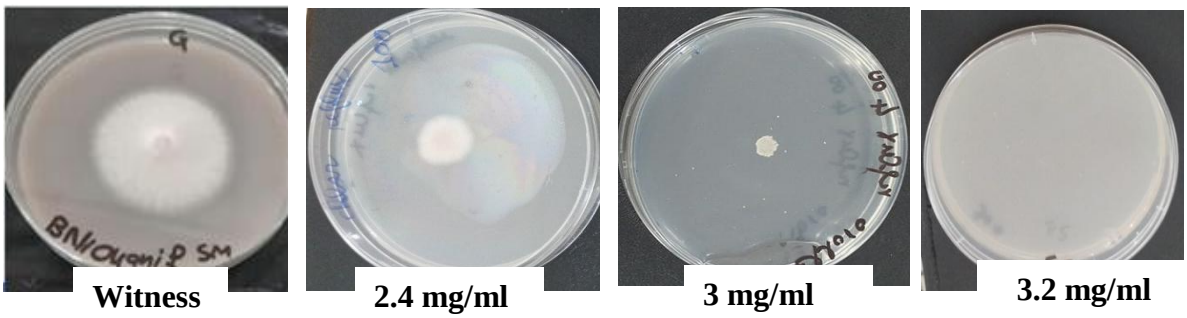

Fig. 5 Effect of *Punica granatum* L chloroform extracts with various concentrations against S6 of FOA

Table 6. Activity for each extracts of *Punica granatum* L. on fungal strains

	Strains	S 1	S 2	S 3	S 4	S 5	S 6	S 7	S 8	S 9	S 10
Methanol extracts	MIC	2	1.8	1.4	2.2	1.6	1.2	2.4	2	2.2	2.6
	MFC	2.2	2	1.6	2.4	1.8	1.4	2.6	2.2	2.4	2.8
	MFC/ MIC	1.10	1.11	1.14	1.09	1.125	1.16	1.08	1.10	1.09	1.07
	Activity	Fungicide	Fungicide	Fungicide	Fungicide	Fungicide	Fungicide	Fungicide	Fungicide	Fungicide	Fungicide
Aqueous extracts	MIC	2.2	2.4	2.2	2.4	2	1.6	2.6	1.8	2	2.8
	MFC	2.4	2.6	2.4	2.6	2.2	1.8	2.8	2	2.2	3
	MFC/ MIC	1.09	1.08	1.09	1.08	1.10	1.125	1.07	1.11	1.10	1.07
	Activity	Fungicide	Fungicide	Fungicide	Fungicide	Fungicide	Fungicide	Fungicide	Fungicide	Fungicide	Fungicide
Ethyl acetate extracts	MIC	3.2	3.8	3.6	3.4	3.6	2.8	3.4	3.2	4	3.8
	MFC	3.4	4	3.8	3.6	3.8	3	3.6	3.4	4.2	4
	MFC/ MIC	1.06	1.05	1.05	1.05	1.05	1.07	1.05	1.06	1.05	1.05
	Activity	Fungicide	Fungicide	Fungicide	Fungicide	Fungicide	Fungicide	Fungicide	Fungicide	Fungicide	Fungicide
Chloroform extracts	MIC	3.4	3.6	3.8	3.8	3.4	3	3.2	4	4.2	3.6
	MFC	3.6	3.8	4	4	3.6	3.2	3.4	4.2	4.4	3.8
	MFC/ MIC	1.05	1.05	1.05	1.05	1.05	1.06	1.06	1.05	1.04	1.05
	Activity	Fungicide	Fungicide	Fungicide	Fungicide	Fungicide	Fungicide	Fungicide	Fungicide	Fungicide	Fungicide

According to the direct contact method on liquid medium, the determination of the antifungal parameters (MIC and MFC) of our extracts of *Punica granatum* L was carried out only on the strains that have presented sensitivity by the aromatogram test. The results obtained are summarized in Table 6.

4. Discussion

Reflux extraction is more efficient than percolation or maceration and requires less extraction time and solvent. It cannot be used for the extraction of thermolabile natural products (Ribeyre, 2014).

As observed in table 1 above, a significant difference in the extraction yield using different solvents. Among solvents tested, aqueous and methanol extracts resulted in the highest extraction yields (54%; 42%) respetively, followed by ethyl acetate (1.15%) and chloroform (0.50%), indicating that the extraction efficiency favors the highly polar solvents. In our study, the extraction yield of *Punica granatum* L extracts are low compared to those obtained by Bapodara et al. (2011); Ismail et al. (2014); Wapwera et al. (2021). This variation of yield extraction may be due to the difference in climate, soil, and other environmental conditions for plant growth in the current study (Bechar area).

Other research workers have also reported that *Punica granatum L* extract obtained by hot methods including reflux and soxhlet method have highest extract yields followed by cold method including static maceration and dynamic maceration (shaking) and this difference might be due to the higher temperature, which may have increased the strength of salvation (El Khetabi *et al*., 2020).

According to these *in vitro* results, (Tables 2, 3, 4, 5 and 6) and the inhibition rates of each extract of *Punica granatum* L, it is generally noted that the aqueous and

methanolic extract have a stronger inhibitory activity than the ethyl acetate and the chloroform extracts.

However, they have a great and good inhibitory antifungal activity against all the investigated fungi. The concentration inhibition is between 1.2 and 4,2 mg/ml. The diameters of inhibition growth zone ranged from 20 to 55 mm with the highest inhibition zone values observed against the strain of *Fusarium oxysporum* f. sp. *Albedinis* (6) FOA(6) (55 mm). However, the studied fungi did not show the same sensitivity to different solvents.

The difference in the degree of this effect is possible due to:

The nature of the secondary metabolites extracted and/or their quantities, the major compounds in the fruits of *Punica granatum* L are polar substances such as flavonoids and tannins (Doostkam *et al*., 2020; Karimi *et al*., 2020; Akuru *et al*., 2022), It is possible that the antifungal effect is linked to these secondary metabolites (this difference depends on the nature of the solvent used and their polarities; water and methanol are more polar than ethyl acetate and chloroform).

These data indicate that the activity of *P. granatum* probably occurs in the compounds present in the pericarp and peel of this fruit. Analyses of the four extracts of *P. granatum* detected the presence of similar substances, such as melatonin and simple sugars (Anibal *et al*., 2013).

Therefore, punicalagin and gallagyldilacton are apparently the major compounds involved in antifungal activity observed in the extracts of *P. granatum*. The possibility of occurring synergism between the compounds is a fact that cannot be discarded as several substances such as simple sugar and conjugated fatty acids are present in large quantities. These data corroborate the observations of some searches. Catão et al. (2006) and Fetrow and Avila (2000) indicated tannins, such as punicalin, punicalagin, gallagyldilacton, pedunculagin, tellimagrandin I and corilagin, to be responsible for the antimicrobial activity. Saroj et al. (2020) observed elevated activity of the isolated compound punicalagin against fungy, indicating this substance as a potent antifungal agent, without however, elucidating the mechanism of action.

Vasconcelos et al. (2006) assigns to the tannins, the ability to inhibit the growth of fungy species due to their action in the cell, specifically in the cell membrane, precipitating proteins.

Other research workers (Hanani *et al*., 2019; Magangana *et al*., 2020; Abd el-Rady *et al.,* 2021; Haghighian *et al*., 2021; Malek Mahdavi *et al*., 2021), have also proposed different mechanisms to explain that the antimicrobial activity is due to the secondary metabolites. These include (i) inhibition of extracellular microbial enzymes; (ii) deprivation of substrates and metal ions required for microbial growth and (iii) direct action on microbial metabolism through inhibition of oxidative phosphorylation. In addition, Huang et al. (2005) proposed that tannins are able to complex with other molecules, including macromolecules such as proteins and polysaccharides.

5. Conclusion

From the present study, one can conclude that the extract of pomegranate fruits, which contains bioactive compounds, is useful for the treatment of several infections and vegetables inflammatory disorders due to fungi of *Fusarium oxposum* fsp *albedenis*. The *Punica granatum* L extracts have a good antifungal activity. The strong antifungal activity against an array of filamentous fungi strains is an indication of the

broad spectrum of the antifungal potential related to this type of extract. This could make it a promising element of natural compounds for the development of safer antimicrobial agents.

These results suggest the possibility of using this raw material in pharmaceuticals as a fertilizer powder. These results represent new perspectives for future research in continuation to this study, where information such as the identification of bioactive compounds could contribute to the management of alternative therapies against these organisms.

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