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Analysis of Phytochemical Constituents by using LC-MS, Antifungal and Allelopathic Activities of Leaves Extracts of *Aloe vera*

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Abstract

This study was conducted to investigate the phytochemical compounds of *Aloe vera* leaves by using LC-MS and evaluate their antifungal and allelopathic activities *in vitro.* The ethanol extract of *Aloe vera* leaves was subjected to evaluate their antifungal activity against three plants pathogenic fungi, *Fusarium roseum*, *Fusarium oxysporum lycopersici* and *Botrytis cinerea* by using the agar plate diffusion plate method. The aqueous extract of *Aloe vera* leaves was subjected to evaluate the allelopathic potential on germination and seedling growth of durum wheat *Triticum durum* and *Amaranthus hybridus* which is an advantice species of cereals. We used the bioassay of germination and seedling growth of both species. By LC-MS analysis, 11 bioactive phytochemical compounds were identified in ethanol extract:5-((S-2'-oxo-4'-hydroxypentyl-2(ß-glucopyranosyl-oxy-methyl)chromone, isoaloerisin D, aloenin, aloeninB, aloenin-2`-p-coumaroyl ester, aloe-emodin-diglucoside, 10-hydroxyaloin B, 10-hydroxyaloin A, aloin B, aloin A, aloveroside B. The antifungal activity showed that the ethanol extract *has an inhibitory activity against all the mycelial strains.* The allelopathic effect of different concentrations slowed the kinetics of germination of *Triticum durum* and *Amaranthus hybridus* and significantly decreased the rate of final germination of *Amaranthus hybridus* (100% at C10% and C25%), and a weak effect of inhibition of the germination was noticed for *Triticum durum* (40% at C25%). The aqueous extract has an inhibitory effect on the length of the roots (98% at C25%) and the height of the stems (100% at C25%) of *Triticum durum.* All concentrations of the aqueous extract have an inhibitory effect on the root length of *Amaranthus hybridus.* These results showed that *Aloe vera* would be suggested as a new potential source of natural herbicides and fungicides.

Keywords: *Aloe vera*, allelopathic potential, antifungal activity, extract, LC-MS.

1. Introduction

Aloe vera is a medicinal and ornamental plant belonging to *Lilaceae* family, usually originating in the dry regions of Africa, Asia, and Southern Europe, especially in the Mediterranean regions (Urch, 1999; Rodr´iguez *et al.* 2010). It is being cultivated in other areas with different climatic conditions (Rodr´iguez *et al.* 2010). Mexico is the main producer of *Aloe vera*, followed by Latin America, China, Thailand, and the United States (Rodr´iguez *et al.* 2010). Vitamins, enzymes, minerals, starch, lignin, anthraquinones, saponins, salicylic acid, and amino acids are among the more than 75 nutrients and 200 active compounds contained in *Aloe vera* (Park and Jo, 2006).

Previous research has revealed that phenolics such as chromone, anthrone, and phenyl pyrone are the most common secondary metabolites found in Aloe species' exudates. It is noteworthy that C-glycosylated chromones are found to represent a class of naturally occurring secondary metabolites that are known to be unique compounds in aloe, not having been reported in other plants (Franz and Grün, 1983).

This plant has several biological properties: anti-inflammatory (Afzal *et al*., 1991; Malterud *et al*., 1993), immuno-stimulatory (Ramamoorthy and Tizard, 1998), antiviral (Khalon *et al.,* 1991), cell growth stimulatory activity (Tizard *et al*., 1994), and antifungal (Kawai *et al.,* 1998).

Fungal diseases of crops are usually controlled using resistant cultivars, fumigants and long rotations, but mainly by using fungicides (Rongai *et al.,* 2015). The widespread use of fungicides to combat plant diseases has resulted in the accumulation of toxins in both humans and the environment. (Cherkupally *et al.*, 2017).

In an effort to minimize the use of synthetic fungicides, alternative methods to combat fungal diseases have been investigated using compounds obtained from plant sources. More than 500 plant species have recently been tested for antifungal activity. (Rongai *et al.*, 2012). Just

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3% of the plants studied had significant antifungal activity. Many authors have also studied the importance of secondary metabolites in antifungal activity (Rongai *et al.*, 2015). The relationship between antifungal activity and total phenolic content has also been reported (Stanković *et al.*, 2012; El-Khateeb *et al.*,2013).

The presence of weeds in a cereal field can be harmful in many ways. The phenomena of competition between weeds and crops, in particular, contribute to yield losses (Le Bourgeois and Merlier, 1995) by directly affecting crop growth. To control these weeds, massive quantities of chemical herbicides are used around the world (Turk and Tawaha, 2003). Synthetic herbicides, on the other hand, are often harmful and trigger environmental issues (Sharifi Rad *et al*., 2014; Khanh *et al.*, 2004; Sodaeizadeh *et al*., 2009). Mechanical weeding methods used in biological agriculture offer an alternative (Mason and Spanner, 2006; Bond and Grundy, 2001; Jordan, 1993), but their limit is linked to energy costs and working time for the farmer. Allelopathy is considered a promising technique for biological control (Ravlić *et al.*, 2017; Lovett, 1991). Allelopathy is a biological process in which plants interact with one another, either negatively or positively, by releasing allelochemicals into the environment. (Ravlić *et al.*, 2017; Rice, 1984). Allelochemicals are found in the stems, leaves, seeds, inflorescences, fruits, roots, and seeds of plants as end products, by-products, and metabolites (Sisodia and Siddiqui, 2010).

Other organisms, such as plants, animals, and microorganisms, are affected by the release of these chemical compounds into the atmosphere, and their behavior is either inhibited or stimulated (Fujii et al., 2003). There is mounting evidence that these plant chemicals can inhibit weed species' germination and development (Mohsenzadeh *et al*., 2011; Singh *et al.*, 2003; Turk and Tawaha, 2003; Sampietro and Vattuone, 2006).

Allelopathy's role in the natural management of weeds and crop growth is now widely acknowledged. (Sharifi Rad *et al.*, 2014; Mohsenzadeh *et al.*, 2010; Khan *et al*., 2009). In recent years, medicinal plants have been increasingly explored for their allelopathic potential (Modallal and Al-Charchafchi , 2006; Anjum *et al.*, 2010).

The present study aims to investigate the *Aloe vera* phytochemical compounds by using LC-MS and to evaluate their antifungal and allelopathic activities *in vitro.*

2. Materials and Methods

2.1. Chemicals and Reagents

Ethanol, methanol, acetonitrile, DMSO and water were obtained from Sigma Chemical Co (Sigma-Aldrich GmbH, Stern-heim, Germany). Formic acid was obtained from Biochem Chemopharma.

2.2. Plant materials

The leaves of *Aloe vera* were collected in October 2016 from the *Aloe vera* farm in Almeria (Spain). Then, the leaves of the plant were cleaned, dried and ground into powder.

Triticum durum and *Amaranthus hybridus* seeds were collected in El Tarf (Algeria) and stored in paper bags.

2.3. Fungal agents

Three plant pathogenic strains, *Fusarium roseum*, *Fusarium oxysporum lycopersici* and *Botrytis cinerea* were obtained from Biotechnology Research Center (CRBT), Laboratory of Mycology, Constantine, Algeria.

2.4. Preparation of ethanol extract

10 g of leaf powder was mixed with 100 mL of ethanol/water (80:20, v/v) for 24 h at room temperature. The extraction was performed 3 times with renewal of the solvent. A double-layered muslin cloth was used to filter the contents, followed by Whatman filter paper n°1. The bacteria were then removed using a micro-filter with a pore size of 0.22 µm. The filtrate was evaporated to dryness using a rotary evaporation at 40° C to yield 19% of the dry weight of residue which was stored at 4° C until use. The extraction yield was calculated and expressed as the percentage of the extract in relation to the mass of crushed leaves (%, w/w).

2.5. Qualitative determination of the phenolic contents of Aloe vera leaves

Qualitative analysis of phenolic compounds in the ethanol extract of *Aloe vera* leaves was carried out according to the protocol described by Bidel *et al.*(2015). 5mg of ethanol extract was mixed with 1mL of the methanol-water mixture (50/50, v/v). The sample was then ultrafiltered (10 min at 1000 rpm on Eppendorf centrifuge). 10 µL of the supernatant solution were injected into the chromatography chain via its automatic injector.

Chromatographic separation was carried out on a XTerra MS equipped with a binary HPLC pump (Waters 1525l, Waters, Manchester, UK), ESCi multimode ionization mass spectrometer (Micromass Ltd, Manchester, UK) equipped with an electrospray ionization ion source (ZSpray MKII) and a Waters 996 photodiode array detector. The tested extract was separated on an Agilent C_{18} column (3.5 µm particle size, 2.1×100 mm) at 40° C. A flow rate of 210 µL/min was used. Mobile phases consisted of water (solvent A) and acetonitrile (solvent B), both acidified with 0.1 % (v/v) formic acid to minimize the ionization of phenolics (around pH 3.0). The capillary voltage was 2.5 kV. Nitrogen was used as the desolation gas (400 L/h) and cone gas (50 L/h). In the first step, spectra were reported in both negative and positive modes in full scan mode over the m/z 50-1200 range. Absorbance spectra in the 210-800 nm range. Mass spectra and absorbance were handled using MassLynx 3.5 software (Micromass Ltd).

2.6. Antifungal activity

The antifungal activity of *Aloe vera* leaves against three plant pathogenic fungi (*Fusarium roseum*, *Fusarium oxysporum lycopersici* and *Botrytis cinerea)* was tested using the agar plate diffusion plate method.

Six dilutions of the ethanol extract were prepared in DMSO (dimethylsulfoxide) (0.15%, 0.31%, 0.62%, 1.25%, 2.5% and 5%). 1 mL of each concentration was added to 15 mL of PDA culture medium (Potato dextrose agar). After solidification of the medium, 5 mm diameter mycelial discs from the margin of seven days old fungal cultures were placed in the center of each petri plate. (1disc/petri plate). Three replicates were performed for each extract concentration. Untreated medium (0%) was

used as control. The petri plates were incubated for 144 hours in the oven at a temperature of 20± 2°C.

Fungal growth was measured by averaging the two perpendicular diameters of each growing colony. The following formula was used to calculate the growth inhibition percentage (%):

Growth inhibition % = [(dc - dt)/dc] × 100

Where dc is the colony diameter in control plates and dt is the colony diameter in treated plates.

The minimum inhibitory concentrations (MIC) of the effective plant extract is defined as the lowest concentration of the antifungal agent that inhibits the fungal growth.

2.7. Allelopathic potential

2.7.1. Preparation of concentrations

Different concentrations (1%, 5%, 10% and 25%) were prepared by maceration the powder from the leaves of *Aloe vera* under agitation in distilled water for 24 hours. The content was then filtered through Whatman filter paper n°1, and then the extract obtained was stored at 4°Cuntil use.

2.7.2. Test for inhibition of germination and growth of Amaranthus hybridus and Triticum durum

Amaranthus hybridus and *Triticum durum* seeds were arranged in petri plates (10 seeds/petri plate) with filter paper moistened with *Aloe vera* aqueous extract at various concentrations. The control was prepared with distilled water. Germination tests were conducted in an oven at a constant temperature of 25°C.

2.7.3. Observations and Measurement

Observations and measurements included the kinetics, seed germination rate and seedling growth (stem height, root length) of *Amaranthus hybridus* and *Triticum durum*. The notations were carried out daily for 8 days. Three repetitions were performed out for each concentration of the extract.

2.7.3.1. Determination of germination percentages

After 8 days of incubation, the germination percentage of each species is determined. Sprouted seeds are any seed that has developed a coleorhiz in monocotyledonous species or a radical in dicotyledonous species.

2.7.3.2. .Determination of inhibition percentages

Inhibition percentages were calculated according to the formula used by Dhima *et al*. (2006) and Chung *et al.* (2003):

Germination inhibition %= [(G-g) / G] × 100

Where G is the germination in the control (distilled water) and g is germination in different concentrations of the extract.]

Growth inhibition % = [(H-h) / H] × 100

Where H is stem height or root length in the control (distilled water) and h is stem height or root lengthin different concentrations of the extract.

2.8. Statistical analysis

All assays were estimated in triplicates and the results were presented as means ± standard deviation (SD). Statistical analysis was performed using Variance Analysis (ANOVA, SAS), and averages were compared according to the student test. The results are considered to be significant when $p < 0.05$.

3. Results

3.1. Qualitative determination of the phenolic contents of Aloe vera leaves

Chromatogram of ethanolic extract by LC-MS analysis was shown in Figure 1 and the characterized compounds were presented in Table 1, identified with the numbers 1-11 considering the elution order. The compounds were divided into four classes in this study: chromones, phenyl pyrones, naphthalene derivatives, and anthrones.

The determination of phenolic compounds classes found were identified using their UV spectra obtained by LC-MS data, and then confirmed by comparison with literature data.

Two peaks 2 and 3 from 5-((S-2'-oxo-4'-hydroxypentyl-2(ß-glucopyranosyl-oxy-methyl)chromone and isoaloerisin D, respectively) were identified as chromones. These compounds have been identified by Wu *et al.*(2013); Fanali *et al*. (2010). Three phenyl pyrones (peaks 4, 8 and 11) eluting at 23.2, 32.8 and 55 min, giving [M− H]− ions, were identified as aloenin, aloenin B and aloenin-2`-p-coumaroyl ester, respectively (Wu *et al*., 2013).

Five Peaks 1, 5, 6, 9 and 10 (from Aloe-emodin-diglucoside,10-Hydroxyaloin B, 10-Hydroxyaloin A, Aloin B, Aloin A, respectively) were characterized as anthrones. The results were in accordance with those reported by Wu *et al.*(2013); Fanali *et al.* (2010); Quispe *et al.*(2018). Peak 7 was characterized as aloveroside B (Naphthalene derivative), similar to previously reported aloveroside A (Wu *et al.*, 2013; Yang *et al*., 2010).

Table 1. Peak assignments of metabolites identified by LC-MS of ethanol extract of *Aloe vera* leaves.

Peak number[a]	Retention time (min)	Molcular formula	MS^1 ion		Fragments MS^2	Tentative Identification	References
1	14.97	$C_{27}H_{29}0_{15}$	593	[M-H]-	377	Aloe-emodin-diglucoside	(Quispe*etal*.,2018)
2	15.6	$C_{21}H_{26}O_{10}$	461	[M+H]+	443, 417, 399, 249, 185	5-((S-2'-oxo-4'-hydroxypentyl2(ß- glucopyranosyl-oxy-methyl)chromone	(Wu *et al.*, 2013)
3	21	$C_{29}H_{32}0_{11}$	557.13	[M+H]+	437,513,393, 291,247,217	isoaloerisin D	(Wu *et al.*, 2013; Fanali*etal.*, 2010)
4	23.2	$C_{19}H_{22}O_{10}$	409.11	[M−H]−	247, 203, 171	Aloenin	(Wu *et al.*, 2013)
5	23.9	$C_{21}H_{22}O_{10}$	433	[M−H]−	270	10-Hydroxyaloin B	(Wu *et al.*, 2013)
6	27.9	$C_{21}H_{22}O_{10}$	433	[M−H]−	270	10-Hydroxyaloin A	(Wu *et al.*, 2013)
7	29.5	$C_{30}H_{40}O_{17}$	695	[M+H]+	549, 335	Aloveroside B	(Wu *et al.*, 2013)
8	32.8	$C_{34}H_{38}O_{17}$	717	[M-H]-	247, 555	Aloenin B	(Wu *et al.*, 2013;Fanali *etal.*, 2010)
9	48.5	$C_{21}H_{22}O_9$	417	[M-H]-	297, 268, 239	Aloin B	Wu *et al.*, 2013 ;Fanali *etal.*, 2010 ;Quispe*etal.*, 2018.)
10	53	$C_{21}H_{22}O_9$	417	[M-H]-	297, 268, 239	Aloin A	Wu *et al.*, 2013 ;Fanali *etal.*, 2010 ;Quispe*etal.*, 2018.)
11	55	$C_{28}H_{28}O_{12}$	555	[M-H]-	247, 391, 307	Aloenin-2`-p-coumaroyl ester	(Wu *et al.*, 2013)

[a]: The notation for peak number refers to figure 1.

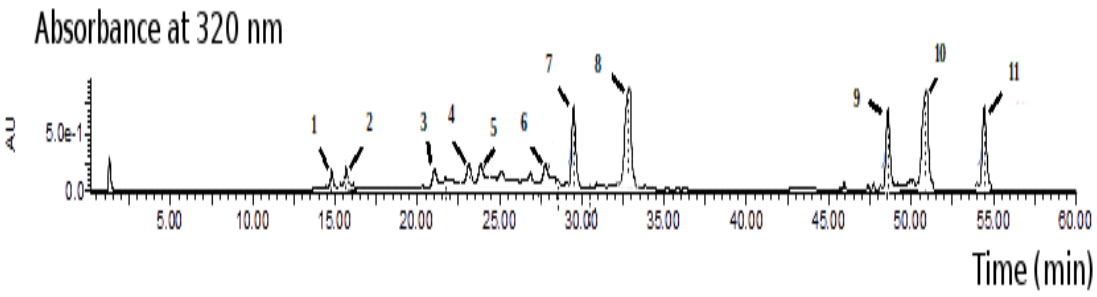

Figure 1. LC-MS chromatogram of ethanol extract of *Aloe vera* leaves at 320 nm expressed in absorbance units (AU).

3.2. Antifungal activity of Aloe vera

Antifungal activity of ethanol extract was determined against three plant pathogenic fungi, Fusarium roseum, Fusarium oxysporum lycopersici and Botrytis cinerea. The results of the *Growth inhibition percentage* of *ethanol extract* show that the ethanol extract has an inhibitory activity against the tested mycelial strains (Figures 2 and 3).

It is noted that the ethanol extract at concentrations (5%, 2.50%, 1.25% and 0.62%) partially inhibited the growth of *Fusarium roseum* with an inhibition rate (92%±2.64, 85%±3.60, 77.33%±1.15 and 51%±2.64, respectively), and at the concentrations (5% and 2.50%) partially inhibited the growth of *Fusarium oxysporum lycopersici* with an inhibition rate (71%± 4.58and 55%±1.63).

Results showed that the ethanol extract at the concentrations (5%, 2.50% and 1.25%) totally inhibited the growth of *Botrytis cinerea* (100%±0.00) and MIC of about 1.25%. The ethanol extract at concentrations (0.62% and 0.31%) partially inhibited the growth of this plant pathogenic fungi with an inhibition rate (72%±2.16 and 63.67%±0.57, respectively).

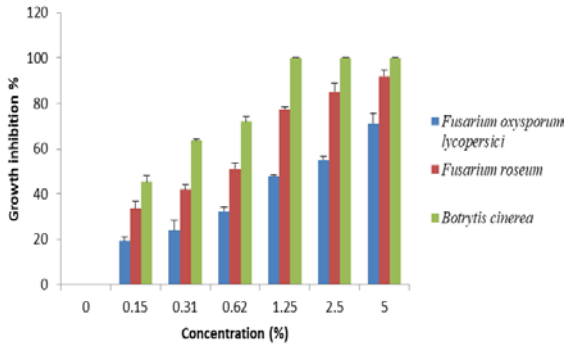

Figure 2. Growth inhibition percentage *of* ethanol extract concentrations *of Aloe vera* leaves.

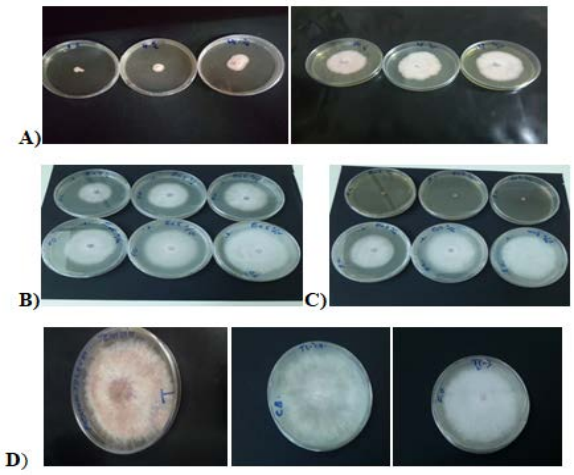

Figure 3. *Fusarium roseum* (A), *Fusarium oxysporum lycopersici* (B) and *Botrytis cinerea (C)* colonies grown on PDA medium supplemented with ethanol extract of *Aloe vera* leaves, tested at six concentrations (5%, 2.5%,1.25%, 0.62%, 0.31% and 0.15%,) recorded after 144 hours of incubation at 20°C. Negative control (D): Untreated control (PDA + DMSO).

3.3. Allelopathic potential

3.3.1. Effects of the aqueous extract of Aloe vera on germination of Triticum durum and Amaranthus hybridus

Different concentrations of the aqueous extract of *Aloe vera* leaves slow the germination kinetics of *Triticum durum* and *Amaranthus hybridus* seeds (Figures 4 and 5) and significantly reduce the rate of final germination of *Amaranthus hybridus*.

The germination inhibition effect of *Amaranthus hybridus* reaches a maximum value of 100% for C10% and C25%. However, a weaker germination inhibition effect (40%) was observed for *Triticum durum* at the concentration C25%.

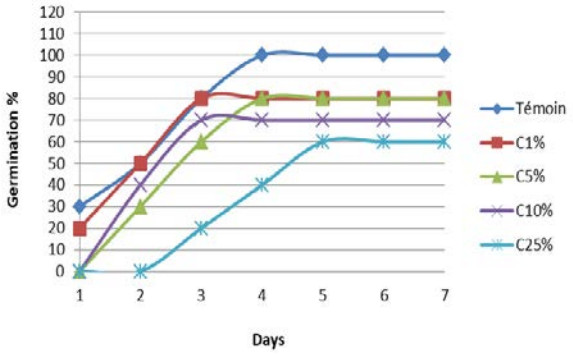

Figure 4. Effect of the aqueous extract of *Aloe vera* leaves on the germination kinetics of *Triticum durum.*

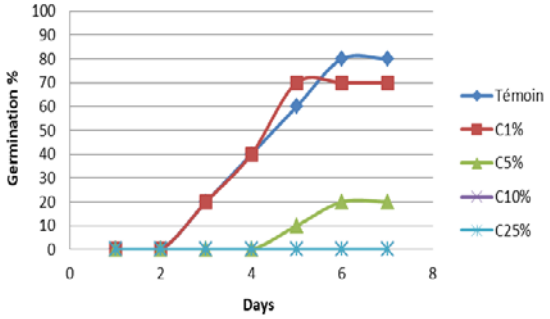

Figure 5. Effect of the aqueous extract of *Aloe vera* leaves on germination kinetics of *Amaranthus hybridus*

Most of the studied concentrations of aqueous extract have an important inhibitory effect on root length and stem height of *Triticum durum* and *Amaranthus hybridus* seedlings (Table 2). The aqueous extract has an inhibitory effect on the length of the roots and the height of the stems of *Triticum durum* from the concentration C5% and reaches a maximum value at the concentration C25% (98.08%, 96.97% for roots and stems). At the C1% concentration, however, the aqueous extract does not represent an inhibitory effect on the root length and height of *Triticum durum* stems, which is respectively 7.20± 0.16cm, 4.4± 0.96cm.

The aqueous extract from the leaves of *Aloe vera* has an inhibitory effect on the length of the roots of *Amaranthus hybridus* at all concentrations and completely inhibits root growth (100%) at C10% and C25% concentrations.

Table 2. Effect of the aqueous extract of *Aloe vera* leaves on the growth of the roots and stems of *Triticum durum* and *Amaranthus hybridus.*

Concentrations	Length of *Triticum durum roots*		Height of the stems of *Triticum durum*		Length of *Amaranthus hybridus* roots	
	cm	Inhibition%	cm	Inhibition%	cm	Inhibition%
Control	7.8±0.28^{a}	0.0±0.00	5.5 ±0.70^{a}	0.0±0.00	5.16±0.62^{a}	0.0±0.00
C1%	7.20±0.16^{a}	8.05±1.41	4.4±0.96^{a}	23.02±3	1.8 ±0.28^{b}	64.44±3.84
C5%	1.83±0.28^{b}	75.97±4.57	0.63±0.15^{b}	88.56±1.25	0.16±0.05^{c}	93.84±4.31
C10%	1.7±0.36^{b}	79.13±4.39	0.16±0.05^{c}	90.1±2.15	0.00±0.00^{d}	100±0.00
C25%	0.15±0.05^{c}	98.08±0.62	0.1±0.05^{c}	96.97±1.00	0.00±0.00^{d}	100±0.00

In the same column, the means followed by the same letter are not significantly different according to the student test at $p<0.05$. Each value represents a mean standard deviation (n=3).

4. Discussion

Evaluation of the antifungal potential of *Aloe vera* leaves in our study is consistent with previous work reports. It should be noted that Casian *et al*. (2007) showed that the *Aloe vera* leaf extract inhibit the mycelial growth of *Penicillium gladioli, Fusarium oxysporum, Heterosporium pruneti* and *Botrytis gladiolorum.* Jasso *et al*. (2005) assessed the antifungal potential *of Aloe vera* leaf extract against the mycelium development of *Rhizoctonia solani, Fusarium oxysporum* and *Collectotrichum coccodes* and found positive results. Saks and Barkai-Golan (1995) tested the antifungal potential of *Aloe vera* leaf on four plant pathogenic fungi, *Botrytis cinerea, Alternaria alternata, Penicillium digitatum* and *penicillium expansum;* the result of this study showed that the *Aloe vera* leaves suppress the mycelial growth of all mycelial strains tested. In our study, the *Aloe vera* leaves completely inhibited the growth of *Botrytis cinerea* (100%). However, Saks and Barkai-Golan (1995) showed that *Aloe vera* leaves partially inhibited the growth of *Botrytis cinerea* (67%), The difference between these results may be due to geographical and climatic conditions, which may affect the phytochemical composition of the plant and its antifungal activity (Jeyasakthy e*t al*., 2017).

Bajwa and Shafique (2007) used *Aloe vera* extract against plant pathogenic strains, *Alternaria alternata, Alternaria citri* and *Alternaria tenuissima*. The results of this study reflect that *Aloe vera* has an inhibitory effect on mycelial growth and the proliferation of these fungi.

Cooposamy and Magwa (2007) also proved that *Aloe vera* extract had an antifungal effect on *A. flavus, A. glaucus, C. albicans, C. tropicalis, T. mentagrophytes* and *T. rubrun*. The study by Sitara *et al.*(2011) showed that *Aloe vera* gel extract completely inhibited the growth of *Drechslera hawaiensis* and *Alternaria alternata*.

Eugene *et al.* (2011) showed that aloin and aloe-emodin have a significant inhibitory effect on the growth of *C.gloeosporioides* and *C.cucumerinum.* Aloin and aloe-emodin are anthraquinone derivatives, and antifungal activity of several anthraquinone derivatives in other plants has been reported by Agarwal *et al.*(2000); Singh *et al.* (2006). These studies were consistent with our study.

Results obtained on seed germination and development of the seedlings of *Triticum durum* and *Amaranthus hybridus* show that the aqueous extract of *Aloe vera* delays seed germination to the point of interruption.

Kruse *et al*. (2000) showed that when susceptible plants are exposed to allelochimical substances, seed germination is delayed. For some seeds, germination stops in the swelling stage of the seed. For others, germination stops at the beginning of the radicle's appearance.

When seed germination is not inhibited, other effects on seedling development have been observed. In the case of inhibition, we noted effects on the root and stem or both. In some cases, the root development is delayed; in other cases, the root development stops. For the aerial part, the effect is manifested by the delay of development or by the absence of stem.

In most of the tests carried out in our study, the aqueous extract's inhibitory effect is more important on the growth of the seedlings (root length and aerial length) and increases when the concentration of the extract increases, Arslan *et al.* (2005), Uremis *et al.* (2005), Turk and Tawaha (2003) and Batish *et al.* (2002), Baličević *et al.* (2014) also showed that inhibition increases with increased concentration of extracts.

Seed germination of the adventitious species *Amaranthus hybridus* is completely inhibited by *Aloe vera* extract at C10% and C25% concentrations. We also tested a variety of wheat to see if the allelopathic plant also affects wheat crops or not. Based on the results obtained, the aqueous extract has no effect on wheat seed germination. However, the aqueous extract affects the development of the roots of the species *Triticum durum* at concentrations C5%, C10% and C25%. These results were in agreement with those of Hussain *et al.* (2007), which showed that the extract (C10%) of *Cassia angustifolia* inhibits root length but does not affect wheat seed germination. Another study by Alipoor *et al* (2012) showed that *Aloe vera* leaf extract exhibited a significant inhibitory effect on the seed germination and seedling lengths of *Triticum aestivum, Secale cereale , Lepidium sativum, Amaranthus retroflexus, Taraxacum officinalis.* Wheat seeds *(Triticum aestivum)* were least affected by the *Aloe vera* extract. Seed germination and seedling growth of *Lepidium sativum, Amaranthus retroflexus,* and *Taraxacum officinalis* were almost completely inhibited at 2.5% of leaf extract.

In our study, seed germination of the adventitious species, *Amaranthus hybridus*, is completely inhibited by the aqueous extract at concentrations C10% and C25%. However, other studies have reported negative effects of aqueous extract from *Aloe vera* leaves on crops such as the results observed by Lin *et al*. (2004); they reported a negligible effect on germination of *Lactuca sativa, Raphanus raphanistrum, sativus, Brassica rapa* and *Oryza sativa.* Also, the study by Ravlić *et al*. (2017) showed that the aqueous extract from the leaves of *Aloe vera* does not affect germination of *Amaranthus retroflexus L, Tripleurospermum inodorum L, Abutilon theophrasti.*

The inhibitory effect of *Aloe vera* leaf extract on seed germination and seedling development may be related to the presence of allelochimic compounds, including tannins, flavonoids and phenolic acids. In addition, toxicity may be caused by a synergistic effect of several secondary metabolites (Saharkhiz *et al.* 2009; Nourimand *et al.* 2011).

5. Conclusion

In this study, the analysis of Phytochemical Constituents attested to the presence of chromones, phenyl pyrones, naphthalene derivative, and anthrones in ethanol extract of *Aloe vera* leaves. Our results bring new information to the literature data about the antifungal activity of *Aloe vera* extract against *Botrytis cinerea;* the ethanol extract at the concentrations (5%, 2.50% and 1.25%) completely inhibited the growth of these mycelial strains. The aqueous extract of *Aloe vera* inhibits the germination of a wheat weed species (*Amaranthus hybridus*) without affecting the germination of durum wheat. Total germination inhibition of *Amaranthus hybridus* is noted at C10% and C25%.

The isolation and purification of several polyphenolic components of *Aloe vera* are necessary for the development of natural derived herbicides and fungicides. Their use would minimize the use of synthetic herbicides and fungicides over time, which would be a significant economic benefit to farmers and would also reduce adverse impacts on the environment.

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References

Afzal MRA, Hassan H, Sweedan N and Dhami MSI. 1991. Identification of someprostanoids in *Aloe vera* extracts. *Planta Medica*., **5:**38-40.

Aggarwal BB, Vijayalekshmi RV and Sung B. 2009. Targeting inflammatory pathways for prevention and therapy of cancer: short-term friend, long-term foe.*Clin. Cancer Res*., **15:**425-430.

Alipoor M, Mohsenzadeh S, Teixeira da silva JA and Niakousari M. 2012. Allelopatic potentiel of *Aloe vera*. *Med Aromat Plant SciBiotechnol*., **6(1):**78-80.

Anjum A, Hussain U,Yousaf Z, Khan F and Umer A. 2010. Evaluation of allelopathic action of some selected medicinal plant on lettuce seeds by using sandwich method. *J. Med. Plants Res*., **4:**536-541.

Arslan M, Uremis I and Uludag A. 2005. Determining bio-herbicidal potential of rapeseed, radish and turnip extracts on germination inhibition of cutleaf ground-cherry (*Physalisangulata*L.) seeds. *J. Agron.*, **4:**134-137.

Bajwa R and Shafique S. 2007. Apprasial of antifungal activity of *Aloe vera*. *J. Mycopath.*, **5(1):**5-9.

Baličević R, Ravlić M, Knežević M and Serezlija I. 2014. Allelopathic Effect of Field Bindweed (*Convolvulus arvensis*L.) Water Extracts on Germination and Initial Growth of Maize. *J. Anim. Plant Sci*., **24(6):**1844-1848.

Batish DR, Singh HP, Kohlir K, Saxena DB and Kaur S. 2002. Allelopathiceffects of parthenin against two weedy species, *Avena fatua* and *Biden spilosa. Environ Exp Bot*., **4(2):**149-155.

Bidel LP, Chomicki G, Bonini F, Mondolot L, Soule J, Coumans M, La Fisca P, Baissac Y, Petit V, Loiseau A, Cerovic ZG, Gould KS and Jay Allemand C. 2015. Dynamics of flavonol accumulation in leaf tissues under different UVB regimes in *Centellaasiatica* (Apiaceae). *Planta*., **242:**545-559.

Bond W and Grundy. 2001. Non-chemical weedmanagement in organic farming systems. *Weed Res*., **41:**383-405.

Casian OR, Parvu M, Vlase L and Tamas M. 2007. Antifungal activity of *Aloe vera* leaves. *Fitoterapia*., **78(3):**219-222.

Sharifi Rad j , Sharifi Rad M and Teixeira da Silva JA. 2014. Evaluation of allelopathic effects of methanolic extracts from *Salicornia herbacea* seed and leaves on germination and seedling growth *in vitro* of two medicinal plants and two weeds. *Environ. Exp. Bot***., 12:** 83-8

Cherkupally R, Reddy Kota S, Amballa H and Narasimha Reddy B. 2017. In vitro antifungal potential of plant extracts against Fusarium oxysporum, Rhizoctoniasolani and Macrophominaphaseolina. *Ann.Plant. Sci*., **6(9):**1676-1680.

Chung IM, Kim KH, Ahn JK, Lee SB, Kim SH and Hahn SJ. 2003. Allelopathy: Comparison of Allelopathic Potential of Rice Leaves, Straw and Hull Extracts on Barnyardgrass. *J. Agron*., **95:**1063-1070.

Coopoosamy RM and Magwa ML. 2007. Traditional use, antibacterial activity and antifungal activity of crude extract of *Aloe excelsa. Afr. J. Biotechnol*., **6(20):** 2406-2410.

Dhima KV, Vasilakoglou IB, Eleftherohorinos IG and Lithourgidis AS. 2006. Allelopathic potential of winter cereal cover crop mulches on grass weed suppression and sugarbeet development. *Crop Sci*., **46:**1682-1691.

El-Khateeb AY, Elsherbiny EA,Tadros LK, Ali SM and Hamed HB. 2013. Phytochemical analysis and antifungal activity of fruit leaves extracts on the mycelial growth of fungal plant pathogens. *J Plant Pathol Microb*.,**4 :**199.

Esteban-Carrasco A, López-Serrano M, Zapata JM, Sabater B and Martín M. 2001. Oxi-dation of phenolic compounds from Aloe barbadensis by peroxidase activity: possible involvement in defence reactions. *Plant Physiol Biochem*., **39:** 521–527.

Eugene S, Nidiry J, Girijia G and Lokecha AN. 2011. Antifungal of some extractives andcostituents of *Aloe vera*. *J. Med. Plant Res*., **5(2):**196-200.

Fanali S, Aturki Z, D'Orazio G, Rocco A, Ferranti A, Mercolini L and Raggi MO. 2010. Analysis of Aloe-based phytotherapeutic products by using nano-LC-MS. *J Sep Sci*., **33:**2663-2670.

Franz G and Grün M. 1983. Chemistry, occurrence and biosynthesis of C-glycosyl com-pounds in plants. *Planta Medica*. **47:**131-140.

Fujii Y, Parvez SS, Parvez MM, Ohmae Y and Iida O. 2003. Screening of 239 medicinal plant species for allelopathic activity using sandwich method. *WeedBiol. Manag.*, **3:** 233-241.

Hussain S, Siddiqui SU, Khalid S, Jamal A, Qayyum A and Ahmad Z. 2007. Allelopathic Potential of Senna (*Cassia Angustifolia*vahl.) on Germination and Seedling Characters of Some Major Cereal Crops and their Associated Grassy Weeds. *Pak. J. Bot.*, **39(4):**1145-1153.

Jasso de Rodriguez D, Hernandez-Castillo D, Rodriguez-Gracia R and Angulo-Sanchez JL. 2005. Antifungal activity *In vitro* of *Aloe vera* pulp and liquid fraction against plant pathogenic fungi. *IndCrops Prod*., **21(1):**81-87.

Jeyasakthy S, Rosdan S, Irfan M and Azian H. 2017. Antifungal Effect of Malaysian *Aloe vera* Leaf Extract on Selected Fungal Species of Pathogenic Otomycosis Species in In Vitro Culture Medium. *Oman Med. J.*, **32(1)**: 41–46.

Jordan, N. 1993. Prospects for weed control through crop interference. *Ecol Appl*., **3:**84-91.

Kahlon J, Kemp MCX, Yawei N, Carpenter RH, McAnalley HR, Shannon WM and Mc Daniel BH. 1991. In evaluation of the synergistic antiviral effects of acemannan in combination with azidothymidine and acyclovir. *Mol Biother.*, **3:** 214-223.

Kawai K, Beppu H,Simpo K, Chihara T, Yamamoto N, Aggatsu T, Ueda *H* and Yamada Y. 1998. *In vivo* effects of *Aloe arborescens Miller* var*natalensis Berger* (Kidachi aloe) on Experimental TineaPidis in guinea pig feet. *Phytother. Res*., **12:** 178-182.

Khan AL, Hussain J, Hanyun M, Shinwari ZK, Khan H, Kang YH, *Kang* SM, Lee IJ. 2009. Inorganic profile and allelopathic effect of endemic *Inula koelzii* from Himalaya Pakistan. *Pak. J. Bot*., **41(5):**2517-2527.

Khanh MA, Marwat KB, Hassan Z. 2004. Allelopathic potential of some multipurpose trees species (MPTS) on the wheat and some of its associate's weeds. *Int. J. Biol. Biotech*., **1:**275-278.

Kolai N, Saiah Fand Boudia A. 2012. Effet inhibiteur *in vitro* de l'huile essentielle d'*Artimesia herba alba* sur deux souches de *Fusarium oxysporum f. sp*. *radicis-lycopersici*. *AJAE*., **2:**71-76.

Kruse M, Strandberg M and Strandberg B. 2000. Ecological Effects of Allelopathic Plants- a Review. NERI Technical Report N°315. National Environmental Research Institute, Silkeborg, Denmark.

Le Bourgeois T and Merlier H. 1995. Adventrop: Les adventices d'Afrique soudano-sahélienne. Quae Editions. Paris, France.

Lovett JV. 1991. Changing perceptions of allelopathy and biological-control. *Biol. Agric. Hortic.*, **8:**89-100.

Lin D, Tsuzuki E, Sugimoto Y, Dong Y, Matsuo M and Terao H. 2004. Allelopathic effects of aqueous *Aloe vera*leaf extracts on selected crops. *Allelopathy J* .,**13:**67-74.

Malterud KE, Fabrot TL and Huse AE. 1993. Antioxidant and radical scavenging effects of anthraquinones and anthrones. *J. Pharmacol*., **47:**77-85.

Mason HE and Spanner D. 2006. Competitive ability of wheat in conventional and organic management systems: a review of the literature. *Can. J. Plant Sci*., **86:**333-343.

Modallal M and Al-Charchafchi FMR. 2006. Allelopathic effect of Artemisia harba alba on germination and seedling growth of *Anabasis setifera*. *Pak J Biol Sci.* **9(9)**: 1795-1798.

Mohsenzadeh S, Gholami M and Teixeira da Silva JA . 2011 Allelopathic potential of *Ephedra*. *Med Aromat Plant SciBiotechnol*., **5(2):**160-162.

Mohsenzadeh S, Zaboli J, Teixeira da Silva JA . 2012. Allelopathic Potential of Ajwain (*Trachyspermum copticum*). *Med Aromat Plant Sci Biotechnol.*, **6(1):** 72-74

Nourimand M, Mohsenzadehn S, Teixeira da Silva JA and Saharkhiz MJ. 2011. Allelopathicpotential of fennel (*Foeniculum vulgare* Mill.). *Med Aromat Plant SciBiotechnol.*,**5(1):**54-57.

Okamura N, Hine N, Harada S, Fujioka T, Mihashi K and Yagi A., 1996. Three chromone components from *Aloe vera* leaves. *Phytochemistry*., **43:** 495-498.

Park YI and Jo TH. 2006. Perspective of industrial application of *Aloe vera*. In: Park YI and Lee SK (Eds.), New Perspective on Aloe. Springer Verlag, New York, USA, pp. 191-200

Quispe C, Villalobos M, Borquez J and Simirgiotis M. 2018. Chemical Composition and Antioxidant Activity of *Aloe vera* from the Pica Oasis (Tarapac´a, Chile) by UHPLC-Q/Orbitrap/MS/MS. *J. Chem*., 1-12.

Turk MA and Tawaha AM. 2003. Allelopathic effect of black mustard (*Brassica nigra*L.) on germination and growth of wild oat (*Avenafatua* L.). *Crop Prot.*, **22(4):**673-67.

Ramamoorthy L and Tizard IR. 1998. Induction of opoptosis in a macrophage cell line RAW 264.7. *Mol. Pharmacol.*, **53:**415-421.

Ravlić M, Baličević R, Visković M and Smolčić I. 2017. Response of weed species on allelopathic potential of *Aloe vera* (L.) Burm.F. *Herbologia*., **16(2):**49-55

Rice EL. 1984. *Allelopathy*. Academic Press, London, England.

Rodriguez Rodriguez E, Darias Martin J and Diaz Romero C. 2010. *Aloe vera* as a functional ingredient in foods. *Crit Rev Food Sci Nutr*., **50(4):**305-326.

Rongai D, Pulcini P, Pesce B and Milano F. 2015. Antifungal activity of some botanical extracts on *Fusarium oxysporum. Open Life Sci.,* **10:**409-416.

Rongai D, Milano F and Scó E. 2012. Inhibitory Effect of Plant Extracts on Conidial Germination of the Phytopathogenic Fungus *Fusarium oxysporum*. *Am J Plant Sci.,* **12:**1693-1698.

Saks y and Barki-Golan R. 1995. *Aloe vera* gel activiy against plant pathogenic fungi. *Postharvest Biol. Technol.,* **6(1-2):**159-165.

Saharkhiz MJ, Ashiri F, Saleh MR, Ghaemaghami J and Mohammadi SH. 2009. Allelopathic potential of essential oils from *Carum copticum* L.,*Cuminum cyminum*L., *Rosmarinus officinalis* L. and *Zataria multiflora* Boiss. *Medicinal Aromatic Plant Sci Biotech.,* **3:**32-35.

Sampietro DA and Vattuone MA. 2006. Sugarcane straw and its phytochemicals as growth regulators of weed and crop plants. *Plant Growth Regul*., **48:**21-27.

Singh Ahlawat K and Singh Khatkar B. 2011. Processing, food applications and safety of aloe vera products: a review. *J. Food Sci. Technol*., **48(5):**525-533.

Singh HP, Batish DR, Kaur S and Kohli RK. 2003. Phytotoxic interference of *Ageratum conyzoides*with wheat (*Triticum aestivum*). *J Agron Crop Sci*.,**189:**341-346.

Sisodia S and Siddiqui B. 2010. Allelopathic effect by aqueous extracts of different parts of *Croton bonplandianum* Baill. On some crop and weed plants. *Agric Ext Rural Dev.,* **2:**22-28.

Sitara U, Hassan N and Naseem J. 2011. Antifungal activity of *Aloe vera* gel against plant pathogenic fungi. *Pak. J. Bot.,* **43(4):** 2231-2233.

Sodaeizadeh H, Rafieiolhossaini M , Havlk J and Damme PV. 2009. Allelopathic activity of different plant parts of *Peganum harmala* L. and identification of their growth inhibitors substances. *Plant Growth Regul.,* **59(3):**227-236.

Stanković MS, Stefanović O, Čomić L, Topuzović M, Radojević I and Solujić S. 2012. Antimicrobial activity, total phenolic content and flavonoid concentrations of *Teucrium* species. *Cent Eur J Biol*., **7:**664-671.

Uremis I, Arslan M and Uludag A. 2005. Allelopathic effects of some brassica species on germination and growth of cutleaf ground-cherry (*Physalis angulata*L.) seeds. *J Biol Sci*., **5:**661-665.

Urch D. 1999. *Aloe vera*– Nature's gift. Blackdown Publications, Bristol, England.

Tizard I, Busbee D , Maxwell Band Mc K. 1994. Effect of Acemannan, a complex carbohydrate, on wound healing in young and aged rats. *J.Wounds*., **6:**201-209.

Turk MA and Tawaha AM. 2003. Weed control in cereal in Jordan. *J.Crop.Prot.,* **22:**239-246.

Wu X, Ding W, Zhong J, Wan J and Xie Z. 2013. Simultaneous qualitative and quantitative determination of phenolic compounds in *Aloe barbadensis* Mill by liquid chromatography–mass spectrometry-ion trap-time-of-flight and high performance liquid chromatography-diode array detector. *J Pharm Biomed Anal.,* **80:**94-106.

Yang QY, Yao CS and Fang WS. 2010. A new triglucosylatednaphthalene glycoside from *Aloe vera* L. *Fitoterapia.,* **81(1):**59-62.