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Antimycotoxigenic and antifungal activities of *Citrullus colocynthis* seeds against *Aspergillus flavus* and *Aspergillus ochraceus* contaminating wheat stored

GACEM Mohamed Amine[1]*, OULD EL HADJ KHELIL Aminata[1], GACEMI Bouabdallah[2], HALLA Noureddine[3], DJERBAOUI Amina Nesrine[1], BOUDERHEM Amel[1], HADEF Sawsen[1], BENREGUIEG Mokhtar[2,4] and ADLI Djalal Eddine Houari[4]

[1]Laboratory of Protection of Ecosystems in Arid and Semi-arid Area, University of Kasdi Merbah, Ouargla (30000), Algeria.
[2]Laboratory of Technology and Animal Production, University of Abdelhamid Ibn Badis, Mostaganem (27000), Algeria.
[3]Laboratory of Antibiotics Antifungal, Physical Chemistry, Synthesis and Biological Activity, University of Abou Bekr Belkaïd, Tlemcen (13000), Algeria.
[4]Departement of Biology, University of Tahar Moulay, Saida (20000), Algeria.

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Plant extracts and their constituents have a long history as antifungal agents, but their use in biotechnology as preservatives, due to the increasing resistance of fungi to fungicides, has been rarely reported. The aim of this study was to assess *in vitro* antifungal and antimycotoxigenic power of methanolic and aqueous extracts of *Citrullus colocynthis* seeds, an aromatic and medicinal plant, of Algerian flora, against two toxigenic species of the genera *Aspergillus* responsible of contamination of wheat stored. The antifungal and antimycotoxigenic activity of methanolic and aqueous extracts were screened against *Aspergillus ochraceus* and *Aspergillus flavus.* Dillution method was used to investigate the antimicrobial and antimycotoxigenic activity. These bioassays are preceded by a phytochemical screening. The phytochemical analysis of seeds extracts revealed the presence of some chemical groups (polyphenols, steroids and alkaloids) which can express the desired activities. The results suggest that the extracts showed a very good antifungal activity against *A. ochraceus*, but for *A. flavus* any antifungal activity was recorded. The extracts have good antiochratoxigenic power in liquid medium. This evaluation confirms that the extracts of *C. colocynthis* seeds used at low concentration may have significant potential for biological control of fungi and theirs toxins.

Key words: *Citrullus colocynthis,* methanolic extract, aqueous extract, phytochemical screening, antifungal activity, antimycotoxigenic activity, antiochratoxigenic activity.

INTRODUCTION

Fungi are the main infectious agents in plants, causing alterations during developmental stages including post-harvest. In fruit and vegetables, there is a wide variety of fungal genera causing quality problems related to aspect,

*Corresponding author. E-mail: biologieamine@yahoo.fr.

Abbreviations: **AFs,** Aflatoxins; **OTA,** ochratoxin A; **TLC,** thin-layer chromatography; **DRBC,** Dichloran Rose-Bengal chloramphenicol agar; **CDA,** Czapek dextrose agar; **PDA,** potatoes dextrose agar; **YES,** yeast extract sucrose; **MIC,** minimum inhibitory concentration; **MFC,** minimum fungicidal concentration.

nutritional value, organoleptic characteristics and limited shelf life (Yanes et al., 2012). In addition, fungi are responsible for allergic or toxic disorders among consumers because of the production of spores or mycotoxins (Dellavalle et al., 2011).

Mycotoxins are secondary metabolites produced by five fungal genera namely *Aspergillus*, *Penicillium*, *Fusarium*, *Alternaria* and *Claviceps*, they are synthesized under favorable conditions of temperature and humidity. They may be developed in several stages, in the field before harvest, during storage and even in the production chain (Petzinger and Weindenbach, 2002). These toxic substances are carcinogenic, nephrotoxic, hepatotoxic and immunosuppressive (Dellavalle et al., 2011; Korhonen et al., 2012). They are found in many food products such as coffee, cereals, wine and fermented products (Cynthia et al., 2012). Aflatoxins (AFs) are the most dangerous mycotoxins.

Five types of aflatoxins are known; AFB1, AFB2, AFG1, AFG2 and AFM, these toxins are produced by *Aspergillus flavus, Aspergillus parasiticus, Aspergillus nomius, Aspergillus pseudotamarii, Aspergillus bombycis, Aspergillus toxicarius, Aspergillus minisclerotigenes, Aspergillus parvisclerotigenus* and *Aspergillus arachidicola* (Samson et al., 2006; Pildain el al., 2008). *Aspergillus flavus* and *Aspergillus parasiticus* are the major producers of AFB1 (Gourama and Bullerman, 1995). Ochratoxin A (OTA) is the second important mycotoxin with fumonisin, zearalenone and trichothecene. OTA is produced by *Penicillium verrucosum, Aspergillus ochraceus, Aspergillus alliaceus, Aspergillus carbonarius, Aspergillus niger* and *Aspergillus melleus* (Da Rocha Rosa et al., 2002; Accensi et al., 2004; Bau et al, 2005; Bayman and Baker, 2006).

Fungi are generally controlled by synthetic fungicides; however, the use of these is increasingly restricted due to the harmful effects of pesticides on human health and the environment (Hermiche et al., 2012). The increased risk of high-level toxic residues in the products and the emergence of pathogens resistant to the products employed, justifies the search for novel actives molecules and new control strategies. Thus, there are a growing interest on the research of possible use of the plant extracts for control of the pest and diseases in agriculture which is less harmful to the health and environment (Nwosu and Okafor, 2000; Logardia et al., 2012).

Several works have demonstrated in laboratory trials that plants tissues, such as roots, leaves, seeds and flowers posses inhibitory properties against bacteria, fungi and insects (Thembo et al., 2010; Benariba et al., 2013). In front these very serious health problems, use of medicinal plants in biomedical research received great interest. This is because herbs are an inexhaustible source of bioactive natural compounds and fewer side effects than drugs (Dramane et al., 2010; Satyavani et al., 2012). Medicinal plants are now an endless source of interesting molecules for scientists and industry, which occur as secondary metabolites (Lozoya and Lozoya, 1989; Karthikeyan et al., 2009). They are grouped as alkaloids, glycosides, flavonoids, saponins, tannins, carbohydrate and essential oils. Molecules from these plants have similar active ingredients which have specific properties giving them an intrinsic behavior (Evon, 2008). A wide spectrum of biological substances extracted from medicinal plants, including oils were tested to replace some of the ways to fight against fungi. In this section, several authors have confirmed the effectiveness of the oils on toxigenic fungi (Ziyada and El Hussein, 2008; Yingying et al., 2008).

Citrullus colocynthis (Schrad), belonging to the family of *Cucurbitaceae*, is an endemic in the south of Algeria. This medicinal plant popularly known as *Handhal, Hdaj* or *Dellaa El-Wad*, is widely used in Algerian folk medicine for treating many diseases such as rheumatism, hypertension hyperglycemia and various contagious diseases, including dermatological problems and gynaecological, urinary and pulmonary infections (Le Flock, 1983; Boukef, 1986; Marzouk et al., 2009; Gurudeeban et al., 2010). .

The objective of this work is to demonstrate the antifungal, antiaflatoxigenic and antiochratoxigenic effect of methanol and aqueous extracts of *C. colocynthis* seeds, after determining their chemical composition, against two toxigenic fungal strains namely: *A. flavus* and *A. ochraceus* isolated from wheat stored.

MATERIALS AND METHODS

Plant

C. colocynthis Schrad. fruits were collected in December (2010) near Ouargla, Algeria in the area of Oued N'sa. The identification was performed according to the flora of Tunisia (Pottier-Alapetite, 1981) and the botanists of Faculty of Biology of Saida University (Algeria).

Extraction protocol

The extractions were performed on the seeds of *C. colocynthis*. Plant materials were washed with tap water, disinfected by immersion in 2% sodium hypochlorite solution for 30 min, rinsed with sterile distilled water to eliminate residual hypochlorite. Afterwards, the seeds are ready for extraction (Jasso de Rodriguez et al., 2005). In this study, water and methanol are the two solvent used for extraction. These two solvent are polar and they can extract the maximum of bioactive substances.

Methanol extract

Twenty grams of seeds were ground with a mixer and added to 100 ml of methanol. After 3 h of maceration with continuous stirring at 200 rev/min, the mixture was then filtered using filter paper (Whatman No 1). This operation is repeated four times after each filtration with renewal of the solvent in order to exhaust the marc and increase the yield. At the end of extraction, the fractions obtained were collected in a vial and then were evaporated by rotavapor at a specific temperature to the solvent (Senhaji et al., 2005).

Aqueous extract

The aqueous extract is prepared by soaking 20 g of the ground seeds in 100 ml of cold distilled water for 3 h with continuous agitation. The mixture was then centrifuged at 3600 g for 30 min. The supernatant was recovered and then filtered through Whatman filter No. 1. This operation was repeated four times after each filtration with renewal of the solvent. At the end of extraction, the fractions obtained were collected in a vial, then, lyophilized or dried in the drying oven, giving the dry aqueous extract (Senhaji et al., 2005).

Determination of extraction yield

The yield is determined by the ratio of the weight of the dry extract after evaporation on the weight of the plant material used for extraction, multiplied by 100%.

$Rd\ \% = (m_1 \times 100) / m_0$

Where, m_1 is the Mass in grams of the dry extract; m_0 is the mass in grams of dry plant material; Rd is the yield.

Qualitative phytochemical screening

Tannins

One milliliter of extract was mixed with 10 ml of distilled water and filtered. Three drops of ferric chloride ($FeCl_3$) reagent (1% prepared in methanol) was added to the filtrate. A blue-black or green precipitate confirmed the presence of gallic tannins or catechol tannins, respectively (Karumi et al., 2004).

Saponins

Ten milliliters of extract were placed in a test tube shaken for 15 s and then deposited for 15 min. A persistent foam height greater than 1 cm indicates the presence of saponins (N'Guessan et al., 2009).

Steroids

After addition of 5 ml of acetic anhydride to 5 ml of hot extract, the mixture was added to 0.5 ml of concentrated sulfuric acid. After stirring, the appearance of a purple or violet ring turning blue to green indicates the presence of steroids (Edeoga et al., 2005).

Flavonoids

Flavonoids were detected by reaction with cyaniding. 2 ml of each extract were evaporated and the residue was taken in 5 ml of alcohol hydrochloric dilute 2 times. By adding 2 to 3 magnesium chips, there is a heat release and an orange-pink coloration or purplish. The addition of 3 drops of isoamyl alcohol has intensified this color which confirmed the presence of flavonoids (N'Guessan et al., 2009).

Alkaloids

Alkaloids have been characterized using reagents of Mayer. 10 milliliters of extract were evaporated until a volume of 0.2 ml was obtained on which, 1.5 ml of HCl (2%) was added. After stirring the acid solution, 1 to 2 drops of reagent were added, and the appearance of a yellowish white precipitate indicates the presence of alkaloids (Mojab et al., 2003).

Anthraquinones

The method cited by Trease and Evans (1996) was used for the detection of anthraquinones. The presence of violet colour in the ammoniacal (lower) phase indicated the presence of free hydroxy anthraquinones (Trease and Evans, 1996).

Coumarins

Coumarins were found from 5 ml of extract placed in a tube brought to boiling until obtaining a volume of 1 ml, this volume is added to 1 ml of hot water. After stirring, the total volume is divided into two volumes, one as a control and the other is added to 0.5 ml of NH_4OH (10%) and examined under a UV lamp. The fluorescence emission indicates the presence of coumarins (Rizk, 1982).

Antifungal activity of plant extracts

Fungal isolation

Dilution plating was used as isolation technique (Pitt and Hocking, 2009). 10 g of the sample were added to 90 ml of 0.1% peptone water. This mixture was then shaken on a rotary shaker for approximately 15 min and diluted 10^{-2}, 10^{-3} and 10^{-4} fold. Aliquots composing of 0.1 ml of each dilution were spread (in triplicate) on the surface of the dichloran Rose-Bengal chloramphenicol agar (DRBC), Czapek dextrose agar (CDA) and potatoes dextrose agar (PDA). All plates were incubated for 5 to 7 days at 28°C in the dark and under normal atmosphere. The identification of fungal strain is realized on the basis of morphological characteristics, under the microscope (Barnett and Hunter, 1972; Pitt and Hocking, 2009), and single spore method by colony characteristics after their culture on different culture media (Pitt, 1973; Pitt and Hocking, 2009).

Identification of strains producing mycotoxins

The strains of *A. flavus* and *A. ochraceus* identified were reseeded separately on 50 ml of yeast extract sucrose (YES) medium. After 14 days of incubation at 27 ± 2°C, the biomass formed is removed by filtering the medium through Whatman filter paper No. 01. The 50 ml of the filtrate are added to 100 ml of chloroform, the mixture is thoroughly stirred for 10 min and then allowed to settle by using a separating funnel. This operation is repeated by adding successively 50 and 30 ml of solvent to the aqueous phase recovered at each separation. The chloroform phase thus obtained is filtered through Whatman paper No. 01 and then concentrated by evaporation under vacuum using a rotary evaporator type (Heidolph efficient Laborota 4000) until a volume of 2 to 3 ml.

Thin-layer chromatography (TLC) is performed on a silica gel plate (silica gel 60 F254). The plate is then placed in a chromatographic tank dipped in a mixture of elution solvent consisting of toluene, ethyl acetate and formic acid with volume (5: 4: 1), respectively. After migration and evaporation of the elution product dry, the plate is examined under a UV lamp at a wavelength of 365 nm (Frayssinet and Cahagnier, 1982).

Evaluation of antifungal and antimycotoxigenic activity of organic extracts

The study of the antifungal and antimycotoxigenic activity of methanol and aqueous extracts were tested against two species *A ochraceus* and *A. flavus* on YES medium in order to be able to extract the mycotoxins produced. On an individual basis, each of

Table 1. Extraction yields (%) and phytochemical screening of *C. colocynthis* seeds.

Extract	Extraction yields (%)	Phytochemical substance						
		Flavonoid	Steroid	Alkaloid	Anthraquinon	Coumarin	Saponosid	Tannin
Methanol	4.89	+	+	+	-	-	+	+
Aqueous	2.72	+	+	+	-	-	-	+

+, Presence; -, absence.

Table 2. Identification of *Aspergillus ochraceus* and *Aspergillus flavus* by single spore method.

Genera species	Medium	Reading (Color)
Aspergillus flavus	MEA 25°C	Pistachio green
	CYA 37°C	Dark brown
	G25N 25°C	Greenish yellow
	AFAP	Orange back
Aspergillus ochraceus	MEA 25°C	Yellow gold
	CYA 37°C	Yellow
	G25N 25°C	Yellow

the two extracts (*C. colocynthis* seeds) was added to 50 ml of YES medium but to varying final concentrations are in the order of 1 to 25 mg/ml. After rigorous agitation, different media are inoculated with discs of 0.6 cm of diameter containing youth cultures of 3 to 7 days of *A. ochraceus* and *A. flavus*. Control tests are made for strains and for each test series (Ezzat and Sarhan, 1991; Al-Rahmah et al., 2011). After an incubation period of 14 days at 27 ± 2°C, the same steps mentioned above for the extraction and the revelation of mycotoxins have been followed. The biomass of the filtered mycelium was determined after drying at 70°C for 4 days till their weights remains constant. The percentage inhibition is calculated by the following formula:

Percentage of mycelial inhibition = [C - T / C] × 100

Where, C and T are the mycelial dry weight (mg) in control and treatment, respectively.

RESULTS

Extraction yield and phytochemical screening

The calculation of the chemical extractions yields relative to the total weight of the dry powder used displayed in Table 1 shows that the *C. colocynthis* seeds gave dry extracts masses greater than 1 g/100 g seed powder. From the point of view profitability by weight, methanolic extract gave the highest proportions by comparing it with the aqueous extract. On same Table 1, the qualitative chemical analysis tests that are designed to demonstrate the different phytochemical families existing in both extracts revealed a slight difference in the composition of the extracts. This difference is noticed by the lack of saponins which are absent in the aqueous extract while they are present in the methanol extract. Both extract react negatively with tests revealing anthraquinons and coumarins, whereas for other photochemical tests, the two types of extracts reacted positively.

Identification of fungal strains

The different microscopic and macroscopic aspects of both fungal strains searched are demonstrated in Figures 1 and 2. The aspects of fungal colonies of the same strains by single spore method on different culture media are shown in Table 2. The results revealed strains producing mycotoxins on TLC which showed that the strain *A. flavus* is producing AFB1 and strain *A. ochraceus* is producing the OTA.

Antifungal and antimycotoxigenic activity of organic extracts

The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) were employed by poisoned food technique to assess fungistatic and fungicidal properties of the effective plant extract. As illustrated in Figure 3, the inhibitory plant extracts showed that the fungal strain *A. ochraceus* is very sensitive to both types of extracts. Beyond 15 mg/ml of methanol extract and 20 mg/ml of aqueous extract, the latter did not develop biomass in YES medium. Transplanting these mycelial discs that could not grow in the presence of extracts on other PDA medium (without extracts) did not provide any radial growth after 14 days of incubation at 25 ± 2°C, which explains that CMF is 15 mg/ml for

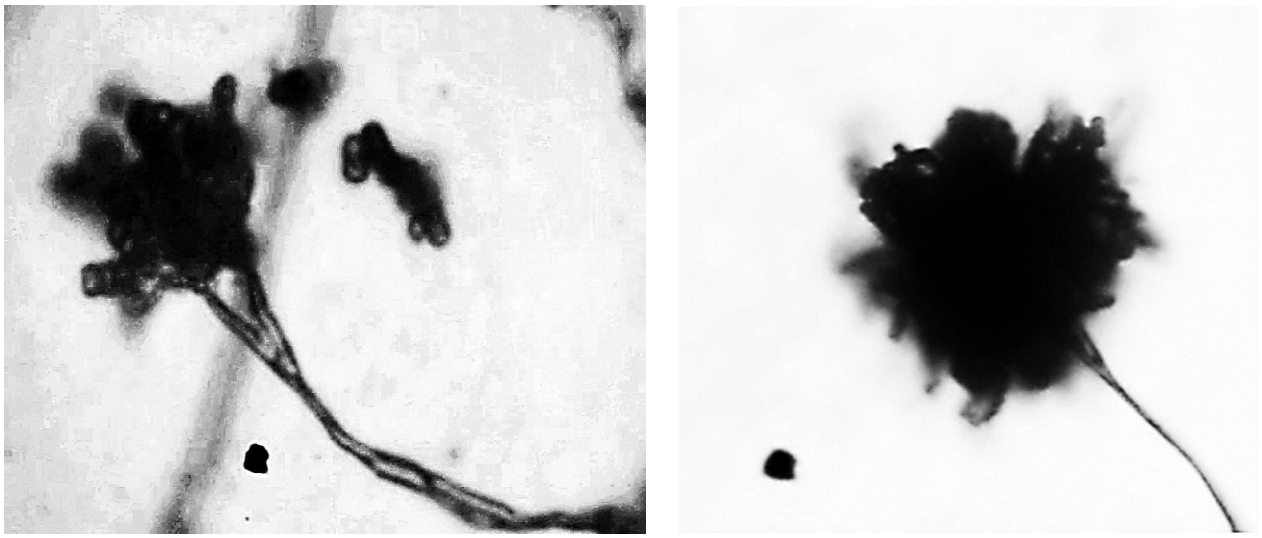

Figure 1. Identification of the genus *Aspergillus* by micro-culture method.

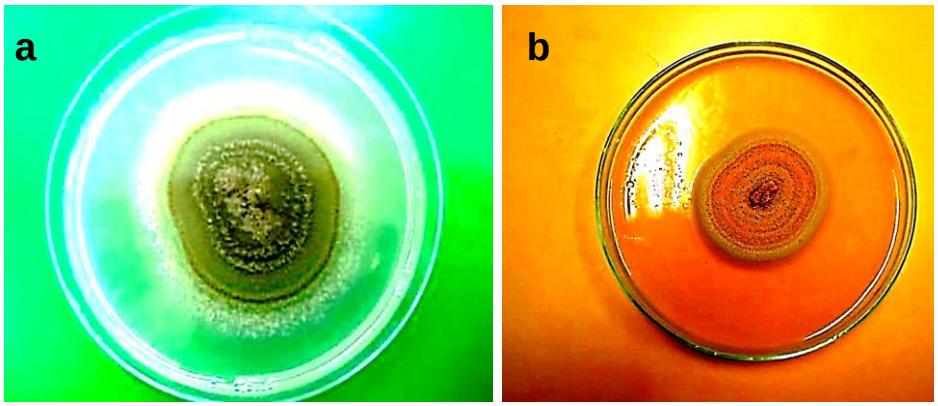

Figure 2. Identification of fungal species by Single Spore method. **a,** Colonies of *Aspergillus flavus* on PDA medium; **b,** colonies of *Aspergillus ochraceus* on PDA medium.

methanolic extract and 20 mg/ml for the aqueous extract. MICs are 10 mg/ml and 15 mg/ml for methanolic and aqueous extract, respectively. Below these two concentrations, the antifungal activity begins to decrease. Figure 4 showed that the strain *A. flavus* has proved highly resistant to two extracts of *C. colocynthis* seeds and no antifungal activity was recorded.

The results displayed in Table 3 achieve the last objective of this study by demonstrating that methanol and aqueous extracts tested against *A. flavus* to determine the power synthesis inhibitor of AFB1 showed no inhibitory activity against this toxin and TLC revealed the presence of a similar spot to the standard of pure AFB1 (Figure 5). For antiochratoxigenic activity (Table 3), methanol extract was able to reduce the synthesis of OTA produced by *A. ochraceus* from 10 mg/ml of extract in the YES medium explained by the reduction of the size of the spot toxin on the TLC plate. For the aqueous extract, the TLC detected a reduction of the synthesis of the toxin produced from 15 mg/ml of extract in the medium. At 15 mg/ml of methanol extract and 20 mg/ml aqueous extract, OTA was not detected on the TLC plate (Table 3).

DISCUSSION

Fungi are ubiquitous in the environment, and infection due to fungal pathogens which has become more common. The genus *Aspergillus* is widely distributed in nature and its species are among the most common destroyers of foodstuffs and grains during storage. It includes species that may damage crops in the field or cause post-harvest decay (Sun et al., 2012). In addition, the

Table 3. Antimycotoxigenic activities of methanol and aqueous extracts of *C. colocynthis* seeds.

Parameter	Concentrations (mg/ml)						
	0	1	5	10	15	20	25
Production of AFB_1 in the presence of Me. E	+	+	+	+	+	+	+
Production of AFB_1 in the presence of Aq. E	+	+	+	+	+	+	+
Production of OTA in the presence of Me. E	+	+	+	-/+	-	-	-
Production of OTA in the presence of Aq. E	+	+	+	+	-/+	-	-

AFB_1, Aflatoxine B_1, OTA, ochratoxine A; Me. E, methanol extract; Aq. E, aqueous extract; +, presence; -, absence.

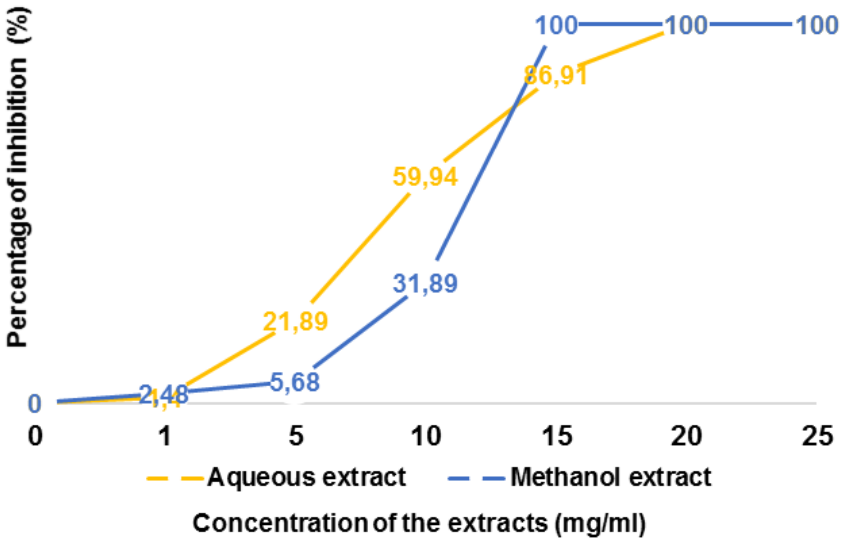

Figure 3. Antifungal activity of methanol and aqueous extracts against *Aspergillus ochraceus* on YES medium.

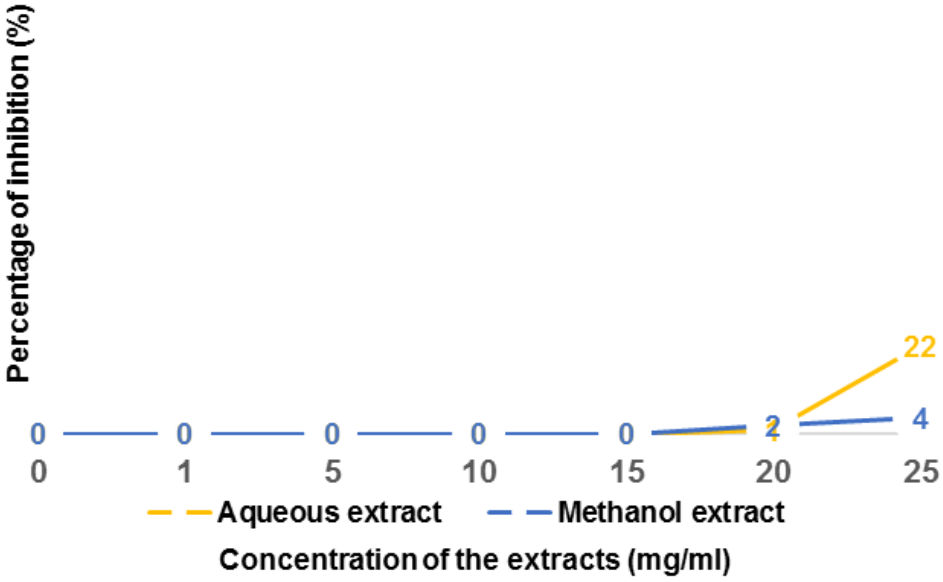

Figure 4. Antifungal activity of methanol and aqueous extracts against *Aspergillus flavus* on YES medium.

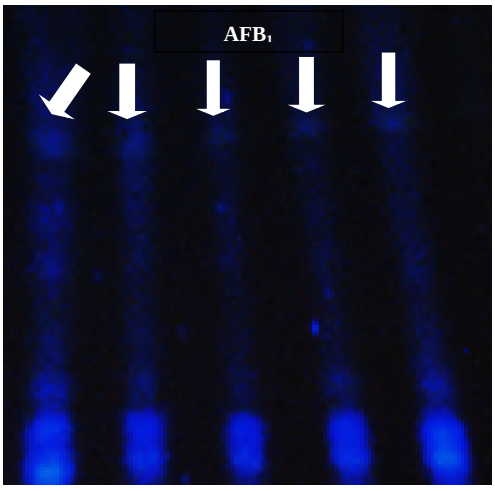

Figure 5. Antiaflatoxigenic activity of methanol extracts against *Aspergillus flavus*.

genus products mycotoxins and studies in the last decade have emphasized its toxicogenic properties. Indeed, the palette adverse effects of mycotoxins on the human and animal health is very extensive and sometimes unknown (Brochard and Le Bacle, 2009; Cynthia et al., 2012). Besides, acute toxic effects or chronic hemorrhagic, immunotoxic, hepatotoxic, nephrotoxic, neurotoxic and teratogenic, some mycotoxins have shown mutagenic and carcinogenic effects in laboratory animals and humans (Korhonen et al., 2012).

Over the last decades, concerns were expressed about the increasing prevalence of pathogenic fungi that are resistant and more precisely those producing mycotoxins. But the problem posed by the high cost and the increased toxic side effects of some synthetic substances coupled with their failure to be treated cannot be underestimated. For this reason, this last decade witnessed increased intensive studies of extracts and biologically active compounds isolated from natural plants (Mabrouk, 2012; Elamathi et al., 2012).

Despite numerous studies on the use of colocynth in the culinary field and that of traditional medicine, little work has been done on the antifungal effect of *C. colocynthis* seeds against fungal strains spoilage of the physicochemical and mycological quality of stored wheat (Gacem et al., 2013). Facing this situation, the aim of this work was to evaluate *in vitro* the antifungal and antimyctoxigenic potential of *C. colocynthis* seeds against toxigenic fungi producing mycotoxins namely *A. flavus* and *A. ochraceus* in order to check possible inhibition activity.

The extraction method performed on the powder of *C. colocynthis* seeds conducted at an ambient temperature can extract maximum compounds and prevent their denaturation or probable modification (Yagoub, 2008). The phytochemical screening of methanol and aqueous extracts reveal the richness of *C. colocynthis* seeds from a qualitative point by secondary metabolites such as steroids, flavonoids, alkaloids and tannins. These compounds have been reported in the *C. colocynthis* by several studies (Gurudeeban et al., 2010; Adebayo-Tayo et al., 2010).

Outcomes related to phytochemical screening of the last class of secondary metabolites show that the alkaloids are present in both extracts from seeds of *C. colocynthis*. This result is confirmed by Sultan et al. (2010) and Benariba et al. (2013) who detected the alkaloids in seeds of this species and Marzouk et al. (2010) who showed that the seeds of *C. colcoynthis* contain 1.64 mg of alkaloid per 100g of material dry. The absence of antraquinons is cited in the study of Suman (2010).

Ethanolic and aqueous extracts from *C. colocynthis* leaves and fruits contain alkaloids, flavonoids, glycosides and saponosides (Najafi et al., 2010). Likewise, the entire *C. colocynthis* plants contain 1.39 mg flavonoids, 0.52 mg saponosides, 1.64 mg alkaloids, 1.64 mg phenolic compounds and 30.12 mg ascorbic acid per 100 g (Sultan et al., 2010). Another study by Gill et al. (2011) documented the presence of alkaloids, steroids, terpenoids, flavonoids, as well as coumarins, glycosides in methanolic and hydromethanolic extracts of *C. colocynthis* seeds. The polyphenol compounds, represented in majority by tannins and flavonoids, are presently a major axis of research, because they are considered as potent antioxidants, anti-inflammatory, anti-bacterial, antiviral and anti-cancer agents (Oliver Chen and Blumberg, 2008).

For instance, Afifi et al. (1973) reported the presence in the whole plant of three alkaloids ($C_{10}H_{15}NO_3$, $C_{20}H_{32}NO$ and $C_{16}H_{24}NO_7$. Hatam et al. (1990) documented the presence of two sterols ($C_{29}H_{48}O$ and $C_{29}H_{50}O$) in a *C. colocynthis* fruits collected in Basra area in Iraq. Thus, *C. colocynthis* contain flavonoids such as quercetin, myricetin and kaemoferol (Oliver Chen and Blumberg, 2008).

Several study identified cucurbitacins in a methanol extract of *C. colocynthis* fruits (Sonja and Hermann, 2000; Seger et al., 2005; Nayab et al., 2006). Such cucurbitacins are relevant to the bitterness and toxicity of the plants, as well as their anti-inflammatory, purgative and anti-cancer activities, such as the inhibition of cell adhesion resulting from the cytoskeleton destabilizing in cancer cells exposed to cucurbitacin E (Jian et al., 2005).

Incidentally, minor differences between the results of distinct studies could be related to differences in local climate and soil composition. The distribution of phytoconstituents such as saponins, tannins, flavonoids and alkaloids, may also vary in distinct parts of *C. colocynthis*, in leaves, fruits, roots and seeds. The study of Gacem et al. (2013) revealed a good activity against strains of *Aspergillus*. The tests of antifungal activities of colocynth in YES medium against strains *A. flavus* and *A. ochraceus* isolated from wheat stored revealed effective-

ness of methanol extract of the seeds of this plant against *A. ochraceus*. The experiment revealed that the methanol extract has a more antagonistic effect than the aqueous extract. This effect is explained by the high yield of methanolic extraction, which is due to the presence of bioactive substances with high quantity. This strong antifungal activity of methanol extract was also reported by several authors (Hadizadeh et al., 2009; Gurudeeban et al., 2010; Gacem et al., 2013).

Chang et al. (2008) and Abdel Ghani et al. (2008) join the antifungal activity of extracts from *C. colocynthis* seeds with bioactive substances of the plant. The power of these phytochemicals compounds to exert higher activity is depending to their concentrations in the extracts (Yan et al., 2008). Among the phytochemicals compounds with antifungal activity, mainly cites alkaloids, polyphenols and steroids (Yan et al., 2008; Oliver Chen and Blumberg, 2008). The antifungal activity of the extracts of the plant depends on its composition, the plant organ to be tested, the nature of the extract and the fungal strains selected (Veldhuizen et al., 2006; Dan et al., 1998).

Several studies have been conducted to understand the mechanism action of plant extracts. Many researchers attribute this feature to phenolic compounds. These compounds can interfere with bio-membranes causing cell damage and causing leakage of cellular materials and finally the death of microorganisms (Veldhuizen et al., 2006; Abdel Ghani et al., 2008). This is a possible mechanism by which the mycelial growth can be reduced or completely inhibited by the effect of extracts acting on the function and structure of the cell membrane. Saponins are a special class of glycosides with a soapy characteristic and very good antifungal activity (Sikkema et al., 1995).

Flavonoids are also responsible for the inhibition of resistant microbes. They are responsible for the scavenging process or chelators and may disrupt microbial membranes. Furthermore, alkaloids contain a detoxifying effect and have a very good antifungal activity (Kessler et al., 2003). Terpens (steroids) affect not only the permeability but also other functions in cell membranes. These compounds can penetrate cell membranes, enter the interior of the cell, and interact with critical sites such as intracellular enzymes and proteins, leading to cell death (Omidbeygi et al., 2007).

The extracts obtained from the upper parts of plants have the ability to suppress the growth of toxigenic fungi and therefore toxin production (Thanaboripat et al., 1997). They can also completely block the biosynthesis of mycotoxins while fungal growth is not affected (Bhatnagar and Mccormick, 1988). These seed extracts of *C. colocynthis* are less important relative to the extracts of *Eucalyptus globolus*, *Olea europea* and *Thymus vulgaris* described in the study of Al-Rahmah et al. (2011), which proved a complete inhibition of AFB1 synthesis and the study of El-Nagerabi et al. (2012) who demonstrated the effect of *Hibiscus sabdariffa* extract and *Nigella sativa* oil for inhibiting the synthesis of AFB1. The phenolic compounds of *C. colocynthis* seeds cannot inhibit the biosynthesis steps of AFB1, explained by their absence in the lipids of the fungal cell wall membrane and mitochondria, disturbing their structure and rendering them more permeable. Leaking of ions and other cell contents can then occur (Cox et al., 2000; Burt, 2004).

Contrariwise, the methanolic extract of *C. colocynthis* seeds showed a very good inhibition of OTA and this extract is ranked higher than other extract as *Ferronia eluhantum, Lawsona innermis* and *Azadirachta indica* causing a reduction of the synthesis only (Warke et al., 2006). The use of this extract is best looked for other substances that have the same effect such as 4-hydroxy-3-(p-toluoyl)-6-(p-tolil)-2H-pyrane-2-one (DHT) and 5-bromo-4-hydroxy-3-(p-toluoyl)-6-(p-tolil)-2H-pyrane-2-one (BrDHT) (Durakovic et al., 1989). The advantage of herbal extracts is their bioactivity, a feature that makes them attractive for the protection of stored products such as cereals against fungal attack.

Conclusion

The results obtained are encouraging and confirm the value of the use of *C. colocynthis* seeds as an antifungal agent and in biotechnology as a preservative for the fight against toxigenic fungi and their mycotoxins. It is therefore interesting to continue this study in order to determine the mode of action of extracts on mold.

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