Research Article

Phytochemical screening, antifungal and antioxidant activities of three medicinal plants from Algerian steppe and Sahara (preliminary screening studies)

Mohamed Amine Gacem[1,2] · Alia Telli[1] · Hiba Gacem[3] · Aminata Ould-El-Hadj-Khelil[1]

Received: 4 October 2019 / Accepted: 26 November 2019 / Published online: 28 November 2019

Abstract
The contamination of foodstuffs by fungi and their mycotoxins, emergence of resistance to fungicides and unwanted side effects of pesticides on human and animal health currently present a challenge for researchers to create and innovate new and better solutions to fight against mycotoxigenic fungi with less harmful effects on the consumer and the environment. In the context of the valorization of natural products, this study aimed, first, to determine the phytochemical composition of methanolic and aqueous extracts of *Pistacia lentiscus, Artemisia herba-alba* and *Citrullus colocynthis* and, second, to evaluate the antifungal potential and antioxidant capacity of these extracts. The extraction of crude active compounds from selected plant species was carried out by methanolic and aqueous maceration. Antifungal activity against five fungal strains (*Aspergillus flavus, Aspergillus parasiticus, Fusarium graminirium, Aspergillus ochraceus* and *Penicillium expansum*) was evaluated by the dilution method in solid medium. Analysis of antioxidant activity was carried out by three tests, namely, the inhibition of $ABTS^{\cdot+}$ radical cation, trapping of $DPPH^{\cdot}$ radical and reduction of Fe^{2+} (FRAP). The results show that the crude extracts were rich in bioactive compounds and that the crude methanolic and aqueous extracts of *A. herba-alba* were the most active against the tested fungal strains and had a percentage of inhibition exceeding 8%. Regarding antioxidant activity, all the extracts exhibited remarkable antioxidant potential in the tests used. The methanolic extract of *A. herba-alba* had the highest iron-reducing capacity. The results show that the studied medicinal plants could be a good alternative for protecting foodstuffs against toxigenic fungi.

Keywords *Pistacia lentiscus* · *Artemisia herba-alba* · *Citrullus colocynthis* · Antifungal activity · Antioxidant activity

1 Introduction

Mycotoxins are secondary metabolites produced by six fungal genera, namely, *Aspergillus*, *Alternaria*, *Claviceps*, *Fusarium*, *Penicillium* and *Stachybotrys*. These metabolites commonly occur in foodstuffs and cause diseases in consumers [1]. Depending on the administered dose, the toxicity can spread to all vital human and animal organs and cause oxidative stress [2], which leads to nephrotoxicity [3], genotoxicity [4], neurotoxicity [5], hepatotoxicity [6], immunotoxicity [7], gastrointestinal toxicity [8], and cardiotoxicity [9]. Long periods of exposure to mycotoxins contribute to cancer development [10].

To limit the presence of mycotoxinogenic fungi in food and the mycotoxicoses caused by the consumption of contaminated foods, a control policy has been applied in recent years that is based on prevention of contamination, on decontamination and on protection against the

✉ Mohamed Amine Gacem, biologieamine@yahoo.fr | [1]Laboratory of Ecosystems Protection in Arid and Semi-Arid Area, University of Kasdi Merbah, 30000 Ouargla, Algeria. [2]Department of Biology, Faculty of Science, University of Amar Tlidji, 03000 Laghouat, Algeria. [3]Epidemiology Service and Preventive Medicine, Hassani Abdelkader University Hospital Center, Faculty of Medicine, University of Djillali Liabes, 22000 Sidi-Bel-Abbès, Algeria.

toxicities of mycotoxins by the application of several physical, chemical and biological techniques [11]. However, it is very difficult to destroy mycotoxins by physical techniques without altering food quality [12]; moreover, the use of chemical processes also has undesirable effects because chemical compounds participate in the generation of free radicals and, therefore, the induction of oxidative stress [13, 14]. At the same time, the massive use of antibiotics in agriculture is a cause for alarm with respect to public health [15] due to antibiotic-induced oxidative stress and to the emergence of multiresistant strains [16–18]. To resolve this problem, several studies investigated the use of essential oils and medicinal plant extracts as a safe alternative in the field of phytoprotection, which is the fight against toxigenic moulds and mycotoxicoses [19, 20]. Reddy et al. [21] showed that aqueous extracts of some plant species (*Allium cepa*, *Allium sativum*, *Curcuma longa*, *Ocimum sanctum*, *Syzigium aromaticum*, etc.) are rich in bioactive chemical compounds that inhibit the biosynthesis of aflatoxin. A study conducted by Elsherbiny et al. [22] demonstrated that the essential oils of *Ocimum basilicum* completely inhibited the growth of several species of *Bipolaris* and the germination of *Bipolaris hawaiensis* spores. However, the use of medicinal plants from arid and semi-arid regions of Algeria as an alternative in the process of food product decontamination has not been extensively studied.

Many medicinal plant species from steppe and desert regions are known for their therapeutic virtues and are traditionally used by the local populations to treat various diseases. These steppe and Saharan species, which include *Pistacia lentiscus* (*Anacardiaceae*), *Artemisia herba-alba* (*Asteraceae*) and *Citrullus colocynthis* (*Cucurbitaceae*) have remarkable antidiabetic, antibacterial, anti-tumoural, anti-inflammatory and anticancer properties [23–29]. Therefore, the objective of this study was to determine the phytochemical composition of the aqueous and methanolic extracts of *P. lentiscus, A. herba-alba* and *C. colocynthis* and to evaluate their antifungal and antioxidant potency.

2 Materials and methods

2.1 Harvest and preparation of medicinal plants

The plants that are the focus of this study are known for their therapeutic virtues in traditional Algerian medicine [30]. The leaves of *P. lentiscus* were harvested in the region of Tifrit (34° 56′ 04.2″ N, 0° 22′ 48.9″ E) in December 2017. For the second species, the aerial part of *A. heba-alba* was harvested in the region of Maamoura (34° 42′ 06.7″ N, 0° 28′ 58.7″ E) in March 2017. Regarding *C. colocynthis* species, fruit were harvested in December 2016 from the desert region of Oued N'sa (32° 48′ 13.7″ N, 3° 48′ 13.6″ E). The geographical location of harvesting sites is illustrated in Fig. 1. The botanical identification of plant species was carried out by botanists from the plant biology laboratory of Saida University (Algeria). To achieve the targeted aim of the study, the plant parts used were the leaves of *P. lentiscus* and *A. heba-alba* and the seeds of *C. colocynthis*.

2.2 Extraction and phytochemical screening

P. lentiscus and *A. heba-alba* leaves and *C. colocynthis* seeds were first washed with distilled water and disinfected for 30 min by immersion in a 2% solution of sodium hypochlorite. The plant material was then rinsed with distilled water to remove residual hypochlorite. After open air drying away from light, the plant material was crushed roughly using a grinder (Moulinex Grinder AR110027) [31], and the powders obtained from each species were divided into two quantities for subsequent methanolic and aqueous extractions.

A quantity of 20 g of each plant powder was mixed with 100 ml of solvent (methanol [Sigma-Aldrich-Germany] or water) for 3 h under continuous stirring (200 rpm) in MS Orbital Shaker at room temperature. The mixture was then filtered using Whatman filter paper (No 4). This operation was repeated four times with renewal of the solvent to deplete the marc and increase the yield. At the end of the extraction, the fractions of each extraction were collected, and the methanolic extracts were evaporated to dryness using a rotavapor (Heidolph-Germany) [32], while the aqueous extracts were lyophilized. Extract powders were kept in dark bottles at 4 °C until use. Figure 2 shows the methanolic and aqueous extraction steps followed in this study. The qualitative detection of alkaloids, saponins, flavonoids, and tannins was carried out following the protocols described in the study done by Al-Daihan et al. [33].

2.2.1 Test for alkaloids

Each dry extract powder (100 mg) was dissolved in 5 ml of methanol and then filtered. 5 ml of hydrochloric acid (1%) was mixed with 2 ml of the filtrate, and then 1 ml of the mixture was taken separately in two test tubes. Few drops of Dragendorff's reagent (potassium iodide-bismuth nitrate) were added in the tube and appearance of orange-red precipitate was taken as positive. Few drops of Mayer's reagent (composed of mercuric chloride and potassium iodide dissolved in distilled water) were added to the second tube and appearance of buff-colored precipitate designates the existence of alkaloids.

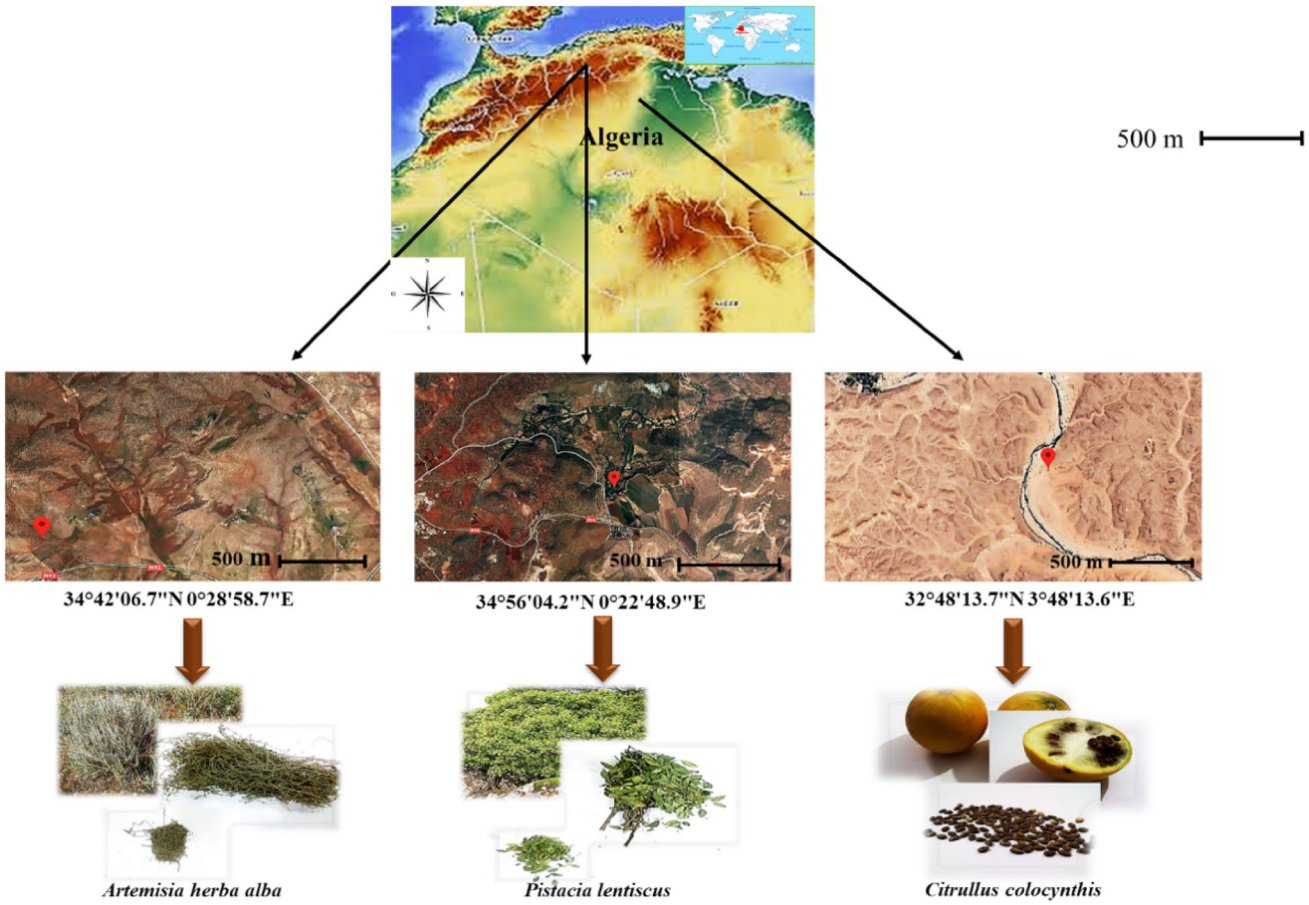

Fig. 1 Geographic location of medicinal plant harvesting stations

2.2.2 Test for saponins

10 ml of distilled water was mixed with 1 g of powdered dry extract and then boiled and filtered. The filtrate was mixed again with 3 ml of distilled water and shaken for 5 min. Appearance of foam after shaking designates the existence of saponins.

2.2.3 Shinoda's test for flavonoids

500 mg of powdered dry extract was added to 5 ml of ethanol, the mixture is slightly heated and then filtered. The filtrate was added to some pieces of magnesium chips, a few drops of concentrated HCl was then added to the mixture. Occurrence of a pink, orange, or red to purple coloration designates the existence of flavonoids.

2.2.4 Test for tannins

500 mg of powdered dry extract was added to 10 ml of distilled water, the mixture is then filtered and few drops of 1% ferric chloride solution are added to the filtrate. Occurrence of a blue-black, green or blue-green precipitate designates the existence of tannins.

2.3 Assessment of antifungal activity

Five fungal strains were selected to evaluate the antifungal activity of the different extracts: *A. flavus* CECT 20802 (producing aflatoxin B_1 (AFB_1), AFB_2, AFM_1 and AFM_2), *A. parasiticus* CBS 100926, *F. graminirium* CECT 2150, *A. ochraceus* NRRL 3174 (producing ochratoxin) and *P. expansum* CECT 2278 (producing patulin and citrinin). The purity of the strains was verified by the microculture chamber (slide culture) with some modifications [34], this technique takes place in a few steps; (1) using a sterile blade cut out the PDA medium (10 × 10 mm) to fit under a coverslip, (2) deposit the PDA block on a sterile blade, (3) inoculate the four sides of the PDA block with spores of the strain to be grown, (4) cover the PDA block with a flamed coverslip, (5) incubate the plate at 30 °C during 15 days, (6) transfer the coverslip on a new clean glass slide containing a small drop of lactophenol blue solution, (7) examine the slides under optical microscope.

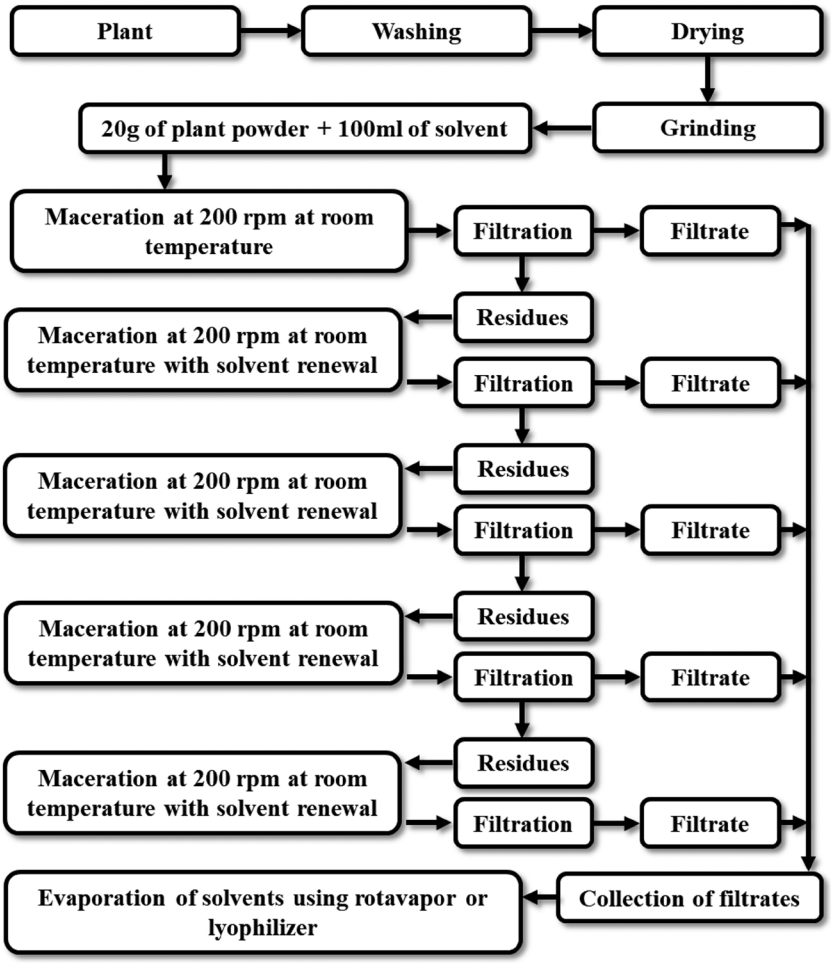

Fig. 2 Methanolic and aqueous extraction protocol carried out on the plants

Antifungal activity was measured by the protocol described by Magnusson and Schnürer [35] with some modifications. As described by Espinel-Ingroff and Cantón [36], spore solutions were prepared from seven-day-old cultures of each strain, and their concentrations were adjusted with a spectrophotometer (Jenway 6315- United Kingdom) set to 530 nm to obtain suspensions of 5×10^6 viable conidia or sporangiospores per milliliter. A volume of 100 µl of each suspension (diluted to approximately 5×10^4 CFU.ml^{-1}) was used to flood-inoculate PDA medium that had been solidified in Petri dishes. A dilution series (25, 50, 75 and 100 mg ml^{-1}) was prepared for each plant extract, and 40 µl of extract dilution was injected into the wells of inoculated PDA medium. The dishes were incubated for 5 days at 27 ± 2 °C. Antifungal activity was calculated by dividing the surface area of growth inhibition due to the extract by the total surface area of the petri dish and multiplying by 100% [35]. The results were categorized as follows:

- If the inhibition was between 0.1 and 3%, the antifungal activity was considered low.
- If the inhibition was between 3 and 8%, the antifungal activity was considered average.
- If the inhibition was greater than 8%, the antifungal activity was considered to be high.

2.4 Determination of antioxidant activity

2.4.1 ABTS radical scavenging assay

To measure the antioxidant capacity by monitoring inhibition of the ABTS$^{\cdot+}$ radical cation, the method described by Surveswaran et al. [37] was followed. The generation of ABTS$^{\cdot+}$ radical cation was carried out by a chemical reaction between a solution of ABTS (7 mM) and a solution of potassium persulfate (2.45 mM) incubated for 16 h in the dark at room temperature. The resulting ABTS$^{\cdot+}$ solution was diluted with methanol (at a ratio of 1/50) in order to obtain an absorbance of 0.700 ± 0.005 at a wavelength of 734 nm. A volume of 100 µl of plant extract solution prepared at different concentrations (10, 5, 2, 1, 0.66 or 0.4 mg ml^{-1}) was added to 3.9 ml of ABTS$^{\cdot+}$ solution (absorbance equal to 0.700 ± 0.005). After 10 min of

reaction, the absorbance was measured by a spectrophotometer (Jenway 6315-United Kingdom) set to 734 nm. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as standard antioxidant at a concentration range of 0–500 µM. The results were expressed in µM Trolox equivalent per gram of extract (µM TE g^{-1}).

2.4.2 DPPH radical scavenging assay

The antiradical activity of plant extracts was determined by the DPPH radical scavenging assay according to the method adopted by Orphanides et al. [38]. A volume of 3.9 ml of $DPPH^{\cdot}$ solution (0.3 mM) was mixed with 100 µl of plant extract prepared at different concentrations (10, 5, 2, 1, 0.66 or 0.4 mg ml^{-1}) and incubated for 30 min at room temperature. The absorbances were then measured by a spectrophotometer (Jenway 6315-United Kingdom) set to 517 nm. Trolox was used as a standard antioxidant at a concentration range of 0–500 µM. The results were expressed in µM TE g^{-1}.

2.4.3 Ferric reducing ability (FRAP)

The ability of the various extracts to reduce ferric iron (Fe^{+3}) to ferrous iron (Fe^{+2}) was determined according to the protocol described by Orphanides et al. [38]. Dilutions of plant extracts and Trolox were prepared at the same concentrations as used in the antioxidant assays above. A volume of 100 µl of extract was mixed with 3.9 ml of a freshly prepared FRAP solution [0.3 M acetate buffer, pH = 3.6, 10 mM TPTZ (2,4,6-tri-(2-pyridyl)-s-triazine) and 20 mM $FeCl_3 \cdot 10H_2O$ at a ratio of 10:1:1 (v/v/v)]. The mixture was then incubated at 37 °C for 4 min, and the absorbance was measured at a 593 nm. Trolox was used as a standard antioxidant at a concentration range of 0–800 µM. The results were expressed in µM TE g^{-1}.

2.4.4 Statistical analyses

Each experiment was performed in triplicate, and the results were expressed as the mean ± standard deviation. Variance analysis was used to test the differences between extracts and selected plant species as well as among the various techniques used to evaluate the antioxidant activity. Linear regression was used to determine the IC_{50} and CE_{50}. All analyses were done by XLSTAT 2009.

3 Results

3.1 Extraction yield and phytochemical analysis

Water and methanol are the most polar solvents used in phytochemical extraction methods. Both solvents are also recommended in the literature to extract a maximum of bioactive compounds from plants [39, 40]. According to the results presented in Table 1, the highest yields were recorded for the methanolic and aqueous extracts of *P. lentiscus* leaves, which were 22.30% and 17.44%, respectively, followed in descending order by the methanolic and aqueous extracts of *A. herba-alba* and *C. colocynthis* seeds, which had the lowest yields with percentages of 8.14, 5.25, 3.20 and 2.26%, respectively. Concerning the phytochemical screening of the studied species, Table 1 indicates all sought bioactive substances (alkaloids, flavonoids, tannins and saponosides) were detected in *A. herba-alba*; however, saponosides were not detected in *C. colocynthis* seeds, while alkaloids were not detected in *P. lentiscus* leaves.

3.2 Antifungal activity

The results confirming the purity of the selected fungal strains are shown in Fig. 3. An examination of micro-culture slides stained with lactophenol blue solution under an optical microscope clearly demonstrated that fungal strains were pure with no other microbial or fungal contamination.

In the assays measuring the antifungal effects of the plant extracts against five fungal strains, the aqueous and methanolic extracts of *A. herba-alba* were the most active (Figs. 4, 5, 6), and at concentrations greater than or equal to 50 mg ml^{-1}, growth inhibition exceeded 8% (Figs. 4, 5). The most sensitive fungal strains were *P. expansum* and *A. ochraceus* followed by *F. graminearum* (Figs. 4, 5). According to the same figures, aqueous and methanolic extracts of the selected plant species had no effect on the growth of *A. parasiticus* and *A. flavus* except for the aqueous and methanolic extracts of *P. lentiscus*, which had an average

Table 1 Extraction yield and phytochemical screening

	Extraction yield %		Alkaloids	Flavonoids	Tannins	Saponins
	Met	Aq				
Plant						
P. lentiscus	22.30	17.44	–	+	+	+
A. herba-alba	8.14	5.25	+	+	+	+
C. colocynthis	3.20	2.26	+	+	+	–

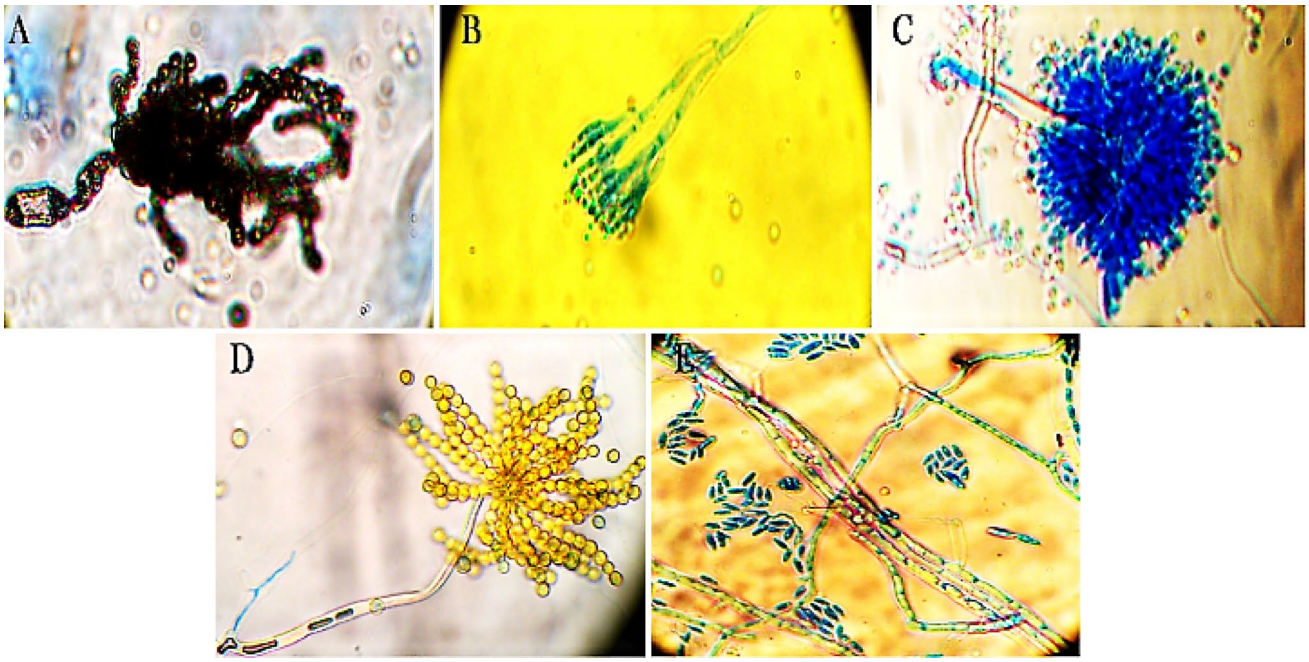

Fig. 3 Morphological appearance of fungal strains under optical microscope. **a** *A. flavus CECT 20802*, **b** *P. expansum CECT 2278*, **c** *A. parasiticus CBS 100926*, **d** *A. ochraceus NRRL 3174*, **e** *F. graminirium CECT 2150*

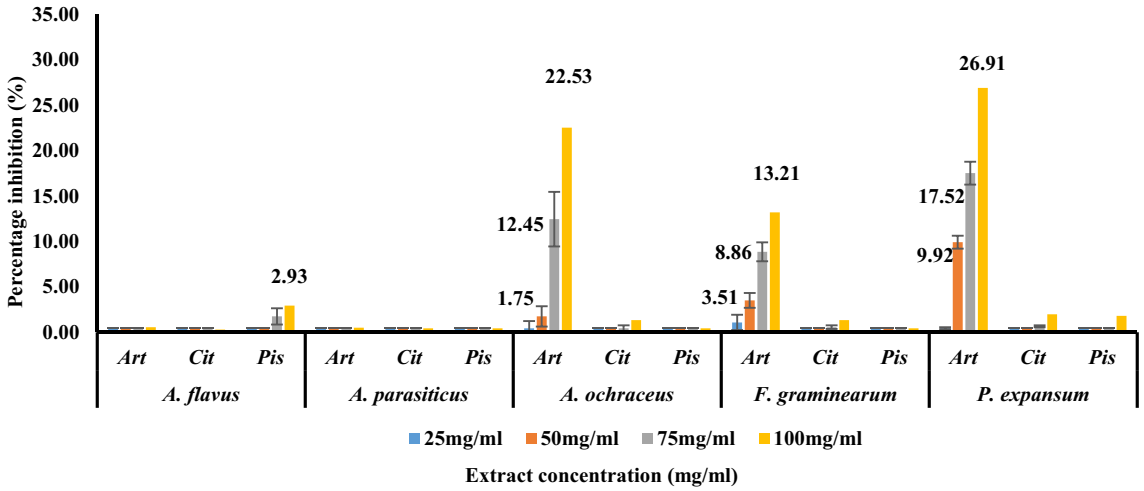

Fig. 4 Antifungal activity of aqueous extracts of the plant species. (*Art*: *A. heba-alba*, *Cit*: *C. colocynthis* and *Pis*: *P. lentiscus*)

inhibitory activity not exceeding 4% against *A. flavus*. A summary of the antifungal activity of plant extracts is shown in Table 2.

3.3 Antioxidant activity

The antioxidant activity of methanolic and aqueous extracts of the plants was determined in vitro by three techniques, namely, the ABTS, DPPH and FRAP assays. According to the results shown in Figs. 7, 8 and 9, the plant extracts exhibited remarkable antioxidant activity expressed in μM TE g^{-1}. The methanolic and aqueous extracts of *A. herba-alba* showed the highest antioxidant activity in the FRAP assay; their reducing powers were 6080.67 μM TE g^{-1} and 5315.11 μM TE g^{-1}, respectively. The antioxidant potentials of *P. lentiscus* leaves and *C. colocynthis* seeds measured by the same assay were lower in comparison with *A. herba-alba,* the values were 5300.48 μM TE g^{-1}, 4123.33 μM TE g^{-1} and

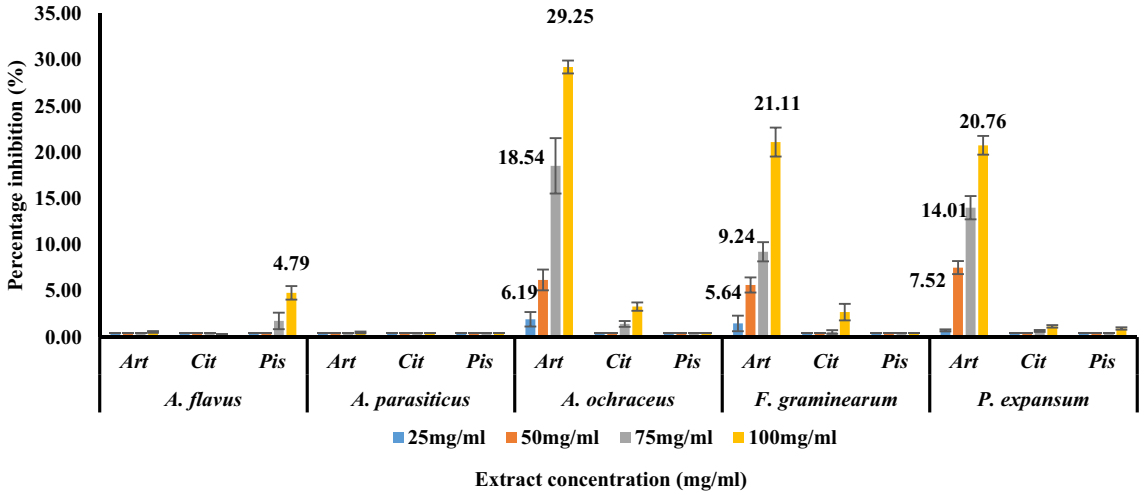

Fig. 5 Antifungal activity of methanolic extracts of the plant species. (*Art*: *A. heba-alba*, *Cit*: *C. colocynthis* and *Pis*: *P. lentiscus*)

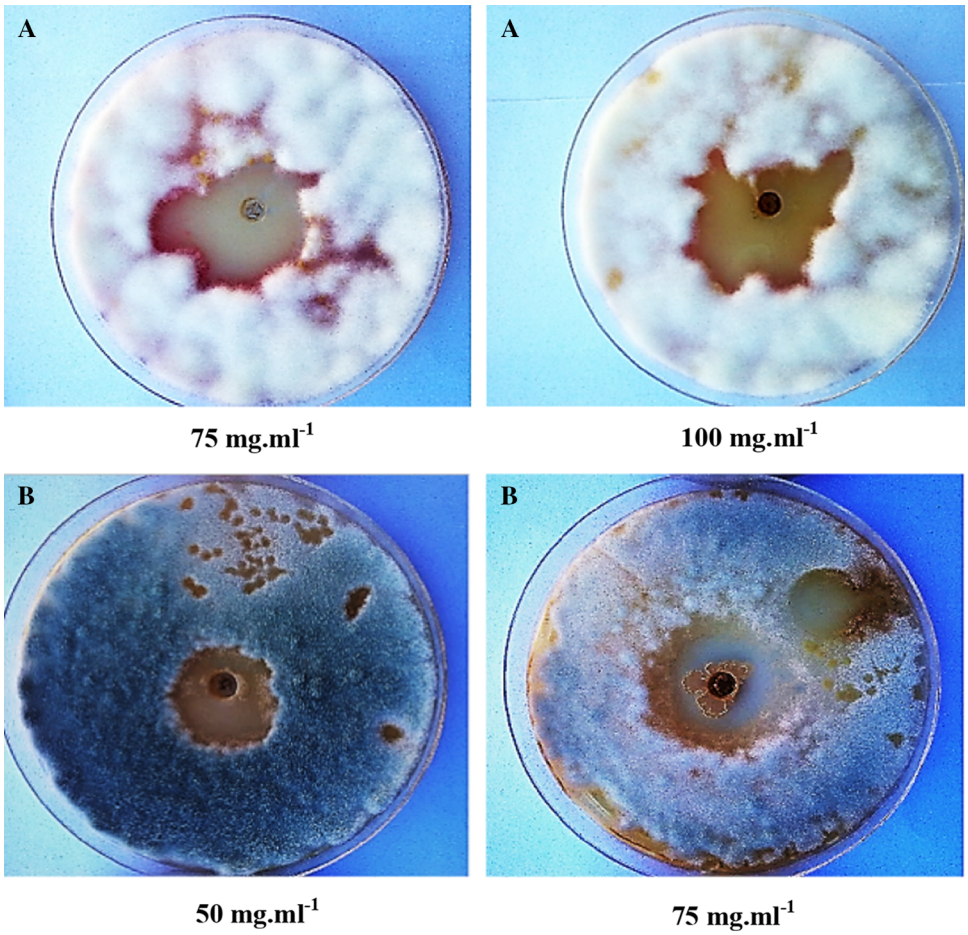

Fig. 6 Antifungal activity of *A. herba-alba* methanolic extract. **a** *F. graminirium CECT 2150*, **b** *P. expansum CECT 2278*

4150.22 µM TE g^{-1}, 3749.11 µM TE g^{-1} for the methanolic and aqueous extracts, respectively in the FRAP assay.

For all extracts, the lowest IC_{50} were observed in the methanolic extracts of *A. herba-alba* in the FRAP assay, and IC_{50} values was 0.91 ± 0.92 mg ml^{-1} followed by the methanolic extracts of *P. lentiscus* leaves with IC_{50} 2.12 ± 0.49 measured by FRAP assay. The methanolic extracts of *C. colocynthis* seeds showed an IC_{50} values of 3.03 ± 0.51

Table 2 Antifungal quality of methanolic and aqueous extracts

	Aqueous extracts concentration									Methanolic extracts concentration								
	25 mg			50 mg			75 mg			100 mg			25 mg			50 mg			75 mg			100 mg		
	Art	*Col*	*Pis*	*Art*	*Col*	*Pis*	*Art*	*Col*	*Pis*	*Art*	*Col*	*Pis*	*Art*	*Col*	*Pis*	*Art*	*Col*	*Pis*	*Art*	*Col*	*Pis*	*Art*	*Col*	*Pis*
A. flavus NRRL 3251	–	–	–	–	–	–	–	–	+	–	–	+	–	–	–	–	–	–	–	–	+	–	–	++
A. parasiticus CBS 100926	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
F. graminirium MUCL 53452	+	–	–	++	–	–	+++	–	–	+++	+	–	+	–	–	++	–	–	+++	–	–	+++	+	–
P. expansum MUCL 29192	–	–	–	+++	–	–	+++	–	–	+++	+	+	+	–	–	++	–	–	+++	–	–	+++	+	+
A. ochraceus NRRL 3174	–	–	–	+	–	–	+++	–	–	+++	+	–	+	–	–	++	–	–	+++	+	–	+++	++	–

– no activity, + low antifungal activity, ++ average antifungal activity, +++ high antifungal activity, Art: *A. heba-alba*, Cit: *C. colocynthis* and Pis: *P. lentiscus*

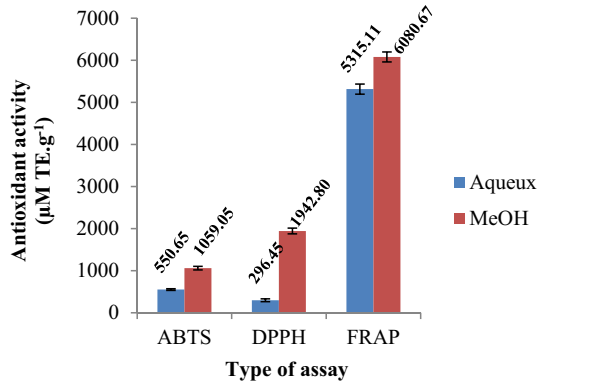

Fig. 7 Antioxidant activity of *Artemisia herba-alba* methanolic and aqueous extracts

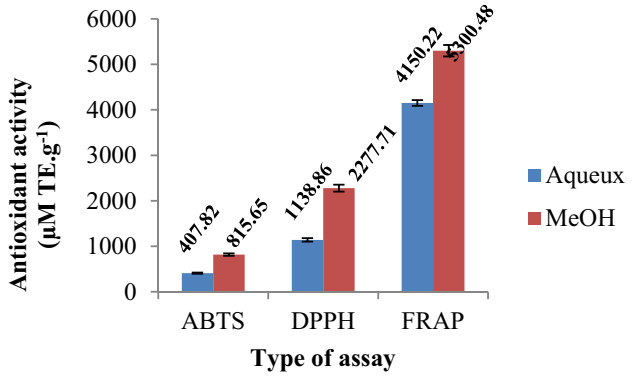

Fig. 8 Antioxidant activity of *P. lentiscus* methanolic and aqueous extracts

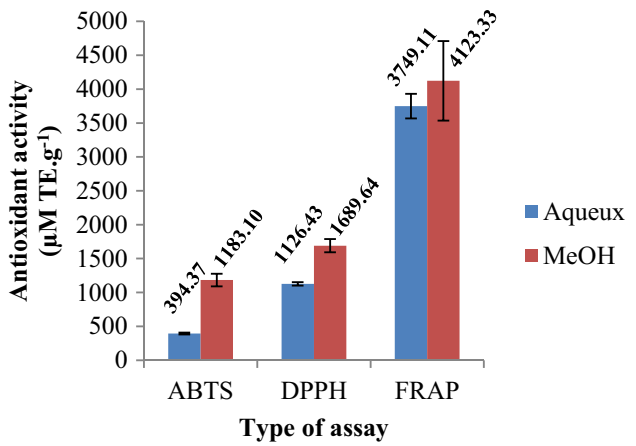

Fig. 9 Antioxidant activity of *C. colocynthis* methanolic and aqueous extracts

determined by the ABTS radical scavenging assay. For aqueous extracts, *A. herba-alba* and *C. colocynthis* seeds had the highest antioxidant potential with a lower IC_{50}

(2.42 ± 0.11 and 3.89 ± 0.44, respectively) followed by *P. lentiscus* leaves with IC_{50} values of 4.48 ± 0.52. However, the values of antioxidant activities evaluated by ABTS, DPPH and FRAP assays of all extracts were lower than the standard antioxidant used in this study (Trolox) (Table 3).

Variance analyses indicated that there are highly significant differences ($P < 0.01$) among the results of three antioxidant activity assays for the same species. Differences were also recorded among the three studied species ($P < 0.05$). The observed differences in the antioxidant activity among the different plant species is probably due to differences in their chemical composition. The differences among the assays seem to be due to the use of a different reaction mechanism by each assay.

4 Discussion

The fight against toxigenic fungi and mycotoxins is one of the difficulties of concern to scientists, and the results of extensive research in the field of mycotoxin toxicity are alarming and disturbing due to the health damage caused by mycotoxicosis and the economic losses caused by decreased food quality [41, 42]. As a result, researchers are abandoning conventional approaches and are exploring new biological control strategies against fungi and mycotoxicosis that include the use of medicinal plants [19, 20]. The three plants selected for this study were collected from semi-arid and arid regions of Algeria, which occupy more than 87% of the country's surface area. The desert regions are characterized by arid climates, in which rainfall is almost absent, with high temperatures, and in some areas of these desert regions, the climate is characterized by extreme aridity. In terms of flora, these arid and semi-arid areas are habitats for more than 2800 species [43], and this biodiversity is why these sites were chosen for plant sampling.

The study of the biological activities of aromatic and medicinal plants is based on extraction, and its principle, which is the crucial stage of any study, is that well-described precautions must be taken into consideration in order to avoid the loss, deformation or destruction of extracted molecules; in this case, the choice of solvent and extraction conditions depends on the targeted bioactive compound and it nature [44]. Regarding yield, the study of Barbouchi and his collaborators [45] demonstrated that the infusion extraction, or Soxhlet, method gave a higher yield without neglecting the nature of the solvent or the area where the leaves were harvested. Gupta and his team obtained a lower extraction yield compared to our yield for *C. colocynthis* seeds [46].

The organic extractions of this study were carried out by maceration, and the two solvents used were of polar

Table 3 IC_{50} of *P. lentiscus*, *A. herba-alba* and *C. colocynthis* methanolic and aqueous extracts obtained by FRAP, ABTS and DPPH assay

	ABTS				DPPH				FRAP			
	Art	Pis	Cit	Trolox*	Art	Pis	Cit	Trolox*	Art	Pis	Cit	Trolox
IC_{50} (mg ml^{-1}) of aqueous extract	6.74 ± 0.84	12.33 ± 1.29	5.33 ± 0.59	0.98 ± 0.1	7.58 ± 0.22	8.98 ± 1.34	3.89 ± 0.44	025 ± 0.03	2.42 ± 0.11	4.48 ± 0.52	5.12 ± 0.64	0.35 ± 0.04
IC_{50} (mg ml^{-1}) of methanolic extract	5.13 ± 0.63	9.78 ± 1.16	3.03 ± 0.51		3.27 ± 0.64	6.89 ± 0.88	3.23 ± 0.38		0.91 ± 0.92	2.12 ± 0.49	3.98 ± 0.5	

nature; consequently, the metabolites indentified were polar nature (alkaloids, flavonoids, tannins and saponins). In this context, Barbouchi's study demonstrated that organic extracts of *P. lentiscus* leaves are rich in catechic tannins, gallic tannins, flavonoids and saponins but not alkaloids [45]. Khlifi's study demonstrated that the hydromethanolic extract of *A. herba-alba* is rich in compound phenolics, flavonoids, tannins and anthocyanins [27]. Regarding the seeds of *C. colocynth*, Benariba et al. [47] revealed that catechic tannins and flavonoids are abundant in hydromethanol extracts, and according to Najafi et al. [48], the ethanolic extract contains tannins, alkaloids, flavonoids, and saponins. These differences in results may be attributed to differences in harvesting region, soil composition, climate, harvest season, solvents and experimental conditions of extraction.

In our assays, *A. herba-alba* exhibited the highest antifungal activity, followed in descending order by *C. colocynthis* seed and *P. lentiscus* leaf extracts. *F. graminearum, P. expansum* and *A. ochraceus* were the most sensitive moulds. *F. graminearum* is an opportunistic pathogen of cereals responsible for yield losses and contamination by its mycotoxins [49]. *P. expansum*, also known as blue mould, is responsible for the most important diseases of pome fruits and causes significant economic losses [50]. *A. ochraceus* is responsible for chronic granulomatous diseases [51]. *A. flavus and A. parasiticus* are known in mycology for their negative impact on health and for economic damage [41, 52]. In this study, these two fungi were the most resistant to the various prepared extracts.

Our results are in agreement with previous studies. *P. lentiscus* is considered to be an antimicrobial plant [53, 54], and ethanolic extracts of its fruits and leaves are used as ingredients in pork sausage to reduce microbial spoilage [55]. Some studies found that organic extracts of *C. colocynthis* are more active in comparison to aqueous extracts [47] and that hydromethanolic extracts are less toxic against *F. oxysporum* and its enzymes (CMCase, pectinase, and protease) [56] and exhibit a moderate antifungal activity against *A. fumigatus* DSM790 and *A. niger* DSM1988 [57].

The antifungal activities of the extracts are largely attributed to the solvent extraction capacity and the concentration of the active compounds in the crude extracts [58]. The sensitivity of microorganisms is due to the intolerance of cells to phytochemicals and their interactions with fungal cell components, which can cause, for example, the inhibition of cell wall formation, rupture of the cell membrane, mitochondria dysfunction, inhibition of cell division, inhibition of DNA replication, inhibition of protein synthesis or inhibition of efflux pumps [59]. However, the resistance of fungal strains may be due to degradation of bioactive compounds by fungal enzymes, removal of bioactive substances by efflux systems, insensitivity of the site targeted by the molecule [60] or by mutations in the genes encoding fungicide targets [61]. Tannins due to their structure interact with ergosterol and damage cell membranes [62]. Flavonoids and alkaloids are also known for their antimicrobial and spasmolytic activities [63, 64].

The complexity and multifunctionality of phytochemicals make the use of a single test insufficient to evaluate the antioxidant activity [65, 66]. The antioxidant activity assays carried out in this study made it possible to measure the capacities of the various bioactive molecules present in the crude extracts to donate an electron to a radical or to an ion, which leads to the inhibition or reduction of oxidants. The plant extracts prepared in this study showed good antioxidant potential in the three assays, and the ability of these extracts to reduce free radicals can be attributed to their phenolic and flavonoid compounds [67, 68], whose chemical structures and concentrations determine the antioxidant activity [69].

The results of the antioxidant activity assays in this study are consistent with those of a recent study done on *A. herba-alba* from another region [70], and another study supports our results and demonstrates that this plant reduces the oxidative damage induced by alloxan [71]. Khlifi et al. [27] reported that the hydromethanolic extract had IC_{50} values of 20.64 ± 0.84 mg ml^{-1} and 36.60 ± 1.03 mg ml^{-1} in the DPPH and ABTS assays, respectively.

Several other studies support our results and state that *P. lentiscus* has good antioxidant activity revealed by the DPPH and ABTS assays [72–75]. Barbouchi et al. [45] reported that the antioxidant activity of *P. lentiscus* leaves varies according to the location of harvest and observed IC_{50} values ranging from 0.50 ± 0.00 to 0.09 ± 0.00 mg ml^{-1} and from 0.57 ± 0.00 to 1.13 ± 0.00 mg ml^{-1} for the aqueous and methanolic extracts, respectively. Botsaris et al. [55] demonstrated that the methanolic extracts prevent lipid oxidation. Some authors showed that this activity is strongly due to the presence of gallic acid, 1,2,3,4,6-pentagalloyl glucose, 5-*O*-galloyl, 3,5-di-*O*-galloyl and 3,4,5-tri-*O*-galloyl and observed an IC_{50} equal to 3.9 µM [76, 77].

For *C. colocynthis*, several in vivo and in vitro studies reported good antioxidant activity in the fruits [47, 78]. Marzouk et al. [79] stated that the IC_{50} of aqueous seed extracts is between 0.020 and 0.021 mg ml^{-1} and attributed this difference to the different stages of fruit ripening. In contrast, Jayaraman and Christina [80] found that the maximum inhibition of DPPH radicals by the methanolic extract was approximately 62% at 0.8 mg ml^{-1}, whereas we observed only 57.11% of DPPH radical scavenging at 10 mg ml^{-1} methanolic extract. Tannin-Spitz et al. [81] reported that cucurbitacin B glucoside and cucurbitacin

E glucoside widely detected in *C. colocynthis* are powerful antioxidants.

5 Conclusion

Ethnopharmacological surveys based on scientific approaches and research oriented towards the exploitation of medicinal plants have been growing steadily in recent years due to pharmaceutical and medical interest in the bioactive substances isolated from these plants and characterized by less lethal side effects. These preliminary studies are of great importance in the development of plant-based pharmaceuticals and in the progression of green nanotechnology leading to the synthesis of new complex nanoparticles doped with bioactive substances active against multiresistant fungal strains. The results of the current study confirm that *Artemisia herba-alba*, *Pistacia lentiscus* and *Citrullus colocynthis* are potential sources of bioactive substances that could be used in the control of food contamination and of oxidative stress and the prevention of mycotoxicoses. Additional studies are in progress to characterize the structure of bioactive compounds and to elucidate their antifungal mechanisms in vitro by molecular docking.

Acknowledgements We are thankful to the researchers from the Laboratory of Ecosystems Protection in Arid and Semi-Arid Area, University of Kasdi Merbah-Ouargla, Algeria for their support during this work.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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