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Screening for *in vitro* antioxidant activity and antifungal effect of *Artemisia campestris* L.

Noui Hendel[1,*] **Djamel Sarri**[2] **Madani Sarri**[2]

Mounir Selloum[1] **Faiza Boussakra**[1] **Ouahiba Driche**[1]

[1]University Mohamed Boudiaf of M'sila, Faculty of Sciences, Department of Microbiology and Biochemistry, M'sila 28000, Algeria.
[2]University Mohamed Boudiaf of M'sila, Faculty of Sciences, Department of Nature and Life Sciences, M'sila 28000, Algeria.

[*]Corresponding Author: noui.hendel@univ-msila.dz

Abstract

In this study, the methanolic extract (ME) and the essential oil (EO) of the medicinal plant *Artemisia campestris* L. were investigated for their antioxidant activity and their antifungal efficacy on the postharvest storage decays; *Botrytis cinerea* Pers. and *Penicillium expansum* Link. The total polyphenolic and flavonoid contents were determined. The ME had higher total polyphenolic and flavonoid contents (400.64 µg GAE/mg and 43.13 ± 0.14 µg QE/mg, respectively) than EO (27.47 ± 0.44 µg GAE/mg and 14.04 ± 0.82 µg QE/mg, respectively). The ME presented higher radical scavenging power than the BHT and its IC_{50} values were 11.71, 40.96 and 23.32 µg/mL for the DPPH, β-carotene bleaching and reducing power respectively. In the antifungal activity, the EO had the stronger effect on both molds, particularly at concentrations > 15 µL, ≥ 800 µL/L and ≥ 15 µL by fumigation, incorporation and disc-diffusion methods respectively, resulting in higher than 80% inhibition of *B. cinerea* mycelial growth, and from 50 to > 80% inhibition on *P. expansum* mycelial growth. Methanolic extract showed nearby 50% inhibition on both fungi. The EO MIC was less than 2.5 µL/mL which was shown as MFC for both molds. The bio-autography test has shown separated compounds of the ME having an inhibitor effect on spore germination. These results offer an advantage of suggesting *A. campestris* could be used as a material for extraction of certain antifungal chemicals for preventing spoilage in food items.

Keywords: Antifungal activity, antioxidant activity, *Artemisia campestris, Botrytis cinerea, Penicillium expansum*

Introduction

Yield losses and food decay caused by insects, fungi, bacteria and viruses are of a great economic importance in crop and food production (Kordali et al., 2008; Zabka et al., 2009). *Penicillium expansum* Link (blue mold is the most common postharvest rot of apple fruit in storage, transit and market (Mari et al., 2002; Larous et al., 2007). It produces mutagenic toxins (Leggott et al., 2000). Among other, patulin is of high hazard to human health (Andersen et al., 2004; Quaglia et al., 2011). *Botrytis cinerea* Pers. (gray mold) causes disease in over 200 plant species around the world (Nakajima and Akutsu, 2014). The application of synthetic fungicides remains the main strategy to control these moulds, but other methods are required, due to public concerns about human health and environmental risks, besides the continuous appearance of fungicide resistant strains (Spadaro et al., 2004; Bi and Yu, 2016).

The plants become the suitable and a promising source of natural compounds as effective alternatives to synthetic chemical pesticides (Zabka et al., 2011). Plant extracts have

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Doi: https://doi.org/10.31015/jaefs.2021.3.1
Orcid: Noui Hendel: 0000-0002-6577-925X, Djamel Sarri: 0000-0003-3617-1162, Madani Sarri: 0000-0003-3617-1162, Mounir Selloum: 0000-0002-0521-6842, Faiza Boussakra: 0000-0002-1961-0429, Ouahiba Driche: 0000-0001-7904-7546
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been used worldwide for control of plant disease and extracts from many plant species were found to be involved in many pathogenic fungi control. (Apisariyakul et al., 1995; Zabka et al., 2009; Prakash et al., 2012; Askarne et al., 2013; Ozbek et al., 2020; 2021).

Artemisia campestris L. is widespread in Algeria and commonly known as "D'gouft" (Quezel and Santa, 1963). The traditional use of this plant includes the infusion, decoction or powder forms to treat digestive, respiratory, metabolic, allergic, cutaneous disorders (Dib et al., 2017), and for the antimicrobial, antiinflammatory, antivenom, antirheumatic and antioxidant properties (Akrout et al., 2001; Nikolova et al., 2010; Ghlissi et al., 2016; Nigam et al., 2019).

In Algeria, apple production reached 400.000 tons in 2011, and pears were estimated to be 211.000 tons in 2012. The strawberry cultivation has increased markedly (Agroligne, 2014). Although gray and blue molds affecting these products are reported, there is no statistics available.

The objective of this work was to determine the antioxidant activity of wild-growing *Artemisia campestris* L. in Algeria and to evaluate the susceptibility of blue and gray molds of Algerian apples and strawberries to its essential oil and methanolic extract, using different methods.

Materials and methods

Plant material

Artemisia campestris L. spontaneously growing in Kef Maâfer, at the station 35°50'58.7"N 5°23'58.4"E, was harvested in September 2015 during its flowering period. The plant identification was done by the botanist Dr SARRI Djamel and voucher specimen (N°: AC15M19) was deposited in the herbarium of the nature and life sciences department. After being dried in the shade in a dry and ventilated room for 15 days, the plant aerial parts were recovered and stored in paper bags and retained protected from light and heat until use.

Extraction of essential oil (EO)

Plant material (100 g) was subjected to the extraction of the essential oil by hydrodistillation, for 3h, in a Clevenger type apparatus. Recovered EO (yield: 0.45 %, v/w) was dehydrated over anhydrous sodium sulfate and stored in dark glass vial at - 4 °C until tested.

Preparation of the plant methanolic extract (ME)

Powdered plant material (50 g) was extracted with 500mL of methanol using Soxhlet extractor for 8 h at 40 °C. The methanol extract was filtered through Whatman filter paper and then concentrated at 40 °C in a rotary evaporator until dryness. The final obtained extract (4.8 g) was stored at 4 °C until use (Erkan et al., 2008).

Total phenolic content

The total phenolic content of the ME or EO was determined by the Folin-Ciocalteu reagent (FCR), according to the method described by Maity et al. (2013) with slight modification, using gallic acid as standard. 40 µL of methanolic solution of the ME or EO were mixed with 200 µL of the FCR and 1160 µL of distilled water. After 3min incubation, 600 µL of 7.5% Na_2CO_3 solution was added to the mixture which was subsequently kept in the dark for 2 h at room temperature. The absorbance was measured at 760 nm. The results were expressed as µg of gallic acid equivalents per mg (µg GAE/mg) of ME or EO.

Total Flavonoid content

The total flavonoid content was determined by the aluminum trichloride method reported by Meda et al. (2005) with minor modification. Briefly, 600 µL of $AlCl_3$ (2% in methanol) were mixed with the same volume of the methanolic solution of ME or EO. After 15 min, the absorbance was measured at 415 nm against a blank composed of the same volumes without $AlCl_3$. The total flavonoid content was determined using a standard curve with quercetin and was expressed as µg quercetin equivalents per mg (µg QE/mg) of ME or EO.

Antioxydant activity

DPPH radical scavenging activity assay

The DPPH free radical scavenging activity was carried out as described by Hazzit et al. (2009). 50 µL of different concentrations of ME (10, 20, 50, 10, 200, 300, 400, 500 µg/mL) or EO (1, 2, 4, 8, 12, 16, 20µL/mL), prepared in methanol, were added to 2 mL of 60 µM methanolic solution of DPPH. After 30 min incubation in the dark at ambient temperature, the absorbance was measured at 517 nm against a control (same amount of methanol and DPPH solution without EO or ME). Butylated hydroxytoluene (BHT) was used as positive control. The tests were carried out in triplicate. The inhibition of DPPH free radical was calculated as follows:

$$\% \text{ Inhibition} = (A_c - A_t / A_c) \times 100$$

where, A_c is the absorbance of the control sample, and A_t is the absorbance of the tested sample. Percentages of inhibition were plotted against concentrations of EO or ME to calculate the concentration providing 50% inhibition (IC_{50}).

β-Carotene bleaching assay

This assay was applied as described by Shukla et al. (2012). A stock solution of ß-carotene and linoleic acid was prepared (0.5 mg of ß-carotene in 1ml chloroform, 25 µL linoleic acid and 200 mg Tween 40). The chloroform was evaporated under vacuum and 100 mL of aerated distilled water was then added to the residue. The resulting mixture was vigorously stirred. Aliquots (2.5 mL) of the ß-carotene-linoleic acid emulsion (freshly prepared before each experiment) were transferred to test tubes, each containing 350 µL of various concentrations of the plant ME or EO diluted in methanol. The absorbance was measured at 470 nm immediately (zero time). The test tubes were incubated in a hot water bath at 50 °C together with blanks, BHT as a positive control and the other contained the same volume of methanol instead of extract as a negative control. After 120 min incubation the absorbance was measured again. All tests were carried out in triplicate and inhibition percentages were averaged. Antioxidant activity (inhibition percentage, I%) values were calculated using the following formula:

$$I\% = (A_t - C_t / C_0 - C_t) \times 100$$

where A_t and C_t are the absorbance of the sample and control at 120min, respectively, and C_0 is absorbance of the control at t = 0 min. The results are expressed as IC_{50} values, the concentration required to cause 50% inhibition of β-carotene bleaching.

Reducing power assay

The reducing power was determined by evaluating the

transformation of Fe^{3+}– Fe^{2+} according to Esmaeili and Sonboli (2010). The ME or EO (0.75 mL) at various concentrations (in methanol) was mixed with 0.75 mL of phosphate buffer (0.2 M, pH 6.6) and 0.75 mL of potassium ferricyanide [$K_3Fe(CN)_6$] (w/v, 1%). The mixture was incubated at 50 °C for 20 min followed by the addition of 0.75 mL of trichloroacetic acid (10%) and then centrifuged at 3000 rpm for 10 min. A volume of 1.5 mL of the upper layer of the solution was collected and mixed with 1.5 mL of distilled water and 0.1 mL of ferric chloride ($FeCl_3$) solution (0.1%, w/v) for 10 min. The absorbance at 700 nm was measured as the reducing power. The EC_{50} (RP) value, which represents the concentration at which absorbance is 0.5, was calculated for ME, EO and BHT.

Evaluation of the antifungal activity

The antifungal effect of *A. campestris* ME and EO was carried out on potato dextrose agar (PDA) or potato dextrose broth (PDB) media depending on the applied method.

Fungal strains

Botrytis cinerea and *P. expansum* molds were isolated from decayed strawberry and apple fruits respectively. They were identified according to the identification technique of Pitt & Hocking (2009) based on the cultural characteristics on Czapek Yeast Extract Agar (CYA), Malt Extract Agar (MEA) and 25% Glycerol Nitrate Agar (G25N), and keys for determination (colony diameter, color and texture; microscopic characteristics: hyphae and conidiophore appearance, size and shape of vesicles, metulae, phialides, and conidia…) described elsewhere (Botton et al., 1990). They were cultured on PDA slants and kept at 4 °C until use.

Preparation of inocula

The conidial suspension was prepared as described by Hendel et al. (2016); conidia were harvested from 10-day-old culture using sterile 0.01% Tween 80 saline solution. After well mixing the conidial suspension, the final concentration was adjusted to 10^4 conidia/ml using a Haemocytometer. The fungal discs were taken from the margins of 7 and 4 days fungal cultures grown on PDA at 25 °C of *P. expansum* and *B. cinerea* respectively.

Measurement of inhibition average

The inhibition of spore germination was evaluated by measuring the inhibition zone diameter (mm) and the percentage of mycelial growth inhibition (I%) was measured according to the formula (1).

$$I\% = (DC-DT) / DC \times 100 \text{ --------(1)}$$

Were

DC: Diameter of the control fungal colony (mm)

DT: Diameter of the treated fungal colony (mm)

Antifungal activity assays

Essential oil fumigation assay

The Fumigation bioassay was carried out as described by (Li et al. 2014); PDA medium (15 mL/Petri dish 90 mm) was inoculated at its center by a 6 mm fungal disc. The petri dishes were reversed and a 9 mm diameter sterile filter paper disc was placed in the center of the lid and soaked with the EO. Different volumes (10, 15, 20 µL) were applied. The dishes were immediately sealed with parafilm and then incubated at 25 °C for 7 days. The paper discs in the control dishes were treated with distilled water instead of the EO. Each test was performed in triplicate. Colony diameters were recorded daily until the 7th day. Growth inhibition was calculated according to formula (1).

Contact assay

This test was applied according to Shukla et al. (2009). The ME or EO, dissolved in dimethyl sulfoxide (DMSO) or Tween 80 (0.05%), was incorporated into a melted PDA to obtain the desired final concentrations (100, 200, 400 µg/ml for the ME and 400, 800, 1200 µL/L for the EO). After solidification in a Petri dish, the medium was aseptically seeded at its center by a fungal disc (6 mm). The control plates were supplemented with DMSO instead of ME or Tween 80 (0.05%) instead of EO. Each test was performed in triplicate. Colony diameters were recorded daily up to the 7th day. Growth inhibition was calculated according to formula (1).

Agar-well diffusion assay

For the effect of the plant on the mycelial growth of fungi, a fungal disc (6 mm) was deposited in the center of PDA Petri plate. Three wells (8 mm) were made using a cork borer, at three points equidistant from the center and the edge of the Petri plate (90mm), then each well was filled by 20 µL of the ME (100, 200, 400, 600 and 800 mg/mL, in DMSO). Each plate represents a triplicate and each test was carried out twice. The ME was replaced by DMSO in the control. Colony diameters were recorded daily up to the 7th day (Talibi et al., 2012).

Disc diffusion assay

The PDA was seeded by spreading 100 µL of a spore suspension (10^4 spores/mL). Sterile filter paper discs (6 mm) were placed at three points equidistant from the center and the edge of the Petri plate (90 mm), then each was impregnated with 20 µL of the ME (200, 400, 600 and 800 mg/ml, in DMSO). Each plate represents a triplicate and each test was carried out twice.

To test the effect of the EO, the PDA was seeded as above. On a single disc, placed in the center of the Petri plate, the EO was applied at different volumes (10, 15, 20, and 25 µL). In the controls, the ME was replaced by DMSO and discs without EO were applied. The test was carried out three times. The inhibition diameters around the discs were measured after incubation of the fungi at 25 °C for 48 h (Shirazi et al., 2008).

Bioautography

To detect biologically active compounds with antifungal activity in ME or EO, bioautography was carried out on silica gel-based thin-layer chromatography plates (TLC) (silica gel 60 F254, 0.2 mm thick) according to Kumar et al. (2012). Twenty microliters of the ME (800 mg/mL, in MeOH) or pure EO were spotted onto the activated TLC plate and then developed in the hexane: ethyl acetate (60:40) system. After the total evaporation of the solvent from the chromatogram, a spore suspension (10^4 spores/mL) of the tested fungi in the PDB was plated on the dried chromatogram and incubation was carried out in a sterile moist chamber for 24 to 72 h at 25 °C. The clear inhibition zones on the TLC plate are indicative of the antifungal activity of the separated compounds on the TLC plate.

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Micro-well dilution assay and determination of the minimal inhibitory concentration (MIC) and the minimal fungicidal concentration (MFC)

This test was conducted using the EO. This latter was dissolved in DMSO then diluted to a concentration of 10 µL/mL in the PDB, and then serial two-fold dilutions were made to obtain concentrations ranging from 10 to 0.312 µL/mL in 5 mL sterile test tubes containing PDB. MIC values of the EO against fungal isolates were determined on the basis of the micro-well dilution method as described by Sokmen et al. (2004). Ninety-five microliter of PDB and 5 µL of the inoculum (10^4 spores/mL) were dispensed into each well of a 96-well plate. An aliquot (100 µL) of initially prepared EO stock solution at the concentration of 10 µL/mL was added to the first wells. From the prepared serial dilutions, 100 µL was transferred into the five consecutive wells. The control contained 195 µL of PDB without EO and 5 µL of the inoculum. The final volume of each well was 200 µL. The plate was covered with parafilm and incubated at 25 °C for 24 to 48 h. The MIC was defined as the lowest concentration of EO at which the tested fungus exhibited no visible growth. To determine the MFC, samples from the broth of each well, showing no growth were plated on PDA and incubated at 25 °C for 48 h. MFC was defined as the lowest concentration of EO at which the incubated fungus was completely killed. Each test was performed in duplicate.

Statistical analysis

All data are expressed as mean ± SD (n = 3). The analysis of variance (ANOVA), Tukey's and Sidak's multiple comparison were considered significant at $p < 0.05$. (Statistical analyses were done using GraphPad prism 6.05 for Microsoft Windows).

Results

The ME revealed high levels of polyphenols and flavonoids (400.64 ± 12.97 µg GAE/mg and 43.13 ± 0.14 µg QE/mg) and low contents were registered in the EO (27.47 ± 0.44 µg GAE/mg and 14.04 ± 0.82 µg QE/mg).

This research investigated the antioxidant efficacy of *A. campestris*' essential oil and methanolic extract by *in vitro* testing. As Table 1 presents, the ME showed more capacity to scavenge the DPPH free radical followed by the standard BHT and the EO. In contrast, regarding the effect of the linoleic acid oxidation inhibition and reducing power test, the BHT appeared showing higher activity than the ME and the EO.

The evaluation of *A. campestris* antifungal activity was conducted by measuring the mould mycelial growth under the effect of progressive concentrations of the EO or the ME, applied by different methods. The inhibition percentage was calculated on the 7th day by the measurement of the inhibition zones, and the EO MIC values were determined. Table 2 summarizes the inhibition percentages of the tested moulds by the EO or the ME, applied at higher concentrations and by different methods.

The EO of *A. campestris* applied as fumigant had the very effective effect on the growth of *B. cinerea*, reaching 87.06% with total inhibition till the 6th day (with 20µL), and had the less effective effect on *P. expansum*, reaching 49.75% . Incorporation of the EO into the culture medium results in 3 to 5-days total inhibition of *B. cinerea*, according to the concentration tested, to up to 80.39% on the 7th at the higher concentration (1200 µL/L). The mycelial growth of *P. expansum* was delayed for up to 4 to 5-days with 81.44% reduction at the end of the incubation period. It should be noted that *B. cinerea* was sensitive to EO applied by both methods and exhibited mycelial constriction with very weak sporulation and secretion of exudates and pigments on and into the culture medium. *Penicillium expansum* exhibited yellow exudates and pigments and was more sensitive when EO was incorporated than fumigated.

The ME of *A. campestris* applied by incorporation into the culture medium had strongly reduced *B. cinerea* mycelial growth, reaching 56.47% inhibition, to a stable inhibitory level from the 6th day for all applied concentrations. *Penicillium expansum* mycelial growth was reduced up to 51.96% at the 7th day. In agar-well diffusion assay *B. cinerea* showed a slow mycelial growth rate with a reduction value up to 56% on the 4th day. The mould reached its maximum growth from the 5th day to the end of the incubation period without registered inhibition. All concentrations showed low inhibitory effect towards *P. expansum*; around 30% with no significant difference between the applied concentrations. It should be noted that the well-diffusion technique has shown less effectiveness when compared to the incorporation technique.

In the disc diffusion test, the *A. campestris* EO was less effective against spore germination of both moulds when applied at 10 µL/disc (inhibition zones were 11.66 mm and 9.42 mm for *P. expansum* and *B. cinerea* respectively), to highly effective at 15 µL/disc on *P. expansum* (59 mm) and cause complete inhibition of *B. cinerea*. The other concentrations caused a total inhibition of both moulds. The extract caused a slight inhibition of the spore germination of both moulds. The 200 and 400 mg/mL concentrations had no inhibitory effect. The 600 mg/mL concentration had a low effect on both mould spores (the respective inhibition zones were 11.17 mm and 13.42 mm for *P. expansum* and *B. cinerea*) and 800 mg/mL was significant on *P. expansum* (16.42 mm) but had the same effect as 600 mg/mL on *B. cinerea* (13.53 mm).

In the micro-well dilution test only the EO was tested for its sporostatic or sporicidal potency by applying regressive concentrations (10 - 0.312 µL/mL) in micro-wells. The results indicated that the MIC is strictly lower than 2.5 µL/mL and greater than 1.25 µL/mL for both moulds since no visible growth was observed at 2.5 µL/mL and the complementary test (plating from this concentration showed no growth on PDA free of EO), confirmed that this concentration (2.5 µL/mL) is the MFC of both moulds.

The bio-autography test allowed well separation of ME components. A sporal germination inhibition of both fungi was observed. We registered clear inhibition zones at the R_f values 0.85, 0.79, 0.61, 0.32 and 0.17. No evident separation was observed with the EO.

Discussion

Our plant ME shows high polyphenol and flavonoid contents compared to those obtained by Djeridane et al. (2007) from *A. campestris* aqueous ethanol (80%) extract (103.40 mg

Table 1. Antioxidant activities of the methanolic extract and the essential oil of *A. campestris*, and the synthetic antioxidant BHT measured in DPPH, b-carotene–linoleic acid and RP assays. [a,*]

	DPPH (IC_{50}, µg/mL)	β-carotene bleaching (IC_{50}, µg/mL)	Reducing Power (EC_{50}, µg/mL)
Methanolic extract	11.71 ± 0.22 [b]	40.96 ± 0.93 [b]	23.32 ± 0.18 [b]
Essential oil	2713 ± 293[c]	6613 ± 60	2613 ± 186 [c]
BHT	26.07 ± 0.75 [d]	4.54 ± 0.13 [d]	5.56 ± 0.66 [d]

[a] Values are means ± SD (n = 3).
[*] Values with the same superscript letter in each row are not statistically different at 5% level, according to the Tukey's multiple comparisons test.

Table 2. Percentage of the growth inhibition of *B. cinerea* and *P. expansum* at the 7th day incubation by the EO and the ME of *A. campestris* applied by different methods at different concentrations each.

Test method	Concentration[#]	Percentage of the growth inhibition [a,*]	
		B. cinerea	*P. expansum*
EO Fumigation (µL)	10	26.67 ± 0.68 [b]	28.42 ± 1.71 [b]
	15	65.10 ± 1.36	42.30 ± 1.13
	20	87.06 ± 0.00	49.75 ± 4.99
EO incorporation (µL/L)	400	52.55 ± 0.68	75.49 ± 3.05
	800	72.55 ± 0.68	81.44 ± 3.13
	1200	80.39 ± 1.80 [c]	81.44 ± 3.13 [c]
ME incorporation (µg/mL)	100	55.69 ± 2.45	35.51 ± 1.64
	200	56.86 ± 1.80	40.13 ± 0.99
	400	56.47 ± 1.18 [d]	51.96 ± 2.59 [d]
ME agar-well diffusion (mg/mL)	200	0	16.08 ± 0.51
	400	0	14.23 ± 2.70
	600	0	14.23 ± 2.70

[a] Values are means (n = 3) ± SD.
[#] In fumigation method, the EO was used without being diluted.
[*] Values with the same superscript letter in each row are not statistically different at the 5% level, according to the Sidak's multiple comparisons test.

GAE/g) and those determined by Al Jahid et al. (2016) from hydro-alcoholic maceration (72 h) of the same plant and mentioned as optimal amounts obtained, among some tested processes and solvents (≈124 µg GAE/mg and ≈ 25 µg QE/mg). Our plant also presented higher polyphenolic content than the halophyte Tunisian one (158.75 ± 12.5 µg GAE/mg) (Megdiche-Ksouri et al., 2015). Akrout et al. (2011) found higher phenolic content (463.2 µg EAG/mg) in 50% ethanol extract. These differences may be attributed to climatic conditions, altitude, soil characteristics and extraction methods. For isolation and identification of the extract compounds, further studies should be conducted.

High antioxidant activity was also registered as estimated by 3 methods. The high polyphenol content of the ME may be responsible for this phenomenon. Flavonoids and phenolics form a group of plant chemicals well known as contributors to the antioxidant activity (Baykan Erel et al., 2012; Pereira et al., 2018). These phytochemicals prevent the lipid oxidation by acting as hydrogen atom donors to free radicals (Al Jahid et al. 2016). Although the effect on linoleic acid oxidation was weaker, ME showed strong free radical scavenging power. This is mainly due to its richness in phenolic compounds. These form a low content of the essential oil. The ME activity towards the radical DPPH was shown to be higher when compared to that obtained by Nikolova et al. (2010) (DPPH IC_{50} = 225 µg/mL), nevertheless the activity of the ethyl acetate fraction issued from this latter ME was comparable to that obtained from our ME (DPPH IC_{50} = 12.50 µg/mL). These extracts were found rich in flavonoids with antioxidant properties. Our plant also showed high reducing power compared to the halophyte one studied by Megdiche-Ksouri et al. (2015). Akrout et al. (2011) pointed out a positive relationship between the polyphenol and flavonoid levels and the antioxidant and the antitumor activities of *A. campestris*.

Plant extracts and essential oils have been listed as a significant means of preventing spoilage of food (Dib and El Alaoui-Faris, 2019). Our results evidently proved the potent antifungal activity of *A. campestris* EO and ME against two most important decay moulds; *P. expansum* blue mould and *B. cinerea* gray mould. The EO was better than the ME since its application by the above different methods resulted in stronger inhibition of the mycelial growth besides the total inhibition of spore germination by disc diffusion method and its fungicidal effect at 2.5 µL/mL by well-dilution test. This latest result is clearly in concordance with that mentioned by Houicher et al. (2016) about *P. expansum* and other pathogenic and toxicogenic fungi. Secondary metabolites of plants have been tested for their antimicrobial activity and a significant number of plant extracts and essential oils have been showen to possess antimicrobial activity (Rahman et al., 2011). *Artemisia campestris* EO, having high levels of germacrene D and β-pinene, has shown clear antibacterial activity (Al Jahid et al., 2016). This is partially in concordance with our findings since our *A. campestris* EO, analysed previously (Sassoui et al., 2020), contains 15.2% of β-pinene and 9.0% of germacrene D, but also other important levels of α-pinene, myrcene (Z)-β-ocimene, and γ-curcumene. Nevertheless, the essential oil biological activity might be attributable to their major components or synergistic/antagonistic interaction between oil components; the main component of *Callistemon lanceolatus* EO (1,8-cineole), used alone has no completely inhibition on *Aspergillus flavus*, but the EO causes total fungal inhibition (Shukla et al., 2012). *Artemisia campestris* aqueous extract has shown to be effective against clinical isolates of yeasts, dermatophytes and other filamentous fungi (Webster et al., 2008). Zabka et al. (2011) investigated the antifungal activity of 46 medicinal plants and found that 14 plant species, among them *A. campestris*, have shown to possess the fungicide character. Methanolic extract of *A. campestris* has a median inhibitory concentration (MIC_{50}) higher than 2 mg/mL on 6 significant pathogenic and toxicogenic fungal species namely, *P. expansum*, *P. brevicompactum*, *Fusarium oxysporum*, *F. verticillioides*, *A. fumigatus* and *A. flavus*. In our study, the application of the ME by the well-diffusion method resulted in low effect on mycelial growth compared to the application by incorporation technique; this may be attributed to the low diffusion capacity of the ME from the well into the culture medium (It remains concentrated at the agar well). Flavonoids are found mainly in plants and may play an important role in controlling fungi. Isolated flavonoids from citrus species were found to have an inhibitory effect on fungal food contaminants *A. parasiticus*, *A. flavus*, *F. semitectum* and *P. expansum* (Salas et al., 2011). *Artemisia campestris* is also known to have a high inhibitory effect on bacteria and yeasts (Naili et al., 2010; Karabegović et al., 2011; Ghorab et al., 2013; Al-Snafi, 2015), and the EO is commonly most effective antimicrobial than the other extracts.

Conclusion

The results of this work have shown that the essential oil and the methanolic extract of *A. campestris* possess significant antioxidant and antifungal properties. Knowing that *A. campestris* is a local medicinal plant with a great abundance in Algeria, it will form a source of natural antimicrobials and antioxidants since public continued demands for reduced pesticide usage and the emergence of fungicide resistant pathogens. Qualitatively, our plant has shown to contain compounds with promising antifungal activity against grey and blue molds. Further study is needed to obtain pure compounds and to reveal more quantitative data. Furthermore, testing the biological activities under *in vivo* conditions is also necessary to confirm the above-cited properties and prevent against these important moulds.

Compliance with Ethical Standards

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Not applicable.

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Data availability

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