An Acad Bras Cienc (2024) 96(Suppl. 3): e20240703 DOI 10.1590/0001-3765202420240703
Anais da Academia Brasileira de Ciências | *Annals of the Brazilian Academy of Sciences*
Printed ISSN 0001-3765 I Online ISSN 1678-2690
www.scielo.br/aabc | www.fb.com/aabcjournal

MICROBIOLOGY

Polyphenolic contents, antioxidant, and antimicrobial activities of *Saccocalyx satureioides* Coss. & Dur. essential oil and methanol extracts

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Abstract: The whole plant *Saccocalyx satureioides*, an endemic medicinal plant in Algeria, was evaluated for its polyphenolic contents, antioxidant and antimicrobial activities. The polyphenolic contents of the plant methanolic extracts ranged from 170.47 to 285.56 (µg GAEs/mg extract) and from 25.39 to 82.68 (µg QEs/mg extract), respectively. High antioxidant activity was registered: DPPH average IC_{50} = 14.68 µg/mL, and β-carotene average IC_{50} = 21.08 µg/mL. The essential oil and methanolic extracts from the aerial parts and roots were tested against bacteria and molds. Different methods, including disk diffusion, well diffusion, and microdilution tests for bacteria, were used. The essential oil (February and June harvest periods) value 1.25 µL/mL was the MIC and the MBC for all bacterial strains, except *E. coli*, *K. pneumonia* and *Ps. aeruginosa*. The average fungal inhibition of the methanol extracts ranged from 5.10 to 71.25%, except for *F. oxysporum*. The essential oil effect was 6.67-83.53% and 17.01-90.57% by the food poisoned and fumigation methods, respectively. The preliminary phytochemical evaluation demonstrated the high polyphenolic contents and effective antioxidant power of the plant extracts. This may have an essential role in the antimicrobial effectiveness and indicate that the plant contains high-quality bioactive molecules in addition to the essential oil.

Key words: *Saccocalyx satureioides*, essential oil, methanol extract, antioxidant activity, antimicrobial activity.

INTRODUCTION

Plants have long been known to treat human ailments. They are still an important source of drugs used in medicine, thanks to their beneficial biological activities (Muntean & Vulpie 2023). Bacteria, fungi, and viruses are known to cause diseases in humans, animals, and plants. Microbial food spoilage causes food waste and loss. Moreover, foodborne disease continuously poses a significant safety concern for public health (Gonelimali et al. 2018).

Various economically important plants are targets of *Fusarium* species. This pathogenic fungus is well known to generate a variety of mycotoxins (Ajmal et al. 2023). *Aspergillus niger* and *A. ochraceus* are more prevalent in stored foods. They were isolated from fruits, oilseeds, and cereals. They have been reported to produce ochratoixin A. *Cladosporium cladosporioides* is commonly present on plant materials and in the air. It causes decay in various fruits and vegetables. It also causes diseases in humans and animals (Hocking 2006, Ma et al. 2021)

The extensive use of antibiotics to save human health from microbial diseases has resulted in the emergence of drug and multidrug resistance in microbes (Srikacha & Ratananikom

2020). Multidrug resistance and virulence, as well as the potential toxicity of antimicrobial chemicals, enhanced the search for new therapeutic strategies, including the search for bioactive molecules such as polyphenols and essential oils (Veiko et al. 2023, Magri et al. 2023). Phenolic acids and flavonoids have been reported to serve as potent antioxidants, neutralizing free radicals and preventing oxidative stress, while also effectively inhibiting microbial growth (Cosmulescu et al. 2017, Oulahal & Degraeve 2022). Essential oils contain terpenes and terpenoids that are very good at killing microbes by breaking down their cell membranes. These compounds have been particularly effective against a broad range of pathogens, including bacteria and fungi (Masyita et al. 2022, Karnwal & Malik 2024).

The species *Saccocalyx satureioides* Coss. & Dur. (belonging to the Lamiaceae), known in Algeria as '*Zaâter*' and '*Azir-el-Bel*', is an endemic species that grows in the dunes of the Algerian pre-desert area. This plant has upright stems and grows to a height of 20 to 100 cm. Its tiny, ovoid, hispid, lanceolate leaves are ciliate, and it can have red, pink, or white flowers (Quezel & Santa 1963). It is traditionally used to treat hypertension, diabetes, kidney problems, and colon and stomach ailments (Allali et al. 2008, Benaradj & Boucherit 2022); it is also recommended to treat colds (Yabrir et al. 2018) and has Antileishmaia (Mostefa Sari et al. 2020), insecticidal (Sassoui et al. 2020) and corrosion inhibition properties (Benahmed et al. 2016). The leaves, flowers, fruit, and seeds of this plant are the parts usually used. But the use of the plant roots is not reported in traditional medicine.

This work aimed to evaluate the polyphenolic contents, antioxidant activity, and antibacterial and antifungal activities of the essential oil and, for the first time, the methanolic extracts of the aerial parts and roots of *Saccocalyx satureioides* from the center-east of Algeria.

MATERIALS AND METHODS

Plant material

The plant material of *S. satureioides*, from the El Houamed region (M'sila), was collected at two periods: the aerial parts (F) and the roots (R) were collected in February 2019, and the aerial parts (J) were collected in June 2020. The plant identification was done by the botanist, Dr. SARRI Djamel, and a voucher specimen (N°: SS2331QS28) was deposited in the herbarium of the Department of Nature and Life Sciences, Faculty of Sciences, M'sila university. After being dried, the leaves and roots were recovered and then stored until use.

Essential oil isolation and obtainment of the methanolic extracts

The plant aerial parts (100 g) were subjected to hydrodistillation for 3 h in a Clevenger-type apparatus. The oil yields, estimated on a dry weight basis (v/w), were 2.8% (EO_J) and 2.55% (EO_F). After being dried over anhydrous sodium sulfate, the essential oil (EO) was stored at 4 °C until tested.

The plant methanolic extracts (ME) were obtained by Soxhlet: 50 g of the plant leaves (or roots) were extracted with 500 mL of methanol at 40 °C for 6 hours. Each extract was recovered after concentration by a rotary evaporator, then dried in an oven, weighted, and finally stored at 4° C until its use.

Total phenolic and flavonoid contents

The total phenolic content was determined according to Ruiz-Navajas et al. (2013): 300 µL the methanolic solution of the methanol extract (ME) or the EO was mixed with 1000 µL of the Folin-Cioclateu reagent (10 times diluted in distilled

water) and 2000 µL of sodium carbonate (7.5%). After being incubated for 5 min at 50 °C, the absorbance, at 760 nm, was measured against a blank. The total polyphenols were determined as µg gallic acid equivalents (GAE) / mg based on a calibration curve.

The total flavonoid content was evaluated according to Hendel et al. (2016): 1 mL of 2% aluminum chloride ($AlCl_3$) was mixed with 1 mL of the methanolic solution of the extract or the EO. After being incubated for 15 min at ambient temperature, the absorbance, at 415 nm, was measured against a blank. The total flavonoids were determined as µg quercetin equivalents (QE) / mg based on a calibration curve.

Antioxidant Activity Evaluation

DPPH test

Fifty microliters of the extract or EO (at different concentrations in methanol) were added to 2 mL of a 0.004% of DPPH methanolic solution. The mixture was incubated to react in the dark for 30 min. The control does not contain extract or EO. The BHT was used as standard. The absorbance of the samples was read at 517 nm (Tepe 2008). The percentage inhibition was calculated by the following formula:

$$I\ (\%) = [(A_c - A_s)/\ A_c] \times 100$$

where A_c is the absorbance of the control; A_s is the absorbance of the sample. The concentration of the sample providing 50% inhibition (IC_{50}) was calculated from the graph representing the percentage of inhibition against the concentration of the extract or the oil.

β-carotene bleaching test

The ß-carotene/linoleic acid bleaching test was carried out as described by Shukla et al. (2012): an emulsion stock solution (a mixture of 0.5 mg of ß-carotene, 01 mL of chloroform, 25 µL of linoleic acid, 200 mg of Tween 40, and 100 mL of distilled water added after evaporating the chloroform) was prepared. Aliquots (2.5 mL) of this solution were transferred to test tubes each containing 350 µL of different concentrations, extracts or EO, diluted in methanol. The absorbance was measured immediately at 470 nm and the test emulsion was incubated in a water bath at 50 °C for 120 min where the absorbance was measured again. The same procedure was applied to BHT as a positive control, and to methanol instead of the extract as a negative control. The relative antioxidant activity (I%) was evaluated in terms of β-carotene bleaching according to the formula:

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

Where A_t and C_t are the absorbance values measured for the test sample and the control respectively, after 120 min of incubation, and C_0 is the control absorbance value measured initially. Results were expressed as IC_{50} values (µg/mL); the concentration required to cause 50% inhibition of β-carotene bleaching.

Antimicrobial activity

Microorganisms

Reference bacterial strains were subjected to the effect of the extracts and the EO: *Bacillus subtilis* ATCC6633, *Escherichia coli* ATCC8739, *Staphylococcus aureus* ATCC6538, *Pseudomonas aeruginosa* ATCC9027, *Proteus mirabilis* ATCC 35659, *Klebsiella Pneumoniae* ATCC 700603.

The fungal isolates to be tested for the action of the extracts and the EO include *Aspergillus ochraceus* ATCC 3174, *Aspergillus niger* (isolate AnGr61), *Fusarium culmorum* (isolate Fc13), *Fusarium oxysporum* (isolate Fob36), and *Cladosporium cladosporioides* (isolate PeCc321), available at the microbiology laboratory of the

department of Microbiology and Biochemistry, M'sila university.

Preparation of the inocula

- Bacterial suspension: from 18- to 24-hour cultures, the bacterial suspension (prepared in a sterile saline solution) was well homogenized and adjusted to 0.5 of the McFarland scale opacity.
- Spore suspension: from 10-day fungal cultures (on potato dextrose agar, PDA, 25 °C), 3 mL of sterile saline (0.05% tween 80 added) were added to each Petri plate. After the culture was scraped, the spores were recovered and adjusted to 10^4 spores per mL using a hemocytometer.
- The filamentous inocula were prepared as 6 mm-disks taken from the margins of a 7-day-old culture on PDA medium.

Measurement of the inhibition

The fungal inhibition (I%) was evaluated according to the formula:

$$I\% = [(DC - DT) / DC] \times 100$$

Where DC is the diameter of the control colony (mm), and DT is the diameter of the treated colony (mm).

Agar-well diffusion and disk diffusion assays

Agar-well diffusion assay was conducted to test the effect of the MEs on both bacteria and fungi. After the Mueller-Hinton agar medium (MHA) was seeded with bacterial suspension, wells (Ø 6 mm) were done on the medium at 3 points, then each was filled with 20 µL of the ME solution (150, 300, and 450 mg/mL of dimethyl sulfoxide (DMSO)). The DMSO replaced the ME in the controls. After the plates were incubated at 37 °C for 24 hours, the inhibition zone (IZ) diameters were measured. The sensitivity degree was based on the IZ diameter: sensitive (8 mm < IZ ≤ 14 mm), very sensitive (14 < IZ ≤ 20mm) and extremely sensitive (IZ ≥20 mm).

After PDA medium was seeded with 6 mm-fungal-disk, wells (Ø 6 mm) were done on the medium at 3 points, then each was filled with 20 µL of the ME solution (150, 300, and 450 mg/mL of DMSO). The DMSO replaced the ME in the controls. The inoculated plates were incubated at 25 °C. The growth was checked daily by measuring the diameters of the colonies until the 7^{th} day. The percentage inhibition (I%) was calculated by the above formula (Hendel et al. 2021).

Disk diffusion test was conducted to test the effect of the EO on both bacterial and fungal strains. Plates containing MHA and PDA media were seeded with bacterial (0.1 mL of 10^7 germ/mL) and fungal (0.1 mL of 10^4 spore/mL) suspensions, respectively. A Whatman-paper disk (Ø 6 mm), placed at the center of the Petri plate was soaked with 10 µL of the EO. The EO was replaced by DMSO in the controls. Ofloxacin (OFX5) was used as positive control. The seeded plates were parafilm sealed and then incubated at 37 °C/ 24 h (bacteria) and 25 °C/24-48 h (fungi), and the IZ diameters were then measured. The sensitivity was described as above.

Determination of the EO bacterial minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC)

The EOs were first dissolved in 10% DMSO and serial dilutions in Mueller-Hinton broth medium (MHB) were prepared separately. The MIC values were determined by the micro-dilution method according to Gulluce et al. (2007). Briefly, the 96-well microplates were prepared by adding 5 µL of inoculum (10^7 germ/mL) and 95 µL of the MHB to each well. The wells were filled with 100 µL of each prepared EO according to the prepared serial dilutions. The final concentrations in the wells were 5 – 0.312 µL/mL. After incubation at 37 °C for 24 h, concentrations in wells with

no visible growth were considered MICs. 0.1 mL of each well considered MIC concentration was seeded on MHA and placed for further incubation to determine the MBC values.

Antifungal activity of the essential oil

Vapor contact assay

Following Li et al. (2014), petri plates of PDA medium were inoculated with a 6mm fungal disk at the center, and the petri plate was inverted. A sterile filter paper disk (Ø 9 mm) was deposited at the center of the plate lid and soaked with EO (2.5, 5, 10 µL) and distilled water in controls. The plates were then parafilm-sealed and incubated at 25 °C for 7 days. Fungal colonies were examined daily, and their diameters were measured on the 7th day. The growth inhibition was calculated.

Poisoned food assay

Following Shukla et al. (2012), the ME (in DMSO) or EO (0.05% Tween80) was incorporated into a melted PDA to the final concentrations: 150, 300, and 450 mg/mL for the ME and 50, 10, 300, and 500 ppm for the EO. Controls received DMSO and Tween 80 (0.05%) instead of ME and EO, respectively. Then the plates were parafilm-sealed and incubated at 25 °C for 7 days. Fungal colonies were daily examined, and their diameters were measured on the 7th day. The growth inhibition was calculated.

Statistical analysis

All experiments were conducted in triplicate and data expressed as mean ± SD. The analysis of variance (ANOVA) and Tukey's multiple comparisons were considered significant at $p<0.05$. The statistical analysis was carried out with GraphPad Prism 6.05 software.

RESULTS AND DISCUSSION

Extraction yields

The hydrodistillation yielded 2.55 and 3.1% (w/w) of a yellow EO with thyme odor for the February (EO_F) and June (EO_J) collections, respectively. The methanol extraction's yields were 23.07 and 26.1% for February and June aerial parts collections, respectively, and the root extraction yielded 12.3%. According to the studies on this Algerian endemic plant, the EO yield varies from 1.96 to 3.5%, depending on the time and site of collection. This was observed in this study; the time of collection focused on the flowering and non-flowering periods. Others have reported 2.3% (w/w) from the plant collected in June from the north-west (Bendahou et al. 2008) and 2.53% (w/w) from the plant leaves collected in April from the north-center region (Mostefa Sari et al. 2020).

Total phenolic and flavonoid contents

The total polyphenolic and flavonoid contents of the different samples (ME_R: methanol extract from roots; ME_F: methanol extract of February period; ME_J: methanol extract of June period) were determined by the Folin-Ciocalteu and aluminum trichloride methods, respectively. The obtained results (Table I) showed that the phenolic contents ranged from 59.46 to 285.56 µg GAEs/mg extract, and the flavonoid contents were between 31.82 and 82.68 µg QEs/mg extract. The ME_J holds the highest contents of polyphenols and flavonoids. The ascending order of the total phenolic content was: $ME_F<ME_R<ME_J$ and $EO_J<EO_F$ for oils; that of the total flavonoid content was: $ME_R<ME_F<ME_J$ and $EO_J<EO_F$. These results indicate a high polyphenolic content of the *S. satureioides* roots in addition to that of the aerial parts, which is somewhat higher compared to the data presented by others: 45.28 ± 0.7 mg QE/g of dry hydro-ethanolic extract (Mostefa Sari et al. 2020), 171.34 ± 1.43 mg GAE/g,

Table I. Total phenolic, flavonoid contents, and antioxidant activity (DPPH and β-carotene tests) of the methanolic extracts and the essential oils from *S. satureioides*.

Sample	Phenolic content (µg GAEs/mg extract)	Flavonoid content (µg QEs/mg extract)	DPPH (IC_{50} values, µg/mL)	β-carotene (IC_{50} values, µg/mL)
ME_F	170.47 ± 0.48	61.13 ± 0.31	11.26 ± 0.41*	13.94 ± 0.58
ME_J	285.56 ± 0.56	82.68 ± 1.05	17.49 ± 0.29[a]	20.15 ± 1.54
ME_R	235.98 ± 1.58	25.39 ± 0.57	15.30 ± 0.54[a]	29.16 ± 0.37
EO_F	83.42 ± 0.50	43.12 ± 0.89	210.73 ± 4.25[b]	382.26 ± 48.81
EO_J	59.46 ± 0.55	31.82 ± 0.33	192.46 ± 0.34[b]	645.10 ± 15.41
BHT	–	–	59.06 ± 1.05	4.49 ± 0.07

[*] Values with the same superscript letter are not statistically different according to the two-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test, $p<0.05$. Values are means (n = 3) ± SD.

and 18.6 ± 0.46 mg QE/g of dry extract of the hydro-methanolic extract (Mehlous et al. 2020), 244 ± 4 mg GAE/g, and 54 ± 1 mg QE/g from the lyophilized plant infusion (Ziani et al. 2015).

Antioxidant activity

The stable DPPH free radical is often used to determine the antioxidant power of different plant extracts. Based on this technique, our results (Table I) showed that ME_F presented the highest DPPH-free radical scavenging power. The antioxidant capacity of the different methanol extracts, expressed as IC_{50} values, ranges from 11.26 to 17.49 µg/mL and can be presented in the order ME_F > ME_R> ME_J; the IC_{50} values of the EOs were 382.26 ± 48.81 and 645.10 ± 15.41 for EO_F and EO_J, respectively. It is notable that all the methanolic extracts have better antiradical activity compared to the positive control BHT (IC_{50} = 59.06 µg/mL). It is obvious that the EOs antioxidant activity values are not statistically different, likewise for the EM_J and EM_R extracts.

The β-carotene bleaching under radical effect is another method used to test the antioxidant activity. Based on this technique, our results (Table I) showed that the IC_{50} values ranged from 13.94 to 29.16 µg/mL for the methanolic extracts, with a significant difference between all values ($p<0.05$). Similarly, the EO_F was more effective (IC_{50} = 382.26 µg/mL) than the EO_J (IC_{50} = 645.10 µg/mL).

Our plant, originating from the center-Est region, exhibited higher power in terms of DPPH radical scavenging compared to the same plant from the center and the western regions. From plants of the center region, the methanolic extract showed an IC_{50} of 30µg/mL (Mehlous et al. 2020), and the EO IC_{50} values ranged from 1292 to 1880µg/mL depending on the harvesting time (Souadia et al. 2020). However, close values to ours were observed with chloroform and ethyl acetate extracts; those of the DPPH radical scavenging power were 23.93 ± 4.15 and 20.81 ± 3.52 µg/mL, respectively; and those of the β-carotene bleaching test were 40.32 ± 5.32 and 57.12 ± 1.55 µg/mL, respectively (Kherkhache et al. 2020). The aqueous extract of *S. satureioides,* originating from the south-west region, presented lower antioxidant activity with IC_{50} values of 236 ± 5 and 256 ± 15 µg/mL for DPPH and β-carotene, respectively (Ziani et al. 2015). Mohamadi et al. (2015) isolated and identified 17 compounds from different *S. satureioides* extracts. They tested their scavenging DPPH free radical, and these compounds were of higher activity (2.42 ± 0.12 to 66.95 ± 1.01 µg/mL). These

isolated phenolic compounds and glycoside derivatives may have an important therapeutic indication.

Antibacterial activity

In this study, the disk diffusion and agar-well diffusion methods were utilized to assess the antibacterial effect of the EO and ME, respectively. The inhibitory effect was measured in terms of the bacterial inhibition zone diameter (IZ).

As shown in Table II, the disk diffusion method allowed us to notice that all the tested bacterial strains were sensitive to *S. satureioides* EO and the OFX5 antibiotic. On the other hand, the tested bacterial strains were extremely sensitive to the EO_J versus the EO_F and OFX5. Besides the high sensitivity to the antibiotic OFX5, it is significant that the bacterial strains presented different degrees of sensitivity to the EO_F; *Ps. aeruginosa* and *B. subtilis* were sensitive (8 mm < IZ ≤ 14 mm); *K. pneumoniae*, *Pr. Mirabilis*, and *S. aureus* were very sensitive (14 < IZ ≤ 20 mm); and *E. coli* was extremely sensitive (IZ > 20 mm).

Table III illustrates the antibacterial activity of *S. satureioides* methanol extracts in terms of inhibition zone diameter. It is clear to notice that the IZ diameter increases proportionally with the extract concentration. Based on the sensitivity criterion, the overall bacterial strains presented increased sensitivity, and this varies between the tested bacterial strains; *Streptococcus aureus* seems to be most resistant to all the methanol extracts and at all concentrations tested (statistically, there is no significant difference between the extracts at all concentrations, $p<0.05$). *Bacillus subtilis* is more sensitive to ME_F and ME_J, then accordingly the bacterial strains *Ps. aeruginosa*, *K. pneumonia*,

Table II. Inhibition zones (mm) showing the antimicrobial activity of *S. satureioides* essential oils EO_J, EO_F, and the antibiotic OFX5.

Microorganisms	EO_J (10µL/disk)	EO_F (10µL/disk)	OFX5
Bacterial strains			
B. subtilis	45.33 ± 1.15 ^{a*}	11.67 ± 0.58 ^{a*}	33.00 ± 0.00 ^{a*}
E. coli	50.00 ± 2.00 ^{b}	23.00 ± 1.00	26.33 ± 0.58
K. pneumoniae	42.00 ± 1.73	17.00 ± 2.00 ^{b}	31.67 ± 0.58 ^{ab}
Ps. aeruginosa	37.67 ± 1.53	10.67 ± 0.58 ^{a}	30.33 ± 0.58 ^{b}
Pr. mirabilis	46.67 ± 2.31^{a}	17.33 ± 1.15 ^{b}	23.33 ± 0.58
S. aureus	46.00 ± 2.00 ^{a}	17.67 ± 0.58 ^{b}	33.00 ± 0.00 ^{ab}
Fungal strains			
A. niger	56.67 ± 3.51	50.33 ± 2.52 ^{c}	–
A. ochraceus	50.33 ± 1.53 ^{bc}	46.00 ± 1.73	–
C. cladosporioides	76.67 ± 2.31	62.00 ± 2.00	–
F. culmorum	45.67 ± 2.08 ^{a}	41.33 ± 2.31	–
F. oxysporum	53.00 ± 1.73 ^{c}	50.67 ± 1.15 ^{c}	–

– not tested.

* Values with the same superscript letter in each column are not statistically different according to the two-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test, $p<0.05$. Values are means (n = 3) ± SD.

P. mirabilis, and *E. coli*. The *Ps. aeruginosa* strain is notably resistant to ME_R.

According to the efficacy of extract concentrations, the EM_J and the EM_R at levels of 300 and 450 mg/mL were the most effective; four of the six tested bacterial strains were very sensitive (14 < IZ ≤ 20 mm). For the other extracts and concentrations, all bacterial strains were weakly to moderately sensitive (9 < IZ ≤ 14 mm), except *Ps. aeruginosa*. Referring to the statistical analysis, there is no difference between the tested EM_F concentrations; the antibacterial effectiveness seems to be similar, except for *Ps. aeruginosa*.

Table IV summarizes the MIC and MBC values of the *S. satureioides* EO on the tested bacterial isolates. It can be seen that the MIC values of the two EOs are equal to 1.25 µL/mL for all bacterial strains, except *K. pneumoniae,* with an EO_F MIC of 0.31 µL/mL. Besides *K. pneumoniae* that has an MBC greater than 5µL/mL, it is notable to qualify the MIC values as MBC values for the EO_F. The same applies to the EO_J, with the exception

Table III. **Inhibition zones (mm) showing the antibacterial activity of *S. satureioides* methanol extracts.**

Extract	mg/mL	*B. subtilis*	*E. coli*	*K. pneumonia*	*Ps. aeruginosa*	*Pr. mirabilis*	*S. aureus*
ME_F	150	22.33 ± 2.08 ^{a*}	8.00 ± 0.00 ^{a*}	12.67 ± 2.08 ^{a*}	13.33 ± 1.53 ^{a*}	13.67 ± 1.15 ^{a*}	8.00 ± 0.00 ^{a*}
	300	25.67 ± 2.52 ^{ab}	10.33 ± 0.58 ^{ab}	13.67 ± 2.08 ^{ab}	16.33 ± 2.52 ^{ab}	13.33 ± 1.53 ^{a}	9.67 ± 1.53 ^{a}
	450	28.33 ± 1.53 ^{b}	11.67 ± 1.53 ^{bc}	15.00 ± 1.00 ^{ac}	22.00 ± 2.00 ^{c}	14.00 ± 1.00 ^{a}	10.67 ± 0.58 ^{ab}
ME_J	150	18.33 ± 0.58 ^{c}	10.67 ± 0.58 ^{acd}	15.33 ± 0.58 ^{ad}	16.00 ± 1.00 ^{ad}	10.33 ± 0.58 ^{a}	9.00 ± 0.00 ^{ac}
	300	23.00 ± 1.00 ^{a}	11.67 ± 0.58 ^{bd}	17.00 ± 2.00 ^{bcde}	17.33 ± 1.15 ^{bd}	17.67 ± 0.58 ^{bc}	10.67 ± 0.58 ^{ad}
	450	24.67 ± 2.08 ^{a}	12.67 ± 1.15 ^{bde}	18.67 ± 0.58 ^{df}	21.67 ± 0.58 ^{c}	21.00 ± 1.73 ^{bd}	11.67 ± 0.58 ^{bcde}
ME_R	150	11.67 ± 0.58	15.33 ± 1.53 ^{e}	17.33 ± 0.58 ^{cdg}	8.00 ± 0.00 ^{e}	17.00 ± 1.73 ^{ac}	8.00 ± 0.00 ^{a}
	300	16.33 ± 1.15 ^{c}	22.00 ± 2.00 ^{f}	18.67 ± 1.15 ^{dh}	8.00 ± 0.00 ^{e}	21.00 ± 1.73 ^{cd}	10.00 ± 1.00 ^{aef}
	450	21.33 ± 1.15 ^{ac}	23.00 ± 1.00 ^{f}	20.33 ± 1.53 ^{efgh}	8.00 ± 0.00 ^{e}	22.00 ± 2.00 ^{d}	12.00 ± 1.73 ^{bcdf}

*** Values with the same superscript letter in each column are not statistically different according to the two-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test, $p<0.05$. Values are means (n = 3) ± SD.**

Table IV. **Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of *S. satureioides* essential oil.**

Bacterial strains	MIC (µL/mL)		MBC (µL/mL)	
	EO_J	EO_F	EO_J	EO_F
E. coli ATCC8739	1.25	1.25	2.5	1.25
B. subtilis ATCC6633	1.25	1.25	1.25	1.25
K. pneumoniae ATCC9027	1.25	0.31	> 5	> 5
S. aureus ATCC6538	1.25	1.25	1.25	1.25
Ps. aeruginosa ATCC9027	1.25	1.25	> 5	1.25
Pr. mirabilis ATCC 35659	1.25	1.25	1.25	1.25

EO_J: Essential oil of June period; EO_F: Essential oil of February period.

of *E. coli* (MBC = 2.5 µL/mL), *K. pneumonia*, and *Ps. aeruginosa* (MBC > 5 µL/mL each).

Because *S. satureioides* is an endemic plant in Algeria, little research has been done on its antimicrobial properties. Laouer et al. (2006) have reported a significant antibacterial effect of *S. satureioides* EO (half diluted) on *E. coli*, *B. subtilis,* and *S. aureus*. The antibacterial effect ranged from 32 and 35 mm IZ diameter on *B. subtilis* and *S. aureus* to total inhibition of *E. coli*. Bendahou et al. (2008) mentioned a moderate antibacterial activity of the *S. satureioides* EO from the west of Algeria. The IZ diameters ranged from 7 to 16.5 mm, with an average diameter of 11 mm on *B. subtilis*, *S. aureus*, *E. coli*, *Ps. aeruginosa*, *Pr. mirabilis*, *Listeria monocytogenes*, *Enterococcus faecalis*, *Klebsiella pneumonia*, *S. typhimarium*, and a particular resistance of *Ps. aeruginosa* (6 mm). Kherkhache et al. (2018) tested the aerial part extracts (with solvents petroleum ether, chloroform, ethyl-acetate, and n-butanol) on the bacterial strains *B. cereus*, *S. aureus*, *E. coli*, *Ps. aeruginosa*, *Pr. mirabilis*, and *Enterobacter aerogenes*. The ethyl-acetate and n-butanol extracts were the most active against all bacterial strains. A particular resistance was observed in *Ps. aeruginosa*. Basically, all the above results are in concordance with ours. In our study, we multiplied the test techniques and tested the root extract additionally, with varied concentrations. The *S. satureioides* EO we tested (from the center-east of Algeria) from the harvested plant at two different stages (non-flowering: February and flowering: June) had a stronger antibacterial effect on all tested bacteria. The EO for the June period was the most effective. Moreover, all the methanolic extracts have antibacterial effects according to the extract type and extract concentration. We note, as in the above studies, the particular resistance of *Ps. aeruginosa* to EO_J and ME_R, in addition to *K. pneumonia* being resistant to both EOs. This bacterium had an EO MIC of 0.31–1.25 µL/mL but a MBC higher than 5 µL/mL. Bacterial sensitivity to plant extracts varies according to bacterial species and the plant extract type. Srikacha & Ratananikom (2020) tested different extract types (hexane, ethanol, and aqueous) from five medicinal plants: *Cissampelos pareira*, *Stemona tuberosa*, *Barringtonia acutangula*, *Piper betle,* and *Artocarpus lacucha,* against *S. aureus*, *E. coli*, *Ps. aeruginosa,* and *Salmonella typhimurium*. The extracts from *Cissampelos pareira* were totally inactive. The ethanolic extract from *Piper betle* was effective against all bacterial species; that from the other plants was effective against at least one bacterial species. Hexane and aqueous extracts were inactive. Our previous study on the antimicrobial methanolic extract from *Lavandula dentata* showed an antibacterial effect against 17 bacterial strains on 18 tested bacterial strains (Hendel et al. 2023). It is noteworthy that the chemical composition of extracts from *S. satureioides* was conducted in only two studies. Mohamadi et al. (2015) isolated phenolic compounds from chloroform, ethyl acetate, and n-butanol extracts of hydro-ethanol extract from *S. satureioides* aerial parts. Among these phenolic compounds are vanillin, caffeic acid, and quercetin, which are well known as antimicrobial agents. Likewise, Kherkhache et al. (2018) isolated compounds such as indoles, glycoside alkaloids, flavone aglycones, and one monoterpene glycoside from *S. satureioides* ethyl acetate extract. This latter has shown strong antibacterial activity. Chen et al. (2023) explored the mechanism of action of vanillin against *E. coli* O157:H7. After treatment, the results showed damage and depolarization of the membrane, as well as leakage of nucleic acid and protein. Additionally, there was disruption to energy metabolism. In addition to its affection for the microbial membrane and leakage of intracellular components, caffeic acid

inhibits fungal mycelial growth and mycotoxin production and has an impact on enzymatic activity related to nucleic acid synthesis, radical generation, and protein synthesis (Khan et al. 2021). Quercetin also inhibits the growth of Gram-positive and Gram-negative bacteria and fungi. Besides its action on the cell membrane and the synthesis of nucleic acids and proteins, it affects mitochondrial function, prevents biofilm formation, and inhibits the growth of various drug-resistant microorganisms (Nguyen & Bhattacharya 2022).

Antifungal activity

The Agar-well diffusion assay was performed to check for the inhibitory effect of the methanol extracts on the fungal mycelial growth. The inhibition values were determined as percentages.

As can be seen from Figure 1, the *F. oxysporum* mold is the most resistant to all the tested methanol extracts, except for the ME_J and ME_F at 450 mg/mL concentrations, with growth inhibition not exceeding 28%. *Fusarium culmorum* seems to be resistant to all root extract concentrations but sensitive to the other extracts, with an inhibition rate ranging from 20 to 56% (figure 4a). In contrast, *C. cladosporioides* was the most sensitive to all methanol extracts, with inhibition ranging from 43 to 71%. *Aspergillus ochraceus* was more resistant to the root methanol extract (ME_R) compared to the other extracts, and its inhibition was between 15 and 62%. Unlike the other tested strains, *A. niger* was more sensitive to the ME_R and was inhibited up to 32%.

Regarding the antifungal effect of the extracts, inhibition increases with increasing concentration, depending on the fungal strain. Excluding *F. oxysporum*, it can be noted that the ME_J presented the highest inhibition degree (from 11 to 71%), followed by the ME_F (from 20 to 64%) and ME_R (from 5 to 55%). Unlike its low inhibitory effect, the ME_R presented the highest effect on *A. niger* for all its concentrations (notably with no significant difference, $p<0.05$).

The Disk diffusion assay was used to assess the effect of the *S. satureioides* EO on the fungal spore germination by means of the measurement of the spore inhibition zone

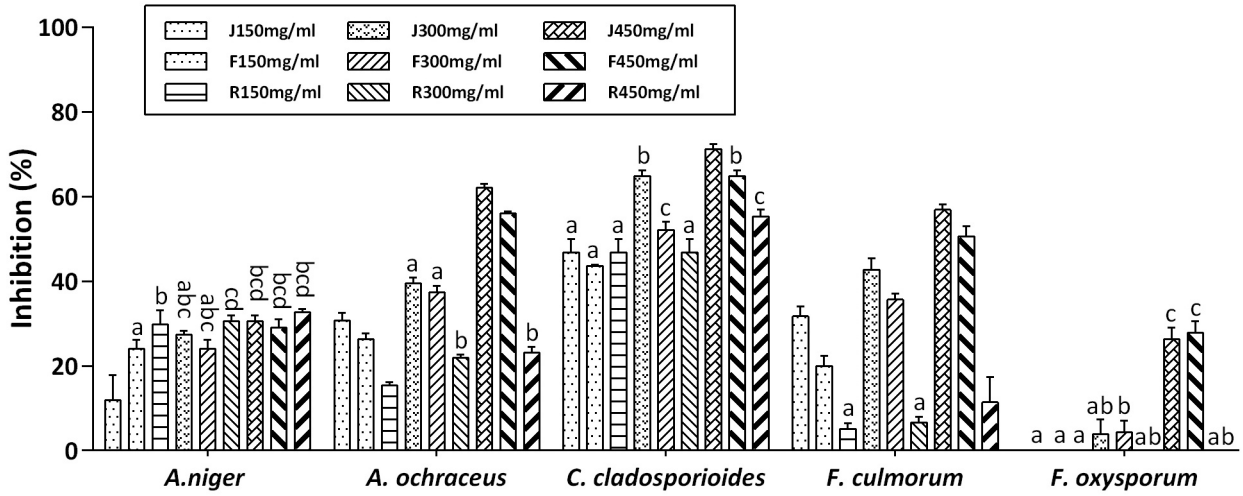

Figure 1. The inhibition of mycelial growth caused by the *S. satureioides* methanol extracts on the 7th day of incubation. (J:ME June; F: ME February; R: ME Root). No significant difference ($p<0.05$) between means of the same letter above the histogram bars of each mold.

(IZ) diameter. The results presented in Table II indicate the high power of the *S. satureioides* EO to inhibit spore germination. All the tested fungal spores can be classified as very sensitive to both EO_J and EO_F; the IZ diameter varies from 41 to 76mm. It is noteworthy that EO_J is slightly more powerful than EO_F. In terms of fungal sensitivity, *C. cladosporioides* is the most sensitive and *F. culmorum* is the most resistant. It can be noted that sporulation was strongly affected (Figure 5a-d).

The poisoned food method was used to assess the effect of the incorporated EO on the fungal growth. Four concentrations of each EO were tested (50, 100, 300, and 500 ppm). The results are shown in Figure 2. From this figure, it can be easily seen that the lower EO concentrations (50 ppm) have a weak to no effect on the overall molds. The EO_F at 100 ppm has no effect on *F. oxysporum*. Based on the percentage inhibition values, a progressive increase is proportional to the EO concentration can be seen. The inhibition percentage ranged from 18 to over 80%, and the EO_J was the most effective on all fungal strains compared to the EO_F. In a global view, *A. ochraceus* and *A. niger* were the least sensitive compared to the other species (Figure 4b). The maximum inhibitory effect of EO_J is clear for the maximal concentration (500 ppm) and varies from 54% on *A. ochraceus* to 89% on *C. cladosporioides*. The EO_F maximal effect ranged from 61% on *A. ochraceus* to 75% on *F. oxysporum*. It is notable that the EO_J concentrations of 300 and 500 ppm are not statistically different in their effect on the *Fusarium* species (p<0.05). On the other hand, we note a fungal growth delay of 48 h for *A. ochraceus* and 4 days for the other fungi under the effect of both EOs.

The EO fumigation test is based on the effect of EO volatilization against the fungal mycelial growth in a close environment. The fungal strains were thus exposed to the progressive volumes of 2.5, 5, and 10 µL. After measurements were done, the results were represented in Figure 3. The inhibition percentage values ranged from nearly 17% to 71% for the two EOs at 2.5 µL. The EOs at 5 and 10 µL presented the same effect on almost all fungal strains, with an inhibition degree ranging from 68 to 90%, except for the

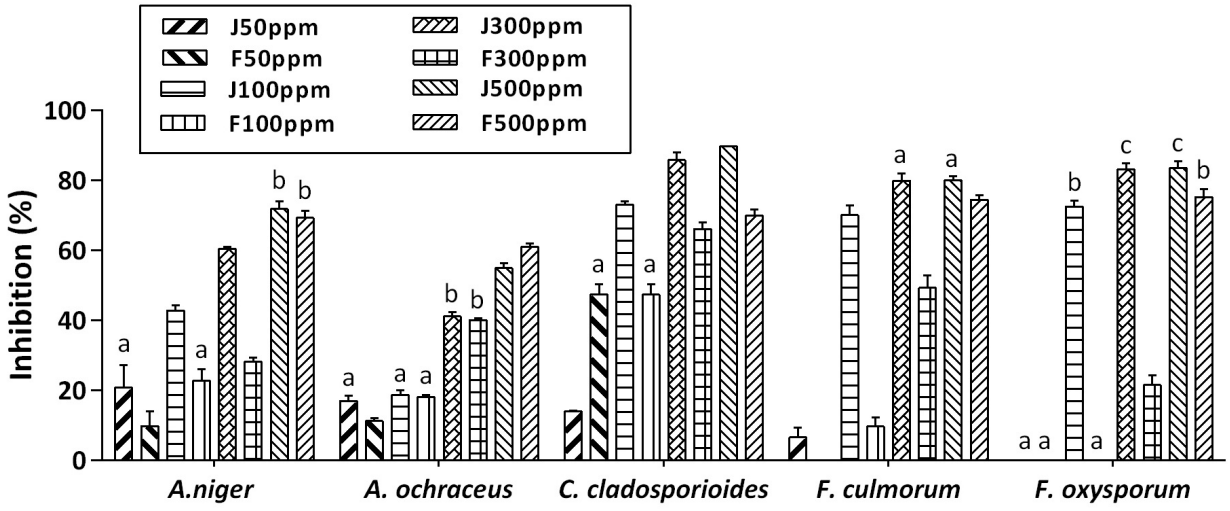

Figure 2. Mycelial growth inhibition registered on the 7th day of incubation. The *S. satureioides* EOs were applied by the poisoned food method. (J: EO June; F: EO February). No significant difference (p<0.05) between means of the same letter above the histogram bars of each mold.

EO_J on *A. ochraceus*. It is clear that *Aspergillus* species were the least sensitive compared to the other species. It can be noted that the *Fusarium* species were the most sensitive. The minimal EO volume delayed the growth of all fungi by at least 48 hours. The effect of the EOs was generally manifested by changes in the macroscopic appearance, namely sporulation weakness and pigmentation (Figure 4c).

Regarding the antifungal activity, the same studies mentioned above were done with the use of *Candida albicans*, but there is no clear discussion related to the filamentous fungi *A. niger* and *A. fumigatus* in the Laouer et al. (2006) study. In summary, these studies showed inhibitory activity on the *C. albicans* yeast of about 12 to 23 mm inhibition zones (Bendahou et al. 2008, Kherkhache et al. 2018) and total inhibitory activity on *C. albicans*, *A. niger,* and *A. fumigatus* (Laouer et al. 2006). In a study on the effect of *S. satureioides* EO on the fungus *Ascochyta rabiei*, Zerroug et al. (2011) mentioned no growth of the fungus at 3 mg/mL, and reduced growth by 50% at the essential oil concentration of 0.0187 mg/mL. Khaldi et al. (2017) tested the *S. satureioides* EO from Nâama (west of Algeria) against the mycelial growth of the molds *Aspergillus niger*, *A. flavus*, *A. ochraceus*, *Penicillium expansum*, *Fusarium oxysporum* f. sp. *albedinis*, *Alternaria alternata*, and *Cladosporium* sp. using the contact method. They found that all molds were totally inhibited at the concentration of 1/370 (v/v). At 1/1500 (v/v) of the EO (≈ 667 ppm), *Aspergillus niger*, *A. flavus*, *A. ochraceus,* and *Penicillium expansum* were inhibited to 56-76%, and the other molds were totally inhibited. These results are in concordance with ours at the EO concentration of 500 ppm, so that the mycelial growth inhibition ranged between 61 and 75%. Plant extracts have been shown to have an inhibitory effect on molds. In an *in vitro* study on the effect of plant extracts on *Fusarium* species, Kursa et al. (2022) found that ethanolic leaf extracts from *Achillea millefolium*, *Tanacetum vulgare, Artemisia absinthium* and *Salvia officinalis* caused fungal mycelial growth inhibition, and this varied according to extract type and concentration. The highest inhibition coefficient ranged from 63.82% to 83.53% at a plant extract concentration of 20%. Aqueous extracts from medicinal plants *Artemisia herba alba, Cotula cinerea, Asphodelus tenuifolius*, and

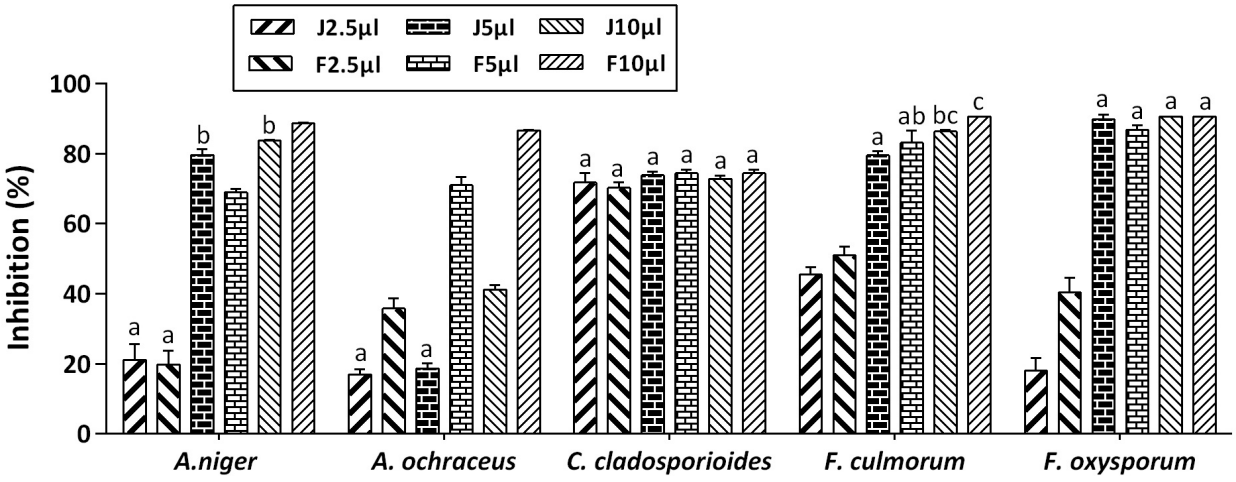

Figure 3. Mycelial growth inhibition registered on the 7th day of incubation. The *S. satureioides* EOs were applied by the fumigation method. (J: EO June; F: EO February). No significant difference ($p<0.05$) between means of the same letter above the histogram bars of each mold.

Euphorbia guyoniana also showed antifungal action against *Fusarium graminearum* and *Fusarium sporotrichioides* at concentrations of 10% and 20% in the culture medium. These plants were found to contain tannins, flavonoids, saponins, steroids, and alkaloids (Salhi et al. 2017). Essential oil (85% eugenol) from clove (*Eugenia caryophyllata*) showed a total *in vitro* inhibitory effect on *Fusarium oxysporum* at concentration of 500 ppm and from 400 to 500 ppm on *Aspergillus niger*. On tomato fruits, the fungal growth was reduced from 50% to 70% and to 40% for *A. niger* and *F. oxysporum*, respectively (Muñoz Castellanos et al. 2020). Some of the most important postharvest fungi, *Alternaria alternata*, *Botrytis cinerea*, and *Penicillium italicum*, were effectively inhibited by aromatic or medicinal plant EOs from *Cinnamomum verrum*, *Syzygium aromaticum*, *Gautheria fragrantissima*, *Cupressus sempervirens*, *Cymbopogon nardus*, *Pelargonium asperum*, *Mentha piperita*, and *Foeniculum vulgare* at 0.5 mg/mL. The antifungal effect varied depending on the EO source and fungus resistance (Allagui et al. 2024). Qu et al. (2022) found reduction in the ergosterol and aflatoxin B1 contents, in the thickness of hyphae, and inhibition of the mycelial growth and spore germination, following treatment of *Aspergillus flavus* with carvacrol concentrations up to 200 µg/mL. Xing-dong & Hua-Li (2014) studied the effect of *Zanthoxylum bungeanum* EO and its major constituent α-pinene on *Fusarium sulphureum*. The hyphal normal structure was affected, such as hyphal

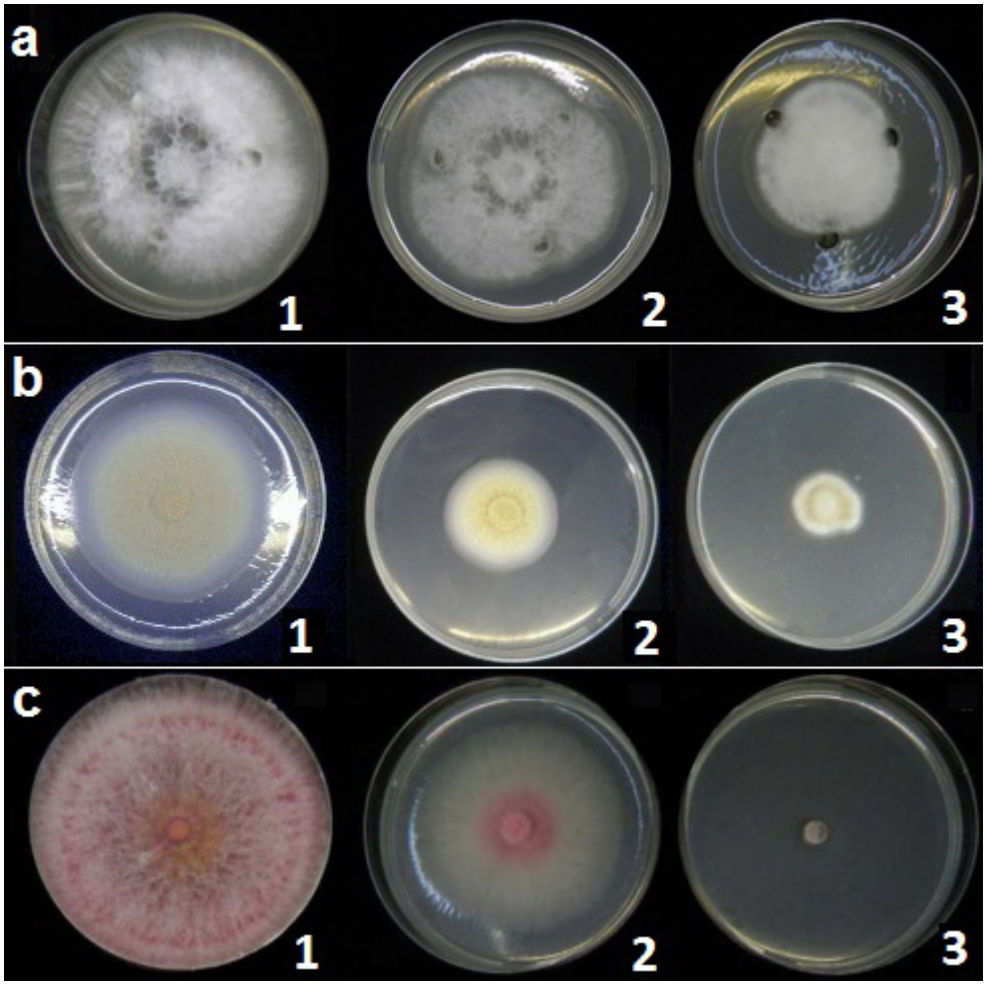

Figure 4. Antifungal effect of *S. satureioides* on *F. culmorum*, *A. ochraceus*, and *F. oxysporum*. a) Effect of the ME_F on *F. culmorum* (Well-diffusion method): 1) control; 2) 300mg/mL; 3) 450mg/mL. b) Effect of the EO_J on *A. ochraceus* (Poisoned food method): 1) control; 2) 300ppm; 3) 500ppm. c) Effect of the EO_J on *F. oxysporum* (Fumigation method): 1) control; 2) 2.5µL; 3) 5 µL.

swelling with more secretions at the surface and cytoplasmic disorder with massive vacuolation and osmiophilic granule formation. The dry rot of potatoes inoculated with *F. sulphureum* was well controlled, and the EO was more interesting than α-pinene.

We found previously that our *S. satureioides* EO is composed mainly of thymol (25.6%), α-terpineol (24.6%), borneol (17.4%), and *p*-cymène (11.4%) (Sassoui et al. 2020). These compounds form 79% of our tested EO and are the basic components of the *S. satureioides* EO cited in the literature, taking into consideration that this plant is endemic to Algeria (Biondi et al. 2006, Laouer et al. 2006, Bendahou et al. 2008, Aouf et al. 2020, Souadia et al. 2020). Thymol, a phenolic component in EOs, was reported to have an antibacterial effect. Due to its pleasant smell and low toxicity, this compound will be used as a food additive in order to prevent bacterial spoilage (Memar et al. 2017). It is an effective antioxidant and reduces oxidative stress and the risk of associated disorders (Rathod et al. 2021). The α-terpineol is known for its praiseworthy and useful properties, including the antimicrobial activity (Ben Akacha et al. 2024). Natural borneol is well known in traditional Chinese medicine, and it possesses antifungal activity. It has been shown to inhibit the *C. albicans* germ tube and biofilm formation (Wang et al. 2022). The *p*-Cymene is a precursor for thymol and carvacrol. It possesses antibacterial and antifungal activities, and it is considered "generally recognized as safe" by the U.S. Food and Drug Administration (Balahbib et al. 2021). These four reputable compounds make *S. satureioides* EO of high quality and suitable for use in the food and pharmaceutical fields.

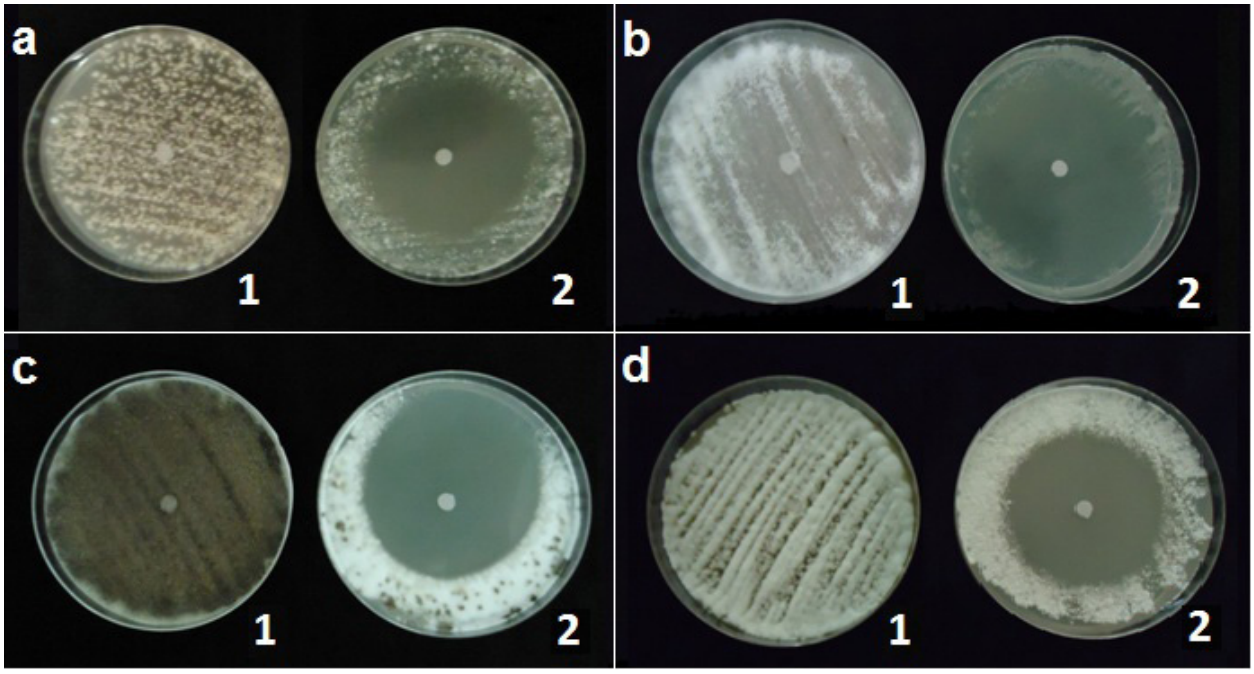

Figure 5. Antifungal effect of *S. satureioides* essential oil (EO_1) on the fungal spore germination (1: control; 2: test). a) *A. ochraceus*; b) *F. oxysporum*; c) *A. niger* ; d) *F. culmorum*.

CONCLUSIONS

The results of this study showed that *S. satureioides* is endowed with great antimicrobial activity against bacteria and filamentous fungi, in addition to antioxidant properties. This is the first report on the bioactive effect of the *S. satureioides* roots. Root extract had an antibacterial effect similar to that of the aerial parts on almost all bacterial strains tested. It also had an antifungal effect, but it was lower than the aerial parts extracts. It presented a particularly high inhibition effect on *A. niger* at the lowest concentration. We note the resistance of the *Fusarium* species to the plant extracts, particularly at low concentrations. In contrast, *Aspergillus* species were less sensitive to the *S. satureioides* essential oil compared to the other fungal strains. Further advanced analyses and tests are needed to identify and quantify bioactive compounds in these plant extracts, to determine their effect under field conditions, and to verify whether biopreparations may be produced to carry less risk to the environment and to humans.

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How to cite

HENDEL N, SARRI D, SARRI M, GHAFSI AA & BENSEGHIR A. 2024. Polyphenolic contents, antioxidant, and antimicrobial activities of *Saccocalyx satureioides* Coss. & Dur. essential oil and methanol extracts. An Acad Bras Cienc 96: e20240703. DOI 10.1590/0001-3765202420240703.

Manuscript received on June 28, 2024;
accepted for publication on October 8, 2024

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Noui Hendel, Achwaq Ali Ghafsi and Aicha Benseghir participated in the preparation of the extracts, microbiological analyzes of the plant extracts, Djamel Sarri and Madani Sarri participated in the plant collection and identification. Noui Hendel, Achwaq Ali Ghafsi and Aicha Benseghir participated in the analysis and interpretation of the data. All authors participated in writing the article and have approved the final version for publication.