Journal of Applied Biological Sciences
E-ISSN: 2146-0108 17(1): 20-38, 2023
DOI: https://doi.org/10.71336/jabs.1047
Research Article

ANTIFUNGAL AND ANTIOXIDANT ACTIVITIES OF ACTINOBACTERIA ISOLATED FROM ALGERIAN DESERT SOILS

Habiba Belgacem[1*], Mokhtar Benreguieg[2],
Djallel Eddine Houari Adli[2], Amel Benzerga[2]

[1]*University of Saida Dr- Moulay Tahar, Laboratory of Biotoxicology, pPharmacognosy and Biological Valorization of Plants, Saida, Algeria*
[2]*University of Saida Dr- Moulay Tahar, Faculty of Sciences, Department of Biology, Saida, Algeria*

**Corresponding Author:*
E-mail: habiba.belgacem@univ-saida.dz

(Received 29th January 2022; accepted 03rd October 2022)

ABSTRACT. *Actinomycetes* are gram-positive filamentous bacteria that are thought to be the best candidates for producing biologically active secondary metabolites. Our research aims to identify antifungal and antioxidant activity, as well as purify and characterize the components involved in this phenomenon in isolated actinomycetes derived from the Algerian desert soil. Eighteen isolates were studied. The antagonism tests are performed by the agar cylinder technique against two gram-positive bacteria, *Staphylococcus aureus* and *Listeria monocytogenes*, and five fungi, which are known for their importance in human infectious pathologies. 14 isolates showed inhibitory activity against the two bacteria, and 15 isolates showed antifungal activity against the five fungal species: *Fusarium graminearum, Aspergillus flavus, Aspergillus ochraceus, Penicillium expansum, and Aspergillus parasiticus*. The results allowed us to choose two strains of Actinomycetes coded A10 and A7, both of which have strong inhibitory abilities. That was used for solid-state fermentation (AF)**.** After 14 days of incubation at 28 °C, methanol is used for the extraction of the metabolites produced in the medium. The methanolic extract was effective against the five target fungi species targets even at low doses of 2 mg/ml. The ergosterol degradation test revealed that strain A7 generates an antifungal agent capable of changing membrane sterols, but strain A10 had no impact. A characterization of the inhibiting agent using chromatographic techniques was carried out. The antioxidant activity of the two crude extracts was measured using DPPH radical scavenging. The results demonstrate that the A10 strain extract has good activity, with a percentage inhibition of 58% and an IC50 test of 8.10. The study of the morphological, cultural, physiological, and biochemical characteristics of the two isolates, A7 and A10, allowed us to link them to the genus *Streptomyces***.**

Keywords: *Actinomycete, active secondary molecules, antifungal activity, antioxidant activity, TLC.*

INTRODUCTION

Invasive infections produced by pathogenic fungi are associated with a high death rate. The number of immunocompromised patients, such as those with cancer, HIV infection, or solid organ transplantation, has grown substantially in the last two decades, owing to an increase in the incidence of fungal infections [1, 2, 3, 4]. In addition, more invasive

treatments including chemotherapy and hematopoietic stem cell transplants, greater use of vascular probes, and long-term use of broad-spectrum antibiotics are all risk factors for deep mycosis. Resistance to fungal infections and the development of new fungal pathogens are other significant variables to consider [5].

The issue is comparable in animal treatment, agriculture, food processing, and other industries where fungi cause havoc, particularly in impoverished nations, and for which there are no effective antifungals or non-toxic, non-polluting, and if feasible, biodegradable fungicides [6, 7, 8].

In the face of this dire scenario, despite the discovery of various antifungal drugs, some are ineffective and others are prohibitively costly. Furthermore, resistance issues prevent many of them from being used. All fermentation products are the finest antifungal agents on the market [5].

Actinomycetes are the primary sources of secondary metabolites produced by anticellulite activity. Streptomyces species account for half of the entire population of soil actinobacteria and produce 75% of the antibacterial molecules. We must continue to look for novel strains capable of producing intriguing chemicals within these taxa [6].

Rare actinobacteria have been found as a potentially significant and understudied source of novel secondary metabolites with antibacterial and antifungal activity [9, 10, 11, 12, 13, 14]. The identification of intriguing novel compounds in the genera *Micromonospora, Actinomadura,* and *Streptosporangium* has resulted from their selective isolation from diverse unknown habitats [15, 16].

Recently, new research has revealed that oxygen free radicals play a critical role in a variety of diseases, including cancer, Alzheimer's disease, many infections, auto-immune diseases, and cardiovascular diseases [17].

Because of the harmful effects of synthetic antioxidants on human health, it has become a hot area of research [18]. Several limits and restrictions will be enforced when using antioxidants and substituting them [19, 20]. Researchers are looking for natural antioxidants produced by plants or microorganisms that are high in secondary metabolites, such as actinomycetes, to tackle this problem [17]. We are interested in the extraction of active molecules from Actinobacteria strains isolated from the El Bayedh region, which is located in southwest of Algeria, by highlighting their antifungal and antioxidant profiles.

MATERIALS AND METHODS

The eighteen actinomycetal strains studied were isolated from samples of Algerian arid soils from the region of Ain Loarak, Sebkha of Bougtob, and El Bayadh. They are coded (A1-A26). (K17-AK) and preserved by freezing in the presence of glycerol in inclined agar tubes containing the ISP2 medium. To revive these strains, 0.1 ml of each bacterial suspension is inoculated on Petri dishes by tight streaks on GYM medium(Yeast extract: 4g, Malt extract: 10g, CaCo3: 2g, Glucose: 4g, Agar: 12g, distilled water: 1000 ml, pH = 7.2), then incubated at 28°C for 7 days. After the Actinomycetes strains have been revived, they are subjected to morphological, cultural, physiological, and biochemical analysis to identify them. The naked eye and a light microscope were used to capture morphological features. Under light microscopy, pure colonies were picked by looking at the thin filaments surrounding the Actinomycete colonies. The colour of the aerial mycelium, substrate mycelium, and diffusible pigments was determined using the ISCC-NBS colour naming chart [21].

Target microorganisms

Pathogenic target microorganisms used were: *Listeria monocytogenes* ATCC 19115, *Staphylococcus aureus* ATCC 25923, *Fusarium graminearum* CECT 2150, *Aspergillus flavus* CECT 20802, *Aspergillus ochrasus* NRRL3174, *Penicillium expansum* CECT 2278, *Aspergillus parasiticus* CBS 100926. The pure cultures of bacteria and fungi were received from Alger's Pasteur Institute (Algeria). These cultures were subcultured every two months on Petri plates with nutrient agar at 4°C.

Antimicrobial activity

The agar cylinder technique was used to test against specified harmful microorganisms. For further research, the isolates with the greatest antibacterial activity were chosen.

For each strain, an inoculum was made on nutrient agar from a 24-hour bacterial culture. This inoculum's cell density was adjusted by diluting it in sterile physiological water and visually comparing it to the Mc Farland solution, which has a final concentration of 106 ufc/ml.

The Agar disc method

The 18 actinomycetal strains are seeded in tight streaks on the surface of 15 ml of GYM (glucose-yeast extract-malt extract) media. After 7 days of incubation at 28°C, 6 mm diameter agar cylinders are taken and placed on the surface of the Mueller-Hinton medium, previously inoculated with test bacteria using a swab. The Petri plates are then placed at 4°C for two to four hours to allow diffusion of the active substances, and then incubated at 37°C for 24 hours. After this incubation, the antibacterial activity was evaluated by measuring the inhibitory distance between target microorganisms and the actinobacterial strain [22].

Study of antifungal activity

The production of antifungal metabolites by Actinomycetes strains is demonstrated by the agar cylinder technique using a Sabouraud medium.

Filamentous fungi are transplanted on a PDA medium and incubated at 28°C for 5 days. A sporal suspension of the target strains (*Fusarium graminearum, Aspergillus flavus, Aspergillus ochraceus, Penicillium expansum,* and *Aspergillus parasiticus*) is prepared by scraping the surface of the culture with a sterile spatula to recover the spores and mixing them by vortex with 10 ml of sterile physiological water.

Actinomycete strains are streaked on ISP2 medium and incubated for 7 days at 28°C. 6mm diameter cylinders are placed on a Sabouraud medium and inoculated with fungal spore suspensions of the test strains using swabs. The plates are left at 4°C for 4 hours for a good diffusion of the antifungal metabolite, then incubated at 25°C for 48 h. After incubation of the target fungus, the widths of the inhibitory zones are determined [12].

Production of secondary metabolites
Fermentation on a solid medium

The strains A7 and A10, selected among the 18 *actinomycete* strains tested after having given important antifungal activities with the agar cylinder method, are inoculated each on 40 petri plates containing sterile AF medium in tight streaks, using a platinum lance, and then incubated at 28°C for 14 to 21 days.

Extraction of metabolites from fermentation on a solid medium

After incubation at 28°C for 14 days, the agar was broken up and transferred to sterile flasks containing 25 ml of methanol for each plate. The flasks are then left at room temperature for 2 hours with vigorous shaking. Extracts are filtered using Whatman paper to remove live cells. The filtrates are centrifuged at 11000tr/10min [22]. The organic components recovered for each strain are concentrated by steam rota evaporation at 40°C after phase separation in separating funnels [23].

Tests of the antifungal activity of extracts

The extracts of the active strains are tested for their antifungal activity by the direct contact technique, according to [24]. This method is based on the study of the effect of the incorporation of different concentrations of methanolic extract of actinomycete strains A10 and A7 in the culture medium on the growth of fungal strains (*Fusarium graminearum, Aspergillus flavus, Aspergillus ochraceus, Penicillium expansum, and Aspergillus parasiticus*).

A series of dilutions from (0.5 to 8 mg/ml) is prepared in suspension with the culture medium from the stock solution by adding variable volumes of the methanolic extract of the actinomycete strains. In flasks containing 250ml of PDA culture medium sterilized by autoclaving (20 minutes at 120°C) and maintained in supercooling at 45°C, 1ml of lactic acid diluted at 25% is aseptically added to inhibit the bacterial strains.
In test tubes, 20 ml of the PDA medium prepared beforehand is poured, and the extract is added at different volumes to obtain the dilutions (0.5, 1, 2, 4, and 8 mg/ml). Controls, containing 20 ml of PDA medium alone, are also prepared.

Then they are suitably shaken with the vortex before being distributed in Petri plates of 90 mm in diameter at a rate of 20 ml of mixture per plate. After solidification, the prepared plates are inoculated in the center of the surface of the agar medium with a disc of mycelium (6 mm diameter) sterilely removed with a needle from the periphery of 7-day-old cultures. The plates were incubated in the dark at 28°C for 5 days. Mycelial growth was monitored by measuring the average of two perpendicular diameters passing through the center of each plate. Fungi toxicity, expressed as a percent inhibition of mycelial growth (I %), is calculated according to the formula [25]. Fungal strains are adjusted to 10-6 using a spectrophotometer.

$$I(\%)=(Di-Di)/Dt\times 100$$

Dt: diameter of the culture of fungi without the extract.
Di: diameter of the culture of the same fungi in a medium in the presence of the extract.

Study of the action of ergosterol on antifungal activity

The two active actinomycetes, A10 and A7, are surface inoculated by tight streaks on AF medium and then incubated for 7 days at 28°C. Using a cookie cutter, agar discs of about 3 mm in diameter containing the cultures of the strains are collected from each culture. Four batches of Petri plates are prepared. The first batch consists of two plates of PDA medium inoculated throughout with *Aspergillus flavus* and containing 50 µg/ml of ergosterol. The second consists of two plates of PDA medium without ergosterol. The agar discs prepared previously are deposited on both types of plates, and then left at +4°C for 4

hours to facilitate the diffusion of the substances. After incubation at 28°C for 48 hours, the diameters of the inhibition zones are measured with a ruler [9].

Preliminary characterization of the antifungal agent by TLC

Ten microliters of each methanol extract of actinomycetal strains A10 and A7 are pipetted onto aluminum TLC plates (6 cm×6 cm) spread with a thin layer of silica gel (indicator 254 nm, lot #BCBR5950V). These plates are then held vertically in TLC tanks containing the following solvent systems:
Toluene-acetone (6 ml, 4 ml) ,Toluene-acetic acid (8 ml, 2 ml) ,N-butanol-acetic acid water (3 ml, 1 ml, 1 ml), Ethyl acetate-methanol (6 ml, 4 ml), Toluene-methanol (5 ml , 5 ml) [22], Dichloromethane-methanol (8 ml, 2 ml) [26]. Chromatography is stopped when the solvent front has traveled a distance from the deposition point. The chromatograms are examined under UV light at two distinct wavelengths, 254 nm and 365 nm, after the solvent has been removed [22].

Semi-purification of the fractions separated by TLC

The two crude extracts are semi-purified on a silica thin layer by chromatography, and this is done to have sufficient quantities of the separated fractions to be able to determine which of them are bioactive.

A volume of 250 to 600 µl of each concentrated extract is deposited in a thin, continuous band on the silica gel plates. These are then developed in the previously selected eluent to accurately locate the spots corresponding to the different separated components. Once the migration is completed, the silica showing spots are then recovered by cutting the sheet into strips at each spot. These are introduced separately into dry tubes, each containing 2 ml of methanol [27]. Once the silica is completely dissolved in the methanol under constant stirring for 30 min at room temperature. The silica is subsequently removed by filtration [28]. The clear methanolic filtrates are collected, and their activity is controlled [29,30].

Control of the activity of each separated component

After extraction and semi-purification of the separated fractions, it is essential to test the inhibitory activity of them by the method of wells on PDA agar inoculated by swabbing according to the NCCLS method with a fungal suspension prepared from a pure culture of the most sensitive target germ. Each well will receive a volume of 20 µl of each fraction separated by chromatography previously. The existence of a control well containing the same volume of methanol is essential to demonstrating whether it has inhibitory activity toward the target strain tested. The plates are then placed at a temperature of 4°C for 2 hours to allow a good diffusion of the substances in the agar. The incubation is then done at 25°C for 48 h and the diameters of the inhibition zones around the wells are measured in mm.

The study of the antioxidant activity of crude extracts

The antioxidant activity of crude extracts was investigated using the DPPH technique of free radical trapping, 50ul of methanolic extract dilutions ranging from 1/2 to 1/64 were added to 1.95 ml of methanolic DPPH solution, stirred with a vortex, and incubated in the dark for 30 minutes at room temperature. The absorbance reading is performed against a blank prepared for each concentration at 517nm using a Spectrophotometer (RAYLEIGH

VIS-723G) [31]. After 30 min of incubation in the dark at room temperature. The positive control is represented by a solution of a standard antioxidant; ascorbic acid whose absorbance was measured under the same conditions as the samples and for each concentration [32]. The percentage of DPPH radical inhibition is calculated using the following equation:

$$\% \text{ inhibition of DPPH} = [(Ac - As)/Ac] * 100$$

Where: Ac: Absorbance of the control
As: Absorbance of the sample

Calculation of IC50

The IC50 is the concentration of the sample that is required to reduce 50% of the DPPH radical. The IC50s are calculated graphically by linear regressions of the plotted graphs, (percentages of inhibition as a function of different concentrations of the tested extracts and the standards).

RESULTS AND DISCUSSION

Macroscopic characteristics of the isolates

Actinomycetes colonies were recognized for their characteristic morphological aspects. They appear dry, rough, and coloured with an irregular contour. They adhere to the agar and present a vegetative and aerial mycelium. Some present only the mycelium of the substrate. The colonies present on the medium have a similar aspect to the fungi, except that the major difference is the size of the colonies and the incubation time, which is very long in actinomycetes; these characteristics are shown in Fig. 1

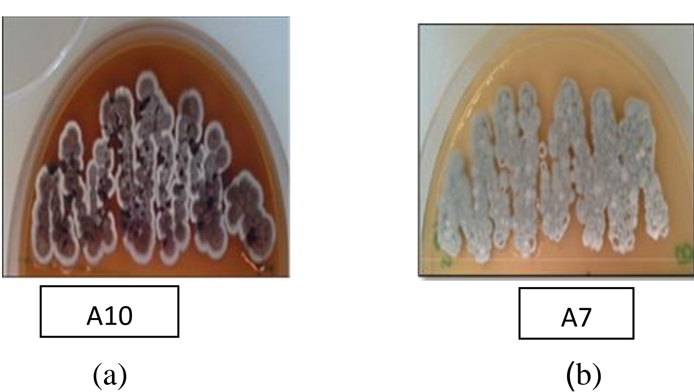


Fig. 1. *Macroscopic aspect of the isolates (a) strain A10 and (b) strain A7.*

Antibacterial activity

Out of a total of 18 isolates purified and tested against 2 indicator bacteria, only 8 showed inhibitory activity against *Listeria monocytogenes* and 13 isolates showed inhibitory activity against *Staphylococcus aureus*. The largest zone of inhibition (17 mm) was given by strain A17 against *S. aureus*. (11 mm) was given by strain A14 against *Listeria monocytogenes*.

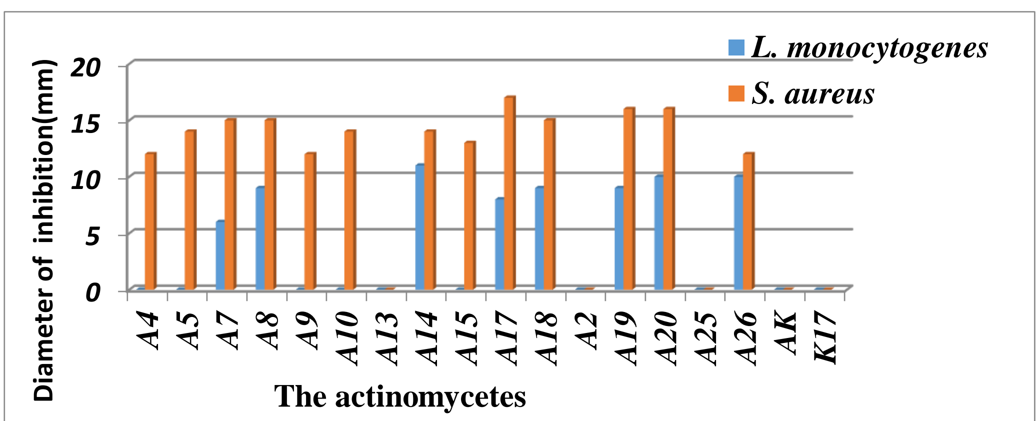


Fig. 2. *Inhibition of Staphylococcus aureus and Listeria monocytogenes by actinomycete isolates.*

Fig. 2 shows that *Staphylococcus aureus* was inhibited by 13 of the 18 active strains. Strain A17 gave the highest zone of inhibition with a diameter of 17 mm. With a diameter of 16 mm, isolates A19 and A20 had less activity, while isolates A7, A8, and A18 had a diameter of 15 mm. The diameters of the zones of inhibition applied by strains A4, A9 and A26 are between 12 and 14 mm.

From the histogram, we can see that 8 strains, out of the 18 tested, showed inhibitory activity against *L. monocytogenes*. The most important inhibitory power is noted in strain A14, with a diameter of 11 mm. Strains A20 and A26 gave an inhibition zone of 10 mm in diameter. The lowest activity, resulting in a diameter of 06 mm, was marked by strain A7. The other actinomycetes gave zones of inhibition between 6 and 10 mm in diameter. Among the 18 isolates, we selected two strains that gave significant inhibitory activity against the two bacterial test species, which are A07 and A10 (Fig. 3).

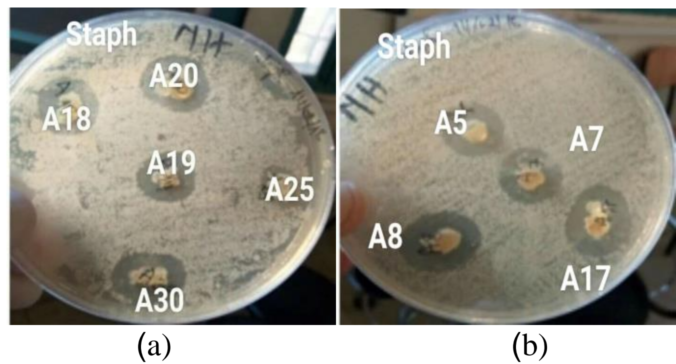


Fig. 3. *Inhibition of Staphylococcus aureus by actinomycete isolates.*

Morphological characterization of active strains

The results of the macromorphological study and the cultural characteristics of the 2 active strains are shown in Table 1.***Table 1.*** *Results of the macromorphological study and cultural characteristics of the active strains.*

Strains	A10	A7
Culture medium	ISP2	ISP2
Colour of Aerial mycelium	Dark grey	White grey
Colour of Substrate mycelium	White	Grey
Pigmentation	Dark brown	Beige

After inoculation on the ISP2 medium, colonies of active actinomycetes strains appeared during two days of incubation at 28°C. On the 14th day, the A10 strain gave powdery and circular convex colonies, humped with wavy and irregular edges, of an average size of 4 to 7 mm, adhering firmly to the culture medium. They are difficult to suspend, with white substrate mycelium, and dark grey aerial mycelium and dark brown pigmentation.

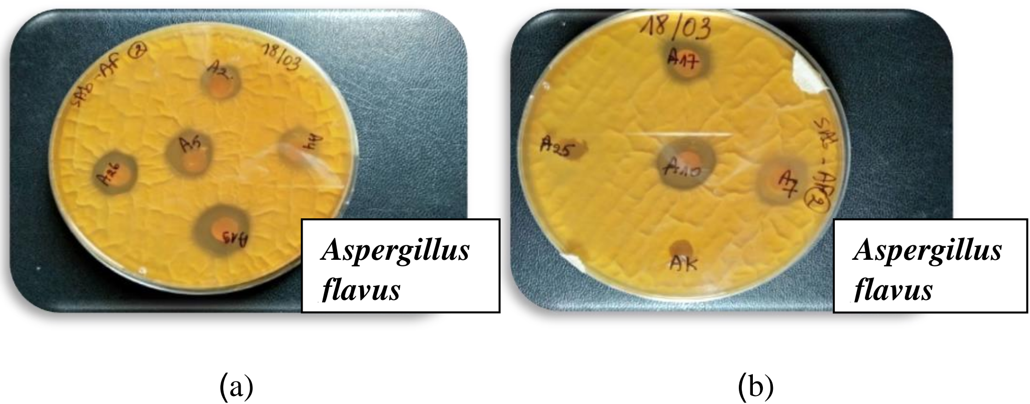
Strain A7 has colonies with wavy and irregular edges, opaque and adherent to the culture medium, grey substrate mycelium, white grey aerial mycelium, and beige pigmentation.

The agar cylinder test is used for the demonstration of antifungal activity, the results of which are represented in Table 2.

Table 2. *Antifungal power of actinomycetes obtained by the agar cylinder technique.*

	Diameter of inhibition zones (mm)				
Actino	***AP***	***AF***	***FG***	***AO***	***PE***
A2	12±04	12±12	08±09	00±02	08±02
A4	13±01	13±08	00±00	08±07	00±08
A5	09±05	07±03	00±00	00±01	08±07
A7	12±02	16±08	12±02	10±01	12±07
A8	13±04	15±03	00±00	08±08	00±00
A10	09±08	17±05	10±05	00±00	12±09
A13	11±05	15±02	00±00	08±02	00±00
A14	13±06	15±08	00±00	10±08	00±05
A15	15±08	15±01	00±00	00±05	00±20
A17	00±03	07±06	00±00	00±06	00±04
A18	00±07	16±07	00±00	14±05	00±00
A19	17±09	15±05	00±00	08±03	08±08
A20	00±01	15±02	00±00	00±03	00±00
A25	00±03	00±01	00±00	00±00	00±03
A26	12±02	14±09	00±00	00±02	06±08
A30	15±01	14±04	00±00	00±08	09±05
AK	00±00	00±05	00±00	00±03	00±08
K17	00±09	00±01	00±00	00±01	00±04

FG-*Fusarium graminearum*; AP- *Aspergillus parasiticus*; AF- *Aspergillus flavus*; AO- *Aspergillus ochrasus*; PE- *Penicillium expansum*; Actino-*Actinomycetes.*



(a) (b)

***Fig. 4**. Result of the agar cylinder technique.*

The results of the antifungal test obtained show that strains A10 and A7 showed significant inhibitory power against the majority of the fungal strains except for the fungal strain *Aspergillus ochrasus,* which was not inhibited by strain A10. The inhibitory zone's distance ranged from 6 to 17 mm (Fig. 4).

Antifungal test of the crude extract of strains A10 and A7:

The results obtained during the study of the effect of different concentrations of the methanolic extract of strain A10 on the mycelial growth of the five fungi after an incubation period of 5 days is shown in the Fig. 5, 6, 7.

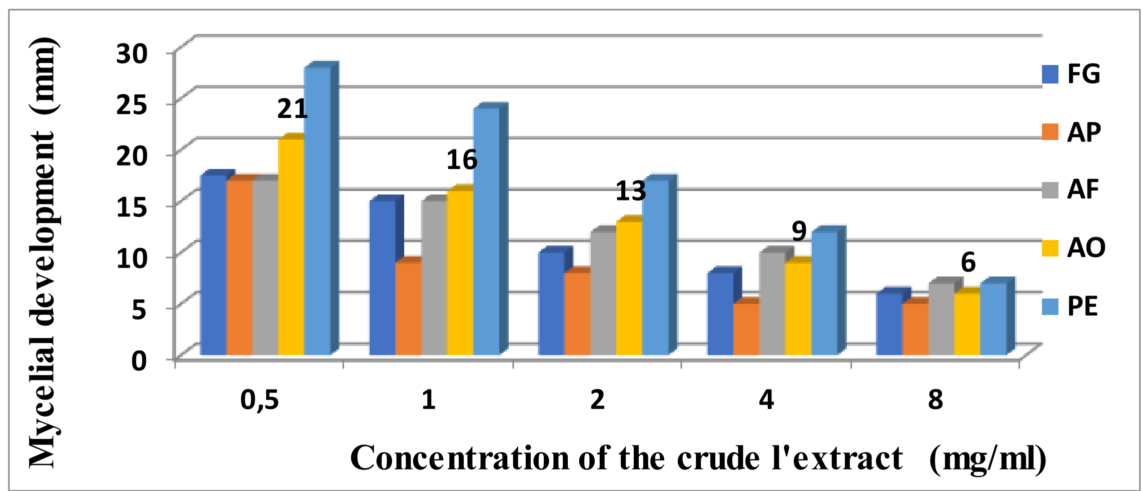


***Fig. 5.** Mycelial growth diameters as a function of the concentration of the crude methanolic extract of strain A10. FG-Fusarium graminearum; AP-Aspergillus parasiticus; AF-Aspergillus flavus; AO-Aspergillus ochrasus; PE-Penicillium expansum.*

The results obtained show that at a concentration of 8mg/ml, all fungal strains are inhibited. In the concentrations (0.5 and 1 mg/ml), the five fungal strains gave a mycelial growth, which increased each time by decreasing the concentrations of the extract. In terms of sensitivity, we notice that the fungal strain *Aspergillus parasiticus* presents very good sensitivity.

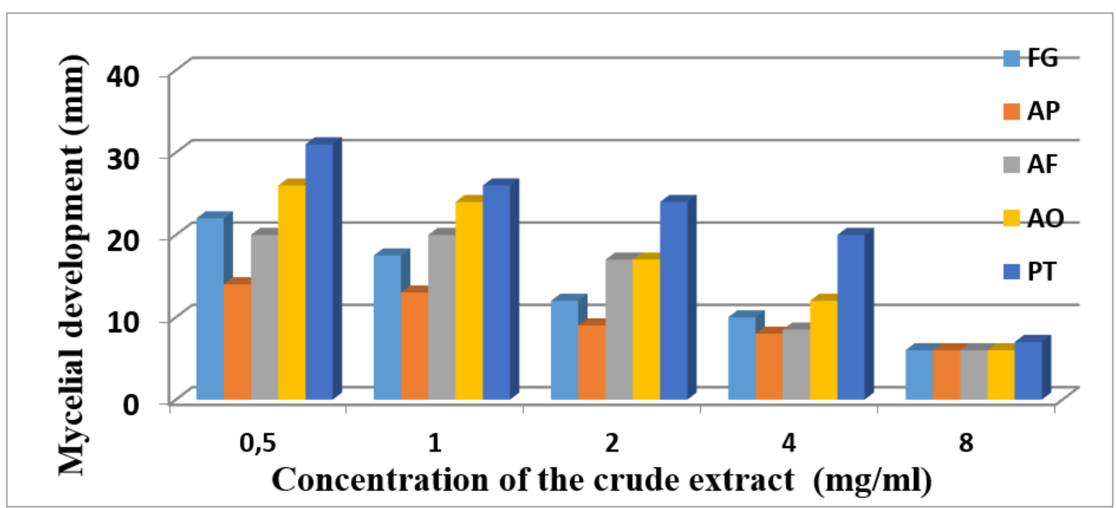


Fig. 6. Diameters of mycelial development as a function of the concentration of the crude methanolic extract of strain A7. FG-Fusarium graminearum; AP-Aspergillus parasiticus; AF-Aspergillus flavus; AO-Aspergillus ochrasus; PT-Penicillium expansum.

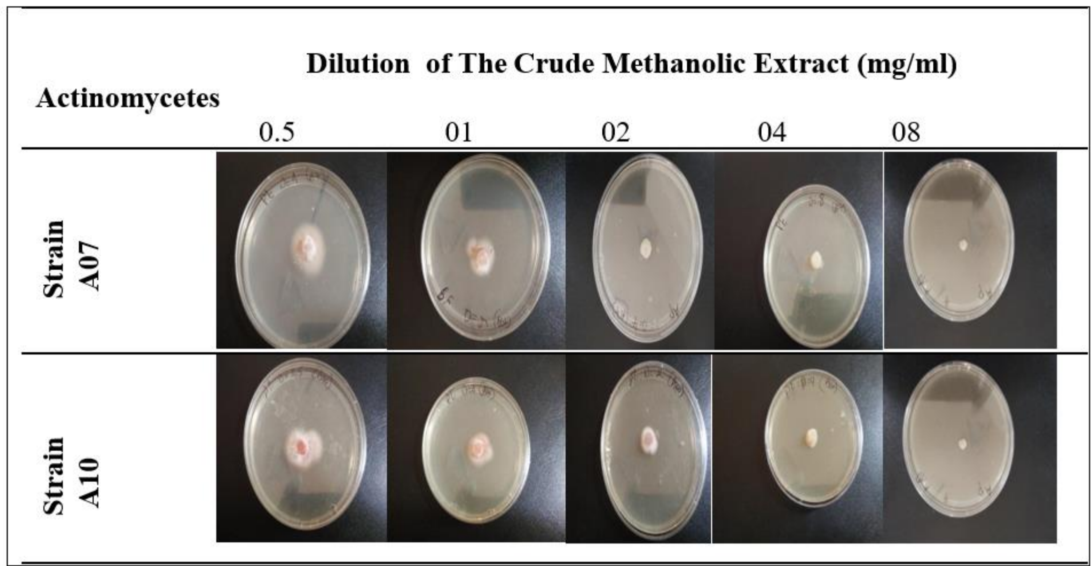


Fig. 7. Mycelial development of the fungal strain Aspergillus parasiticus tested against different concentrations of the extract.

Study of the action of ergosterol on antifungal activity

The ergosterol test consists in revealing the effect of active molecules on membrane sterols based on the diameters of the inhibition zones caused by actinomycetes against fungal strains on a medium with and without ergosterol. The results of this test are presented in the Table 3.

Table 3. *Results of the antifungal effect in the presence of ergosterol.*

Actinomycetes	Without ergosterol	With ergosterol
Inhibition zone given by A07(mm) against AF	10.5	14
Inhibition zone given by A10 (mm) against AF	10	10

AF -Aspergillus flavus

Preliminary characterization of the antifungal agent by thin-layer chromatography

The results showed that the best separation of the extract products was given by the eluent systems Toluene-acetic acid (8.2) and Dichloromethane-methanol (8.2) for the two extracts (Table 4).

Table 4. *Result of thin-layer chromatography system Toluene-acetic acid (8.2).*

The spots	RF (Extract A7)	RF (Extract A10)
1st spot	0.11	0.14
2nd spot	0.21	0.23
3rd spot	0.28	0.30
4th spot	0.35	0.52
5th spot	0.52	0.59
6th spot	0.59	0.67
7th spot	0.67	

The results showed that the last 3 spots of the two extracts have the same front ratios respectively RF= (0.67, 0.59, and 0.52) while. All the spots of both extracts have almost the same colour which is dark yellow.

Purification of bioactive molecules

After the semi-purification of the fractions separated by TLC, the activity of the filtrates is tested on fungal strains (*Aspergillus flavus*) and (*Penicillium expansum*) inoculations on PDA medium. Table 5 shows the frontal ratios of the active fractions, as well as the diameters of the inhibition zones, obtained after 48h of incubation.

Table 5. *Results of the activity test of the semi-purified extracts and the Rf of the active fractions.*

Extracts Crude	Frontal reports (Rf) à 254 nm (UV)	Zones of inhibition (mm)
Methanolic extract A10	0.14	14 mm
	0.23	15 mm
	0.30	13mm
Methanolic extract A7	0.11	12 mm
	0.21	15 mm
	0.28	13 mm

The results showed that the first 3 spots of both extracts have an inhibitory activity, so the bioactive molecules are located in these active fractions, or RF :(0.11-0.21-0.28) for the extract of the strain A7 and RF :(0.14-0.23-0.30) for the extract of the strain A10.

Free radical trapping DPPH

This essay is one of the most widely used and quick techniques for determining the radical activity of biologically active compounds [33]. This radical is purple and has a distinctive absorbance in the region of 512-517nm, although it fades quickly to yellow when reduced. The colour intensity is related to the capacity of antioxidants present in the medium. The percentage of DPPH radical inhibition as a function of the concentrations of the two methanolic extracts A7 and A10 tested is illustrated in the following Fig. 8 and 9.

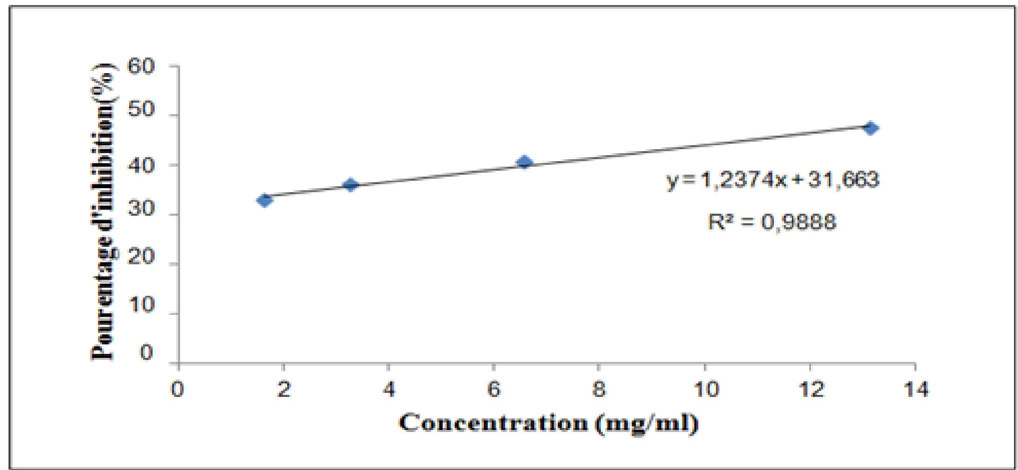


Fig. 8. *Variation of DPPH radical inhibition as a function of concentrations of A7 extract.*

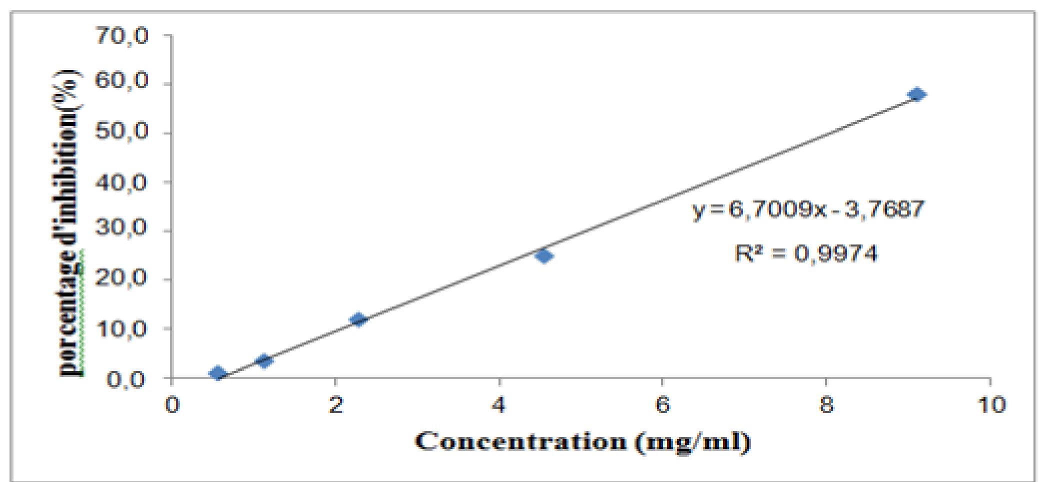


Fig. 9. *Variation of DPPH radical inhibition as a function of concentrations of A10 extract.*

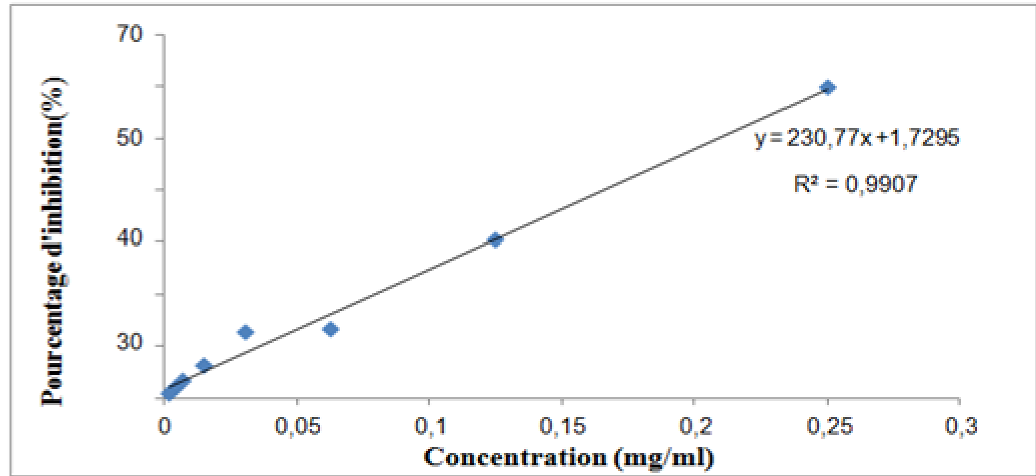


***Fig. 10.** Variation of DPPH inhibition as a function of the concentration of Ascorbic Acid*

The proportion of free radical trapping rises with increasing concentration for both the standard ascorbic acid) Fig. 10) and the two crude extracts A7 and A10, as shown in the results in Fig. 8, 9. Even though our two extracts have poor antioxidant activity when compared to the industry standard, it is clear that they may release hydrogen. As a result of this capacity, they can be utilized as main antioxidants and free radical trappers [34]. The percentages of inhibition were estimated at 47% for strain A7 and 58% for strain A10.

The IC 50 on the radical DPPH:

The IC50 was used to evaluate the antioxidant capability of the various extracts. This is the concentration required to eliminate 50% of the free radicals (DPPH). The lower the IC50 value, the stronger a compound's antioxidant activity [35]. The IC50 for the DPPH test, the two extracts, and the standard are calculated using the curves (Fig. 8, 9, 10). The IC50 values are listed in the table 6.

***Table 6.** IC50 values in (mg /ml) for the DPPH test.*

Extracts and standard	IC 50 (mg/ml) DPPH
A7	14.88
A10	8.10
Ascorbic acid	0.27

The IC50 obtained for the standard is much lower than that of the extracts of the two strains A7 and A10, indicating that the standard has a very high antioxidant activity. The IC50 obtained for strain A10 is lower than that of the extract of strain A7, indicating that strain A10 has high antioxidant activity. The two extracts examined had a less inhibitory impact than the high inhibitory effect of the standard (ascorbic acid), which is owing to the latter's purity. Other writers have observed similar findings [36, 37, 38, 39].

The development of multidrug-resistant organisms has made treating a variety of infectious illnesses more challenging. As a result, novel effective medicines against the aforementioned pathogens are required. Exploration of unexplored and harsh environments can lead to the isolation of novel microbes that create novel bioactive chemicals for this

purpose. Recently, numerous studies have demonstrated the potential of extreme habitats as reservoirs of promising antibacterial drug makers [30, 40, 41, 42, 43, 44].

In light of the findings, microorganisms with promising antibacterial properties were isolated from Algerian Sahara soils as a model of a harsh environment.

Among the 18 actinomycetes isolated from Algerian Sahara soils, two of them named A10 and A7 showed broad-spectrum antimicrobial activities against different bacteria and five pathogenic fungi. The study of morphological, cultural, and physiological characteristics allowed us to link these two isolates A7 and A10 to the genus Streptomyces.

The antibacterial activity of their crude extracts against diverse pathogenic microbes indicates that the isolated strains A7 and A10 are potential sources of bioactive chemicals. These findings are consistent with those reported in many studies. As a result,[45, 43] discovered antimicrobial activity in actinomycetes isolated from Algerian Sahara soils, and [46]discovered the antimicrobial activity and antibiotic production in the most common soil bacteria actinobacteria isolated from soil samples from a mangrove forest in India [47]. Actinobacteria isolated from Egyptian farm soils have been shown to have antibacterial properties. Apart from that, S. Albus G was found to generate an antibiotic substance [48] while S. violoaceoruber VLK-4, which was isolated from soil samples in India, was shown to produce an antimicrobial compound [49] and a bioactive chemical.

Antibiotic action against filamentous fungus was found to be greater than antibacterial activity [50, 51].

The synthesis of bioactive compounds was further confirmed by the secondary screening of actinomycetes ethyl acetate extracts. In the isolates' components Likewise, Bhakyashree and Kannabiran have both submitted reports [52]. Antioxidants are frequently utilized as preservatives in the food and cosmetic industries. Many scandals have recently surfaced as a result of the strong antioxidants employed having the potential to cause cancer and hormone problems. As a result, the hunt for novel natural antioxidants is more important than ever.

The antiradical potential of the extracts was determined using the DPPH method, and the results show that the extract of strain A10 has good activity, indicating that this strain contains molecules that are considered first-class antioxidant agents and can be used for therapeutic applications, given that antioxidants help to prevent diseases like cancer in a very effective way. However, our findings are in line with those of numerous writers who have studied the antioxidant properties of actinomycetes extracts and found that they had lesser activity than the natural oxidant [53, 54]. Specific secondary metabolites, such as phenolic chemicals and terpenes, contribute to a compound's antioxidant activity [55, 56, 57].

Strain A10 exhibited higher antioxidant activity than strain A7(Table 6). These differences can be attributed to their different chemical structures, their disintegration during the extraction process [41], and environmental factors (temperature and pH of the crude extract).

CONCLUSION

Actinomycetes from Algerian Sahara soils have been identified as potential sources of new antimicrobial and antioxidant chemicals, according to the findings. Future studies will be needed to identify the antimicrobial and antioxidant chemicals generated, which will entail purification and the application of several chemical analysis techniques such as

MALDI-TOF MS, HPLC-MS, and NMR, FTIR .and on the other hand, has been shown to be a quick, dependable, and extremely robust method for bacterial identification.

Acknowledgement. The authors are grateful to the Ministry of Higher Education Algeria for supporting the research financially, and the Laboratory of biotoxicology, pharmacognosy and biological valorization of plants, University of Saida Dr- Moulay Tahar. Algeria for research facilities.

Conflict of Interest. The authors declared that there is no conflict of interest.

Authorship Contributions. Concept: H.B., M.B., D.A., A.B., Design: H.B., M.B., D.A Data Collection or Processing: H.B., M.B., A.B Analysis or Interpretation: H.B., M.B., LiteratureSearch: H.B., M.B., D.A Writing: H.B., M.B.

Financial Disclosure. Already stated in the acknowledgement.

REFERENCES

[1] Odds, F. C., Vranckx, L., Woestenborghs, F. (1995): Antifungal susceptibility testing of yeasts: evaluation of technical variables for test automation. Antimicrobial Agents and Chemotherapy 39(9): 2051-2060.

[2] Ascioglu, S., Rex, J. H., De Pauw, B., Bennett, J. E., Bille, J., Crokaert, F., Stevens, D. A., Denning, D.W., Donnelly, J. P., Edwards, J. E., Erjavec, Z., Fiere, D. Lortholary, O., Maertens, J. Meis, J. F., Patterson, T. F. Ritter, J., Selleslag, D. Shah, P. M. (2003): Defining opportunistic invasive fungal infections in immunocompromised patients with cancer and hematopoietic stem cell transplants an international consensus. Проблемы медицинской микологии 5(1): 10-16.

[3] Ampel, N. M. (1996): Emerging disease issues and fungal pathogens associated with HIV infection. Emerging Infectious Diseases 2: 109.

[4] Pfaller, M. A., Diekema, D. J. (2002): Role of sentinel surveillance of candidemia: trends in species distribution and antifungal susceptibility. Journal of Clinical Microbiology 40(10): 3551-355.

[5] Perry, J. J., Staley, J. T., Lory, S. (2004): Microbiology. Edition Dunod, Paris

[6] Larpent, J. P., Sanglier, J. J. (1989): Biotechnology of antibiotics.Masson, Paris

[7] Sanglier, J. J., Haag, H., Huck, T. A., Fehr, T. (1993): Novel bioactive compounds from actinomycetes: a short review (1988–1992). Research in Microbiology 144(8): 633-642.

[8] Sanglier, J. J., Basik, A. A., Juboi, H., Shamsul, S. S. G., Yeo, T. C. (2021): actinomycetes isolated from wetland and hill paddy during the warm and cool seasons in Sarawak, East Malaysia. Journal of Microbiology, Biotechnology, and Food Sciences 2021:774-780.

[9] Hacene, H., Sabaou, N., Bounaga, N., Lefebvre, G. (1994): Screening for non-polyenic antifungal antibiotics produced by rare Actinomycetales. Microbios 79(319): 81-85.

[10] Ouhdouch, Y., Mustapha, B., Finance, C. (2001): Actinomycetes of Moroccan habitats: Isolation and screening for antifungal activities. European Journal of Soil Biology 37: 69-74.

[11] Khattabi, H., Aleyai, L., Lovy, C., Mania, J. (2001): Evaluation of the impact of leachate from a household waste landfill on the physicochemical and bacteriological quality of the water of a Franche-Comté stream. Waste Science and Technology 24: 3-6.

[12] Lemriss S., Laurent F., Couble A., Casoli E., Lancelin J.M., Saint Pierre-Bonaccio D., Rifali S., Fassouane A., Boiron P., (2003): Screening of non-polyenic antifungal metabolites produced by clinical isolates of actinomycetes. Can J Microbio l49:669-74.

[13] Zitouni, A., Boudjella, H., Lamari, L., Badji, B., Mathieu, F., Lebrihi, A., Sabaou, N. (2005): Nocardiopsis and Saccharothrix genera in Saharan soils in Algeria: isolation, biological activities and partial characterization of antibiotics. Research in Microbiology 156(10): 984-993.

[14] Wang, J., Soisson, S. M., Young, K., Shoop, W., Kodali, S., Galgoci, A., Singh, S. B. (2006): Platensimycin is a selective FabF inhibitor with potent antibiotic properties. Nature 441(7091):358-361.

[15] Shearer, W.T., Quinn, T. C., La Russa, P., Lew, J. F., Mofenson, L., Almy, S., Rich, K., Handelsman, E., Diaz, C., Pagano, M., Smeriglio, V., Kalish, L. A. (1997): Viral load and disease progression in infants infected with human immunodeficiency virus type 1. New England Journal of Medicine 336(19): 1337-1342.

[16] Lamari, L., Zitouni, A., Boudjella, H., Badji, B., Sabaou, N., Lebrihi, A., Lefebvre, G., Seguin, E., Tillequin, F. (2002): New Dithiolopyrrolone Antibiotics from Saccharothrix sp. SA 233 I. Taxonomy, Fermentation, Isolation, and Biological Activities. The Journal of Antibiotics 55(8): 696-701.

[17] Lee, D. R., Lee, S. K., Choi, B. K., Cheng, J., Lee, Y. S., Yang, S. H., Suh, J. W. (2014): Antioxidant activity and free radical scavenging activities of Streptomyces sp. strain MJM 10778. Asian Pacific journal of tropical medicine 7(12):962-967

[18] Barlow, S. M. (1990): Toxicological aspects of antioxidants used as food additives. In Food Antioxidants 1990: 253-307.

[19] Frankel, E. N. (1993): In search of better methods to evaluate natural antioxidants and oxidative stability in food lipids.Trends Food Sci Techno l4:220-225.

[20] Moure, A., Cruz, J. M., Franco, D., Domínguez, J. M., Sineiro, J., Domínguez, H., Parajó, J. C. (2001): Natural antioxidants from residual sources. Food Chemistry 72(2):145-171.

[21] Kelly, K. L., Judd, D. B. (1976): Color: universal language and dictionary of names (Vol. 440). Department of Commerce, National Bureau of Standards, Center for Building Technology, Sensory Environment Section.

[22] Boughachiche, F., Reghioua, S., Zerizer, H., Boulahrouf, A. (2012): Antibacterial activity of rare Streptomyces species against multidrug-resistant clinical isolates. In Annales de Biologie Clinique 70 (2): 169.

[23] Boudemagh, A., Kitouni, M., Boughachiche, F., Hamdiken, H., Oulmi, L., Reghioua, S., Zerizer, H., Couble, A., Mouniee, D., Boulahrouf, A., Boiron, P. (2005): Isolation and molecular identification of actinomycete microflora, of some Saharan soils of southeast Algeria (Biskra, EL-Oued, and Ourgla) study of antifungal activity of isolated strains. Journal of Medical Mycology 15(1): 39-44.

[24] Laghchimi, A., Znini, M., Majidi, L., Renucci, F., El-Harrak, A., Costa, J. (2014): Chemical composition and effect of liquid and vapor phase of Lavandula multifida essential oil on mycelial growth of fungi responsible for the rot of apple. Journal of Materials and Environmental Science 5(6):1570-15.

[25] Pandey, D. K., Tripathi, N. N., Tripathi, R. D., Dixit, S. N. (1982): Fungitoxic and phytotoxic properties of the essential oil of Hyptis. Journal of Plant Diseases and Protection 89(6):344–349.

[26] Awaad, A. S., Al-Jaber, N. A. (2010): Antioxidant natural plant. RPMP Ethnomedicine: Source and Mechanism 27: 1-35.
[27] Boucheffa, K. (2011): Screening of actinomycetes strains producing non-polyene antifungals: Identification of the producing strains characterization of the antifungals produced. University of Bouira, Faculty of Sciences, Bouira, Algeria
[28] Lamari, L. (2006): Production of new pyrrhotine antibiotics by a new actinomycete species, Saccharothrix algeriensis. University of Mouloud Mammeri, Faculty of Sciences ,Tizi-Ouzou, Algeria
[29] Silambarasan, S., Murugan, T., Saravanan, D., Balagurunathan, R. (2012): Antibacterial and antifungal activities of Actinobacteria isolated from Rathnagiri hills. Journal of Applied Pharmaceutical Science 2(10): 099-103.
[30] Benreguieg, M., Adli, D. E. H., Hachem, K., Kaid, M'hamed. (2017): Bioactive compound produced from Algerian arid soil Streptomyces MBA07 and its antimicrobial activity. Bioscience Research 14(3):678-685.
[31] Athamena, I. Chalghem, A. Kassah-Laouar, S. Larouiet S. Khebri.(2010): Antioxidant and antimicrobial activity of cuminumcyminum extracts. Lebanese Science Journal 11: 69-81.
[32] Bougandoura, N., Bendimerad, N. (2013): Evaluation of the antioxidant activity of aqueous and methanolic extracts of Satureja Calamintha ssp. Nepeta (L.) Briq. Nature & Technology (9): 14.
[33] Molyneux, R. J., Lee, S. T., Gardner, D. R., Panter, K. E., James, L. F. (2007): Phytochemicals: the good, the bad and the ugly. Phytochemistry 68(22-24): 2973-2985.
[34] Chung, Y. C., Chien, C. T., Teng, K. Y., Chou, S. T. (2006): Antioxidative and mutagenic properties of Zanthoxylum ailanthoides Sieb & zucc. Food Chemistry 97(3):418-425.
[35] Hebi, M., Eddouks, M. (2016): Evaluation of the antioxidant activity of Stevia rebaudiana.Phytothérapie 14(1): 17-22
[36] Kekuda, P. T. R., Shobha, K. S., Onkarappa, R. (2010): Studies on antioxidant and anthelmintic activity of two Streptomyces species isolated from Western Ghat soils of Agumbe, Karnataka. Journal of Pharmacy Research 3(1): 26-29.
[37] Thenmozhi, M., Kannabiran, K. (2012): Antimicrobial and antioxidant properties of marine actinomycetes Streptomyces sp VITSTK7. Oxidants and Antioxidants in Medical Science 1(1): 51-57
[38] Manasa, J., Katzenstein, D., Cassol, S., Newell, M. L., T. (2012): Oliveira, for the Southern Africa Treatment and Resistance Network, Primary drug resistance in South Africa: data from 10 years of surveys. AIDS Research and Human Retroviruses 28(6): 558-565.
[39] Gautham, S. A., Onkarappa, R., Kuppast, I. J. (2013): Pharmacological activities of the metabolite from Streptomyces fradiae strain GOS1. International Journal of Chemical Sciences 11(1): 583-590.
[40] Boubetra, D., Zitouni, A., Bouras, N., Mathieu, F., Lebrihi, A., Schumann, P., Spröer, C., Klenk, H. P., Sabaou, N. (2013): Saccharothrix hoggarensis sp. nov., an actinomycete isolated from Saharan soil. International Journal of Systematic and Evolutionary Microbiology 63(Pt_2):549-553.
[41] Gebreyohannes, G., Moges, F., Sahile, S., Raja, N. (2013): Isolation and characterization of potential antibiotic-producing actinomycetes from water and

sediments of Lake Tana, Ethiopia. Asian Pacific Journal of Tropical Biomedicine 3(6): 426-435.

[42] Singh, L. S., Sharma, H., Talukdar, N.C. (2014): Production of a potent antimicrobial agent by an actinomycete, Streptomyces sannanensis strain SU118 isolated from phoomdi in Loktak Lake of Manipur, India. BMC Microbiology 14(1): 1-13.

[43] Rashad, F. M., Fathy, H. M., El-Zayat, A. S., Elghonaimy, A. M. (2015): Isolation and characterization of multifunctional Streptomyces species with antimicrobial, nematicidal and phytohormone activities from marine environments in Egypt. Microbiological Research 175:34-47.

[44] Shaik, M., Sankar, G.G., Iswarya, M., Rajitha, P. (2017): Isolation and characterization of bioactive metabolites producing marine Streptomyces parvulus strain sankarensis-A10. Journal of Genetic Engineering and Biotechnology 15(1): 87-94.

[45] Mohamed, H., Miloud, B., Zohra, F., García-Arenzana, J. M., Veloso, A., Rodríguez-Couto, S. (2017): Isolation and Characterization of Actinobacteria from Algerian Sahara Soils with Antimicrobial Activities. International Journal of Molecular and Cellular Medicine 6(2): 109–120.

[46] Arumugam, T., Kumar, P. S., Kameshwar, R., Prapanchana, K. (2017): Screening of novel actinobacteria and characterization of the potential isolates from mangrove sediment of south coastal India. Microbial Pathogenesis 107: 225-233.

[47] Elbendary, A., Silhavy, T. J., Kahne, D., Walker, S. (2010): The bacterial cell envelope. Cold Spring Harbor Perspectives in Biology 2(5): a000414.

[48] Majer, J., Chater, K. F. (1987): Streptomyces albus G produces an antibiotic complex identical to paulomycins A and B. Microbiology 133(9):2503- 2507.

[49] Naragani, K., Munaganti, R. K., Sirigiri, C. K., Muvva, V. (2014): Antimicrobial potential of Streptomyces violaceoruber VLK-4 isolated from the South coast of Andhra Pradesh, India. Int J Pharm Sci Rev Res 25: 125-29.

[50] Badji, B., Mostefaoui, A., Sabaou, N., Lebrihi, A., Mathieu, F., Seguin, E., Tillequin, F. (2007): Isolation and partial characterization of antimicrobial compounds from a new strain Nonomuraea sp. NM94. Journal of Industrial Microbiology and Biotechnology 34(6): 403-412.

[51] Lahoum, A., Aouiche, A., Bouras, N., Verheecke, C., Klenk, H. P., Sabaou, N. (2016): Antifungal activity of a Saharan strain of Actinomadura sp. ACD1 against toxigenic fungi and other pathogenic microorganisms. Journal de Mycologie Medicale 26(3): 193-200.

[52] Bhakyashree, K., Kannabiran, K. (2018): Anti-MRSA activity of actinomycetes isolated from marine soil sample of Aryaman beach, Tamil Nadu, India. Research Journal of Pharmacy and Technology 11(5): 2036- 2039.

[53] Rekha, C., Poornima, G., Manasa, M., Abhipsa, V., Devi, J. P., Kumar, H. T. V., Kekuda, T. R. P. (2012): Ascorbic acid, total phenol content and antioxidant activity of fresh juices of four ripe and unripe citrus fruits. Chemical Science Transactions 1(2): 303-310.

[54] Janardhan, A., Kumar, A. P., Viswanath, B., Saigopal, D. V. R., Narasimha, G. (2014). Production of bioactive compounds by actinomycetes and their antioxidant properties. Biotechnology Research International 2014:8.

[55] Katalinic, V., Milos, M., Kulisic, T., Jukic, M. (2006): Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. Food Chemistry 94(4): 550-557.

[56] Marzouk, M. S., Moharram, F. A., Mohamed, M. A., Gamal-Eldeen, A. M., Aboutabl, E. A. (2007): Anticancer and antioxidant tannins from Pimenta dioica leaves. Zeitschrift für Naturforschung C 62(7-8):526-536.
[57] Tokiniaina, A. (2010): Biological and chemical studies of secondary metabolites of telluric actinomycetes Case of the Ankafobe forest. University of Antananarivo, Faculty of Sciences, Antananarivo, Madagascar