Antibacterial and Antifungal Activities of Various Extracts of *Dianthus sylvestris* subsp. *aristidis* (Batt.) Greuter & Burdet

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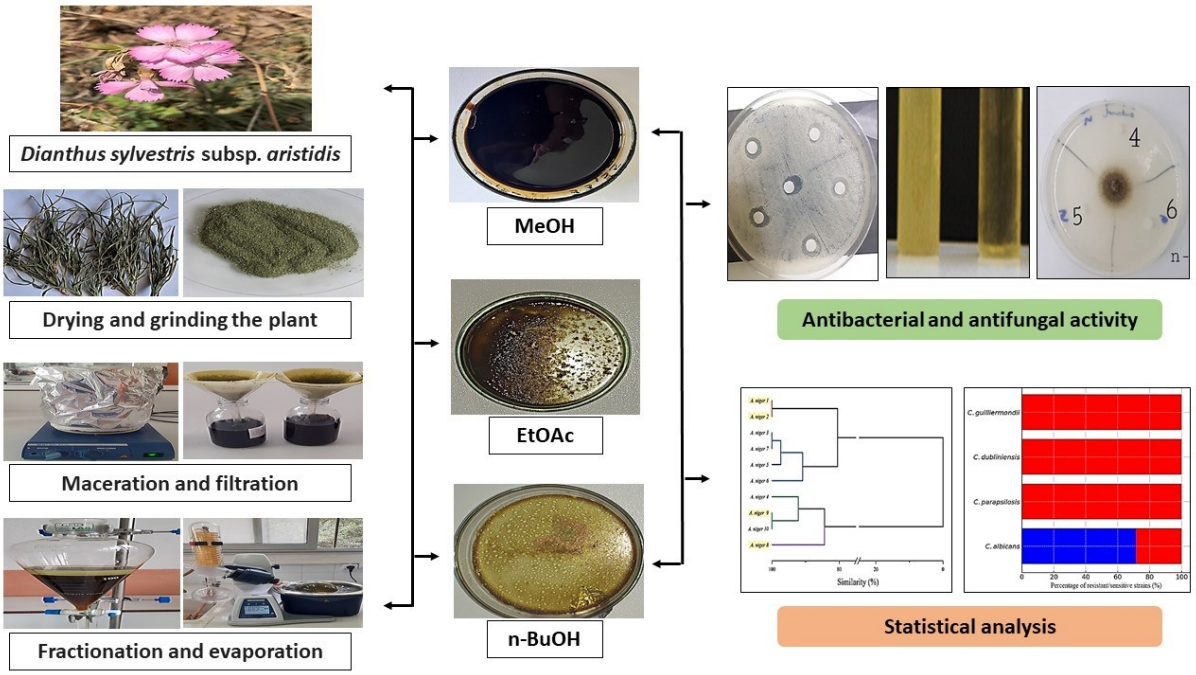
Graphical abstract



Abstract

In this study, we investigated the antibacterial and antifungal activities of hydro-methanolic (MeOH), ethyl acetate (EtOAc), and butanolic (n-BuOH) extracts from the leaves of *Dianthus*

sylvestris subsp. *aristidis* (Batt.) Greuter & Burdet against 78 clinical and 6 reference bacterial and fungal strains using disk diffusion to asses inhibition diameters (ID) and broth dilution methods to determine the minimum inhibitory concentration (MIC). The antimicrobial activity varied the extracts and strains, with interesting antibacterial effects against most tested bacterial strains. Inhibition diameters ranging from 10 to 20 mm, and MIC values varied between 31.25 and 1000 µg/mL. Furthermore, significant antifungal effects were observed, especially against *Candida albicans*, with ID ranging from 10 to 14 mm and MIC values ranging from 31.25 to 1000 µg/mL. All extracts showed growth inhibition percentages up to 100% against *Aspergillus niger.* These findings suggest that *Dianthus sylvestris* subsp. *aristidis* extracts are promising candidates for developing drugs against resistant pathogens.

Key words: Antibacterial activity, antifungal activity, *Dianthus sylvestris* subsp. *aristidis*

1. Introduction

The emergence of multi-resistant pathogens in bacterial and fungal infections, such as urinary infections, candidiasis, aspergillosis, dermatophytosis, and systemic mycoses, becomes a major global health concern (Alajlani, 2023).

The incidence of bacterial and fungal infections has risen due to factors such as the growth of the human population, close contact with animals, climate change, as well as the misuse and insufficient control of antibiotics (Khan *et al*., 2023).

Plant based medicines, containing therapeutic substances, may possess mechanisms that combat pathogenic microorganisms while minimizing the risk of resistance development. The vast diversity of bioactive compounds present in these plants has drawn the attention of researchers towards exploring their potential as natural antibacterial and antifungal agents.

The genus *Dianthus* produces a variety of secondary metabolites, including alkaloids, flavonoids, saponins, terpenoids, and phenolic acids, these compounds have been shown to possess a wide range of pharmacological activities (Jakimiuk *et al*., 2022), such as antimicrobial, anti-inflammatory, and anticancer effects (Yusupova *et al*., 2020; Celik *et al*., 2024).

Several species within the *Dianthus* genus have shown significant promise for their antimicrobial properties. For instance, the ethanolic extract of *D. coryophyllum* exhibited moderate antibacterial activity against multiple bacterial strains, as well as antifungal activity against *Candida albicans* and *Aspergillus niger* (Ertürk, 2006). Similarly, essential oils from *D. carmelitarum* and *D. calocephalus* demonstrated antifungal activity against *C. albicans* (Yucel and Yayli, 2018). These studies suggest that *Dianthus* species, with their rich chemical diversity, could offer a valuable source of bioactive compounds for antimicrobial therapy.

Despite these promising findings, most research on *Dianthus* species has focused on well-known species. In contrast, *Dianthus sylvestris* subsp. *aristidis*, an Algerian endemic plant, remains largely unexplored for its antimicrobial potential. This study aims to fill this gap by evaluating, for the first time, the antibacterial and antifungal activities of the hydro-methanolic (MeOH), ethyl acetate (EtOAc), and n-butanol (n-BuOH) extracts of *D. sylvestris* subsp. *aristidis*. Recognized for its cultural and heritage value (Dobignard and Chatelain, 2011), this

plant has the potential to contribute novel therapeutic agents that could complement current antimicrobial treatments.

Investigating the bioactivity of *D. sylvestris* subsp. *aristidis* contributes to the scientific understanding of underutilized plant species and highlights the importance of preserving plant biodiversity for future pharmaceutical applications.

2. Materiel and methods

2.1. Biological material

2.1.1. Plant material and extraction

The leaves of *Dianthus sylvestris* subsp, *aristidis* were collected in November 2020 from the state of Skikda, Algeria. The extraction procedure, as described by Bouzana *et al*. (2023) involved the use of different polarity solvents. This process yielded three types of extracts: hydro-methanolic (MeOH), ethyl acetate (EtOAc), and butanolic (n-BuOH) extracts.

2.1.2. Extraction Yield

The extraction yield was estimated using the following formula (Stanojević *et al*., 2009):

Extraction yield (%) = (weight of dry extract / weight of dry sample) × 100

2.1.3. Bacterial and fungal strains

In this study, 55 bacterial and 23 fungal strains were used. The bacterial strains included *S. aureus* (10 strains), *E. coli* (14 strains), *K. pneumoniae* (9 strains), *K. oxytoca* (1 strain), *K. ozaenae* (1 strain), *Proteus mirabilis* (5 strains), *P. vulgaris* (1 strain), *Enterobacter* sp. (3 strains), *Serratia* sp. (3 strains), *Salmonella* sp. (2 strains), and *P. aeruginosa* (6 strains). The fungal strains comprised 13 yeast strains of the genus *Candida*, *C. albicans* (10 strains), *C. parapsilosis* (1 strain), *C. dubliniensis* (1 strain), and *C. guilliermondii* (1 strain), along with 10 fungal strains of *Aspergillus niger*. Additionally, 6 reference strains obtained from the Institut Pasteur, Algiers, including *S. aureus* ATCC 25923, *S. aureus* ATCC 19111, *E. coli* ATCC

25922, *K. pneumoniae* ATCC 70603, *P. aeruginosa* ATCC 27853, and *C. albicans* ATCC 21300.

2.2. Isolation and Identification of Bacterial and Fungal Strains

Bacterial and fungal strains were collected from public and private laboratories in Skikda and Annaba, isolated from samples, including pus, urine, stool, vaginal swabs, nails, interdigital spaces, and ear swabs. Bacterial identification was performed using macroscopic and microscopic observations, as well as biochemical characterization with the API Identification System (API 20E, API 20NE, API STAPH). Fungal strains were identified through macroscopic and microscopic analyses, with *C. albicans* confirmed using the serum filamentation test as described by Mackenzie (1962). In cases where the test was negative, the automated Vitek 2 system (BioMérieux) was utilized to identify species other than *C. albicans*.

2.3. *Evaluation of the antibacterial and antifungal activity of D. sylvestris subsp. aristidis extracts against clinical and reference strains*

2.3.1. Solid medium diffusion method

The antibacterial and antifungal activity against bacterial strains and *Candida* strains was determined using the Kirby-Bauer disk diffusion method on Mueller- Hinton agar (CASFM, 2023). Results were interpreted according to the scale of Ponce et al. (2003)

2.3.2. Determination of minimum inhibitory concentrations

The MIC of bacterial and *Candida* strains were determined using the Mueller-Hinton broth dilution method (CASFM, 2023). The activity was visually estimated by comparing the presence or absence of bacterial growth with that of the control tube.

2.3.3. Determination of growth inhibition percentages of ***A. niger***

The antifungal activity against pathogenic molds of the genus *A. niger* was determined using the disk diffusion method on Sabouraud agar with chloramphenicol with minor modifications. The growth inhibition percentage (%) was calculated using the following formula (Hajji, 2016):

$$\text{Growth inhibition \%} = [(dc - dt)/dc] \times 100$$

Where, dc; the colony diameter in control plates and, dt: the colony diameter in treated plates

The results were interpreted according to the interpretation scale established by Abd-Ellatif et al. (2011)

Statistical analysis

The results were analyzed using one-way analysis of variance (ANOVA) with the software OriginPro v.2021 (OriginLab Corporation, 2021). Differences were considered statistically significant at a threshold of 0.05 ($p < 0.05$). Hierarchical Ascendant Classification (HAC, Cluster analysis) was also performed using OriginPro v.2021 (OriginLab Corporation, 2021).

3. Results

3.1. Physical characterization and extraction yield

The extraction yields are calculated relative to 100 g of dry sample and expressed as a percentage (%). The results in Table 1 indicate that MeOH extract has the highest yield with 23%, followed by n-BuOH extract with a yield of 14.75%, and finally, EtOAc extract with a yield of 2.70%.

Table 1: Yields (%) and Physical Characterization of MeOH, EtOAc, and n-BuOH Extracts

	MeOH	EtOAc	n-BuOH
Yield %	23%	2.70%	14.75%
Color	Dark brown	Black	Yellow
Appearance	Paste-like	Paste-like	solid

3.2. Evaluation of the antibacterial and antifungal activity of D. sylvestris subsp. aristidis extracts

3.2.1. Characterization of the studied microorganisms

3.2.1.1. Distribution of microorganisms by species

In our study, 84 species were isolated and identified, primarily represented by: *E. coli* with a percentage of 18%, *S. aureus*, *C. albicans*, and *A. niger*, each with a percentage of 12% (Figure 1).

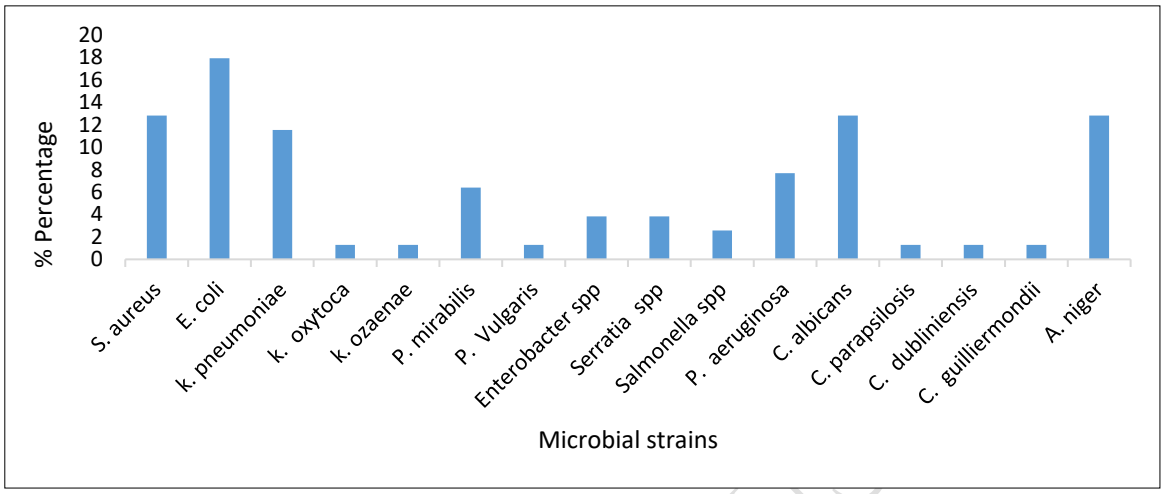


Figure 1: Distribution of isolated microorganisms by species

3.3. Evaluation of the antibacterial activity of D. sylvestris subsp. *aristidis extracts*

The findings of the antibacterial activity of the MeOH, EtOAc, and n-BuOH extracts are presented in Tables 2 and 3

The diameters of the inhibition zones of **MeOH** extract against clinical strains vary between 10 and 20 mm. The smaller diameter (10 mm) was obtained with *S. aureus* 01 and *proteus* sp 04, the greater diameter (20 mm) was obtained with *Serratia* sp 02 *and Pseudomonas aeruginosa* 01. The obtained MIC vary between 31.25 and 1000 µg/mL

The diameters of the inhibition zones of **EtOAc** extract against clinical strains vary between 10 and 16 mm. The smaller diameter (10 mm) was obtained with *Pseudomonas aeruginosa* 01, the greater diameter (16 mm) was obtained with *Staphylococcus aureus* 01. The obtained MIC vary between 31.25 and 1000 µg/mL

The diameters of the inhibition zones of **n-BuOH** extract against clinical strains vary between 10 and 17 mm. The smaller diameter (10 mm) was obtained with *Pseudomonas*

aeruginosa 1 and Escherichia coli 03, the greater diameter (17 mm) was obtained with *Escherichia coli* 01. The obtained MIC vary between 31.25 and 1000 µg/mL.

Table 2: Diameters of inhibition zones and MIC of Gram-positive reference and clinical strains (*S. aureus*) against the tested extracts of *D. sylvestris* subsp. *aristidis*

Extracts / Bacterial strains	MeOH		EtOAc		n-BuOH		GEN
	IZ (mm)	**MIC (µg/mL)**	**IZ (mm)**	**MIC (µg/mL)**	**IZ (mm)**	**MIC (µg/mL)**	
S. aureus ATCC 25923	R	R	R	R	R	R	S
S. aureus ATCC 19111	R	R	R	R	R	R	S
S. aureus 01	11±0.01	31.25	13±0.98	125	11±0.64	500	S
S. aureus 02	10 ±0.20	62.5	10±0.52	31.25	R	R	S
S. aureus 03	13±0.90	500	14±1.20	31.25	R	R	S
S. aureus 04	14±1.20	31.25	R	R	R	R	S
S. aureus 05	15±1.82	125	R	R	R	R	R
S. aureus 06	R	R	R	R	R	R	R
S. aureus 07	R	R	R	R	R	R	S
S. aureus 08	R	R	R	R	R	R	S
S. aureus 09	R	R	R	R	R	R	S
S. aureus 10	R	R	R	R	R	R	S

R : resistant

S : sensitive

GEN : Gentamicine.

Table 3: Diameters of inhibition zones and MIC of Gram-negative reference and clinical strains against the tested extracts of *D. sylvestris* subsp. *aristidis*

Extracts / Bacterial strains	MeOH		EtOAc		n-BuOH		GEN
	IZ (mm)	**MIC (µg/mL)**	**IZ (mm)**	**MIC (µg/mL)**	**IZ (mm)**	**MIC (µg/mL)**	
E. coli ATCC 25922	R	R	R	R	R	R	S
E. coli 01	R	R	13±1.60	31.25	15±0.25	250	S
E. coli 02	12±1.22	31.25	R	R	R	R	S
E. coli 03	14±0.82	1000	R	R	R	R	S
E. coli 04	R	R	R	R	17±2.04	1000	S
E. coli 05	R	R	R	R	10±1.45	125	S
E. coli 06	R	R	R	R	15±1.01	1000	S
E. coli 07	R	R	14±1.75	1000	R	R	S
E. coli 08	R	R	R	R	R	R	R
E. coli 09	R	R	R	R	R	R	S
E. coli 10	R	R	R	R	R	R	S
E. coli 11	R	R	R	R	R	R	S
E. coli 12	R	R	R	R	R	R	S
E. coli 13	R	R	R	R	R	R	S
E. coli 14	R	R	R	R	R	R	S
K. pneumoniae ATCC 70603	10±1.00	62.5	12±2.30	125	11±1.92	62.5	S
K. pneumoniae 01	12±0.92	62.5	11±1.11	31.25	R	R	S
K. pneumoniae 02	R	R	14	31.25	R	R	S
K. pneumoniae 03	R	R	15	62.5	R	R	S

K. pneumoniae 04	14±0.00	31.25	R	R	R	R	S
K. pneumoniae 05	R	R	R	R	12±0.06	1000	S
K. pneumoniae 06	R	R	R	R	R	R	S
K. pneumoniae 07	R	R	R	R	R	R	S
K. pneumoniae 08	R	R	R	R	R	R	S
K. pneumoniae 09	R	R	R	R	R	R	S
K. ozaenae	R	R	R	R	R	R	R
K. oxytoca	R	R	R	R	R	R	R
P. mirabilis 01	R	R	12±0.65	125	15±1.12	500	S
*P. mirabilis*02	13±0.64	125	R	R	R	R	S
P. mirabilis 03	10±0.22	1000	R	R	R	R	S
P. mirabilis 04	R	R	R	R	R	R	S
P. mirabilis 05	R	R	R	R	R	R	S
P. vulgaris	R	R	R	R	R	R	S
Enterobacter sp 01	13±1.22	500	12±1.25	1000	12±0.05	31.25	S
Enterobacter sp 02	15±2.02	1000	15±0.35	500	14±0.58	62.5	S
Enterobacter sp 03	R	R	13±2.01	250	15±0.95	62.5	R
Serratia sp 01	15±0.25	62,5	15±0.39	250	10±1,23	31.25	S
Serratia sp 02	20±0.27	31,25	15±0.90	31.25	14±1.02	31.25	S
Serratia sp 03	13±0.15	500	15±0.89	31.25	15±0.60	1000	S
Salmonella sp 01	R	R	12±0.12	31.25	R	R	S
Salmonella sp 02	R	R	13±1.98	250	R	R	S
P. aeruginosa 27853 ATCC	R	R	12±0.12	31.25	10±0.29	31.25	S
P. aeruginosa 01	20±1.01	31.25	16±0.45	31.25	10±0.96	1000	S

P. aeruginosa 02	14±0.98	31.25	12±0.18	250	13±0.97	250	S
P. aeruginosa 03	13±1.89	62.5	12±0.92	62.5	14±1.15	125	S
P. aeruginosa 04	R	R	R	R	11±096	31.25	S
P. aeruginosa 05	R	R	R	R	R	R	S
P. aeruginosa 06	R	R	R	R	R	R	S

R : resistant

S : sensitive

GEN : Gentamicine.

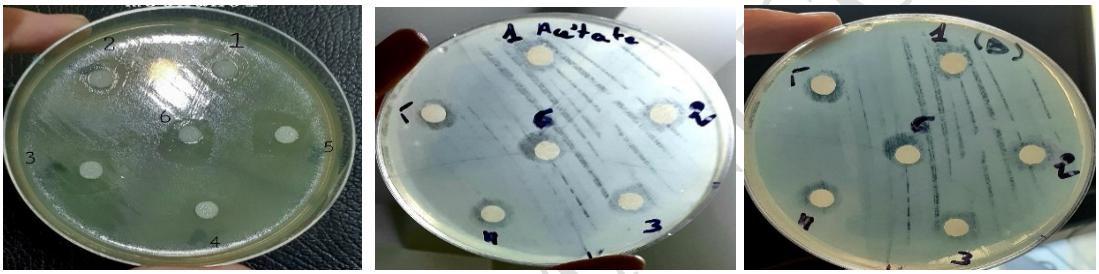


Figure 2: Effect of MeOH, EtOAc, and n-BuOH Extracts on *P. aeruginosa* 01

1: 1000µg/mL; 2: 500µg/mL; **3**: 250µg/mL; **4**: 125µg/mL; **5**: 62. 5µg/mL; **6**: 31. 25µg/mL

3.4. Evaluation of the antifungal activity of D. sylvestris subsp. aristidis extracts

The findings of the antifungal activity of the MeOH, EtOAc, and n-BuOH extracts are presented in Tables 4 and 5

3.4.1. Candida sp strains

The findings showed that *C. albicans* ATCC 21300 exhibited sensitivity to all the tested extracts, with inhibition zone diameters of 12 mm and MIC of 62.5 µg/mL. Additionally, nine *C. albicans* yeasts were also found to be sensitive to the MeOH, EtOAc and n-BuOH extracts, with varying inhibition diameters ranging from 12 to 14 mm, 11 to 14 mm, and 10 to 13 mm, respectively. The corresponding MIC of these yeasts ranged from 31.25 to 62.5 µg/mL, 62.5 to 250 µg/mL, and 31.25 to 1000 µg/mL for the MeOH, EtOAc, and n-BuOH extracts,

respectively. Four yeasts, *C. albicans* 10, *Candida parapsilosis, Candida dubliniensis,* and *Candida guilliermondii* were found to be resistant to all tested extracts.

Table 4: Diameters of inhibition zones and MIC of reference and clinical strains of *Candida* sp. against the tested extracts of *D. sylvestris* subsp. *aristidis*

Extracts / Yeasts	MeOH		EtOAc		n-BuOH	
	IZ (mm)	**MIC (µg/mL)**	**IZ (mm)**	**MIC (µg/mL)**	**IZ (mm)**	**MIC (µg/mL)**
C. albicans ATCC 21300	12±0.02	62.5	12±0.25	62.5	12±0.45	62.5
C. albicans 01	14±0.00	31.25	13±0.26	62.5	10±0.68	62.5
C. albicans 02	12±1.23	62.5	12±0.39	125	12±0.14	31.25
C. albicans 03	12±1.20	62.5	12±0.56	250	12±0.26	125
C. albicans 04	12±0.12	62.5	12±1.26	62.5	10±1.26	62.5
C. albicans 05	12±1.85	31.25	12±1.28	125	12±0.98	62.5
C. albicans 06	13±0.09	31.25	14±0.42	62.5	13±0.00	31.25
C. albicans 07	13±2.01	6.,5	14±0.41	62.5	13±0.16	62.5
C. albicans 08	12±0.56	62.5	11±2.05	125	10±0.23	1000
C. albicans 09	13±0.78	62.5	12±0.36	125	13±0.12	62.5
C. albicans 10	R	R	R	R	R	R
C. parapsilosis	R	R	R	R	R	R
C. dubliniensis	R	R	R	R	R	R
C. guilliermondii	R	R	R	R	R	R

R: resistant

Figure 3: Effect of MeOH, EtOAc, and n-BuOH extracts on *C. albicans* 01

1: 1000µg/mL; 2: 500µg/mL; **3**: 250µg/mL; **4**: 125µg/mL; **5**: 62. 5µg/mL; **6**: 31. 25µg/mL

3.4.2. Aspergillus niger strains

The findings showed that the **MeOH** extract exhibited an excellent activity against *A. niger* strains 01 (Figure 4), 02, 03, and 04, with growth inhibition percentages ranging from 70% to 100%. Moderate activity was observed against *A. niger* strains 06 and 07, with growth inhibition percentages of 50% and 55%, respectively. Low activity was observed against *A. niger* strains 05 and 10, with growth inhibition percentages of 30%. *A. niger* strains 08 and 09 were resistant to this extract.

The **EtOA**c extract also showed excellent activity against *A. niger* strains 01 (Figure 4), 02, 03, 05, 06, 07, and 09, with growth inhibition percentages ranging from 80% to 100%. Low activity was observed against *A. niger* strains 04 and 08, with growth inhibition percentages of 20% and 30%, respectively, while *A. niger* strain 10 was resistant to this extract.

The **n-BuOH** extract showed excellent activity against *A. niger* strains 01 (Figure 4), 02, 03, 05, and 07, with growth inhibition percentages ranging from 75% to 100%. Moderate activity was observed against *A. niger* strain 06, with a growth inhibition percentage of 60%. Low activity was observed against *A. niger* strains 04 and 08, with growth inhibition percentages of 15% and 35%, respectively. *A. niger* strains 09 and 10 were resistant to this extract.

Table 5: Growth inhibition percentage (%) of *Aspergillus niger* against various extracts of *D. sylvestris* subsp. *aristidis*

Extracts / Mold strains	MeOH		EtOAc		n-BuOH	
	Concentration	**inhibition%**	**Concentration**	**inhibition%**	**Concentration**	**inhibition%**
A. niger 01	500	90°°°°	31.25	100°°°°	31,25	95°°°°
A. niger 02	250	100°°°°	1000	80°°°°	31,25	95°°°°
A. niger 03	31,25	100°°°°	62,5	100°°°°	62 ,5	75°°°°
A. niger 04	1000	70°°°°	1000	20°	1000	15°
A. niger 05	1000	30°	32,5	80°°°°	250	80°°°°
A. niger 06	250	55°°	250	90°°°°	500	60°°°
A. niger 07	250	50°°	500	90°°°°	62, 5	75°°°°
A. niger 08	NA	NA	62,5	30°	31,25	35°
A. niger 09	NA	NA	250	80°°°°	NA	NA
A. niger 10	1000	30°	NA	NA	NA	NA

NA : no activity
° : low activity
°° : moderate activity
°°° : good activity
°°°° : excellent activity

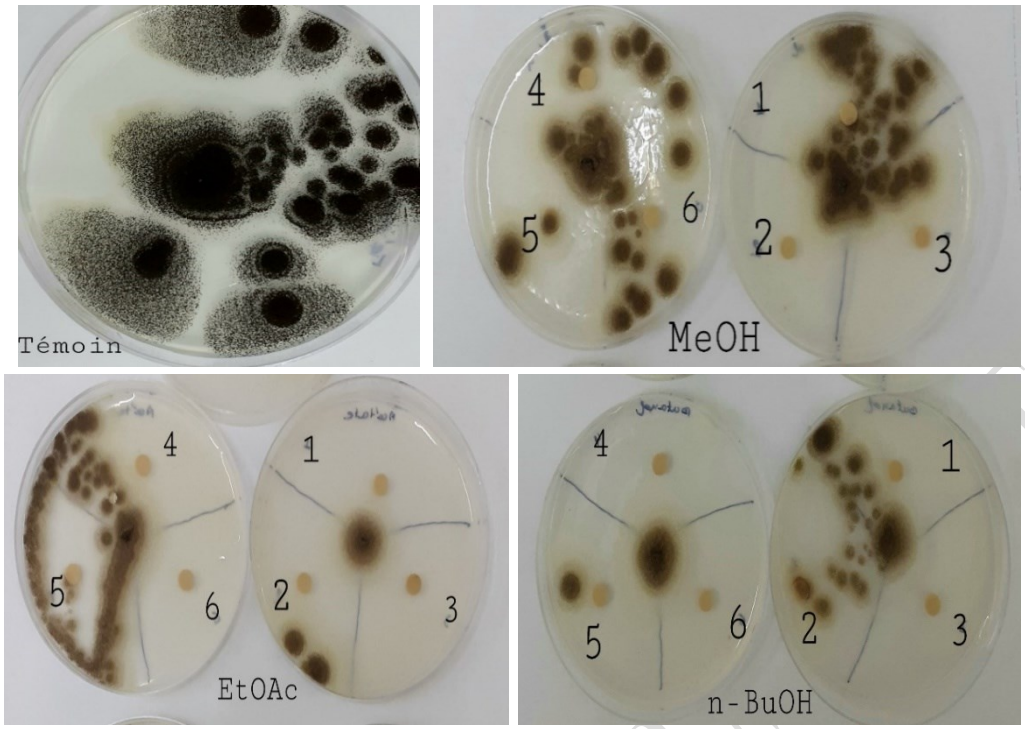


Figure 4: Effect of MeOH, EtOAc, and n-BuOH extracts on *A. niger* 01

1: 1000µg/mL; 2: 500µg/mL; **3**: 250µg/mL; **4**: 125µg/mL; **5**: 62.5µg/mL; **6**: 31.25µg/mL.

3.4.3. Statistical evaluation of the effectiveness of the tested extracts

The effectiveness of the different extracts (MeOH, EtOAc, and n-BuOH) on various microbial strains (bacteria, yeasts, and molds) was compared using one-way analysis of variance (ANOVA). The results showed no significant difference in effectiveness among the three extracts, with p-values >0.05 for Gram-positive bacteria (0.6099), Gram-negative bacteria (0.6134), yeasts (0.4904), and molds (0.617).

Overall, all three extracts acted on a similar number of strains. However, each extract showed activity against different groups of strains. Hierarchical Ascendant Classification (HAC) analysis was performed using OriginPro v.2021, incorporating the inhibition zone diameter and the minimum inhibitory concentration data. This analysis allowed for grouping of the tested strains based on their sensitivity levels to the extracts.

The HAC results as shown in Figure 5 (bacteria), 6 (yeasts), and 7 (molds), indicated that some strains retained consistent sensitivity or resistance profile regardless of the extract used (Figure 8).

For **bacterial strains**, 40% to 60% of the strains of *S. aureus*, *E. coli*, *K. pneumoniae*, and *P. mirabilis* were sensitive to all three extracts. In contrast, *K. oxytoca*, *K. ozonae*, and *P. vulgaris* were resistant to all three extracts.

For **yeasts strains**, the HAC results indicated that 71.42% of the tested strains were sensitive to all three extracts. However, the strains *C. albican*s 10, *C. parapsilosis*, *C. dubliniensis,* and *C. guilliermondii* were resistant to all three extracts.

Regarding **molds**, the HAC results indicated that the EtOAc and n-BuOH extracts showed excellent activity against 60% to 70% of the tested *A. niger* strains, while the MeOH extract showed activity against only 30% of these strains.

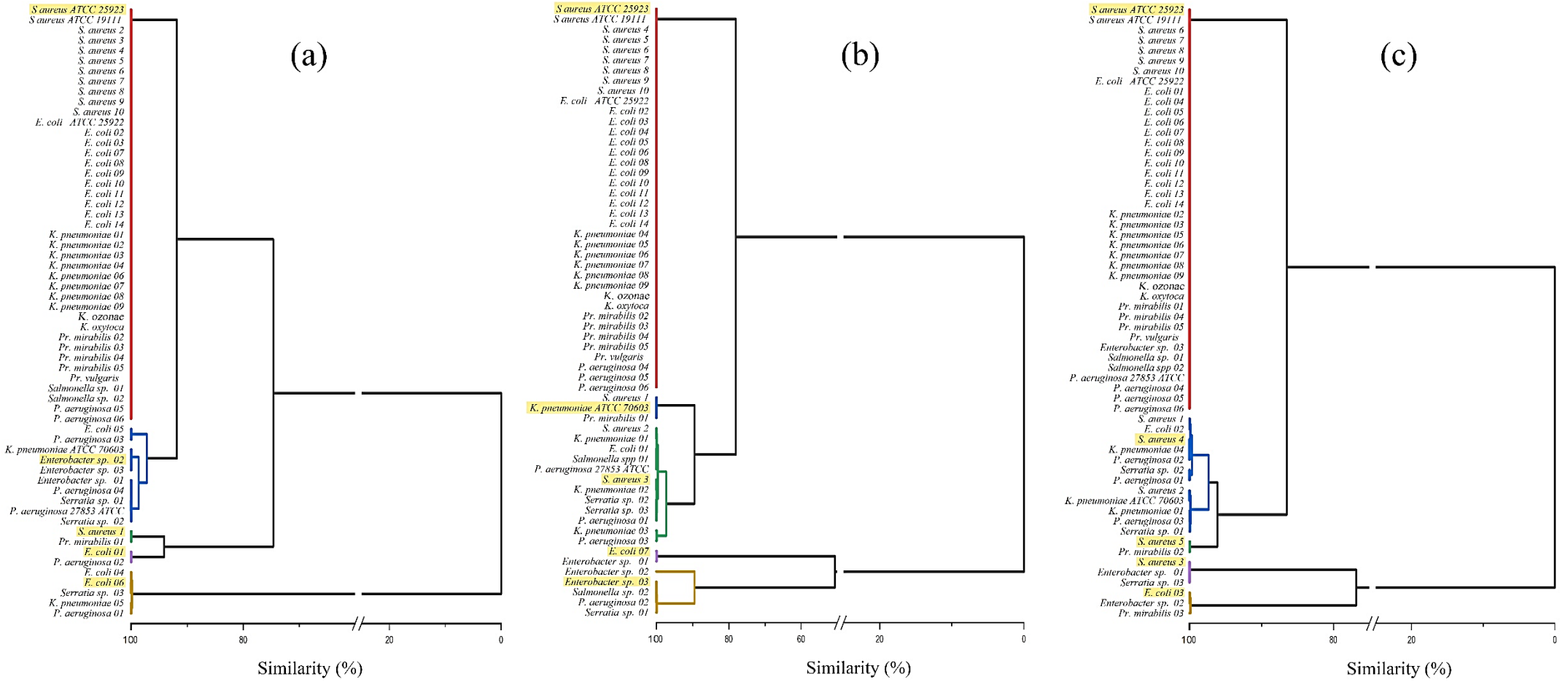


Figure 5: Hierarchical ascendant classification of tested Gram-positive and Gram-negative bacterial strains based on their sensitivity to the studied extracts (a): n-BuOH, (b): EtOAc, (c): MeOH

The strains highlighted in yellow are representative of a cluster.

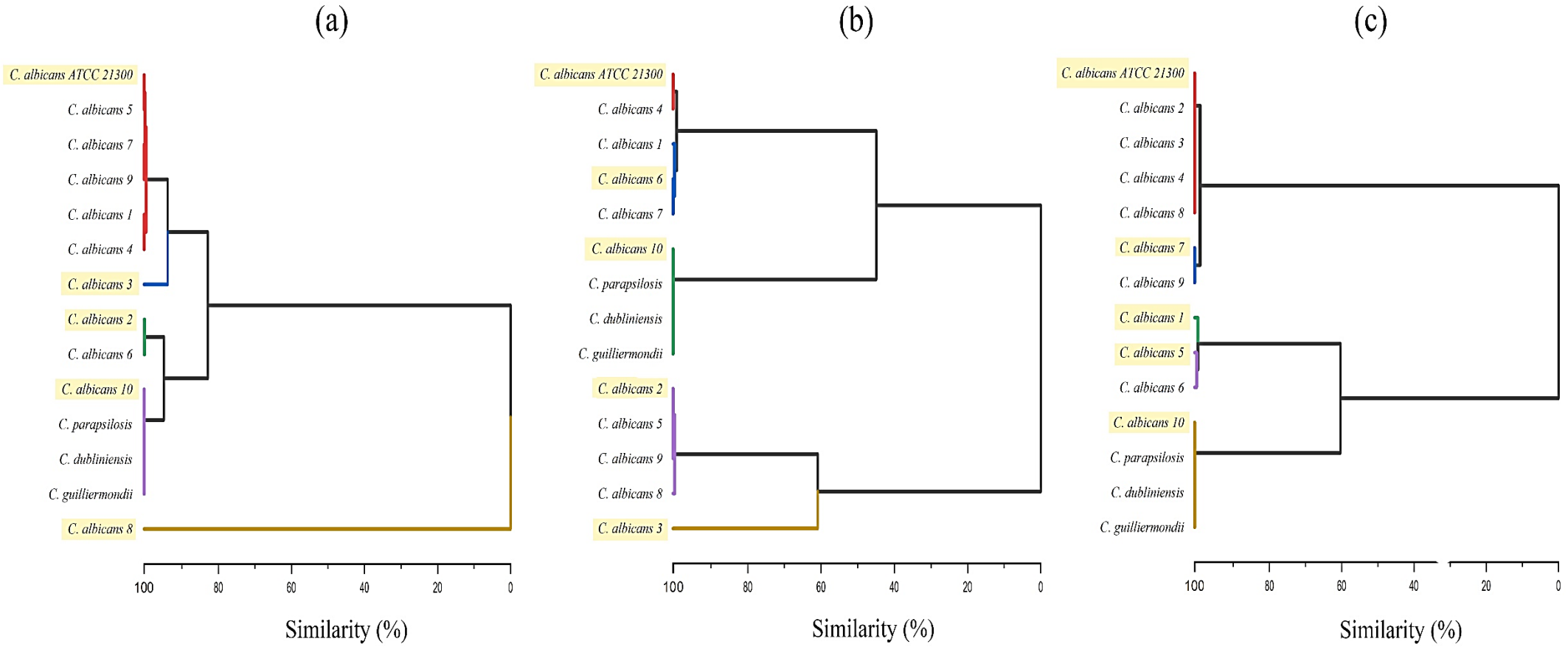


Figure 6: Hierarchical ascendant classification of tested yeasts based on their sensitivity to the studied extracts a): n-BuOH, (b): EtOAc, (c): MeOH

The strains highlighted in yellow are representative of a cluster

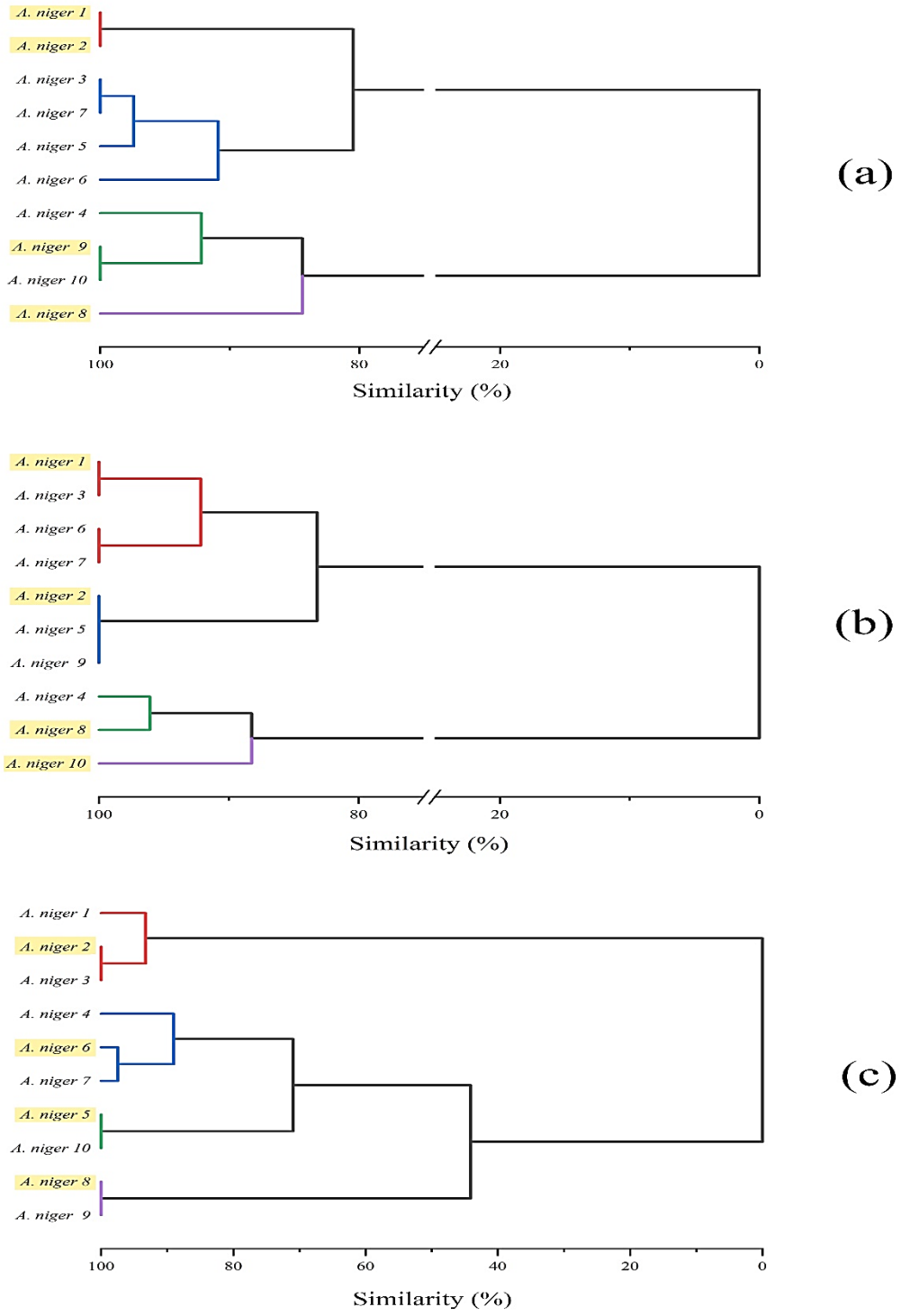
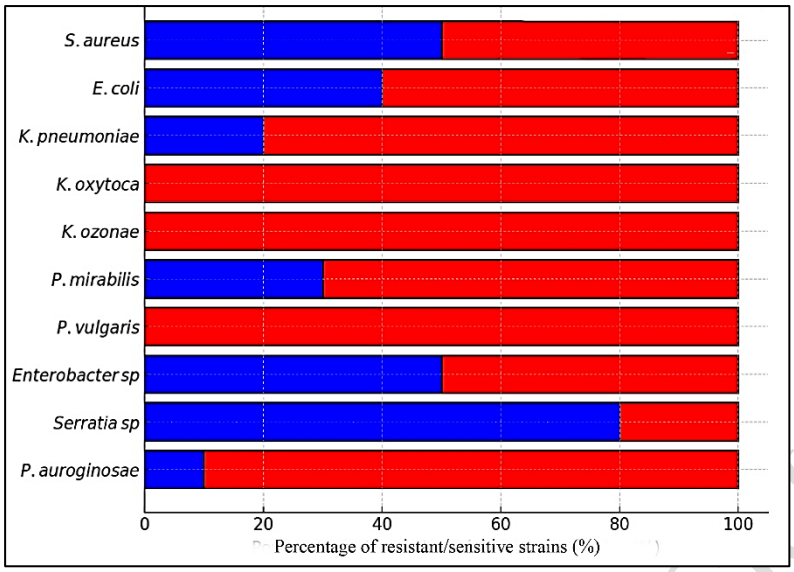
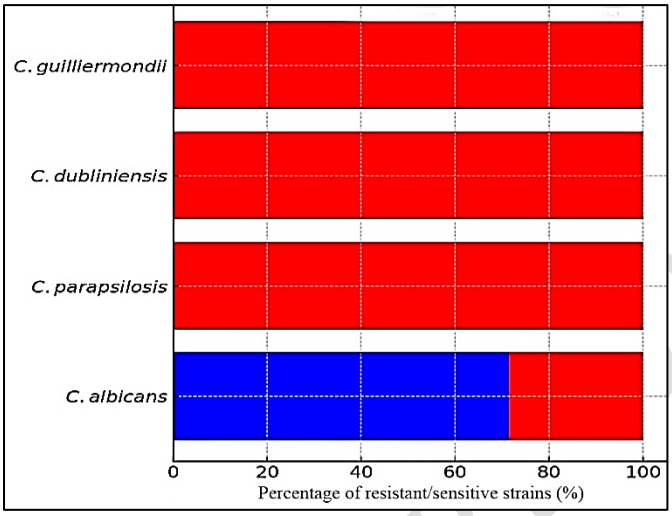


Figure 7: Hierarchical ascendant classification of tested fungal strains (*A. niger*) based on their sensitivity to the studied extracts a): n-BuOH, (b) : EtOAc, (c): MeOH

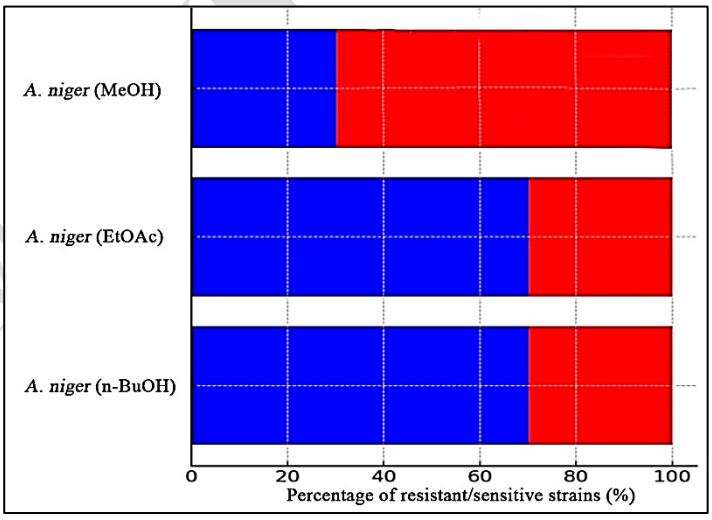
The strains highlighted in yellow are representative of a cluster



(a)



(b)



(c)

Figure 8: Percentage of sensitive/resistant (a) bacterial, (b) yeasts, and (c) mold strains belonging to a single species, regardless of the extract used. Bleu: % of sensitive strains / Red: % of resistant strains

4. Discussion

Antimicrobial resistance, responsible for an estimated 4.95 million deaths annually, emphasizes the need for alternative treatments (Okeke *et al*., 2024; Azad, 2024). Plant-based extracts, such as those from *D. sylvestris subsp. aristidis*, offer a promising solution due to their diverse phytochemical composition and broad-spectrum antimicrobial activity. This study evaluated the antibacterial and antifungal efficacy of three extracts MeOH, EtOAc, and n-BuOH against clinically relevant pathogens, including *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans*, and *Aspergillus niger*. These microorganisms are known to cause common infections such as urinary tract infections (*E. coli*) (Zhou *et al*., 2023), pus-associated infections (*S. aureus*) (Saptoka *et al*., 2019), vaginal candidiasis (*C. albicans*), and onychomycosis (*A. niger*) (Yapar, 2014; Pappas *et al*., 2016; Bongomin *et al*., 2018).

Although statistical analyses (ANOVA) revealed no significant differences between the three extracts ($p > 0.05$) in the total number of sensitive or resistant strains, specific variations were observed across microbial groups. Among bacteria, 40% - 60% of strains of *S. aureus*, *E. coli*, *K. pneumoniae*, and *P. mirabilis* exhibited sensitivity, while *K. oxytoca, K. ozonae* and *P. vulgaris* were resistant to all extracts. For yeasts, 71.42% of the tested strains were sensitive, whereas strains such as *C. parapsilosis*, *C. dubliniensis* and *C. guilliermondii* were completely resistant. Concerning molds, EtOAc and n-BuOH extracts showed superior antifungal activity, inhibiting 60 - 70% of *A. niger* strains, whereas MeOH extract was effective against only 30% of strains. These variations highlight the influence of solvent polarity in the extraction of bioactive compounds, which affects the spectrum and intensity of antimicrobial activity.

The antimicrobial efficacy of *D. sylvestris subsp. aristidis* extracts can be attributed to their rich phytochemical composition (Bouzana *et al*., 2024). Phenolic acids, like coumaric and benzoic acids, disrupt bacterial proteins, polysaccharides, and membrane permeability, leading to cell death (Mostafa *et al*., 2018; Kamelé *et al*., 2019). Flavonoids, such as quercetin,

naringenin, and hesperetin inhibit bacterial and fungal biofilm formation, which is critical for microbial survival and virulence (Rauha *et al*., 2000 ; Rigano *et al*., 2007; Slobodníková *et al*., 2016). Vanillin, another compound identified in the extracts, inhibits bacterial and fungal growth by interfering with quorum sensing mechanisms (Maisch *et al*., 2022). These compounds may act synergistically, enhancing the overall antimicrobial effect of the extracts (Essawi and Srour, 2000).

The inhibition zones (10–20 mm) and MIC values (31.25–1000 µg/mL) align with previous studies on *Dianthus* species. For example, the ethanolic extract of *D. caryophyllus* showed inhibition diameters of 10 to 14 mm against *K. pneumonia,* while the ethanolic extract of *D. coryophyllum* showed activity against *E. coli*, *S. aureus*, and *P. aeruginosa* with MIC values of 15, 25, and 15 mg/mL, respectively (Ertürk, 2006). In contrast, the aqueous extract of *D. carmelitarum* showed antibacterial activity with an MIC of 250 µg/mL against *S. aureus,* and *P. aeruginosa* (Aliyazıcıoğlu *et al*., 2017), but no activity against *E. coli*. In addition, the essential oils of *D. carmelitarum* and *D. calocephalus* showed no antibacterial activity against *S. aureus*, *E. coli,* and *P. aeruginosa* (Yucel and Yayli, 2018).

Regarding the antifungal activity of the genus *Dianthus*, the essential oils of *D. carmelitarum* and *D. calocephalus* showed moderate antifungal activity against *C. albicans* with MIC of 668 µg/mL and 1041 µg/mL, respectively (Yucel and Yayli, 2018). The ethanolic extract of *D. coryophyllum* showed strong antifungal activity against *C. albicans* and *A. niger* with inhibition diameter of 30 and 22 mm, respectively (Ertürk, 2006). On the other hand, the aqueous extract of *D. carmelitarum* showed no antifungal activity against *C. albicans* (Aliyazıcıoğlu *et al*., 2017). These differences highlight the role of phytochemical diversity, solvent properties, and microbial variability in determining efficacy. Environmental and genetic factors such as resistance genes, biofilm formation, pH, and nutrient availability also influence pathogen susceptibility (Woods *et al*., 2021; Saleem *et al*., 2019).

The combination of these extracts with conventional antibiotics or antifungals has the potential to offer synergistic effects, addressing the growing issue of antimicrobial resistance (Manso *et al*., 2021). Further research should explore these synergies and optimize the use of *D. sylvestris* extracts in therapeutic applications.

5. Conclusion

The study highlights the promising antimicrobial properties of the MeOH, EtOAc, and n-BuOH extracts from *Dianthus sylvestris* subsp. *aristidis*. These extracts show potent antimicrobial activity against both Gram-positive and Gram-negative resistant bacteria, as well as fungal strains. Therefore, *Dianthus sylvestris* subsp, *aristidis* stand out as a promising candidate for effectively controlling bacterial infection.

Conflict of Interest

The authors declare no conflicts of interest.

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References

Abd-Ellatif, S., Abdel Rahman, S. M., & Deraz, S. F. (2011). Promising antifungal effect of some folkloric medicinal plants collected from El-Hammam habitat, Egypt against dangerous pathogenic and toxinogenic fungi. *ARPN Journal of Agricultural and Biological Science*, ***6***(9), 26-32.

Alajlani, Muaaz. (2023). Antifungal property of medicinal plants: A comprehensive review. *International Journal of Herbal Medicine.* ***11*** (4) 51-57.

Aliyazıcıoğlu, R., Demir, S., Badem, M., Şener, S. Ö., Korkmaz, N., Demir, E. A., Özgen, U., Karaoğlu, Ş. A., & Aliyazıcıoğlu, Y. (2017). Antioxidant, antigenotoxic, antimicrobial activities and phytochemical analysis of *Dianthus carmelitarum*. *Records of Natural Products*, ***11***(3), 270-284

Azad., Md, Abul Kalam. (2024). Antimicrobial resistance: Real threat for the clinician. *Bangladesh Journal of Medicine*, 131-131.

Bongomin, F., Batac, CR., Richardson, MD., Denning, DW. (2018). A Review of Onychomycosis Due to *Aspergillus* Species. *Mycopathologia*. ***183***(3):485-493.

Bouzana, A., Chekroud, Z., Becheker, I., Sakhraoui, N., Bouzenad, N., & Bensouici, C. (2023). Phytochemical analysis by LC MS/MS and *in vitro* antioxidant activity of the Algerian endemic plant *Dianthus sylvestris* subsp. *aristidis* (Batt.) Greuter & Burdet. *Global NEST Journal*, ***25***(7), 113–119.

CASFM. (2023). Comite de l'antibiogramme de la societe francaise de microbiologie. disponible à : https://www.sfm-microbiologie.org/wp-content/uploads/2023/06/CASFM2023_V1.0.pdf . Consulté le: 18 septembre 2024.Celik, A. K., Usta, N. C., Baba, Y., Cimen, A., & Turker, A. U. (2024). Phenolic characterization, antimutagenic, antioxidant and antibacterial capacities of seven endemic *Dianthus* species from Turkey. *South African Journal of Botany*, ***164***, 39-49.

Dobignard, A., & Chatelain, C. (2011). Synonymic index to the flora of North Africa. Volume 3 : Dicotyledoneae: Balsaminaceae-Euphorbiaceae.

Ertürk, Ö. (2006). Antibacterial and antifungal activity of ethanolic extracts from eleven spice plants. *Biologia*, ***61***(3), 275-278.

Essawi, T., & Srour, M. (2000). Screening of some Palestinian medicinal plants for antibacterial activity. *Journal of ethnopharmacology*, ***70***(3), 343-349.

Jakimiuk, K., Wink, M. & Tomczyk, M. (2022). Flavonoids of the Caryophyllaceae. Phytochem Rev ***21***, 179–218.

Hajji, H. (2016). Evaluation in vitro de l'activité antifongique de quatre plantes médicinales marocaines sur cinq champignons phytopathogènes. *Revue Marocaine de Protection des Plantes*, ***10.***

Khan A, Moni SS, Ali M, Mohan S, Jan H, Rasool S, et al. (2023). Antifungal activity of plant secondary metabolites on *Candida albicans*: An Updated Review. *Curr Mol Pharmacol.* ***16*** (1):15-42.

Manso, T., Lores, M., & de Miguel, T. (2021). Antimicrobial activity of polyphenols and natural polyphenolic extracts on clinical isolates. *Antibiotics*, ***11***(1), 46.

Maisch, N. A., Bereswill, S., & Heimesaat, M. M. (2022). Antibacterial effects of vanilla ingredients provide novel treatment options for infections with multidrug-resistant

bacteria–A recent literature review. *European Journal of Microbiology and Immunology*, ***12***(3), 53–62

Mostafa, A. A., Al-Askar, A. A., Almaary, K. S., Dawoud, T. M., Sholkamy, E. N., & Bakri, M. M. (2018). Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases. *Saudi journal of biological sciences*, *25*(2), 361-366.

OriginLab Corporation (2021). Origin software, Northampton, MA 01060 USA

Pappas P.G., Kauffman C.A., Andes D.R., Clancy C.J., Marr K.A., Ostrosky-Zeichner L., Reboli A.C., Schuster M.G., Vazquez J.A., Walsh T.J., et al. (2016) Clinical Practice Guideline for the Management of Candidiasis: 2016 Update by the Infectious Diseases Society of America. *Clinical infectious diseases*, ***62*** (4):e1–e50.

Ponce, A. G., Fritz, R., Del Valle, C., & Roura, S. I. (2003). Antimicrobial activity of essential oils on the native microflora of organic Swiss chard. *LWT-Food Science and Technology*, ***36***(7), 679 684.

Okeke, I. N., de Kraker, M. E., Van Boeckel, T. P., Kumar, C. K., Schmitt, H., Gales, A. C., ... & Laxminarayan, R. (2024). The scope of the antimicrobial resistance challenge. The Lancet, ***403***(10442), 2426-2438.

Rauha, J.-P., Remes, S., Heinonen, M., Hopia, A., Kähkönen, M., Kujala, T., Pihlaja, K., Vuorela, H., & Vuorela, P. (2000). Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds. *International journal of food microbiology*, ***56***(1), 3-12.

Rigano, D., Formisano, C., Basile, A., Lavitola, A., Senatore, F., Rosselli, S., & Bruno, M. (2007). Antibacterial activity of flavonoids and phenylpropanoids from *Marrubium globosum* ssp. Libanoticum. *Phytotherapy Research*, ***21***(4), 395-397.

Saleem, M., Deters, B., de la Bastide, A., & Korzen, M. (2019). Antibiotics overuse and bacterial resistance. *Annals of Microbiology and Research*, ***3***(1), 93.

Sapkota, J., Sharma, M., Jha, B., Bhatt, CP. (2019). Prevalence of S*taphylococcus aureus* Isolated from Clinical Samples in a Tertiary Care Hospital: A Descriptive Cross-sectional Study. *Journal of Nepal Medical Association,* ***57***(220):398-402.

Slobodníková, L., Fialová, S., Rendeková, K., Kováč, J., & Mučaji, P. (2016). Antibiofilm activity of plant polyphenols. *Molecules*, ***21***(12), 1717.

Stanojević, L., Stanković, M., Nikolić, V., Nikolić, L., Ristić, D., Čanadanovic-Brunet, J., & Tumbas, V. (2009). Antioxidant activity and total phenolic and flavonoid contents of *Hieracium pilosella* L. extracts. *sensors*, ***9***(7), 5702-5714.

Woods, R. J., Barbosa, C., Koepping, L., Raygoza Garay, J. A., Mwangi, M., & Read, A. F. (2021). Genetic determinants of antibiotic resistance and the evolution of trade-offs during adaptation in a single patient. *bioRxiv*, 2021-10.

Yapar, N. (2014). Epidemiology and risk factors for invasive candidiasis. *Therapeutics and clinical risk management,* 95-105.

Yucel, T. B., & Yayli, N. (2018). GC/MS analysis and antimicrobial activity of the volatile compounds from *Dianthus carmelitarum* Reut. Ex Boiss and *Dianthus calocephalus* Boiss. Grown in Turkey. *Ege Üniversitesi Ziraat Fakültesi Dergisi*, ***55***(1), 89-94.

Yusupova, Ugiloy., Usmanov, Durbek., Azamatov, Azizbek., Ramazonov, Nurmurod., Rejepov, Jumadilla. (2020). Phytochemical constituents and biological activities of *Dianthus helenae* Vved., growing in Uzbekistan. *Natural Product Research*. **36**.

Zhou, Y.; Zhou, Z.; Zheng, L.; Gong, Z.; Li, Y.; Jin, Y.; Huang, Y.; Chi, M. (2023). Urinary Tract Infections Caused by Uropathogenic *Escherichia coli*: Mechanisms of Infection and Treatment Options. *International Journal of Molecular Sciences*, ***24***(13), 10537.