



African Journal of Biological Sciences



Evaluation of the antimicrobial activity of root extracts of selected plants growing spontaneously in southern Algeria

Elhadj Kolli^{1*}, Samir Zeroual², Azize Demirpolat³, Hamada Haba⁴

¹Laboratory of LSPN, Faculty of Science and Technology, University of May 8, 1945 Guelma, Algeria

²Laboratory of Genetics, Biotechnology and Valorization of Bio-resources, Faculty of Exact Sciences and Nature Sciences and the Life, University Mohamed Khider, Biskra, Algeria

³Vocational School of Food, Agriculture, and Livestock, Bingol University, Bingol 12000, Turkey

⁴Laboratory of Chemistry and Environmental Chemistry (L.C.C.E), Department of Chemistry, Faculty of Matter Sciences, University of Batna 1, Batna, Algeria

*E-mail: kolli.elhadj@univ-guelma.dz

ABSTRACT

Conducting research on the medicinal properties of plants in the Biskra region, southern Algeria, is indeed a valuable and commendable endeavor. The rich biodiversity of Algerian vegetation provides an excellent opportunity to explore and understand the potential medicinal properties of various plants. Studying the antimicrobial activity of plant extracts is a common approach in microbiology and biochemistry. This method allows researchers to assess the ability of plant compounds to inhibit bacterial growth. For this purpose, we performed, using standard methods, the antimicrobial activity of extracts of six spontaneous plant species: *Astragalus depressus* L., *Atractylis humilus* L., *Atractylis flava* Desf., *Euphorbia guyoniana* Boiss. & Reut., *Euphorbia bupleuroides* Desf. and *Aloe vera* (L.) Burm. F. against nine selected bacterial strains. The obtained results revealed that the different extracts of the studied plants gave different levels of antibacterial activity against all the microorganisms tested and that the *n*-butanol extract of *Euphorbia bupleuroides* at 2 mg showed the highest antibacterial activity with 27.66 mm in zone of inhibition against *E. coli* spp., followed by *n*-butanol extract of *Astragalus depressus* 5 mg with 21.00 mm inhibition zone against *S. aureus* ATCC25923 while the others showed moderate to low activities except for the two extracts *A. humilus* BuOH 5 mg and *Aloe vera* PE 2.5 mg that did not show any effectiveness. From these results we conclude that *S. aureus* ATCC25923 and *S. aureus* ATCC29213 are the most sensitive strains while *E. coli* ATCC25922 is the most resistant strain. However, all studied plant extracts are considered bacteriostatic compared to the MBC / MIC ratio values, which are ≥ 4 . It appears from these results that plant extracts from certain studied plants have potential applications as natural alternatives for preservation or as antimicrobial substances. This is likely due to the presence of active substances in these extracts, which are notable for their specific chemical compositions.

Keywords: Plant extracts, antibacterial activity, *E. guyoniana*, *E. bupleuroides*.

1. INTRODUCTION

Diseases can infect humans, plants and animals, and in most cases bacteria are the main cause. Food poisoning, for example, is usually caused by foodborne bacteria such as *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes*, *Escherichia coli*, and *Salmonella* spp. (Zabrodskii, 2020 and Thongphichai *et al.*, 2023), or some other causes like fungi, parasites, or toxic substance (Abu-Zaida *et al.*, 2022).

Food spoilage is mainly due to several factors, the most important of which is exposure of foods to heat or humidity for a long period of time, in addition to inadequate preservation and failure to maintain the cold chain during the production, storage, and transportation. Foods that are not well preserved are quickly subject to spoilage, and the reason is always their exposure to bacteria or some other microscopic organisms such as fungi, parasites, and molds, these factors can multiply and break down the food's organic matter (Mantegazza *et al.*, 2023).

The ideal way to preserve and protect these foods is to either store them in refrigerators, which helping to prevent it from being exposed to various microorganisms or to use some chemical preservatives to inhibit the growth of these pathogens. Perhaps both of these methods indeed have their own set of advantages and disadvantages, because adding chemicals to food has both positive and negative effects on the human body. For example, the refrigeration process can only extend the shelf life of foods for a certain period, and some foods still have a limited shelf life even when refrigerated. Some chemical preservatives may have negative health effects when consumed in large quantities, such as allergic reactions, or may change the taste, texture, or nutritional value of foods (Piskin and Chanda, 2021; Cheng *et al.*, 2023 and Orlova *et al.*, 2023).

From the early days of humanity to the present day, medicinal herbs have played an active role in human health care. Some plant powders were used for treatment or as preservatives (El-Demery *et al.*, 2016), or were used as creams (Mahboubi *et al.*, 2017), or in the form of infusions (Chaity *et al.*, 2020), without knowing the active ingredients they contain. Indeed, there has been a resurgence of interest in herbal treatments, fueled in part by modern scientific studies that employ analytical methods and laboratory experiments. As technology and research methods advance, the medical world is gaining a deeper understanding of the advantages and significance of experimental recipes derived from natural sources (Kebede *et al.*, 2021 and Lahsissene *et al.*, 2009). Medicinal plants are considered one of the most important sources of pharmaceutical research and drug development involves not only searching for active substances found in plants and using them directly as therapeutic agents, but also as raw materials for drug synthesis or as models for pharmacologically active compounds (Najmi *et al.*, 2022).

On this basis, there is a lot of research conducted in various pharmacological fields, especially antimicrobial activities; this is what prompted us to study the antibacterial activity of extracts of some plants that grow spontaneously in the Biskra region, which are less exploited or not applied by the population in traditional medicine, it's about two species of the family Euphorbiaceae: *E. guyoniana*, *E. bupleuroides*, two species of the family Asteraceae: *A. humilus*, *A. flava*, one species of the family Fabaceae: *As. depressus*, and one species of the family Asphodelaceae: *Aloe vera*.

E. guyoniana has been the subject of many intensive studies, in terms of chemical composition; several secondary metabolites have been isolated from this species (Ahmed *et al.*, 2006; Haba *et al.*, 2013; Haba *et al.*, 2007; Kúsz *et al.*, 2016 and Mohamed-Elamir *et al.*, 2010). On the pharmacological side, numerous studies have been carried out on the different extracts (Boumaza *et al.*, 2018; Boudiar *et al.*, 2010; Nasrine *et al.*, 2013 and Palici *et al.*, 2015). For example, the ethanolic extract of the areal parts of this plant was used against tomato leaf miners in southeastern Algeria and gave positive results, where revealed that it could constitute a good means of managing *Tuta absoluta* Meyrick that might be introduced in sustainable organic agriculture (Lakhdari *et al.*, 2020). Same thing, lots of studies were conducted on the species *E. bupleuroides*, where many natural compounds were isolated in the form of terpenoids (Aichour *et al.*, 2014), another study showed that *E. bupleuroides* latex has an insecticidal effect on *Blattella germanica* (Azoui *et al.*, 2016).

The chemical aspect of the *A. flava* plant was studied by Chabani *et al.*, 2013 and 2016 where Terpene and flavonic compounds have been isolated, whereas the *n*-butanol extract of this plant has shown anticancer and antidiabetic effects (Akram *et al.*, 2019 and Melakhessou *et al.*, 2021). The ethyl acetate and *n*-butanol extracts of *A. humilis* were subjected to antimicrobial study, and the results showed a low antibacterial effect against *E. coli*, *P. aeruginosa*, and *S. aureus* (Mouffouk *et al.*, 2023). Whereas, phytochemical research on this plant led to the isolation of many different secondary metabolites from the same extracts (Sifouane *et al.*, 2020). Some research has been performed on *As. depressus* species regarding its chemical composition has shown the presence of an isoflavane, saponins (Maamria *et al.*, 2015), and an acylated derivative of kaempferol (Shkondrov and Krasteva, 2021). Intensive phytochemical and pharmacological studies have been undertaken on the *Aloe vera* species and have revealed the presence of different Phytoconstituents (Chang-Liang *et al.*, 2011 and Batool *et al.*, 2023), and biological activities (Guerrero-Turriza *et al.*, 2023; Znad and Zghair, 2022).

2. Materials and Methods

Plant material

The roots of the studied plants *As. depressus*, *A. flava*, *A. humilis*, *E. guyoniana* and *E. bupleuroides* were collected at the flowering stage in May 2019 from the Ain ben noui area in Biskra (34°48'25.1" North, 5°39'50.9" Est. Latitude: 34.806970, Longitude: 5.664150), which characterized by dry and hot desert climate, and an estimated altitude of 146 m. While the leaves of *Aloe vera* plant were collected from two different areas in Biskra- ElBranis (35°00'17.1" North, 5°50'53.3" Est. Latitude: 35.004736, Longitude: 5.848148, Altitude: 250 m, climate is dry and hot desert) and El kantra (35°14'24.7" North, 5°45'11.3" Est. Latitude: 35.240205, Longitude: 5.753139, Altitude: 680 m, Climate: dry and hot). All of these plants have been identified and studied, especially in terms of their chemical composition (Maamria *et al.*, 2015; Haba *et al.*, 2013; Aichour *et al.*, 2014; Chabani *et al.*, 2013; Sifouane *et al.*, 2020 and Chang-Liang *et al.*, 2011).

Preparation of extracts

Plant extraction

Organic extracts were prepared according to Zeroual *et al.* (2020) method with small modifications. A mass of Air-dried roots of each selected plants except *Aloe vera*, were macerated at room temperature with EtOH–H₂O (80:20, v/v) for 24 h, three times. After filtration, the filtrate was concentrated then re-dissolve it in distilled water heated with magnetic stirring in order to extract them, using solvents of increasing polarities (Petroleum ether or hexane, chloroform or dichloromethane, ethyl acetate and finally the *n*-Butanol) to obtain various extracts (Table 1).

Table 1. The different extracts of plant with their yields

Plant	Weight of plant material	Extracts					
		1st phase Petroleum ether (PE)	2nd phase Hexane (Hex)	3rd phase CH ₂ Cl ₂ (Dic)	4th phase CHCl ₃ (Chl)		
<i>E. guyoniana</i> Dic	500 g	/	/	2.8%	/	/	/
<i>E. bupleuroides</i> BuOH	800 g	0.44%	/	1.01%	/	1.29%	3.57%
<i>A. flava</i> BuOH	700 g	0.22%	/	0.49%	/	0.28%	2.72%
<i>A. humilis</i> BuOH	130 g	1.09%	/	/	/	1.39%	2%
<i>Aloe vera</i> BuOH	30 g	0.36%	/	/	1%	0.53%	1%
<i>Aloe vera</i> gel	100 g	/	/	/	/	/	/
<i>As. Depressus</i> BuOH	800 g	/	0.375%	/	/	1%	5%

***Aloe vera* extraction**

The leaves of the *Aloe vera* plant were washed; dried and powdered, 30 grams of the vegetable powder were macerated in 20% water compared to methanol for 24 hours, two times. After filtration, the filtrate obtained was concentrated in vacuum at room temperature, and then the methanolic extract was put in a drying oven at a temperature of 37° for a week.

***Aloe vera* gel**

After washing the *Aloe vera* leaves, a cut was made on the green surface of the leaves in order to extract the inner gel, this last follows the same extraction procedure as the leaves, and the extracts obtained are stored at 4°C before carrying out the antibacterial tests.

Antibacterial tests

Microbiological strains

The microbial support used is composed of 9 bacterial strains, 6 reference strains: *E.coli* ATCC25922, *E.coli* ATCC43894, *S. aureus* ATCC25923, *S. aureus* ATCC29213, *P. aeruginosa* ATCC27853, *P. aeruginosa* ATCC700370 and 3 pathogenic strains originate from patients: *E.coli* spp., *Enterobacter* spp., *Leisteria* spp. All these strains are provided by Central Bacteriology Laboratory - CHU - Batna and Hakim Sâadan -Biskra Hospital, these strains are stored at 4°C in tubes containing a conservation medium.

Agar well diffusion method

The first evaluation of the antibacterial activity was carried out using the agar well diffusion method described by (Dulger and Gonuz, 2004; Parekh and Chanda, 2007; Rota *et al.*, 2008). The plant extracts are taken up in dimethyl sulfoxide (DMSO), in order to obtain stock solutions of different concentrations (Table 02). The discs are prepared with 6 mm diameter of Wathman No. 3 paper sterilized in the autoclave (120°C for 30 min), then impregnated with 5 µl, 10 µl and 20 µl of each stock solution in order to obtain a mass of 5 mg/Disc. The discs are delicately placed on the agar medium inoculated with the bacterial inoculum then incubated in the oven at 37°C for 24 hours. Antibacterial activity was determined by measuring the diameter of the inhibition zone around each disc (Doughari *et al.*, 2007).

Table 2.The different concentrations of extracts

Extracts	Stock solution	C1 (mg/Disc)	C2 (mg/Disc)	C3 (mg/Disc)
<i>E. guyoniana</i> Dic	135 mg/270 μ l	/	5	/
<i>E. bupleuroides</i> BuOH	18 mg/54 μ l	2	1	/
<i>A. flava</i> PE	189 mg/450 μ l	2	5	/
<i>Atractylisflava</i> BuOH	189 mg/450 μ l	2	5	/
<i>Atractylis humilis</i> BuOH	594 mg/810 μ l	2	5	15
<i>Aloe vera</i> PE	202.5 mg/450 μ l	2.5	5	/
<i>Aloe vera</i> BuOH	189 mg/450 μ l	2	5	/
<i>Aloe vera</i> Dic	189 mg/450 μ l	2	5	/
<i>Aloe vera</i> gel	189 mg/450 μ l	2	5	/
<i>As. depressus</i> BuOH	189mg/450 μ l	2	5	/

Determination of the minimal inhibitory concentrations

The minimum inhibitory concentrations were determined by the micro dilution method using 96-well microplates (Aouni *et al.*, 2013). Ten concentrations were prepared in the microplates by mixing the bacterial suspension at 10^6 CFU/mL with each studied extract. The obtained concentrations in the microplate were ranged between 32 and 0.063 mg/mL. The MIC is determined after incubation at 37°C for 24 hours, as the lowest concentration of which no growth is observed (Absence of deposit).

Determination of the minimal bactericidal concentrations

The minimum bactericidal concentration corresponds to the lowest concentration of the studied extract capable of eradicating more than 99.9 % of the initial microbial inoculum (Chebaibi *et al.*, 2016).

This method consists of subculturing the microbial suspension obtained from wells showing a complete absence of microbial growth (microplate after the MIC reading) using a streaked swab on Müller-Hinton (MH) agar. The inoculated petri dishes are then incubated at 37°C for 48 hours. The MBC is determined as the lowest concentration that hasn't made any bacterial culture.

Statistical study

The statistical study was carried out using the PAST software. 1.98. (Hammer *et al.*, 2001). Multivariate analysis was performed to determine the structure of variability and to calculate differences between groups. Complete data sets were used for these analyses (Figure3). We chose the first two axes F1, F2; whose cumulative percentage is 74.26 % of the total information (F1, F2) = 74.26 %.

3. Results and Discussion

The antibacterial activities of the extracts (Table 3) are estimated in terms of the diameter of the inhibition zone around the discs containing different concentrations against the selected strains. According to the results obtained we note that the extract of *E. bupleuroides* (2 mg and 1 mg) reveal significant activity against the two strains : *E.coli*. spp. (27.66 mm) and *Leisteria* spp. (25.33 mm), while the extract *As. depressus* 5 mg and *A. humilus* BuOH 15 mg shows average activity against the same strain *S. aureus* ATCC25923 (21.00 mm; 19.66 mm), whose low activity is observed in the other strains tested.

Table 3. Results of antibacterial activities (diameters of the inhibition zone, mm) of all extracts

	<i>E. coli</i> ATCC 25922	<i>P. aeruginosa</i> ATCC27853	<i>S. aureus</i> ATCC25923	<i>E. coli</i> ATCC43894	<i>P. aeruginosa</i> ATCC700370	<i>S. aureus</i> ATCC29213	<i>Enterobacter</i> spp.	<i>Leisteria</i> spp.	<i>E. coli</i> spp.
<i>E. guyoniana</i> Dic 5 mg	-	-	10.00	-	10.66	15.00	-	-	-
<i>E. bupleuroides</i> BuOH 2 mg	9.00	10.33	19.00	16.66	14.66	14.66	14.33	25.33	27.66
<i>E. bupleuroides</i> BuOH 1 mg	7.00	8.33	15.00	11.66	12.00	12.66	13.66	20.00	22.33
<i>A. flava</i> PE 5 mg	-	-	10.00	-	10.66	15.00	-	-	-
<i>A. flava</i> BuOH 5 mg	-	10.00	-	-	12.00	12.00	-	-	10.00
<i>A. humilus</i> BuOH 5 mg	-	-	-	-	-	-	-	-	-
<i>A. humilus</i> BuOH 15 mg	-	7.66	19.66	-	9.66	9.66	16.00	-	-
<i>As. depressus</i> BuOH 5 mg	-	10.00	21.00	-	13.00	15.66	-	-	-
<i>As. depressus</i> BuOH 2 mg	-	10.00	14.33	-	13.00	15.66	-	-	-
<i>Aloe vera</i> gel 5 mg	-	-	6.66	-	-	-	-	-	-
<i>Aloe vera</i> PE 5 mg	-	-	-	-	8.00	8.00	-	-	-
<i>Aloe vera</i> PE 2.5 mg	-	-	-	-	-	-	-	-	-

MIC and MBC of the most active extracts

The minimum inhibitory concentration (MIC) is the lowest concentration of antimicrobial capable of inhibiting all visible growth compared to controls - without extract - after an incubation time of 18 to 24 hours (Moroh *et al.*, 2008). The determination of MIC and MBC was carried out using the microdilution technique in a liquid medium coupled with spreading on a solid medium (Chabert *et al.*, 1985). Analysis of experimental data show that compared to growth control indicators; there is a decrease in the number of colonies of germs studied in the experimental tubes (turbidity of the tubes) gradually as the concentration of the extract increases (Kesinkaya *et al.*, 2020). Thus, our results show on the

one hand, that the *n*-butanol phases of *E. bupleuroides* and *As. depressus* inhibit the growth of bacterial germs in vitro at different doses according to the “dose-response” relationship (Figure 1 and Figure 2).

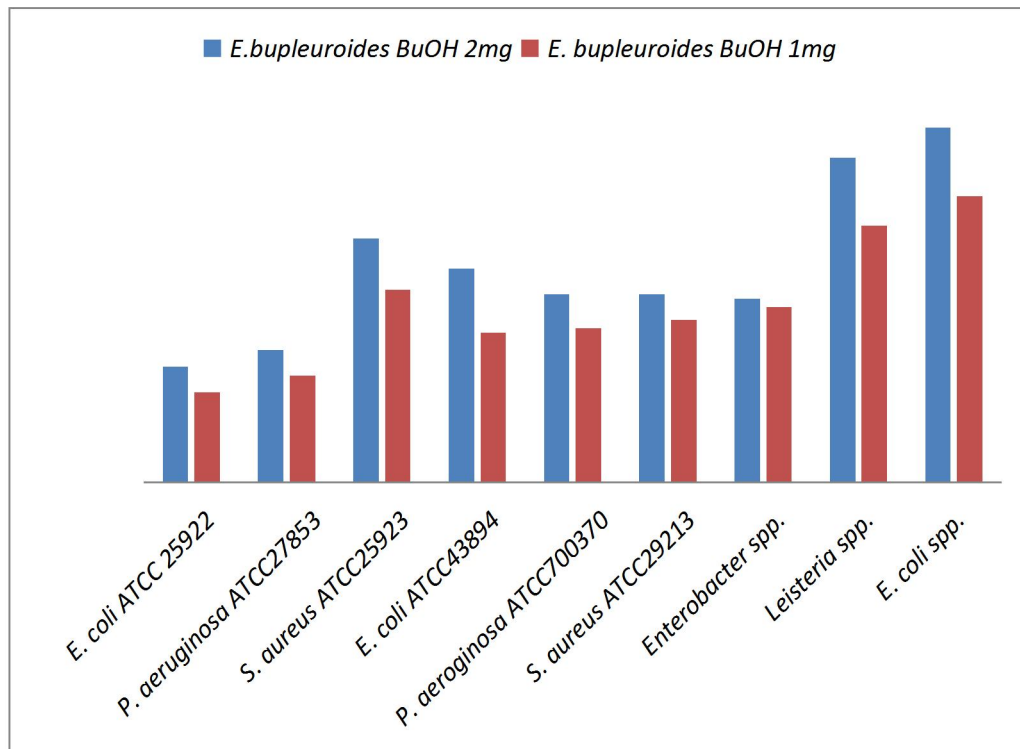


Figure 1. Antimicrobial effects of *E. bupleuroides* 1 mg and 2 mg against selected strains

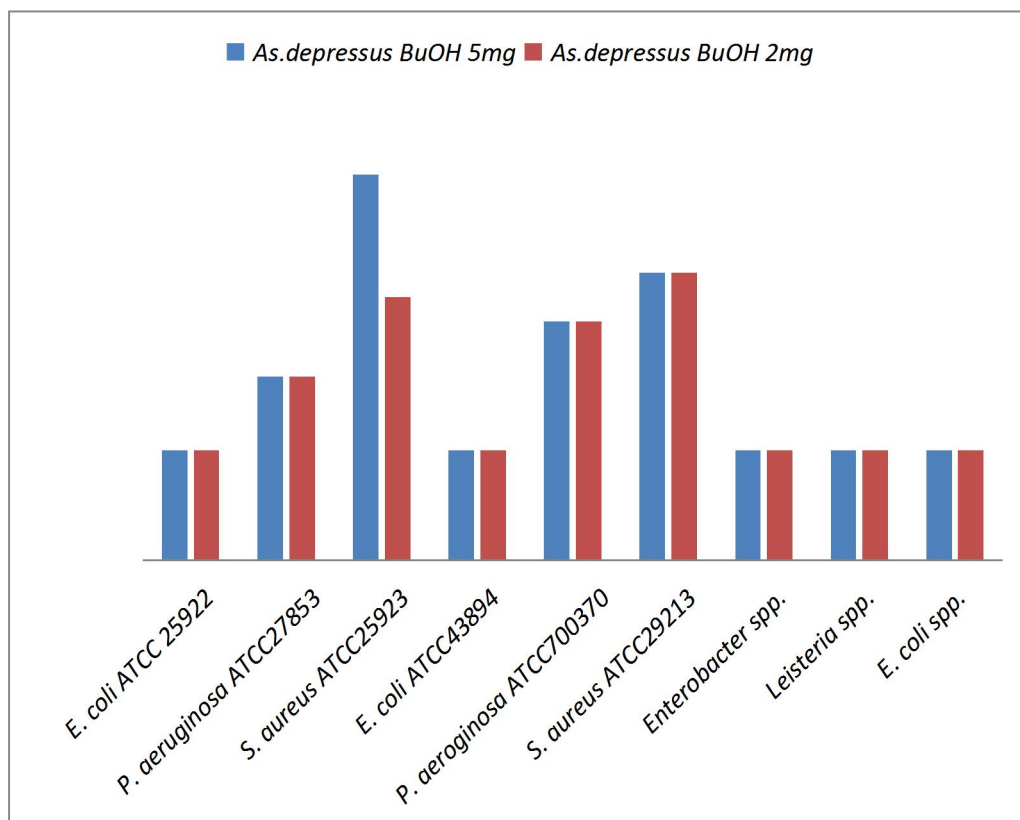


Figure 2. Antimicrobial effects of *As. depressus* 2 mg and 5 mg against selected strains

On the other hand, after reading the MIC, the transplants are carried out in streaks, on new agar; the boxes are then incubated at 37°C for 24 hours; the MBC is where the survival of bacteria is not visible (Table 4), and the MBC/ MIC ratio is then determined (Table 5) to estimate the effect bacteriostatic, the MIC and MBC results of different bacterial strains of *n*-butanol extracts of *E. bupleuroides* 2 mg and *As. depressus* 2 mg (same concentration) are compared in Table 4. We see that the MIC of *As. depressus* 2 mg extract gives a good result 4 mg/ml against *P. aeruginosa* ATCC700370 strain where other values are medium to low.

Table 4. Determination of the MIC and MBC

Plant	Bacterial strains											
	<i>P. aeruginosa</i> ATCC27853		<i>S. aureus</i> ATCC25923		<i>P. aeruginosa</i> ATCC700370		<i>S. aureus</i> ATCC29213		<i>Enterobacter</i> spp		<i>E. coli</i> Spp	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
<i>E. bupleuroides</i> BuOH 2 mg	2 mg/ml	≥ 32 mg/ml	1 mg/ml	32 mg/ml	0.25 mg/ml	32 mg/ml	0.5 mg/ml	≥ 32 mg/ml	0.25 mg/ml	≥ 32 mg/ml	0.5 mg/ml	≥ 32 mg/ml
<i>As. depressus</i> BuOH 2 mg	0.25 mg/ml	≥ 32 mg/ml	0.25 mg/ml	≥ 32 mg/ml	4 mg/ml	≥ 32 mg/ml	0.5 mg/ml	≥ 32 mg/ml	/	/	/	/

Table 5. MBC/ MIC ratio of *n*-butanol extracts of *E. bupleuroides* and *A. depressus*

Extract	Bacterial strains					
	<i>P. aeruginosa</i> ATCC27853	<i>S. aureus</i> ATCC25923	<i>P. aeruginosa</i> ATCC700370	<i>S. aureus</i> ATCC29213	<i>Enterobacter</i> pp.	<i>E. coli</i> Spp.
<i>E. bupleuroides</i> BuOH 2 mg	MBC/MIC					
	16	32	128	≥ 128	≥ 128	64
<i>As. depressus</i> BuOH 2 mg	Bacteriostatic effect					
	MBC/MIC					
	128	128	≥ 8	64	/	/
	Bacteriostatic effect					

Principal component analysis

One of the main objectives of this study is the search for the most effective extract and the most sensitive strain, the interpretation of our results by the Principal Component Analysis (PCA)(Pozzo *et al.*, 2023 and Demirpolat, 2023), demonstrates that the behavior of the

strains studied changes according to the extracts of the plants and vice versa. PCA conducted on these matrices allowed to construct a hierarchical classification calculated from the coordinates of the extracts and the strains on the first two axes. Table 6 shows abbreviation list of extracts used in Figure 3 and 4.

Table 6. Abbreviation list of extracts used in Figure 3 and 4

Extract abbreviations					
Abbreviation	Extract	Abbreviation	Extract	Abbreviation	Extract
<i>E-b</i> 2mg	<i>E. bupleuroides</i> BuOH 2 mg	<i>A-H-BuOH</i> 5mg	<i>A. humilis</i> BuOH 5 mg	<i>A-D</i> -5mg	<i>As. depressus</i> BuOH 5 mg
<i>E-b</i> 1mg	<i>E. bupleuroides</i> BuOH 1 mg	<i>A-V-EP</i> 5mg	<i>Aloevera</i> PE2, 5 mg	<i>A-D</i> -2mg	<i>As. Depressus</i> BuOH 2 mg
<i>A-V-EP</i> 5mg	<i>Aloevera</i> PE 5 mg	<i>A-F-EP</i> 5mg	<i>A. flava</i> PE 5 mg	<i>A-F-BuOH</i> 5mg	<i>A. Flava</i> BuOH 5 mg
<i>A-V-g</i> 5mg	<i>Aloevera</i> gel 5 mg	<i>E-g</i> -5mg	<i>E. guyoniana</i> Dic 5 mg	<i>A-H-BuOH</i> 15mg	<i>A. humilis</i> BuOH 15 mg

The results of principal component analysis (PCA) (Figure 3) demonstrates that the behavior of the extracts changes depending on the strains tested; that is, the extracts behave differently with respect to bacterial sensitivity.

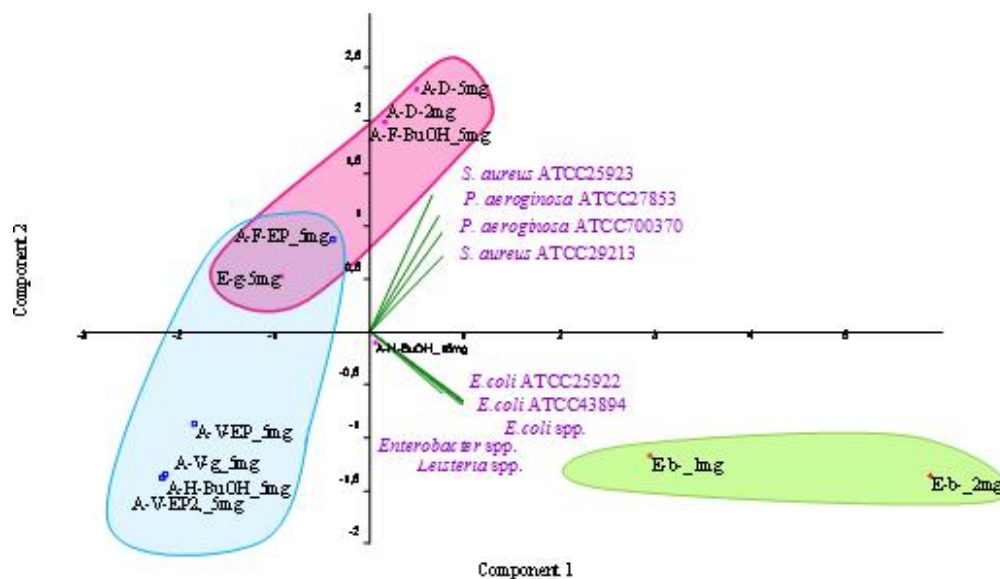


Figure 3. Representation of Principal component analysis (PCA) of different extracts

Group (1): Includes the extracts: *A. humilis* BuOH 15 mg, *A. flava* BuOH 5 mg, *As. Depressus* (2 mg and 5 mg) and *E. guyoniana*.

Group (2): Includes the extracts: *A. humilis* 5 mg, *A. flava* PE (2.5 mg and 5 mg), *Aloe vera* gel 5 mg and *Aloe vera* PE 5 mg.

Group (3): Includes the extracts: *E. bupleuroides* extract (1 mg and 2 mg).

Effectiveness of plant extracts

The dendrogram (Figure 4) confirms the results of the PCA. It differentiates in particular three groups according to the inhibitory effect “diameter of the inhibition zones”.

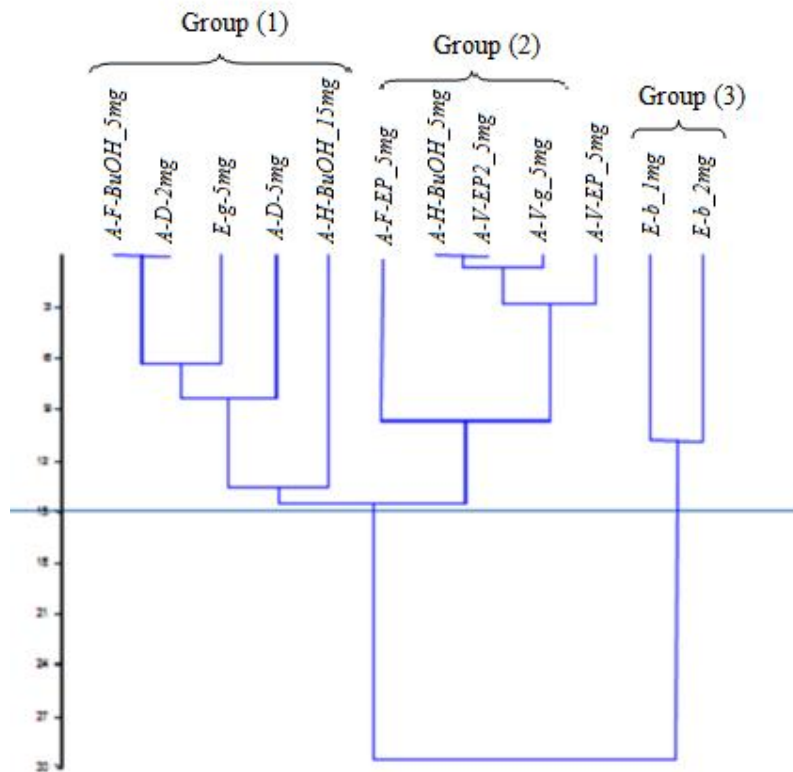


Figure 4. Hierarchical ascending classification (HAC) of PCA

Similarity between groups

The difference in composition of the groups taken two by two and confirmed by ANOSIM using the Bray-Curtis index as a distance measure, since the P values corrected by Bonferroni, obtained after 10.000 permutations, are significant; in all combinations (Table 7).

Table 7. Bray-Curtis index

	Group (3)	Group (2)	Group (1)
Group (3)		0.0488	0.0511
Group (2)	0.0488		0.0071
Group (1)	0.0511	0.0071	

According to the results obtained in this work, we can say that there is a significant difference between group (1) and (2), and between group (1) and (3), while there is no significant difference between group (2) and (3).

Group (1): Does not act on any germs.

Group (2): Acts on “*Staphylococcus aureus* ATCC25923, *Staphylococcus aureus* ATCC29213, *Pseudomonas aeruginosa* ATCC700370 and *Pseudomonas aeruginosa* ATCC29213”.

ATCC27853”

Group (3): Acts on “*E. coli* ATCC25922, *E. coli* ATCC43894, *Enterobacter* spp., *Escherichia coli* spp. and *Listeria* spp.”.

Interpretation of results

The interpretation of these results by PCA shows that the difference between the first group and the second group based on the sensitivity of the bacterial strains “*S. aureus* ATCC25923, *S. aureus* ATCC29213, *P. aeruginosa* ATCC700370 and *P. aeruginosa* ATCC27853” against group (2) extracts, while these strains were resistant to group (1) extracts. The difference is based on the activity of the extracts on the strains, not on the type of germs affected. The existence of a significant difference between the two groups (1) and (3) explained by the intense effectiveness of the extracts of group (3) with respect to the strains: “*E. coli* ATCC25922, *E. coli* ATCC43894, *E. coli* sp., *Enterobacter* spp. and *Listeria* spp.”, moreover these germs were resistant to group extracts (1). The absence of a significant difference between the two groups (2) and (3); indicates the presence of antibacterial activity of extracts from two groups against different germs. The hypersensitivity of the *S. aureus* ATCC25923 and *S. aureus* ATCC29213 strains can be explained by the probability of sensitivity of Gram-positive bacteria to external environmental changes, such as temperature, PH, and nature of extracts due to the absence of the outer membrane (Balentine *et al.*, 2006). The resistance of the *E. coli* ATCC25922 strain can be attributed to the ability of the antibacterial agent to diffuse in the agar (Hayouni *et al.*, 2007).

The values obtained by Herouini *et al.* (2015) on the crude extract of *Euphorbia guyoniana* are somewhat lower than the results we obtained with values ranging from 6.00 to 8.50 mm, while in another study conducted by Boumaza *et al.* on the aqueous extract of the same plant, it gave remarkable results on 12 bacterial strains with CMI values ranging between 6.00 to 61.78 mg/ml (Boumaza *et al.*, 2018) The difference may be due to the concentrations used, the plant parts that were used in the extraction process or in the extraction method itself, as well as the strains used in the study. All of these factors can affect in the results (Boumaza *et al.*, 2018).

In a study on honey from the plant *E. bupleuroides* for its antimicrobial activity by Latifa *et al.*, on strains of *E. coli*, *S. aureus*, *P. aeruginosa*, and *Candida albicans*, the results showed that the volatile fraction had a significant effect against *E. coli* and *S. aureus* with values ranging from 10 to 50 % of the minimum inhibitory concentrations (MIC) (Latifa *et al.*, 2020). The antibacterial activity of petroleum ether, ethyl acetate and *n*-butanol extracts of *Atractylis cancellata* (same genus of *A. humilis* and *A. flava*) against five different strains

showed results similar to those we obtained, as the petroleum ether extract was the more reactive compared to the other extracts and the strain *P. aeruginosa* ATCC 27853 was the most sensitive with 12 ± 0.1 for each of the petroleum ether and *n*-butanol extracts at the same concentration of 100 µg/mL (Sifouane *et al.*, 2020).

The acetone and methanol extracts of the *Astragalus flavescens* (Boissier) plant of the same genus of *As. depressus* collected from Turkey were subjected to antimicrobial activity against 10 different strains of bacteria, giving weak results compared to positive control amikacin where the highest value obtained was 13 mm (Akyil *et al.*, 2013).

The genus *Aloe vera* has known numerous in-depth studies related to the antibacterial activity on various extracts resulting from various parts of the plant or on the hydrogels. In recent studies, two hydrogels prepared by an all-green synthesis method were performed on a range of Gram-positive and Gram-negative strains of *S. aureus* and *P. aeruginosa*. The results showed that the new hydrogels containing green *Aloe vera* have an important antibacterial property (Chelu *et al.*, 2023). In another study, an endophytic bacteria found in *Aloe vera* was isolated and identified. This latter was characterized by the growth of 8 different colonies on Murashige-Skoog medium. It was found that these isolated colonies had a great ability to inhibit the growth of *E. coli* and *S. aureus* bacteria with the inhibition zone ranging from 7 to 20 mm (Missa *et al.*, 2023).

4. Conclusion

This work includes an ethnobotanical and microbiological study of six plants belonging to the families of *Euphorbiaceae*, *Asteraceae*, *Asphodelaceae* and *Liliaceae* growing in southern Algeria. The evaluation of the antibacterial activity was carried out using agar disk diffusion methods allowed us to determine the diameters of the inhibition zone for the different extracts studied and the values of the MIC and MBC of *E. bupleuroides* and *As. depressus* extracts. The majority of the plants studied reveal interesting antimicrobial activity against selected strains with the exception of *A. humilis* BuOH 5 mg and *Aloe vera* PE 2.5 mg extracts; these plant extracts can play a vital role as an antimicrobial agent due to their phytochemical composition. It's important to note that further research and testing would be necessary to fully understand the effectiveness, safety, and practical applications of these plant extracts in different contexts. To our knowledge, this is the first time that these results appear, particularly on the *n*-butanol extract on all of these plants.

5. References

Abu-Zaida, A. A., A. Al-Barty, K. Morsy, H. Hamdi (2022). In vitro study of antimicrobial activity of some plant seeds against bacterial strains causing food poisoning diseases. *Braz J Biol.* 82,

e256409. <https://doi.org/10.1590/1519-6984.256409>

- Ahmed, A. A., N. Gherraf, A. A. El-Bassuony, S. Rhouati, M. H. Gad, S. Ohta, T. Hirata (2006). Guyonianin A and B, Two Polyester Diterpenes from Algerian *Euphorbia guyoniana*. *Nat Prod Commun.* 1(0), 1–7.
- Aichour, S., H. Haba, M. Benkhaled, D. Harakat, C. Lavaud (2014). Terpenoids and other constituents from *Euphorbia bupleuroides*. *Phytochem Lett.* 10, 198–203. <http://dx.doi.org/10.1016/j.phytol.2014.09.011>
- Akram, M. M., B. Naima, M. Salah Eddine, O. Joubert, Z. M. Doumandji, R. Safar (2019). Gene Expression Levels of Selected Factors in Monocytic Leukemia Cell Line THP-1 Upon Treatment with n-butanol Extract of *Atractylis flava* Desf. against Cancer. *J Young Pharm.* 11(1), 36–39. [10.5530/jyp.2019.11.8](http://dx.doi.org/10.5530/jyp.2019.11.8)
- Akyil, D., S. F. Erdoğan, Y. Eren, A. Özkara, E. Korcan (2013). Potential antimicrobial and antimutagenic activities of *Astragalus flavescens*. *Fresen Environ Bull.* 22, 1868–1873.
- Aouni, M., Pelen, F., et Soulimani, R (2013). Étude de l'activité antimicrobienne d'un mélange de 41 huiles essentielles et domaines d'application. *Phytothérapie.* 11(4), 225–236.
- Azoui, I., N. Frah, B. Nia (2016). Insecticidal Effect of *Euphorbia bupleuroides* latex On *Blattella Germanica* (Dictyoptera: Blattellidae). *Int J Pure Appl Zool.* 4(3), 271–276. <http://www.ijpaz.com>
- Balentine, C. W., P. G. Crandall, C. A. O'Bryan, D. Q. Duong, F. W. Pohlman (2006). The pre-and post-grinding application of rosemary and its effects on lipid oxidation and color during storage of ground beef. *Meat Sci.* 73, 413–421.
- Batool, M., M. Khan, M. Mubarak, A. Hussain, M. Shafiq, S. Firdous, A. Saeed (2023). A Wonder Plant *Aloe vera* L. (Liliaceae): An Overview of its Folk Traditional Uses, Phytoconstituents, Biological Activities, and Cosmaceutical Applications. *Proc Pak Acad Sci: B.* 60, 337–365.
- Boudiar, T., L. Hichem, A. Khalfallah, A. Kabouche, Z. Kabouche, I. Brouard, J. Bermejo, C. Bruneau (2010). A New Alkaloid and Flavonoids from the Aerial Parts of *Euphorbia guyoniana*. *Nat Prod Commun.* 5(1), 35–37.
- Boumaza, S., O. Bouchenak, K. Yahiaoui, S. Toubal, D. El Haddad, K. Arab (2018). Effect of The Aqueous Extract of *Euphorbia guyoniana* (Euphorbiaceae) on Pathogenic Bacteria from Land-Based Sources. *Appl Ecol Env Res.* 1–15. DOI: <http://dx.doi.org/10.15666/aeer/>
- Chabani, S., H. Haba, C. Lavaud, M. Benkhaled, D. Harakat (2013). Flavonoid glycosides and triterpenoids from *Atractylis flava*. *Phytochem Lett.* 6, 9–13. <http://dx.doi.org/10.1016/j.phytol.2012.10.004>
- Chabani, S., C. Lavaud, M. Benkhaled, D. Harakat, C. Long, H. Haba (2016). Three new oleanane-type triterpene saponins from *Atractylis flava*. *Phytochem Lett.* 15, 88–93. <http://dx.doi.org/10.1016/j.phytol.2015.11.017>
- Chabert, Y. A., G. L. Daguet (1985). Techniques en bactériologie: Antibiotiques en bactériologie

- médicale. Flammarion, Médecine & Sciences, Sérologie bactérienne, Paris, pp. 244.
- Chaity, A. S., T. Nasrin, K. J. Ferdouse, M. A. Islam, B. Sikdar, M. F. Hasan (2020). Antimicrobial activity of *Kalanchoe blossfeldiana* and *Paederia foetida* plant leaves' extracts against some selected bacterial strains. *Recent Adv Biol Med.* 6(3): 1121418.
<https://doi.org/10.18639/RABM.2020.1121418>
- Chang-Liang, H., F. Ben-Dong, S. Hai-Qing, J. Xiao-Lin, W. Xu-Bin (2011). Fumaric acid, an antibacterial component of *Aloe vera* L. *Afr J Biotechnol.* 10, 2973–2977.
- Chebaibi, A., Marouf, Z., Rhazi-Filali, F., Fahim, M., et Ed-Dra, A (2016). Évaluation du pouvoir antimicrobien des huiles essentielles de sept plantes médicinales récoltées au Maroc. *Phytothérapie*, 14(6), 355–362.
- Chelu, M., A.M. Musuc, L. Aricov, E. A. Ozon, A. Iosageanu, L.M. Stefan, A.-M. Prelipcean, M. Popa, J.C. Moreno (2023). Antibacterial *Aloe vera* Based Biocompatible Hydrogel for Use in Dermatological Applications. *Int J Mol Sci.* 24, 3893. <https://doi.org/10.3390/ijms24043893>
- Cheng, C., L. Jiang, X. Li, H. Song, W. Fang (2023). Can natural preservatives serve as a new line of protective technology against bacterial pathogens in meat and meat products?. *FoodQual.* 49, 1–45.
- Demirpolat, A. (2023). Essential Oil Composition Analysis, Antimicrobial Activities, and Biosystematic Studies on Six Species of *Salvia*. *Life.* 13, 634.
<https://doi.org/10.3390/life13030634>
- Doughari, J. H., M. S. Pukuma, N. De (2007). Antibacterial effects of *Balanites aegyptiaca* L. Drel. and *Moringa oleifera* Lam. on *Salmonella typhi*. *Afr J Biotechnol.* 6(19), 2212–2215.
<http://www.academicjournals.org/AJB>
- Dulger, B., A. Gonuz (2004). Antimicrobial activity of some Turkish medicinal plants. *PJBS.* 7, 1559–1562.
- El-Demery, M., E. M. Elsebaie, N. Zidan, R. Essa (2016). Efficiency of propolis and turmeric powders as natural preservatives in minced beef. *JFDS.* 7(1), 45–50.
- Guerrero-Turriza, H. O., M. C. Soto-Barajas, N. L. Rodríguez-Ávila, D. M. Chan-Uc (2023). Comparison of aqueous extracts of *Citrus xaurantium* and *Aloe vera* L. as fungistatic control of *Fusarium* spp. *Agro product.* 103–110.
- Haba, H., C. Lavaud, H. Harkat, A. Alabdul Magid, L. Marcourt, M. Benkhaled (2007). Diterpenoids and triterpenoids from *Euphorbia guyoniana*. *Phytochemistry.* 68, 1255–1260.
[doi:10.1016/j.phytochem.2007.01.017](https://doi.org/10.1016/j.phytochem.2007.01.017)
- Haba, H., L. Marcourt, M. Benkhaled, C. Long (2013). Minor *Ent*-abietane Diterpenoids from *Euphorbia guyoniana*. *Nat Prod Commun.* 8(11), 1519–1522.
- Hammer, D.A.T., P.D. Harper, P. Ryan (2001). PAST: Paleontological statistics software package for education and data analysis, *Palaeont Electr.* 4(1), 1–9. <http://palaeo->

electronica.org/2001_1/past/issue1_01.htm.

- Hayouni, E. A., M. Abedrabba, M. Bouix, M. Hamdi (2007). The effects of solvents and extraction method on the phenolic contents and biological activities in vitro of Tunisian *Quercuscoccifera* L. and *Juniperusphoenicea* L. fruit extracts. *Food Chem.* 105 (2007), 1126–1134. doi:10.1016/j.foodchem.2007.02.010
- Hegazy, M.-E. F., A. E. H. Mohamed, N. Aoki, T. Ikeuchi, E. Ohta, S. Ohta (2010). Bioactive jatrophanediterpenes from *Euphorbia guyoniana*. *Phytochemistry*.71, 249–253. doi:10.1016/j.phytochem.2009.10.003
- Herouini, A., A. Kemassi, M.D. Ould El Hadj (2015). Etude de l'activité biologique des extraits aqueux *Euphorbiaguyoniana* (Euphorbiaceae) récoltée dans Oued Sebseb (Sahara Algérien). *ElWahat pour les Recherches et les Etudes*. 8(2), 15–25.
- Kebede, T., E. Gadisa, A. Tufa (2021). Antimicrobial activities evaluation and phytochemical screening of some selected medicinal plants: A possible alternative in the treatment of multidrug-resistant microbes. *PLoS ONE*.16(3), e0249253. <https://doi.org/10.1371/journal.pone.0249253>
- Keskinkaya, H. B., C. Akköz, E. Güneş, M. A. F. Albayati, E. Ş. Okudan, S. Karakurt (2020). Determination of antimicrobial activity of different extracts of *Enteromorpha intestinalis* (Linnaeus Nees.1820) against pathogenic microorganisms. *Bull Biotechnol.* 1, 13-18.
- Kúsz, N., P. Orvos, A. Csorba, L. Tálosi, M. Chaieb, J. Hohmann, D. Rédei (2016). Jatrophanediterpenes from *Euphorbia guyoniana* are new potent inhibitors of atrial GIRK channels. *Tetrahedron.* 72, 5724–5728.
- Latifa, H., A. Saada, M. Arezki (2020). Antimicrobial Potential of *Ziziphus* and *Euphorbia* Honeys Harvested In Semi-Arid Region Of Algeria And Their Possible Use In Soft Medicine. *J Microbiol Biotech Food Sci.* 9(6), 1114–1118. doi: 10.15414/jmbfs.2020.9.6.1114-1118
- Lahsissene, H., A. Kahouadji, M. Tijane, S. Hseini (2009). Catalogue des plantes médicinales utilisées dans la région de Zaër (Maroc Occidental):Nouvelle série N° 186. Lejeunia, Belgique, pp. 1-19.
- Lakhdari, W., A. Dehliz, R. Mlik, W. Benlamoudi, H. Hammi, R. Fathallah, I. Benyahia, N. Mekhadmi, F. Acheuk, D. Ouargli (2020). *Euphorbia guyoniana* Ethanolic Extract Efficiency Against Tomato Leaf Miner in Southeastern Algeria. *Res J Med Plants*.14, 1–7. DOI: 10.3923/rjmp.2020.
- Maamria, L., H. Haba, C. Lavaud, D. Harakat, M. Benkhaled 2015. An isoflavane and saponins from *Astragalus depressus* L. *J Serb Chem Soc.* 80, 137–142.
- Mahboubi, M., M. M. Kermani, H. K. Zadeh (2017). Main ingredients in herbal formulation may act as preservative agent. *J Herb Drugs.* 8, 116–120.

- Mantegazza, G., G. Gargari, R. Duncan, F. Consalez, V. Taverniti, P. Riso, S. Guglielmetti (2023). Ready-To-Eat Rocket Salads as Potential Reservoir of Bacteria for the Human Microbiome. *Microbiol Spectr.* 11, 1–14.
- Melakhessou, M. A., S. E. Marref, N. Benkiki, C. Marref, I. Bechecker, L. Khattabi (2021). In vitro, acute and subchronic evaluation of the antidiabetic activity of *Atractylis fava* Desf *n*- butanol extract in alloxandiabetic rats. *Futur J PharmSci.* 7(2006), 1–7. <https://doi.org/10.1186/s43094-021-00358-5>
- Moroh, J. L. A., C. Bahi, K. Dje, Y. G. Loukou, F. Guede-guina (2008). Étude de l'activité antibactérienne de l'extrait acétatique (EAC) de *Morinda morindoides* (Baker) milne- redheat (rubiaceae) sur la croissance in-vitro des souches d'*Escherichia coli*. *Bull Soc R Sci Liège.* 77(2008), 44–61.
- Mouffouk, S., C. Mouffouk, S. Mouffouk, A. H. Mekki, A. M. Messaoud, H. Haba (2023). Anti-inflammatory, antibacterial and antioxidant activities of the medicinal species *Atractylis cancellate*. *J Biol Res.* 96, 11096. doi:10.4081/jbr.2023.11096
- Missa, H., O. D. F. LeloCarion, Y. S. Enggong, A. Djalo, S. Ndukang (2023). Isolations and Antibacterial Tests of *Aloe vera* Endophytic Bacteria (*Aloe barbadensis* Miller) towards *Staphylococcus aureus* and *Escherichia coli*. *Agro Bali Agric J.* 6(1), 47–55. <https://doi.org/10.37637/ab.v6i1.964>
- Najmi, A., S. A. Javed, M. Al Bratty, H.A. Alhazmi (2022). Modern Approaches in the Discovery and Development of Plant-Based Natural Products and Their Analogues as Potential Therapeutic Agents. *Molecules.* 27,349. <https://doi.org/10.3390/molecules 27020349>
- Nasrine, S., S. M. El-Darier, H. M. El-Taher (2013). Allelopathic Effect of *Euphorbia guyoniana* Aqueous Extract and Their Potential Uses as Natural Herbicides. *Sains Malays.* 42(10), 1501–1504.
- Orlova, E. S., S. A. El-Sohaimy, M. B. Rebezov (2023). Evaluation of the antioxidant and antimicrobial activity of plant bioactive compounds as natural preservatives. *Agr Sci.* 373(8), 143–150. DOI: 10.32634/0869-8155-2023-373-8-143-150
- Palici, I. F., E. Iktor-Busa, I. Zupkó, B. Touzard, M. Chaieb, E. Urbán, J. Hohmann (2015). Study of in Vitro Antimicrobial and Antiproliferative Activities of Selected Saharan Plants. *Acta Biol Hung.* 66, 385–394.
- Parekh, J., S. V. Chanda (2007). In vitro antimicrobial activity and phytochemical analysis of some Indian medicinal plant. *Turk J Biol.* 31, 53–58.
- Piskin, B. K., G. V. Seyhan (2021). Natural Preservatives. *Int J Tradit Complement Med Res.* 2(3), 184–192. DOI: 10.53811/ijtcmr.987607
- Pozzo, L., T. Grande, A. Raffaelli, V. Longo, S. Weidner, R. Amarowicz, M. Karamac (2023). Characterization of Antioxidant and Antimicrobial Activity and Phenolic Compound Profile of

- Extracts from Seeds of Different *Vitis* Species. *Molecules*. 28, 4924.
<https://doi.org/10.3390/molecules28134924>
- Rota, M. C., A. Herrera, R. M. Martinez, J. A. Sotomayor, M. J. Jordán (2008). Antimicrobial activity and chemical composition of *Thymus vulgaris*, *Thymus zygis* and *Thymus hyemalis* essential oils. *Food control*. 19(2008), 681–687. doi:10.1016/j.foodcont.2007.07.007
- Shkondrov, A. M., I. N. Krasteva (2021). High resolution LC-MS/MS screening for secondary metabolites in bulgarian species of genus *Astragalus* L. *Quim Nova*. XY, 1–6.
- Sifouane, S., I. Benabdelaziz, M. Benkhaled, S. Gómez-Ruiz, S. Carralero, H. Haba (2020). A new aryl tetralinlignan and other phytoconstituents from *Atractylis humilis*. *Biochem Syst Ecol*. 90, 104018.
- Thongphichai, W., V. Pongkittiphan, A. Laorpaksa, W. Wiwatcharakornkul, S. Sukrong (2023). Antimicrobial Activity against Foodborne Pathogens and Antioxidant Activity of Plant Leaves Traditionally Used as Food Packaging. *Foods*. 12, 2409.
- Zabrodskii, P. F. (2020). Food Poisoning. Bacteria Associated with Food. *Acta sci. microbial*. 3(3), 1. DOI:10.31080/ASMI.2020.03.0503
- Zeroual, S., Daoud, I., Gaouaoui, R., & Ghalem, S. (2020). In vitro and molecular docking studies of DPPH with *Phoenix dactylifera* L.(Deglet-Nour) crude fruits extracts and evaluation of their antioxidant activity. *Asian Journal of Research in Chemistry*, 13(1), 52-59.
- Znad, O. S., Z. R. Zghair (2022). Antimicrobial activity of aloe-emodin from *Aloe-vera* against *Staphylococcus aureus*. *J Pak Med Assoc (Suppl. 8), Iraq Supp.: 2nd ISCOHMS*. S-124–S-128.
<https://doi.org/10.47391/JPMA.IQ-26>