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Characterisation and Antibacterial Activity of the Flavonoids Extracts from *Adiantum Capilus-Veneris, Lavandula Stoechas* and *Ajuga Iva*

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ABSTRACT

Background: Medicinal plants are important natural sources of materials for the treatment of various infectious diseases of human and therefore scientists are vigorously focusing their attention to discover natural compounds from medicinal plants with the aim of introducing new drugs which would be more effective than those available in the market. In recent year, essential oil and herbal extracts have attracted a great scientific interest due to their potential use as a source of natural antioxidants and biologically active compounds. There is a growing tendency all over the world, to shift from synthetic to natural based products including medicinal and aromatic plants. The present study is to investigate the characterisation and antibacterial activity of the main constituents of *Adiantum capilus-veneris, Ajuga iva* and *Lavandula stoechas*.
Methods and Findings: Plant materials were dried for use in preparing flavonoids extracts using organic solvents of increasing polarity. Several extracts were obtained (Crude extracts, Ethyl acetate extract, Butanol and aqueous extracts). Essential oil was extracted by hydrodistillation of the fresh material. The ethyl acetate extract is the richest in polyphenols followed by Butanol extract then crude extracts and finally aqueous extract where *Lavandula* has the highest content followed by *Adiantum* and *Ajuga*. The determination of flavonoids revealed that *Lavandula* has the highest content followed by *Ajuga* and *Adiantum* whose butanol extract represents the most flavonoid-rich fraction, second comes ethyl acetate extract followed by crude extracts and aqueous extract. The Thin-Layer Chromatography showed that the various fractions are very rich in flavonoids. Fractions of different plant species were tested against referenced pathogenic bacterial strains: *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 25853) and *Escherichia coli* (ATCC 25922).
Conclusion: *Pseudomonas aeruginosa* has a very high resistance potential against antibacterial activity of these plants.
KEYWORDS: Antibacterial activity, Essential oils, Flavonoids, Medicinal plants.

1. INTRODUCTION

Adiantum capillus-veneris belonging to the *Adiantaceae* family is one of the most common and widely distributed species [1], which consists of 150 to 200 species worldwide distributed in North America, United States, South Dakota, British Columbia, Canada and India [2]. Ethnomedicinally, the genus has been used as tonic and diuretic; in treatment of cold, fever, cough and bronchial disorders, as stimulant, emollient, purgative, demulcent, general tonic and hair tonic, in addition to skin diseases, tumors of spleen, liver and other viscera [1], in treatment of jaundice and hepatitis [3] and many other uses [4, 5]. The *Lavandula* genus consists of about 20 species of small evergreen shrubs having aromatic foliage and flowers. Lavenders (*Lavandula* spp.) belong to the family *Labiatae* (*Lamiaceae*) and have been used either dried or as an essential oil for centuries for a variety of therapeutic and cosmetic purposes. The plants are useful as decorative hedges in the garden, while the dried flowers are used in potpourris, as cooking herbs [6], and as insect repellents. Lavender essential oil is produced, usually by steam distillation, from both the flower heads and foliage, but the chemical composition differs greatly, with the sweeter and most aromatic oil being derived from the flowers [7]. *Ajuga* species are used in folk medicine of different parts of the world for the treatment of rheumatism, gout, asthma, diabetes, malaria, ulcers and diarrhea and have antibacterial, antitumor, antifeedant and vulnerary properties [8]. In view of the vast potentiality of aromatic plants, the present work deals with the antimicrobial activity of these plants.

2. MATERIAL AND METHODS

2.1. Plant materials: *Adiantum*, *Lavandula* and *Ajuga* were collected in January, March and April 2014 (flowering period) from Mascara (Northwest of Algeria). The plant material was dried to get a better extraction temperature.

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2.2. Extraction of essential oils: The fresh material from three plants (100g) was subjected to hydrodistillation for 3 hours, using a Clevenger-type apparatus (ST15 OSA, Staffordshire, UK). The obtained distillate (100 mL) was extracted twice successively with 100 mL of n-hexane and methanol. The mixture was dried with anhydrous sodium sulphate and the solvent was evaporated using a rotatory vacuum evaporator.

2.3. Extraction of flavonoids: The aerial parts of the three plants have been thoroughly cleaned and dried at room temperature and then milled using a mortar. The method of Merghem et al. [9] was followed for the extraction of flavonoids using organic solvents of increasing polarity. 100 g of the powder was made up to 1 liter with methanol/distilled water (85/15 v/v). The mixture was subjected to stirring overnight at 4°C and then allowed to stand for several hours. The floating fraction was subsequently filtered to give the filtrate 1 and stored at 4°C. The extraction was repeated once and the precipitate was added to 1 liter 50% methanol to yield the filtrate which 2 was mixed with the filtrate 1. Hydro alcoholic macerates are then combined and evaporated to dryness under vacuum using a rotary evaporator. The dry residue is taken up in boiling distilled water (200 mL) which quantitatively solubilizes phenolics compounds; decantation for 12 hours and followed by filtration to eliminate the "sludge" (fats, resin). Crude extracts thus obtained were subjected to several extractions with various organic solvents: diethyl ether (removes chlorophyll pigments, carotenoids and fat, all non-phenolic compounds), ethyl acetate (mono-o-glucoside and partially di-o-glucosides) and butanol (this solvent will cause the rest essentially of di-o-glycoside, tri-o-glycosides and c-glycosides). The aqueous phase and the solvent are mixed thoroughly by leaving out every time the product gases. After standing for an hour and a half, the water phase and the solvent used in charge of its specific compounds are recovered separately. After several washes, it also takes the remaining aqueous phase containing flavonoids. The phases are evaporated to dryness using a rotary evaporator and taken up in methanol for assays and chromatographic diagnosis.

2.4. Determination of total polyphenols and flavonoids content: polyphenols content was determined as previously described by Miliauskas et al. [10]. Determination of flavonoids was carried out by the colorimetric method described by Ardestani and Yazdanparast [11]. Results are expressed in equivalent mg catechin per gram of dry vegetable matter (mg EC/g E).

2.5. Thin-Layer Chromatography (TLC): TLC analysis was made using the elution system consisting of: Toluene/n-butanol/methanol/petroleum ether (40/30/30/05). After elution, the plate was dried at room temperature and examined using UV at 256 nm and 366 nm. Retention values were calculated.

2.6. Evaluation of antibacterial activity

2.6.1. Test bacteria: In this study, we tested extracts of different plant species against referenced pathogenic bacterial strains involved in nosocomial infectious, some of which have acquired resistance to antibiotics. Gram positive bacteria, *Staphylococcus aureus* (ATCC 25923) and Gram negative bacteria, *Pseudomonas aeruginosa* (ATCC 25853) with *Escherichia coli* (ATCC 25922) were kindly provided by the Medical analysis laboratory of the Oran's hospital (western Algeria). All the test bacteria were maintained on nutrient agar slope at 4°C, then sub cultured for 24 hours at 37 ± 1°C before use.

2.6.2. Preparation of test sample and inoculums: The various extracts were evaporated to dryness and dissolved in dimethyl sulfoxide (DMSO). The samples are sterilized by filtration through a 0.45 mm filter paper. A preliminary test was performed to determine effect of different percentages of DMSO on the microbial growth. Inoculum preparation was carried out as described by Atwal [12]. Briefly, incolum was obtained from a culture of 18-24 h of the bacteria cultured on agar medium , suspended in saline (0.85 % NaCl) equivalent to 0.5 McFarland (10^8 CFU/mL). The suspension may be obtained by measuring the optical density equal to 0.1 at 625 nm. The inoculum is then diluted 1/100 in sterile saline to a final concentration of 10^6 CFU/mL. The tests were performed on agar and liquid medium Muller Hinton [13].

2.6.3. The antibiogram: Antibiotics selected were: Erythromycin (E: 15 UI), Gentamicin (G: 10 UI), Tetracycline (TE: 30 µg), Spiromycine (SP: 100 µg), Amoxicillin (AMX: 25 µg) and Ampicillin (AM: 10 µg). We placed on the dried agar (MH) different disks of selected antibiotics, and then the boxes are left dried for 30 minutes at room temperature to get a good spread of the antibiotic. The plates were incubated at 37±2 ºC for 18 to 24 hours. Inhibition zone's diameter allowed us to classify the studied bacteria into three categories: sensitive, intermediate or resistant.

2.6.4. Agar diffusion method: Antibacterial activity of different extracts was evaluated by the disk diffusion method using Mueller-Hinton agar [13]. The sterile disks were separately impregnated with the extract for hours. In sterile *Petri* dishes, culture medium Muller Hinton was poured, left for 15 min for solidification, and then 1 mL of inoculum was deposited. The disks containing various extracts were transferred to the respective plates. The latter were incubated at 37 ± 1°C for 24 h, and the diameter (mm) of inhibitory zones around each disc was measured [14]. The antimicrobial activity is considered positive when the inhibitory zone diameter is greater than 6 mm according to the antibiogram committee of the French Society of Microbiology, 1994 [15].

2.6.5. Determination of MIC and MBC: The MIC and MBC of the tested extracts were determined using agar dilution method [16]. 100 µL of each sample were added to the first well, then a serial dilution was carried out (1/2). 100 µL of the of 18 hours bacterial suspension in the broth Muller Hinton (10^6 CFU/mL) are added to each well. The plates are shaken and incubated at 37°C for 24 hours. The lowest concentration required to completely

inhibit the growth of the tested microorganism was designated as the MIC and is expressed in µg/mL. The MBC is defined as the lowest concentration that kills 99.99% of the burden of the initial inoculums. For the determination of the MBC, 100 µL of each well that showed no change in culture were inoculated on nutrient agar and incubated at 37°C for 48 hours and the lowest concentration showing no growth after incubation is considered the MBC. All experiments were conducted in triplicates and results were expressed as mean ± SD.

3. RESULTS AND DISCUSSION

3.1. Content of total phenols and flavonoids: As shown in table 1, the Ethyl acetate extract (EAE) was the richest in polyphenols followed by Butanol extract (BtE), Crude extract (CrE) and Aqueous extract (AqE). *Lavandula* had the highest content followed by *Adiantum* and *Ajuga*. Similarly, *Lavandula* had the highest content of flavonoids followed by *Ajuga* and *Adiantum*.

Table 1: Polyphenols and flavonoids content in the studied extracts.

		CrE	EAE	BtE	AqE
Ajuga	Polyphenols (mg EGA/g E)	9.57±0.33	159.74±4.39	48.09±0.33	3.49±0.22
	Flavonoids (mg EC/g E)	5.19±0.27	33.60±2.98	40.14±0.33	1.23±0.02
Adiantum	Polyphenols (mg EGA/g E)	23.44±0.14	101.85±2.84	81.90±0.42	10.24±0.69
	Flavonoids (mg EC/g E)	4.66±0.097	31.60±1.48	34.43±0.42	0.18±0.002
Lavandula	Polyphenols (mg EGA/g E)	43.15±0.51	189.16±2.12	104.67±1.98	36.02±0.76
	Flavonoids (mg EC/g E)	35.06±0.31	88.75±1.79	90.83±2.01	14.71±0.29

EAE: Ethyl acetate extracts, BtE: Butanol extracts, CrE: Crude extract, AqE: Aqueous extract.

The concentration of polyphenolic substrates, degradability depends, is likely to be influenced by the method of cultivation, the phenological stage (with time, the plants tend to the garden woodiness and decrease the nutritional value), soil type (dry soils are sandy, infertile and low in nutrients), the climate at the time of sampling (temperature and precipitation) and the sample constitution (sheet - proportion of flower stems) [10, 17]. Variations in our results may be attributed to manipulations (drying method, grinding and storage) [18].

Phenolic compounds are ubiquitous in plants, possessing many biological activities due to their capability to act as free-radical scavengers [19]. The beneficial properties and importance of phenolic compounds may be linked to the well-recognized role of these substances as substrates for oxidative reactions (by both enzymatic and chemical mechanisms), as well as protective agents against the oxidative damage caused by free radicals [20]. The mechanisms responsible for the phenolic toxicity to microorganisms include enzyme inhibition by the oxidized compounds through specific or nonspecific interactions with proteins [21]. Flavonoids are hydroxylated phenolic substances, widely distributed in plants [22]. They can be synthesized by plants in response to microbial infection, and can consequently act as effective antimicrobial substances against a wide array of microorganisms. Flavonoids have been isolated from the genus *Adiantum* [23]. Quercetin, keampferol and their glycosides are the most common flavonols [24]. According to the results obtained by Jakovljević et al. [25], the highest flavonoid content of *Ajuga chamaepitys* subsp. *chamaepitys* was obtained in the ethyl acetate extract (91.76 mg RU/g), followed by the methanol (63.87 mg RU/g) and acetone extracts (61.77 mg RU/g) and the highest total phenolic content determined in the ethyl acetate extract (57.02 mg GA/g). The flavonoid contents in the extracts of *A. capillus veneris* obtained by Pourmorad et al. [26] is 78.3 ± 4.5 mg g^{-1}.

3.2. Chromatography of flavonoids fractions: The TLC showed that the various fractions are very rich in flavonoids. In *Ajuga* (Figure 1, table 2), we identified flavonol, flavone, aurone, phenolic acids and anthocyanidin 3-glycosides. Flavonic pool in this species is dominated by flavonols and phenolic acids. Compounds identified in *Adiantum* (Figure 2, table 2) were flavonols, flavones and phenolic acids with predominance of phenolic acids. For *Lavandula* (Figure 3, table 2), we identified flavones, phenols, flavonols, phenolic acids, anthocyanidin 3-glycoside and anthocyanidin 3,5-diglycosides. The flavonic pool in this species was dominated by flavones and phenolic acids.

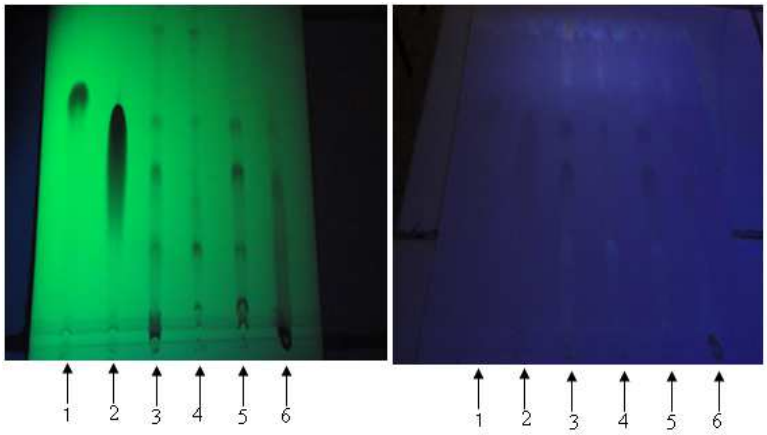


Figure 1: Analysis of flavonoids fractions of *Ajuga iva* by TLC on silica gel (revelation under UV). [1: Catechin, 2: Gallic acid, 3: Crude extract, 4: Ethyl acetate extract, 5: Butanol extract, 6: Aqueous extract].

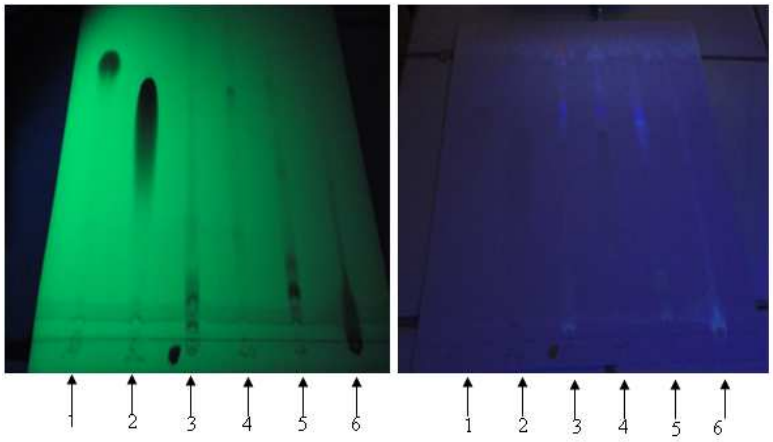


Figure 2: Analysis of flavonoids fractions from *Adiantum capilus-veneris* by TLC on silica gel (revelation under UV). [1: Catechin, 2: Gallic acid, 3: Crude extract, 4: Ethyl acetate extract, 5: Butanol extract, 6: Aqueous extract]

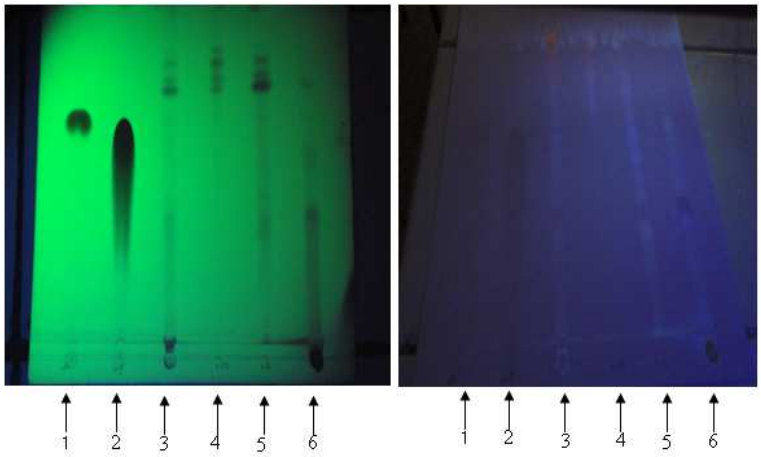


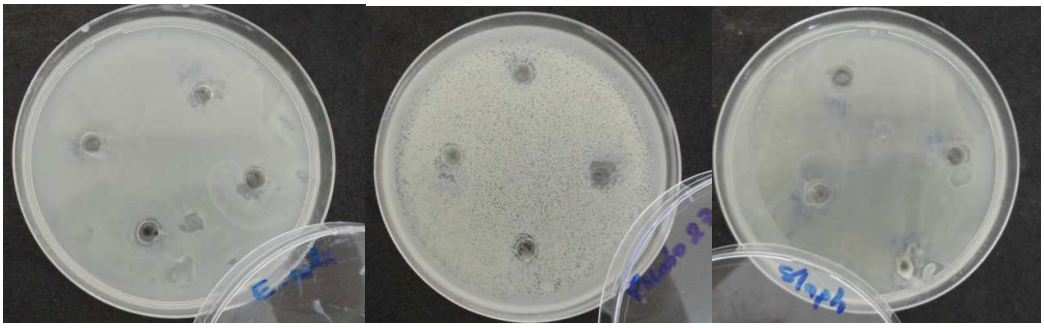
Figure 3: Analysis of flavonoids fractions from *Lavandula stoechas* by TLC on silica gel (revelation under UV). [1: Catechin, 2: Gallic acid, 3: Crude extract, 4: Ethyl acetate extract, 5: Butanol extract, 6: Aqueous extract]

Table 2: Results of TLC analysis

	Ajuga iva		*Adiantum*		*Lavandula*	
	RF	**Flavonoids [9]**	**RF**	**Flavonoids [9]**	**RF**	**Flavonoids [9]**
Crude extract	0.02	Flavonol, Flavone, Aurone	0.04	Flavonol , Flavanone, Aurone	0.02	Phenolic acid
	0.05	Phenolic acid	0.05	Phenolic acid	0.05	Phenolic acid
	0.27	Anthocyanidin 3-glycosides	0.24	Phenolic acid	0.11	Flavone
	0.39	Flavone	0.39	Flavone	0.22	Flavone
	0.43	Flavonol	0.43	Flavonol	0.45	Anthocyanidin 3-glycosides
	0.65	Phenolic acid			0.48	Anthocyanidin 3-glycosides
	0.77	Anthocyanidin 3-glycosides			0.53	Anthocyanidin 3,5-diglycosides
					0.59	Flavone
Ethyl acetate extract	0.02	Flavonol, Flavone, Aurone	0.38	Flavonol	0.38	Flavone
	0.05	Phenolic acid	0.72	Flavonol	0.44	Flavone
	0.27	Flavonol			0.48	Flavone
	0.38	Flavonol			0.53	Phenolic acid
	0.44	Flavonol				
	0.67	Flavonol				
	0.77	Phenolic acid				
Butanol extract	0.02	Flavonol, Flavanone, Aurone	0.05	Phenolic acid	0.25	Flavone
	0.05	Phenolic acid	0.08	Phenolic acid	0.34	Phenol
	0.39	Flavonol	0.19	Flavone	0.37	Phenolic acid
	0.44	Flavone	0.24	Phenolic acid	0.39	Flavone
	0.73	Phenolic acid	0.37	Phenolic acid	0.40	Flavone
			0.73	Phenolic acid	0.41	Flavone
					0.44	Flavonol
					0.51	Flavonol
					0.58	Phenolic acid
Aqueous extract	0.24	Phenolic acid	0.93	Flavone	0.32	Flavonol
	0.43	Flavonol			0.37	Phenolic acid
					0.38	Flavonol , Flavone, Chalcone, Isoflavone, Flavanone
					0.45	Flavonol , Flavone , Chalcone, Isoflavone, flavanone

Warda et al. [27] and Teixeira et al. [28], reported that *Lavandula stoechas* is very rich in terpenes, sterols, flavonoides, sponines, tannins and alkaloid. According to Ez Zoubi et al. [29], phytochemical screening of extract of *L. stoechas* revealed a presence of tannins, catechic tannins, flavonoids, sterols, coumarins, leucoanthocyans and mucilages compounds. However, gallic tannins and quinones were not detected. Ours results are in accordance with other studies related to the *Lavandula* family. The species of this family produce flavonoids, tannins [30] and coumarins [31]. Sixty-seven components consisting 88.22% of total oil of *Adiantum capillus- veneris* were identified by Laleh et al. [32]. Among identified phytochemicals, carvone was the main compound (31.58%). Previous phytochemical investigations of *L. stoechas* revealed the presence of acetylated glucoside of luteolin and flavone glucoside in ethylacetate extracts of the aerial parts [33].

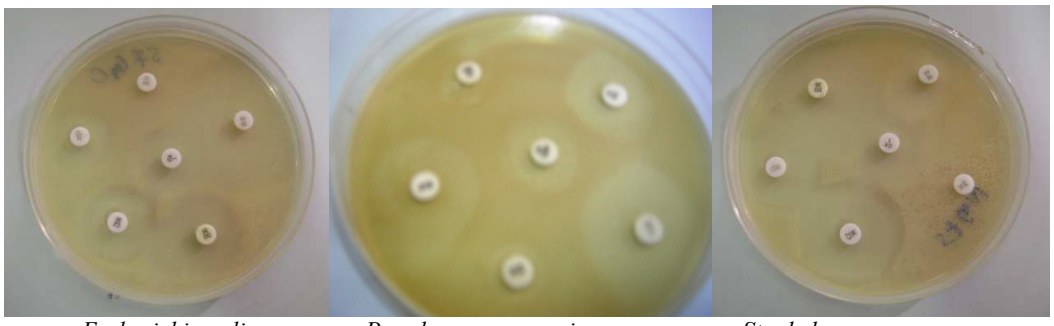
3.3. Antimicrobial activity: The preliminary test using DMSO at various concentrations (100%, 50%, 25% and 12.5%) was performed in order to select the concentration with no effect on normal growth of the bacterial strains. According to our results (figure 4), we found that DMSO at different concentrations had no effect on the bacterial growth. Our results are in consistence with previous studies [16, 34].



Escherichia coli *Pseudomonas aeruginosa* *Staphylococcus aureus*

Figure 4: Effect of DMSO (100%, 50%, 25% and 12.5%) on the growth of the studied bacterial strains

3.3.1. The antibiogram: Considering all these results, it appears that among the most active antibiotics on Gram-negative bacteria, Spiramycin was the most active against the two strains followed by Gentamycin, Tetracycline and Erytromycine. By contrats, Amoxicillin, generally specific antibiotic of Gram-negative bacteria was inactive on all strains. Similar findings were obtained with Ampicillin. For Gram-positive bacteria, Staphylococci were sensitive to the most antibiotics except Ampicillin. All bacterial strains were resistant to antibiotics (Figures 5 and 6).



Escherichia coli *Pseudomonas aeruginosa* *Staphylococcus aureus*

Figure 5: Antibiogram of *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*

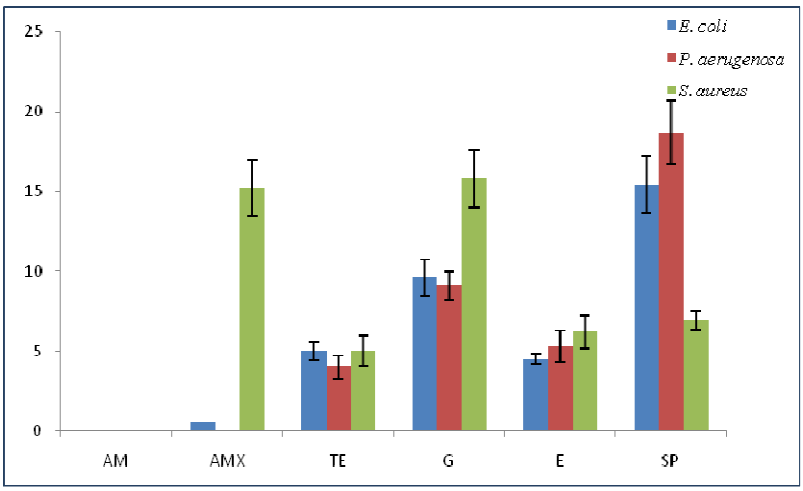
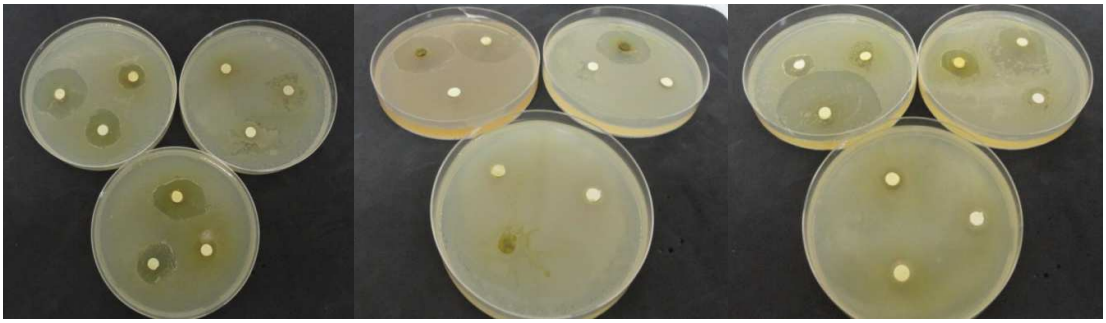


Figure 6: Results of the antibiogram (diameter of inhibitory zone in mm).
[**E:** Erythromycin, **G:** Gentamicin, **TE:** Tetracycline, **SP:** Spiromycine, **AMX:** Amoxicillin, **AM:** Ampicillin].

3.3.2. Disk diffusion method: The inhibitory zones of various strains with different samples are presented in table 3. No inhibition zone was observed around the disks after the end of incubation of *Pseudomonas aeruginosa*. This strain had a very high resistance potential against antibacterial activity of these plants. Essential oil of *Adiantum* showed the lowest activity compared to the other two oils, noting that it was without activity against *P. aeruginosa* and *E. coli.* These results are in line with those reported by Victor *et al.* [2] showing a mild activity against *P.*

aeruginosa of this oil. A low zone of inhibition was observed with *S. aureus* (5 mm). For extracts of *Adiantum*, the aqueous fraction appears without effect on all strains, the butanol and ethyl acetate fractions against *S. aureus* and the crude extract against *E. coli*. Among the three plants, all extracts of *Ajuga* could be considered without antibacterial activity. The essential oil of *Lavandula* showed moderate activity, the diameters of the inhibitory zones did not exceed 13 mm. The highest activity was noted against the *S. aureus* with an average value of 12 mm. 7mm inhibitory zone was obtained with the same oil against *E. coli*. The flavonoid extracts of *Lavandula* exhibited the strongest antibacterial activity, especially against *S. aureus* with an inhibitory zone up to 30 mm. An important activity of the crude extract and ethyl acetate extract against *E. coli* was noticed. The antibacterial activity of essential oils can be attributed to the presence of phenols such as eugenol, carvacrol, isoborneol, thymol, vanillin and salicylaldehyde [35]. According to Sato *et al.* [36], molecules that are endowed with antibacterial power are flavone (luteolin and its derivatives). Luteolin free OH is equipped with a very significant inhibitory effect on *Staphylococcus aureus*, the most sensitive, *Escherichia coli* and finally *Pseudomonas aerogenosa* which has a high resistance. This test is a screening of antibacterial activities of the extracts; it allows us to select for each strain the extract, with the most antibacterial activity among all natural extracts (Figures 7 and 8).



Essential oil Crude extract Ethyl acetate extract

Butanol extract Aqueous extract

Figure 7: The antibacterial activity of the extracts by the disk diffusion method.

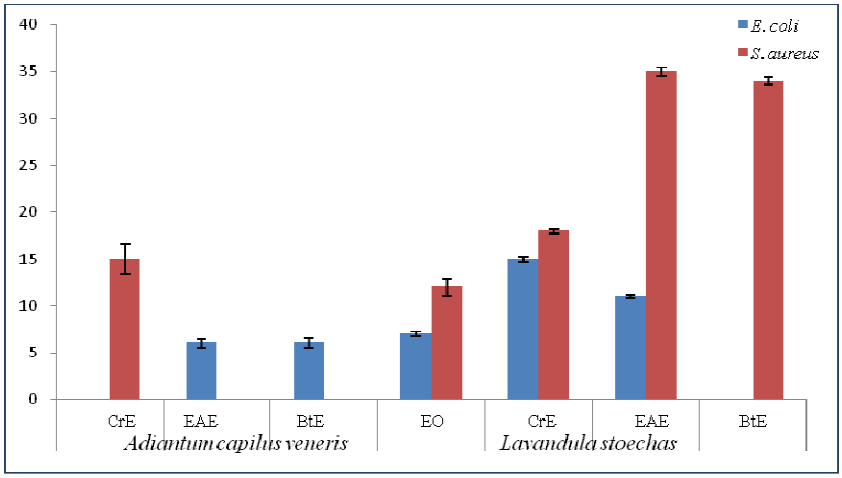


Figure 8: Results of the screening of antibacterial natural extracts made by the disk diffusion method. [CrE: Crude extract, EAE: Ethyl acetate extract, BtE: Butanol extract, EO: Essential oil].

Table 3: Antibacterial activity of essential oil and flavonoids extracts (diameter of the inhibition in mm).

		Essential oil	Crude extract	Ethyl acetate extract	Butanol extract	Aqueous extract
Ajuga iva	*P. aeruginosa*	0.00	0.00	0.00	0.00	0.00
	Escherichia coli	5 ± 0.03	0.00	0.00	0.00	0.00
	S. aureus	2 ± 0.14	0.00	0.00	5 ± 1.1	3 ± 0.4
Adiantum	*P. aeruginosa*	0.00	0.00	0.00	0.00	0.00
	Escherichia coli	0.00	0.00	6 ± 0.4	6 ± 0.48	0.00
	S. aureus	5 ± 0.58	15 ± 1.6	3 ± 0.12	5 ± 0.2	0.00
Lavandula	*P. aeruginosa*	0.00	0.00	0.00	0.00	0.00
	Escherichia coli	7 ± 0.25	15 ± 0.32	11 ± 0.17	0.00	0.00
	S. aureus	12 ± 0.88	18 ± 0.23	35 ± 0.46	34 ± 0.37	0.00

Earlier studies reported that extracts from leaves of *Adiantum latifolium* showed antibacterial activities against *S. aureus, Escherichia coli* and *Pseudomonas aeruginosa* [37 - 39]. The anti microbial activity of the methanolic extract of *Adiantum capillus-veneris* was far more active against the test bacterial strains: *Bacillus subtilis*, *Pseudomonas eurogenosa, Streptococcus faecalis*, *Salmonella typhi* and *Staphylococcus aureus* [40].

Lavenders' antimicrobial activity is usually attributed to their terpenic compounds [41, 42]. Recent studies also report that *L. pedunculata* essential oils were also used to evaluate the antifungal activity and significant results were found against dermatophyte strains [43]. It has also been suggested that essential oils, including lavender, may be useful in treating bacterial infections that are resistant to antibiotics [44]. Ours results of *L. stoechas* antimicrobial activity are confirmed by the work of Warda et al. [27]. Teixeira et al. [28] reported that the majority of the extracts of *L. stoechas* subsp. *luisieri* presented moderate activity (MIC 62 µg/mL) against *Staphylococcus aureus* strains and also against other Gram-positive bacteria tested. On the other hand, against the Gram-negative strains, only *n*-hexane extract showed activity against *K. pneumonia*.

Our results are not confirmed findings of Makni et al. [45] about Tunisian *Ajuga iva* L. According to last authors the methanol extracts of *Ajuga iva* in Tunisia exhibited promising antibacterial and antifungal activities. The interest of the studied species as a source of antimicrobial agents is supported by other data [46] showing that *Ajuga iva* L. extract in Algeria is potentially a good source of antimicrobial agents and demonstrating the importance of such plant in urolithiasis medicine and alternative healthcare. Previous reports show that Gram-negative bacteria are generally less sensitive to herb extracts than Gram-positive bacteria, due to the significant outer layers differences between Gram+ and Gram- bacteria [47]. Gram negative bacteria possess an outer membrane and a unique periplasmic space not found in Gram positive bacteria [48, 49]. According to the results obtained by Ayari et al. [50], the most important antibacterial activity of *Ajuga iva* essential oil was observed against Gram-positive bacterial strains: *Staphylococcus aureus* ATCC 25923 and *Enterococcus faecalis* ATCC 29212. Our results are confirmed by the work of Zerroug et al. [51] and not agreement with the work of Jakovljević et al. [25]. In contrast other workers have demonstrated that aqueous extracts of *Ajuga iva* reduced the formation of calcium oxalate crystals in artificial urine, suggesting that preparations from this plant might be helpful in decreasing the incidence of kidney stones [52].

3.3.3. Determination of MIC and MBC: In a second step, we determined the antibacterial potency by micro dilution method. For this, we selected extracts having a strong antibacterial capacity, for which we have determined the minimum inhibitory concentration (MIC) and minimum bactericidal concentrations (MBC). These antibacterial activities were not due to the presence of a particular substance but only due to the synergistic or antagonistic effect of each component of the extract [53]. We also noted that the bacterial strains group (Gram + or Gram -) does not affect sensitivity. The susceptibility of bacteria is independent effect of Gram [54]. Aligiannis *et al.* [55] proposed a classification of plant material on the basis of MIC results as follows: Strong inhibition: MIC less than 500 µg/mL and moderate Inhibition: MIC ranging from 600 to 1500 µg/mL. The results of the determination of the MIC and MBC are shown in table 4. The ethyl acetate fraction had a better activity compared to the other fractions with MIC below 40 µg/mL for both plants. MIC was found to be 18 µg/mL against *S. aureus* (*Lavandula*) and 25 µg/mL against *E. coli* (*Adiantum*). The butanol and crude extracts also showed good antibacterial activity with MIC and MBC very low compared to standards. Similarly, the essential oil of *Lavandula* appeared to be very active on *S. aureus* and *E. coli* at very low concentrations. The antibacterial activity of plant extracts is due to their richness in phenolic compounds [56]. Most of the work studying the mechanism of action of phenolic compounds, argue that their primary site of action is bacterial plasma membrane [57]. They are able to disintegrate the bacterial cell membrane [58]. The membrane loses its structure and becomes more permeable to ions [59] and may also allow for the dissipation of the pH gradient, the decrease of the membrane potential and the inhibition of ATP synthesis [58]. Currently, these oxygenated monoterpenoids compounds attack not only the wall and cell membrane permeability and destroying its releasing intracellular components, but they are likely to interfere with various cell functions: electron transport, protein and acid nucleic synthesis, enzymatic reactions [57]. The variability of the antibacterial activity of three plants is closely related to the chemical composition of extracts and the synergistic or antagonistic effect of each

component present in low concentration [53]. *Pseudomonas aerogenosa* strain is very resistant, this can be linked either to the heavy inoculums that was used, or to its ability to develop resistance to many antimicrobial agents hence its frequent involvement in hospital infections.

Table 4: Determination of the MIC and MBC (µg/mL).

		Essential oil		Crude extract		Ethyl acetate extract		Butanol extract	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Adiantum	*E. coli*	-	-	-	-	25	>25	85	>85
	S. aureus	-	-	72.5	290	-	-	-	-
Lavandula	*E. coli*	112.26	224.53	33.25	66.5	36	>36	-	-
	S. aureus	28.06	112.26	133	266	18	>36	52.5	105

4. CONCLUSION

The present study aimed to characterize and evaluate the antibacterial activity of the flavonoids extracts and essential oils from *Adiantum capilus-veneris, Ajuga iva* and *Lavandula stoechas*. The ethyl acetate extract was the richest in polyphenols followed by Butanol extract, crude extracts and finally aqueous extract. *Lavandula* had the highest content followed by *Adiantum* and *Ajuga*. *Lavandula* contained the highest content of flavonoids followed by *Ajuga* and *Adiantum*. *Adiantum* butanol extract has been the most flavonoid-rich fraction, followed by ethyl acetate extract, crude extracts and aqueous extract. TLC results showed that the various fractions were rich in flavonoids. Regarding antimicrobial activity, *Pseudomonas aeruginosa* presented a very high resistance potential against the different extracts. These results allow us to conclude that the antibacterial properties of plant extracts are related to their chemical composition. The latter could vary between different species of the same genus and even between samples of the same species.

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