Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2021, 13(1): 33-39
(http://www.derpharmachemica.com/archive.html)

Phytochemical Screening and Evaluation of Antibacterial Activity of Extracts from Leaves and Roots of *Echium Vulgare* of the Region of Tlemcen

Nesrine Bentabet[1,2], Kebir boucherit[1], Boucherit-Otmani zahia[1] and Nadjib Rahmoun[1]

[1]*Faculty of Natural and Life Sciences, Earth and Universe Sciences, Abou Bekr Belkaïd University, BP 119, Imama, Tlemcen, Algeria*
[2]*Faculty of Natural and Life Sciences, Earth and Universe Sciences,University Belhadj Bouchaib, Ain Temouchent*

***Corresponding author:** Nesrine Bentabet, Faculty of Natural and Life Sciences, Earth and Universe Sciences, Abou Bekr Belkaïd University, BP 119, Imama, Tlemcen, Algeria, E-mail:nes_tab200701@yahoo.fr*

ABSTRACT

Objectives: Our work is part of the search for new natural antibacterial agents from a plant that grows wild in the region of Tlemcen (Remchi) called *Echium vulgare* and belonging to the family Boraginaceae.

Methods: Triphytochimical study was conducted using several experimental protocols. The antibacterial activity of the different samples was evaluated by two methods: Disk diffusion method and the dilutions against four bacterial strains Gram positive and four Gram negative. The minimum inhibitory concentrations (MICs) were determined by growth in liquid medium microplate (NCCLS).

Results: Phytochemical tests performed in this study revealed the presence of different families of existing chemicals with varied intensity between the two parts of the plant. Yields are higher in leaves compared to roots. The results show that all extracts are endowed with antibacterial activity with inhibition diameters between 07 mm and 22 mm. The calculation of CMI extracts of *Echium vulgare* against bacteria showed good antibacterial activity (0.95 mg/mL ≤ MIC ≤ 13.5 mg/mL).

Conclusion: The extracts showed variable antimicrobial activities against different bacterial strains tested. This variation can be explained by the variation of the chemical composition of the plant studied.

Keywords: Antibacterial activity, CMI, Echium vulgare, Phytochemical study, Flavonoids

INTRODUCTION

Infectious pathologies constitute a real public health problem worldwide. They are caused by viruses, bacteria or parasitic eukaryotes [1]. Since the discovery of the first antibiotic; penicillin, the fight against infectious diseases with new molecules has undergone a real revolution. However, the appearance of resistant microorganisms and the toxicity of certain anti-infectious currently constitute a real problem of antibiotic therapy [2,3].

Indeed, the emergence of resistance is due on the one hand, to the misuse of antibiotics and on the other hand, to the infinite capacity of germs to mutate under the pressure of antimicrobials, as well as to their speed of replication [4,5].

Although bacterial diseases are getting better and better diagnosed; they always cause a mortality problem such as: pneumonia, tuberculosis, meningitis, septicemia and typhoid. In 2007, the World Health Organization (WHO) estimated more than 3.3 million cases of bacteremia worldwide, including nearly 850,000 cases in Africa and 8,700 cases in Algeria [6].

The use of natural resources in general and medicinal plants in particular then becomes one of the most important and interesting avenues to explore for the search for new, more effective antibacterial products [7,8]. The study of medicinal plants for their antimicrobial activities is an interesting prospect for the discovery of new antimicrobial agents [9,10]. This is why we proposed to carry out this study which consists in evaluating the antibacterial activity of the plant *Echium vulgare* which is traditionally used in Algeria in the treatment of several ailments.

MATERIALS AND METHODS

This work was carried out in the laboratory "Antifungal Antibiotics: physico-chemistry, synthesis and biological activity" Department of Biology-University Abou Bekr Belkaïd, of Tlemcen.

Biological material

Plant material

Our study focused on the plant "Echium vulgare" harvested during the month of March 2013 in the region of Remchi which is located 21 km northwest of the city of Tlemcen (Algeria). The leaves and roots were dried in the shade and protected from humidity in an oven at 25°C for a week. Once dried, these were crushed and then subjected to extraction. For the evaluation of the antibacterial activity of the different plant extracts, we have chosen reference bacterial strains (Table 1). These were supplied by our laboratory. The strains are maintained by successive subcultures and then stored at 4°C on inclined nutrient agar.

Table 1: The bacterial strains used.

Gram positive bacterial strains	Gram negative bacterial strains
Enterococcus faecalis ATCC 25212	*Escherichia coli ATCC 25933*
Staphylococcus aureus ATCC 25923	*Enterobacter cloacae ATCC 13047*
Staphylococcus aureus methicillin resistant ATCC 43300	*Acinetobacter baumannii ATCC 19606*
Bacillus cereus ATCC 25212	*Klebsiella pneumoniae ATCC 70603*

METHODS

Phytochemical screening

Phytochemical examination is necessary to identify the main families of secondary metabolites existing in the two parts of the plant studied. We characterized the presence of this metabolites (saponosides, alkaloids, flavonoids, tannins, coumarins and reducing compounds) by preparing three extracts of increasing polarity (diethyl ether, ethanol and water) at a concentration of 10 % , under heating and with continuous stirring, for 30 minutes according to the experimental protocols of Karumi et al., 2004 , Ciulel, 1982 , Trease and Evans, 1987 and Benmehdi, 2000.

Evaluation of antibacterial activity

Preparation of raw plant extracts

The aqueous extract and the hydromethanolic extract (70%) are prepared by dissolving 1 g of the plant material (leaves and roots) in 10 mL of solvent. After 24-hours of maceration with continuous stirring at room temperature, the mixture is filtered. The operation is repeated 3 times with renewal of the solvent every 24 hours. The three filtered fractions are combined and evaporated to dryness [11].

Selective extraction of flavonoids from the two parts of *Echium vulgare* (Fraction of ethyl acetate and butanol)

The dry residues of the two parts of the plant obtained by the same extraction procedure of the 70 % methanolic extract are treated with boiling water to dissolve the flavonoids. The aqueous phase is filtered and extracted first two (02) times with ethyl acetate (v / v) in a separatory funnel. The ethyl acetate phase is thus recovered, which will be evaporated to dryness (ethyl acetate extract). The aqueous phase resulting from the extraction with ethyl acetate is treated twice (02) twice with 1-butanol and the 1-butanol phase is thus recovered which has been evaporated to dryness (1-butanol extract).

The yield of dry extracts

We can determine the dry extract yield, by calculating the ratio between the weight of the dry extract (powder) in grams, and the weight of the plant material used for the extraction in grams; according to the following equation:

$$\text{Yields (\%)} = [(P1 - P2) / P3] \times 100$$

P1: weight of the flask after evaporation;
P2: weight of the flask before evaporation;
P3: weight of the initial plant material

Study of the antibacterial activity of *Echium vulgare* extracts

The antibacterial activity of the different extracts of *Echium vulgare* was evaluated by two reference methods:
- The diffusion technique on Muller Hinton agar (disc method);
- The microdilution method in liquid medium to determine the minimum inhibitory concentrations (MIC).

It should be noted that DMSO ensures complete safety against the antibacterial activity of our extracts.'

Diffusion technique on Muller Hinton agar (disc method) (EUCAST, 2003)

Obtaining the final inoculum is carried out as follows:

Each bacterial culture must be streaked on a nutrient agar to obtain well isolated colonies. After incubation at 37 °C for 24 hours, a few colonies are transferred using a platinum loop into a test tube containing physiological water. The optical densities are adjusted using a "SPECORD 200 plus" spectrophotometer at a wavelength of 625 nm. The optical density must be between 0.08 and 0.1 the equivalent of 10^8 CFU/mL.

The inoculum thus prepared is diluted 1/100 in physiological water. The final optical density obtained from the inoculum must be equivalent to 10^6 CFU/mL. The petri dishes containing Muller Hinton agar medium are inoculated by flooding. Discs of 6 mm in diameter prepared extemporaneously from filter paper are sterilized by autoclaving, then impregnated with the extracts to be tested dissolved in DMSO (20 µL for each disc). The discs are placed aseptically on the agar previously inoculated. The control discs are successively impregnated with 10 µL of distilled water and 10 µL of pure DMSO. The dishes are incubated at 37°C. for 24 hours.

The method of microdilution in liquid medium (CMI) (EUCAST, 2003)

Muller Hinton broth (MHB) supplemented with cations is widely used as a standard medium for plate microdilution. It allows better growth of most non-demanding pathogenic bacteria, in addition to its weak antagonistic effect against antibiotics. The 96-well microplate makes it possible to determine the MIC of the various plant extracts. In the wells of columns 1 to 12, we introduce using a micropipette 100 µL of Muller Hinton broth (MHB), then we add 100 µL of the plant extract to the 2nd well (which will serve as a negative control) and 100 µL in the 3rd well, then we dilute 100 µL of well to well from the 3rd well, using a micropipette. The factor of ½ is taken into account in the calculation of the concentrations of the products to be tested, so each well contains 100 µL of broth and the product tested in dilution. Then, we add 100 µL of the inoculum (10^6 CFU/mL) to the 96 wells except those in column 2 (negative control), well 1 will serve as a positive control (100 µL of the broth and 100 µL of the inoculum). The microplate is covered and incubated at 37°C for 18 to 20 hours. Reading is carried out with the naked eye knowing that the MIC is the lowest concentration of the test substance, at which no visual disturbance is observed. We used gentamycin as a reference antibiotic at a concentration of 5.12 mg/mL [12].

RESULTS AND DISCUSSIONS

Phytochemical screening

Phytochemical tests consist in detecting the different compounds existing in the two studied parts of the plant by qualitative reactions. The latter are based on precipitation or coloring phenomena by reagents specific to each family of compounds. The experimental results of the phytochemical tests mentioned in Tables 2 and 3 show the presence in both parts of *Echium vulgare*: alkaloids, flavonoids, tannins, reducing compounds, coumarins and quinones in the aqueous extract. The phytochemical screening carried out on the two parts of the plant with the ethanolic extract made it possible to highlight the presence of alkaloids, tannins, reducing compounds but in small quantities compared to the aqueous extract. With regard to screening from the diethyl ether extract, this made it possible to highlight only the coumarins. There is also a complete absence of starch and saponosides in both parts of the plant.

According to previous work, species of the genus Echium are rich in pyrrolizide alkaloids known for their hepatotoxic properties [13]. These data are compatible with our results, since the tests revealed their strong presence not only in the leaves but also in the roots. In addition, *Echium vulgare* contains flavonoids, naphthoquinones [14] which is compatible with our results. The presence of flavonoids and quinones has been proven even in the roots of *Echium lycopsis*and *Echium italicum* [15,16].

Table 2: Results of phytochemical tests of the aqueous extract

Secondry metabolites	Leaves+Steams	Root
Alkaloids	++	++
Flavonoids	++	++
Tanins	+++	+++
Reducing compounds	+	+
Quinones	+	++
Saponins	+/-	-
Strach	-	-
Coumarines	+	+

Table 3: Results of phytochemical tests of the aqueous extract

Secondry metabolites	Leaves+Steams	Root
Alkaloids	++	++
Flavonoids	++	++
Tanins	+++	+++
Reducing compounds	+	+
Quinones	+	++
Saponins	+/-	-
Strach	-	-
Coumarines	+	+

Yields in dry extracts

The extraction of the most abundant phenolic compounds from our plant allowed us to calculate the yield of each extract from the two parts of the plant. The yield which has been determined relative to 20 g of dry vegetable matter is expressed as a percentage (Table 4). A hydro-alcoholic extraction made by Filomena in 2009, shows yields of the raw extract of the leaves of *Echium vulgare* much lower compared to our raw extract (7.5%) [17,18].

Table 4: Yield of the extracts of the two parts of the plant *Echium vulgare*.

The extracts	Solvents	Yields %	
		Leaves+Steams	Root
Aqueous extract	Water	16.53	15.39
Water/Methanol extract	Water-Methanol	17.25	15.75
Flavonoids: Ethyl acetate fraction	Ethyl acetat	0.87	0.45
Flavonoids: Butanolic fraction	1-butanol	1.43	2.16

Evaluation of the antibacterial activity of the different extracts of *Echium vulgare*

Mueller Hinton agar diffusion technique (disc method)

The different extracts were tested on Gram (+) and Gram (-) reference bacteria implicated in severe human pathologies. The results of the qualitative tests of the antibacterial activity of the different extracts of our plant obtained on the eight reference strains are collated in Tables 5 and 6. The values indicated are the means of the triplicates for each test [19,20].

The results show that each of the extracts has a fairly well defined activity on the growth of at least one of the bacteria tested.

• The results obtained in Table 5 show that the extracts of the two flavonoid fractions of the roots of *Echium vulgare* exert a remarkable inhibitory activity with inhibition diameters of up to 22 mm. The crude methanolic and aqueous extracts were less active with inhibition diameters which varied between 7 mm and 9 mm on all the strains tested. Based on the inhibition diameters, it can be said that all bacteria are sensitive to flavonoid extracts.

Table 5: Diameters of the zones of inhibition (mm) of the extracts of the roots of *Echium vulgare*.

Extracts / Strains	Roots			
	Aqueous	Water/Methanol	Ethyl acetate fraction	Butanolic fraction
Staphylococcus aureus ATCC 25923	7	13	15	20
Enterococcus faecalis ATCC 25212	7	8	15	18

Bacillus cereus ATCC 11778	7	12	9	8
Staphylococcus aureus MRSA ATCC 43300	7	11	17	17
Escherichia coli ATCC 25933	8	7	20	22
Acinetobacter baumanii ATCC 19606	/	7	15	15
Klebsiella pneumoniae ATCC 70603	7	9	22	20
Enterobacter cloacae ATCC 13047	7	8	18	17

Disc diameter included/: No effect

In Table 6, we note that the crude methanolic extract exhibits good antibacterial activity with zones of inhibition of 16 and 15 mm in diameter with respect to *Escherichia coli* and *Klebsiella pneumoniae* respectively. The specific extract of the ethyl acetate fraction is the most active with regard to the strains tested. The aqueous extract remains the least active against bacteria.

The good inhibitory activity of most extracts of *Echium vulgare* against the different strains is probably linked to their richness in flavonoids, alkaloids, tannins, sterols, reducing compounds and coumarins which can be qualified as antibacterial molecules. On the basis of the results obtained, we can say that all of the extracts of *Echium vulgare* with the exception of the aqueous extract have a relatively high antibacterial activity with respect to the bacteria tested.

Table 6: Diameters of the zones of inhibition (mm) of extracts of leaves of *Echium vulgare*.

Extracts / **Strains**	**Leaves+Steams**			
	Aqueous	Water/Methanol	Ethyl acetate fraction	Butanolic fraction
Staphylococcus aureus ATCC 25923	/	8	15	12
Enterococcus faecalis ATCC 25212	/	9	12	10
Bacillus cereus ATCC 11778	/	/	11	10
Staphylococcus aureus MRSA ATCC 43300	/	15	/	/
Escherichia coli ATCC 25933	9	8	16	13
Acinetobacter baumanii ATCC 19606	/	9	10	10
Klebsiella pneumoniae ATCC 70603	7	12	15	13
Enterobacter cloacae ATCC 13047	/	8	9	/

Determination of minimum inhibitory concentrations (MIC)

The results relating to the MICs of Gentamycin and of the various extracts of *Echium vulgare* against the eight strains tested are collated in Tables 7 and 8. These concentrations obtained make it possible to compare the activity of our extracts with that of the reference antibiotic. Based on these results we can say that the MIC values vary from one bacteria to another depending on the different extracts tested. The results obtained in Table N°07 show that the flavonoid extract of the ethyl acetate fraction exerts a remarkable inhibitory activity. The flavonoid extract of the n-butanol fraction also shows very good inhibitory activity with MICs ranging from 0.85 mg/mL to 3.42 mg/mL and those against *Escherichia coli, Klebsiella pneumoniae , Staphylococcus aureus and Enterococcus faecalis* . The aqueous extract was found to be the least active with MICs ranging from 9.75 to 13.5 mg/mL on all strains except *Bacillus cereus* and *Acinetobacter baumanii* which have shown no effect. *Klebsiella pneumoniae* has the lowest MIC of all strains, making it the most sensitive followed by *Escherichia coli.* Despite the great sensitivity of these strains towards our extracts, Gentamycin remains an antibiotic having an inhibitory effect superior to that of our extracts.

Table 7: Minimum inhibitory concentrations (MIC) (mg /mL) of extracts of the roots of *Echium vulgare* against bacterial strains.

Extracts / Strains	Roots				Gentamicin (mg/mL)
	Aqueous	Water/Methanol	Ethyl acetate fraction	Butanolic fraction	
Staphylococcus aureus ATCC 25923	12.5 ± 0	6.5 ± 0	3.25 ± 0.22	1.17 ± 0	0.19 ± 0
Enterococcus faecalis ATCC 25212	9.75 ± 0	8.5 ± 0.05	1.12 ± 0	3.42 ± 0	0.78 ± 0
Bacillus cereus ATCC 11778	/	7 ± 0	11 ± 0	15 ± 0	0.19 ± 0
Staphylococcus aureus MRSA ATCC 43300	13.5 ± 0.15	5.62 ± 0	1.34 ± 0.11	4.5 ± 0	0.39 ± 0
Escherichia coli ATCC 25933	7 ± 0	5.5 ± 0	1.03 ± 0.25	0.85 ± 0	0.32 ± 0.11
Acinetobacter baumanii ATCC 19606	/	5 ± 0	2.25 ± 0	5.5 ± 0.11	0.65 ± 0.22
Klebsiella pneumoniae ATCC 70603	11.5 ± 0	2.81 ± 0.75	0.95 ± 0	1.12 ± 0	4.166 ± 1.80
Enterobacter cloacae ATCC 13047	12.5 ± 0	11 ± 0	1.12 ± 0.85	5.5 ± 0	2.603 ± 0.90

In Table 8, we note that the methanolic crude extract exhibits good antibacterial activity with MICs of 0.98 mg/mL and 2.25 mg/ml against *Staphylococcus aureus* MRSA and *Klebsiella pneumoniae* respectively. The flavonoid extract of the 1-butanol fraction was found to be active against *Enterococcus faecalis* and *Escherichia coli* with an MIC=2 mg/mL.

Table 8: Minimum inhibitory concentrations (MIC) (mg/mL) of extracts of *Echium vulgare* leaves against bacterial strains.

Extracts / Strains	**Leaves + Steams**				**Gentamicin (mg/mL)**
	Aqueous	**Water/Methanol**	**Ethyl acetate fraction**	**Butanolic fraction**	
Staphylococcus aureus ATCC 25923	/	3.5 ± 0	2.25 ± 0.1	5 ± 0	0.19 ± 0
Enterococcus faecalis ATCC 25212	/	/	3.25 ± 0.25	2 ± 0	0.78 ± 0
Bacillus cereus ATCC 11778	/	/	11 ± 0	11.75 ± 0	0.19 ± 0
Staphylococcus aureus MRSA ATCC 43300	/	0.98 ± 0	/	/	0.39 ± 0
Escherichia coli ATCC 25933	10.45 ± 0	6.5 ± 0	6.5 ± 0	2 ± 0	0.32 ± 0.11

Acinetobacter baumanii ATCC 19606	/	5.5 ± 0	7.25 ± 0.04	7.37 ± 0	0.65 ± 0.22
Klebsiella pneumoniae ATCC 70603	12.5 ± 0.12	2.25 ± 0	3.12 ± 0.75	4.37 ± 0	4.166 ± 1.80
Enterobacter cloacae ATCC 13047	/	4.5 ± 0	4.5 ± 0	/	2.603 ± 0.90

The specific extracts of flavonoids and the raw aqueous extract of the root part of *Echium vulgare* have shown better antibacterial activity compared to that of the aerial part. However, the raw water/methanol extract from the aerial part was more active than that from the roots. A study carried out by Ayşe and collaborators in 2004, showed that the antimicrobial activity of crude extracts of *Echium vulgare* with respect to the microorganisms tested (*E. coli* and *S. aureus*) exceeded 1 mg/mL (MIC>1 mg/mL). This result confirms ours.

CONCLUSION

In this work, we are interested in the study of the antibacterial effect of crude and specific extracts of the leaves and roots of *Echium vulgare*, a plant widely used in traditional medicine around the world. Qualitative research, through phytochemical tests, of secondary metabolites in our two parts of the plant has made it possible to highlight the presence of alkaloids, tannins, flavonoids, coumarins and reducing compounds. These results confirm the great richness of the leaves and roots of *Echium vulgare* in phenolic substances. This preliminary phytochemical study remains insufficient and does not explain the different results of activity sought, because the antibacterial activity of plant extracts is due to the different chemical agents present in these extracts. The variation in chemical composition therefore explains the variations observed in the antimicrobial activity of extracts from the same plant or from different plants. The extracts of *Echium vulgare* were tested in vitro by the method of diffusion from a solid disc, for their inhibitory power, against a group of pathogenic bacteria. The extracts revealed variable antimicrobial activities against the different bacterial strains tested.

CONFLICTS OF INTERESTS

The authors declare that they have no conflicts of interest regarding this article.

BIBLIOGRAPHIC REFERENCES

[1] L Aubert. Nutrition et diététique.**2009**.
[2] Ayşe Kuruűzűm-Uz, Zuhal Gűvenalp, Karsten Strȍch et al., Biochem Systematic Eco. **2004**, 32: p. 833–836.
[3] Benmahdi H. Thèse de Doctorat à l'université de Béchar **2000**.
[4] I Ciulel Ed I.P.A.C. Romania.**1982**, p. 67.
[5] F Conforti, S Sosa, M Marrelli et al., Food chemistry. **2009**, 112: p. 587-594.
[6] JP Dedet, Dunod, Paris, France. **2007**, p. 050806-8.
[7] SP Denye., NA Hodges and SP Gorman. Pharmaceutical Microbiology. **2004**.
[8] A El shazly, M Abdel-AH M, A Tei. Z naturforsching. **1999**, 54: p. 295-300.
[9] European Committee for Antimicrobial Susceptibility Testing (EUCAST) of the European Society of Clinical Microbiology and Infectious deseases**2003**, 9(8): p. 1-7.
[10] H Fukui, M Tsukada and H Mizukami. Phytochemistry. **1982**, 22(2): p. 252-258.
[11] F Gbadamassi. La pneumonie, première cause de la mortalité infantile en Afrique, Togo. **2007**.
[12] JN Joffin and G Leyral. **2006**, p. 967-971.
[13] Y Karumi, PA Onyeyili and V Ogugbuaja, J Med Sci. **2004**, 4: p. 179-182.
[14] Y Landry. ISBN. **2012**, 058213-6.
[15] VP Papageorgiou, AN Assimoupoulou, EA Coladouros et al., Chimie international ed. **1999**, 38(3): p. 270-301
[16] E Trease and WC Evans. Pharmacognosy, 1987.
[17] TM Upson, RJ Grayer, JR Greenham. **2000,** 28: p. 991-1007.
[18] H Wagner and MG Ulrich. **2009**, 16: p. 97-110.
[19]World Health Organization (WHO). Organisation Mondiale de la Santé. **2012**.
[20] GD Wright and AD Sutherland A.D. Trends Mol Med. **2007**, 13: p. 260-267.