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# Phytochemical screening of Algerian *Borago officinalis L.* and evaluation of its antioxidant and antimicrobial activities against respiratory pathogens

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## Abstract

Context and purpose of the study: Infectious diseases represent a serious problem of public health in countries where resistance of bacteria to antibiotics is spreading alarmingly. Such situation leads researchers to make efforts so they can isolate or synthesize new antimicrobial molecules or molecules that inhibit the resistance mechanisms. Medicinal plants can represent a rich source of such molecules. In this context, *Borago officinalis L.*, a medicinal species which is used traditionally in Algeria to heal infectious diseases of respiratory system is studied. Both of chemical composition and biological activities were explored.

Main findings: GC-MS analysis of the essential oil reveals the Spathulenol as the most abundant component (38.24%). The HPLC applied on flavonoids extract shows the presence of the Caffeic acid, *p*-coumaric acid, Rutin, Rosmarinic acid, Quercetin and the Gallic acid. The test of the antioxidant capacity using the DPPH method reveal an inhibiting effect which is more important with the flavonoid extract with an EC 50 = 4.02± 0.06µg/ml. Otherwise, resistant strains to conventional antibiotics seem to be sensitive to the flavonoids with MICs varying from10.14µg/ml to 81.12µg/ml.

Brief summary and potential implications: This study indicates that *Borago officinalis L.* has an important antimicrobial effect, which justifies its potential use in infectious diseases. This species remains poorly investigated, further refined studies on its pure secondary metabolites are needed, in the perspective of identifying new antimicrobial molecules from this plant.

**Keywords:** - *Borago officinalis L.*, antimicrobial activity, respiratory pathogens, antioxidant activity, essential oil, flavonoids.

## Introduction

Despite serious efforts made by researchers in the hope of synthesizing new molecules, more than 25% of prescribed medicines in developed countries are directly or indirectly derived from plants [1]. However, as a source of medicines, plants remain under exploited and this fact is more remarkable in medical microbiology [2]. Certainly, most of prescribed antibiotics are derived from micro organisms [3], but there are many evidences that antimicrobial agents issued from plantae kingdom have an important place in this category of medicines [4]. Practically, every antibiotic has limited effective lifespan and after a while micro organisms could develop resistance so it becomes less effective or not effective at all. Moreover, in developing countries, these products are expensive, so more than 80% of population use plants to treat many diseases. [2,5]. In these countries, some populations practice self prescription mainly using herb teas [6].

*Boraginaceae* family include some 100 genus and more than 2000 species including bushes, trees, herbaceous plants and liana [7] which are used as decorative plants such as species of genus *Echium, Myosotis* and *Eritrichium*, but also for medical properties, it's the case of *Borago officinalis*, *Symphytum officinale*, *Pulmonaria officinalis* and *Echium vulgare* [8].

In Algeria, *Borago officinalis* is traditionally used for its anti infectious properties, taken as herb tea or eaten in dishes.

This herb native from Syria was naturalized throughout the Mediterranean region, as well as Minor Asia, Europe, North Africa, and South America. Traditionally borage was cultivated for culinary and medicinal uses [9]. According to some authors, this plant is used for regulating metabolism and hormonal system, and is considered to be a good remedy for menopause symptoms such as the hot flash and sometimes indicated to alleviate and heal colds, bronchitis and respiratory infections.

However, no reported studies were interested by the Algerian borage. One study was accomplished in Tunisia and was interested by the chemical composition of the essential oil of this species [10], another one performed in Italy was interested by the antioxidant activity of phenols [11].

The purpose of this study is to highlight the antimicrobial effect of this plant on both reference strains and strains isolated from patients with respiratory infections and also, to study the antioxidant properties of flavonoids extract and essential oil of *Borago officinalis* L.

## Materials and methods

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## Studied plant

The harvest of the aerial part of *B. officinalis* was accomplished at the end of the winter and spring from the area of Tizi Ouzou, in the northeast of Algeria, about 80 km of Algiers, the capital, between 36 43′ 00″ North and 4 03′ 00″ East. To extract flavonoids, collected parts were dried in the open air and sheltered from light, then transformed into powder using an electrical crusher with helix, this powder was maintained in tightly closed glass flasks.

## Tested Bacterial strains

The tested strains was either reference strains or isolated from patients diagnosed with respiratory infections and hospitalized in the department of infectious diseases in the hospital university of Tizi Ouzou, the identification was effected using biochemical galleries Api 20E, Api 20NE,or specific tests.

## Phytochemical Screening

It means a set of colorimetric methods that allow detecting the presence or lack of secondary metabolites and realized on plant powder or infusion. We searched mainly anthocyanins, leuco-anthocyanins, total tannins, irridoids, gallic tannins, catechin tannins, alkaloids, flavonoids, saponosids, senosids, quinons , coumarins and mucilages [12-13].

## Extraction of essential oil

The extraction of essential oil was effected by hydro distillation, for that, 100g of fresh matter was soaked in a recipient of 1l then filled with 600 ml of distilled water, the hole system was boiled during 3 hours. Massed vapors of essential oils were condensed by crossing a refrigerator and collected. Organic state was recovered by adding few milliliters of diethyl ether and the obtained oil was kept in a temperature between 0 C and 4 C.

## GC-MS of essential oils

The analysis of chemical composition of the obtained essential oil was accomplished by
GC-MS. The used device was GC Perkin Elmer 600, SM Perkin Elmer 600C, using also a Rtx-VMS column (60m length, 250µm diameter). The carrier gas was Helium (1ml/min). 0.2µl of the oil to analyze was injected using a syringe.

## Extraction of flavonoids

The extraction of flavonoids was realized according to Bruneton protocol [14]. This extraction is based on the solubility degree of flavonoids in organic solvents. This protocol includes two main steps: the first one is accomplished using methanol to solubilize flavonoids and the second one includes washings using petroleum ether, diethyl ether, ethyl acetate and butanol. The extract obtained after washing using butanol contains the most polar flavonoids.

## Dosage of flavonoids using spectrophotometry method

To realize the dosage of flavonoids, we used the method of aluminum chloride colorimetric method [15]. Absorbance was read by a spectrophotometer (Optizen 2120 UV) at 430 nm. To calculate the concentration of flavonoids in the extract, a calibration range was established using quercetin (1-25µg/ml). The results of dosage are expressed in equivalent micrograms of quercetin for a gram of the extract.

## High performance liquid chromatography (HPLC)

Qualitative analysis of the extract of flavonoids was realized using HPLC. Stationary phase was made with a column of silica (C18 reverse phase), dimensions were 125 mm by 4.6 mm. Mobile phase was a mixture of water/methanol /acetic acid (50:47:2.5), in isocratic system with a flow of 1 ml/min [16]. Extracts and standards were analyzed with concentrations of 0.5 mg/ml. The injected volume was 20 µl. Detection was realized using a UV-Visible detector in a wave length of 254 nm.

## Antimicrobial activity

The antimicrobial activity of essential oil was studied using a device of multiple seeding, Steers machine, according to the recommendation of the French society of microbiology that allowed determining the inhibitory activity of the extract on the growth of bacteria. Essential oil was directly diluted in mediums: Mueller Hinton for bacteria or Sabouraud for fungi then let to solidify. Bacterial suspensions with concentrations of 0.5 Mc Farland was placed using spots. For flavonoids, plates of Mueller Hinton agar for bacteria and Sabouraud agar for fungi were inoculated by swabbing of standardized microbial suspension (0.5 Mc Farland), according to NCCLS recommendations [17]. Then, discs of 6 mm of diameter, impregnated with 10 µL of extract at different concentrations were placed on the surface of agar.
In addition, discs of antibiotics were placed on the center of plate and served as positive controls for bacterial and fungal strains respectively. After incubation at 37 C for 24 h for bacteria or at 28 C for 48 h for fungi, antimicrobial activity of both extracts was determined by measuring the minimal inhibitory concentration. All tests were performed in triplicate.

## Antioxidant activity

## Study of antioxidant properties using the DPPH test

To asses qualitatively the antioxidant activity of both flavonoid extract and the essential oil, we used thin layer chromatography, Huang *et al.* in 2005 [18]. This method is generally based on the inhibition of accumulation of oxidized products. In this case, free radicals are inhibited by adding antioxidant molecules. In this test, both extracts and standard (ascorbic acid) were diluted in pure ethanol with different concentrations: 0.2, 0.4, 0.6, 0.8 and 1 mg/ml. A volume of 10 µL from each concentration and from the

standard was deposed on the silica block, after drying, the block was entirely soaked during 10 seconds in a tray containing a DPPH solution, yellow stains appeared revealing antioxidant activity.

To determine quantitatively the antioxidant effect of extracts, we used the method proposed by Sanchez Moreno *et al.* [19]. This determination consists to prepare concentrations of 0.00125, 0.0025, 0.005, 0.01, 0.031, 0.0625, 0.125, 0.25, 0.5, 0.75 and 1 mg/ml from a stock solution of both extracts obtained by dissolution in methanol. For the essential oil, 1ml from each concentration was mixed with 4ml of DPPH solution witch concentration was 0.0024g/ml. For flavonoids, we realized a mixture of 25 µl of each concentration with 975 µl of the same solution of DPPH. Ascorbic acid was also prepared using the same method. The measure of the variation of absorbance was done 30 minutes after introducing tanks in UV-Visible spectrophotometer (Optizen 2120 UV), drove by an informatics system in the wavelength of 517 nm. The obtained values were transformed to percentages of inhibition using the following formula: $I\% = 100 \times \frac{A\,reference - A\,test}{A\,reference}$

A reference represents the absorbance of the control (containing reactive without the extract to be tested)

$A_{Test}$ is the absorbance of the extract.

## Statistical analysis

Results of the antioxidant activity were expressed as mean ± SD. For the antimicrobial activity, the statistical comparison among the group was performed with one way ANOVA using statistical presentation system, Statistica version 6.

# Results

## Phytochemical screening

Results show that *B. officinalis* is wealthy in tannins, leuco-antocyanins , alkaloids, saponosids, glucosids and mucilage (table 1).

**Table 1: Presence or absence of secondary metabolites in Borage**

| Tests | Abondance |
|---|---|
| Total tannins | ++ |
| Catechin tannins | - |
| Gallic tannins | + |
| Flavonoids | ++ |
| Anthocyanins | + |
| Leuco- anthocyanins | - |
| Alkaloids | ++ |
| Senosids | - |
| Amidon | - |
| Saponins | ++ |
| Irridoids | - |
| Glucosids | ++ |
| Mucilages | ++ |
| Coumarins | - |
| Quinons | - |

++: abundance +: Presence -: Absence

## Extraction of essential oil

For 100 g of dried matter, we obtained a yield of 0.054% of essential oil. The obtained oil is lightly viscous, with a pale coloration (nearly transparent) and a specific odor.

## Extraction of flavonoids

The obtained aqueous extract presents a gelatinous aspect, with a brown color. The yields of different extracts (diethyl ether, ethyl acetate, butanolic and aqueous) vary between 2.066% and 37.6% (table2).

**Table 2: Yields in % of flavonoids extracts**

| Extract | % |
|---|---|
| Diethyl ether extract | 2,066% |
| Ethyl acetat extract | 16,1% |
| Butanolic extract | 18,16% |
| Flavonoid extract | 37,6% |

## Dosage of flavonoids

The content in flavonoids is reported in equivalent µg of quercetin/ml of the extract of the plant. The concentration of the flavonoid in the extract is 81.12 µg/ml. Results are compiled in table 3.

**Table 3: Values of dilution factors and concentrations of different extracts**

| Plant | Factor of dilution | Concentration expressed in µg Equivalent of Quercetin /ml |
|---|---|---|
| *Borago officinalis* | 4,081 | 81,12 µg/ml |

## High performance liquid chromatography

The HPLC analysis showed the presence of the Gallic acid, the Caffeic acid, the *p*-coumaric acid, the Rutin, the Quercetin and the Rosmarinic acid in the flavonoid extract of *B. officinalis* (figure.1, table 4)

**Table 4: Compounds revealed by the HPLC analysis**

| Retention time (min) | Standard |
|---|---|
| 1 | Gallic acid |
| 2 | Caffeic acid |
| 3 | *p*-coumaric acid |
| 4 | Rutin |
| 5 | Rosmarinic acid |
| 6 | Quercetin |

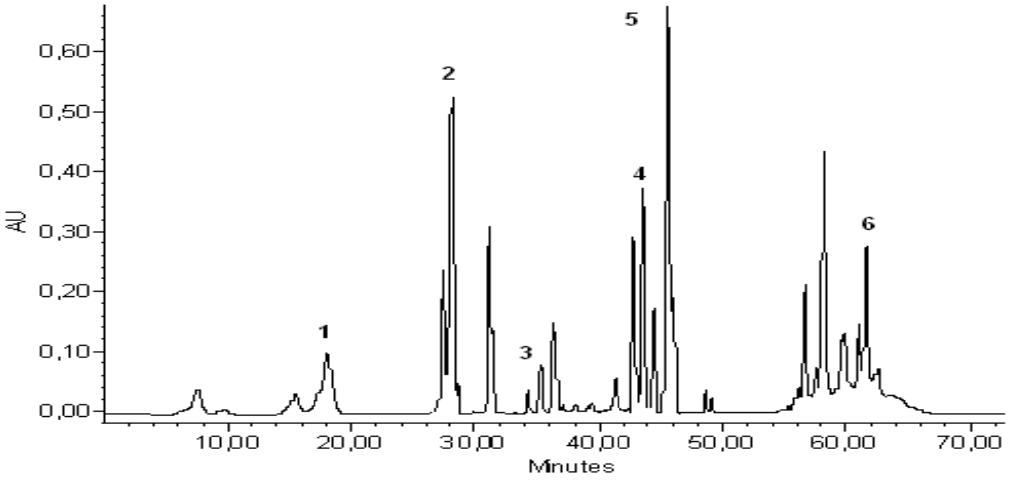



**Figure.1** Chromatogram of the flavonoid extract registered at 254 nm

## GC-MS of Essential Oil of *B. officinalis*

The GC-MS analysis of the essential oil of borage allowed identifying 7 molecules (figure. 2). The Spathulenol constitute the major component (38.24%) (Table 5).

**Table 5:** Main chemical components analyzed by GC-MS

| N | $R_t$ | Name of the compound | % |
|---|---|---|---|
| 1 | 42.55 | Trifluoromethyl | |
| 2 | 45.22 | Thymol | |
| 3 | 48.31 | Verrucarol | 06.93 |
| 4 | 55.87 | Spathulenol | 38.24 |
| 5 | 56.19 | Globulol | 10.02 |
| 6 | 57.60 | Gamma-Himachalène | 07.84 |
| 7 | 59.07 | Guaiol | 12.87 |
| Total | | | 91.70 |

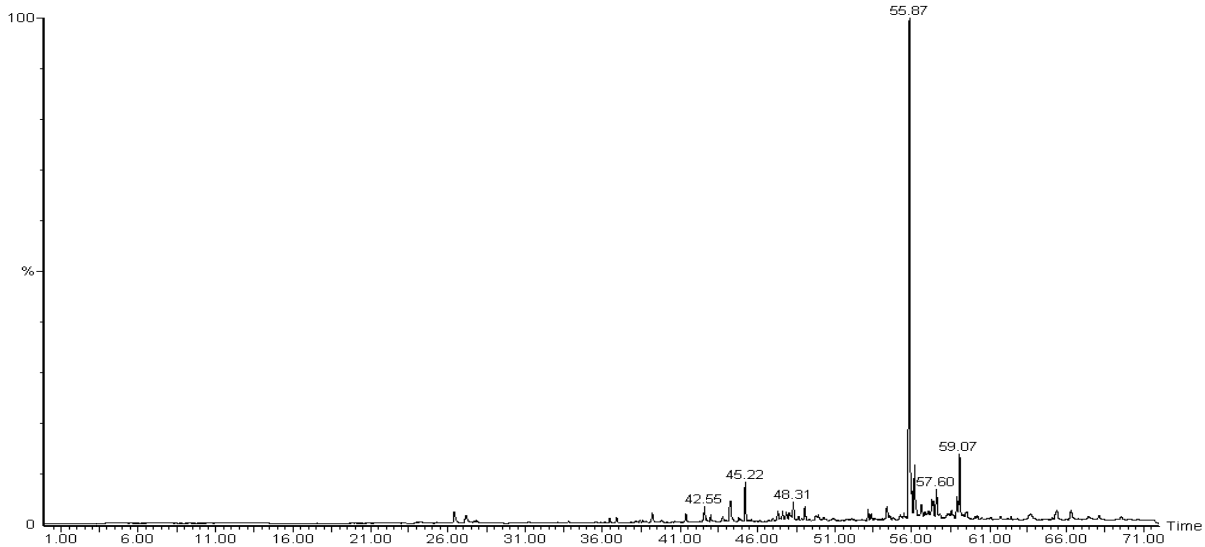



**Figure.2 Chromatographic profile of the analysis of essential oil by GC-MS**

## Antimicrobial activity

The results of the antimicrobial activity of both extracts against reference strains showed an important effect on bacteria of respiratory flora (Table 6).

**Table 6: Antimicrobial activity of *B. officinalis* against reference strains**

| Strain | Reference | $MIC_s$ | |
|---|---|---|---|
| | | E.O (mg/ml) | Flavonoïds (µg/ml) |
| *E. coli* | ATCC 11229 | 0.5 | 40.56 |
| *Enterobacter cloacae* | ATCC 13047 | 0.5 | 40.56 |
| *Staphylococcus aureus* | ATCC 6538 | R | 20.28 |
| *Haemophilus influensae* | ATCC 10211 | 0.125 | 20.28 |
| *Streptocuccus pneumoniae* | ATCC 10015 | R | R |
| *Pseudomonas aeruginosa* | ATCC 15442 | 0.0625 | 10.14 |
| *Klebsiella pneumoniae* | ATCC 13883 | 0.0625 | 20.28 |
| *Candida albicans* | ATCC 10231 | 0.125 | 40.56 |

Extracts were tested on isolated bacteria from respiratory infections. Clinical bacterial strains are isolated from 250 samples of patients with repiratory infections, 70 of samples are ORL infections samples when 180 are bronchitis infections.

Figure 3 shows the frequency of every bacterial strain in the hole of isolated strains.

Isolated strains that showed resistance to some antibiotics were sensitive to flavonoid extract and sometimes also to essential oil, it is interesting to note that *E.coli* (resistant), *Streptococcus pneumoniae* (sensitive to amoxicilline ) and *Klebsiella pneumoniae* (sensitive to Imipeneme) are ssensitives to flavonoids with $MIC_s$ of 40.56 µg/ml (Table 7).

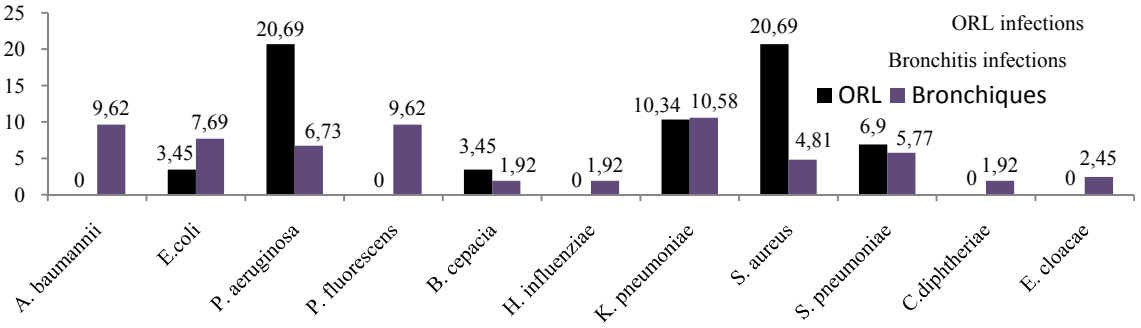



**Figure.3 Frequency of microbial strains isolated from patients with repiratory infections.**

**Table 7: Activity of *B. officinalis* extracts against respiratory pathogens**

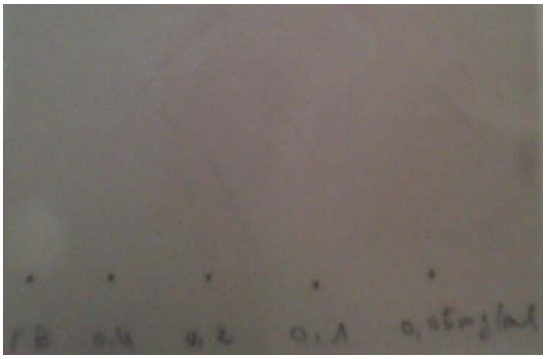
| Strains | Antibiotics | | | | | | | | Essential oils($CIM_s$) | Flavonoids($CIM_s$)µg/ml |
|---|---|---|---|---|---|---|---|---|---|---|
| *Escherichia coli* | Cip | Amo | Ceph | Tic | Trim | Cefo | Cefu | Ceft | R | 40.56 |
| | R | R | R | R | R | R | R | R | | |
| *Pseudomonas Aeruginosa* | Fost | Imi | Levo | Amik | Cet | Cip | / | / | 0.5 | 20.28 |
| | R | R | S | S | S | S | / | / | | |
| *Pseudomonas fluorescens* | Azith | Cipr | Levo | Tic | Trim | Gent | Imi | Cef | R | 20.28 |
| | R | R | R | R | R | S | S | S | | |
| *Burkholderia Cepacia* | Amox | Imi | Gent | Cefo | Col | Cefa | / | / | 0.25 | 10.14 |
| | S | S | S | S | S | R | / | / | | |
| *Haemophylus Influensiae* | Cot | Amo | Ofl | Tet | Cefo | / | / | / | 0.25 | 10.14 |
| | R | S | S | R | S | / | / | / | | |
| *Klebsiella pneumoniae* | Amo | Cef | Gent | Amp | Ami | Col | Imi | Cot | R | 40.56 |
| | R | R | R | R | R | R | S | R | | |
| *Acinetobacter baumannii* | Tic | Pop | Cef | Imi | Ofl | Cip | Col | Tic +Ac | C.E. | 20.28 |
| | R | R | R | R | R | R | S | R | | |
| *Streptococcus Pneumoniae* | Azith | Cefac | Ceft | Cefur | Eryt | Peni | Tetr | Amo | C.E | 81.12 |
| | R | R | R | R | R | R | R | S | | |
| *Staphylococcus aureus* | Vanc | Oxa | Gent | Amp | / | / | / | / | C.E | R |
| | S | S | S | R | | | | | | |
| *Enterobacter Cloacae* | Azith | Oxa | Gent | Amp | Amo | Imi | Tetr | | R | 20.28 |
| | R | R | R | R | R | R | S | | | |
| *Candida albicans* | Azith | Oxa | Gent | Amp | Amo | Imi | Tetr | | R | 20.28 |
| | R | R | R | R | R | R | R | | | |

R : Resistant / S : Sensitive

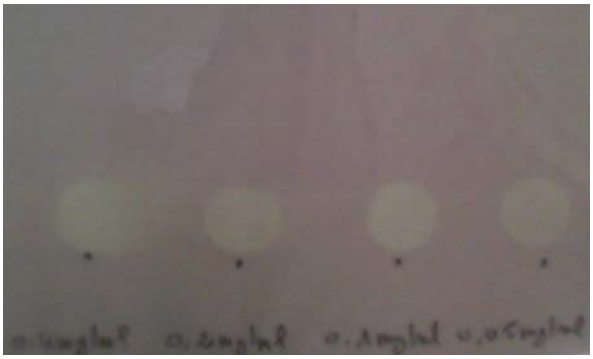
## Antioxidant activity

### Qualitative study

Observation of blocks revealed the presence of a light yellowish stain only for the untreated extract. For flavonoids, the oxidization produced a yellowish stains for all dilutions which makes evidence that flavonoids of borage have a superior antioxidant effect than essential oils (Figure.4).



1mg/ml 0.8mg/ml 0.6mg/ml 0.4mg/ml 0.2mg/ml
Qualitative antioxidant activity of essential oil



1 mg/ml 0.8mg/ml 0.6mg/ml 0.4mg/ml
Qualitative antioxidant activity of flavonoids

### Quantitative study

The free radical scavenging assayed by DPPH discoloration revealed a high activity of flavonoids extract of *B. officinalis* and a very weak activity for her essential oil. Values of $EC_{50}$ of obtained flavonoids (=4.02± 0.06µg/ml), are closer to the ascorbic acid taken as standard ($EC_{50}$= 1,21 µg/ml), in opposition of essential oils with an $EC_{50}$=10.39±0.12 µg/ml(Figure. 5).

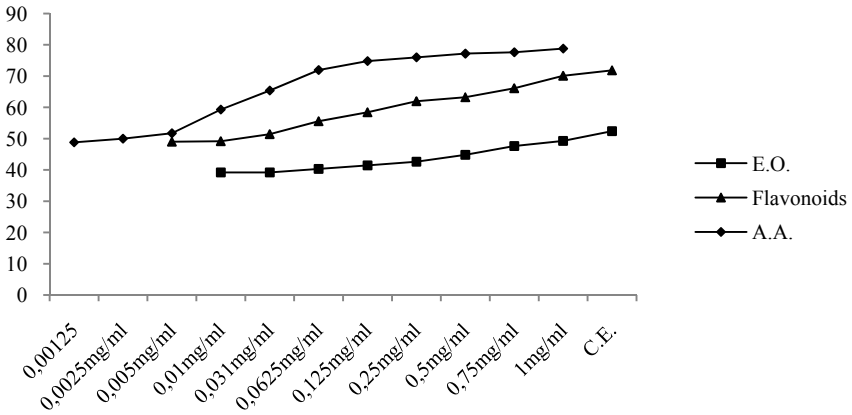



**Figure.5 Graphic of inhibition percentages**

## Statistical analysis

Statistical analysis of the antimicrobial activity gave a value of $p<0.05$ ($p=0.049$), that means that the extract acted significantly against bacterial strains which was resistant to antibiotics.

# Discussion

The phytochemical screening of *Borago officinalis* showed an important wealth in tannins, alkaloids, flavonoids, saponosids , glucosids and mucilage. However, these metabolits were found with a low abundance by Sorooshzadeh *et al.* in 2008 in Iran [21]. Hydrodistillation allowed a yield of 0.37 ± 0.01% of essential oil. This value is lower than the one found by selem *et al.* in the area of Korba (Tunisia) (0.95±0.03%), and lightly higher than the one obtained by the same authors in Beja, Tunisia (0.29±0.03%) [22].

Concerning flavonoids, the yield was 37.6%. Because of lack of studies, we compared our results with those obtained by Javad Aliakbarlu *et al.* in 2012 on polyphenols of borage in Iran; these authors reported a yield of 64.1 mg/g of polyphenols [23].
These differences may be explained by many factors mainly by the biotope of the plant and the season of harvest.
Biochemical characterization of both extracts used in this study shows the presence of Rutin and Quercetin for flavonoids and Spathulenol for essential oil as a major component.
There are no preceding studies that showed presence of Rutin in flavonoids of borage, concerning the chemical composition of essential oil, Baya Mhamedi *et al.*, has shown the presence of β-Caryophyllene as the most abundant component with a rate of 26% [10].
A study of anti microbial activity showed that both methanolic and ethanolic extracts of borage have a very low antimicrobial activity against *Listeria monocytogenes* (inhibition zone = 10.32mm) [23].
The antimicrobial activity of flavonoids issued from borage is more important than the one of essential oil and that can be attributed to the wealth of both extracts in antimicrobial components. Essential oil contains seven molecules and the major component is the spathulenol witch belongs to sesquiterpens oxidized family which is known as having a weak antibacterial activity, this component is more considered as anti fungal agent. Concerning flavonoids, they are wealthy in Rutin and Quercetin which are known as antimicrobial agents.
Furthermore, essential oils of this species seems to be more active against gram negative bacteria than gram positive bacteria unlike flavonoids, they have activity against both Gram positive and Gram negative bacteria.
Many studies established the correlation between the chemical composition and the antimicrobial activity, these works ordered the exercised effect by major components of essential oil as follow: phenols, alcohols, aldehyds, cetons, ether and hydrocarbons [24].
These results of antimicrobial activity of flavonoids are promising and could be a starting point to develop new antimicrobial drugs.
Our study was limited on bacterial strains isolated from respiratory infections, results showed that some resistant strains to standard antibiotics was sensitive to flavonoids extract, these results are promising and could lead to discover new antimicrobial molecules.
Concerning the antioxidant activity, in this work, this property seems to be high for flavonoids, with an $EC_{50}$ of 4.02± 0.06µg/ml, this activity is lower for essential oil of the same plant. This result is the same for DPPH method practiced on the same plant but on a different extract: the hydroalcoholic extract that showed a higher antioxidant activity with an $EC_{50}$ of 58 ± 0.11 µg/ml [11].
The evaluation of the antioxidant and antimicrobial activities of two extracts obtained from Algerian *Borago officinalis* tested in this work showed that this plant, especially her flavonoïds extract, had a remarkable inhibitory effect on resistant respiratory pathogens.
Further studies are needed to develop formulations for pharmaceutical use based on these extracts to fight these pathogens.

## Conclusion

The evaluation of the antioxidant and antimicrobial activities of two extracts obtained from Algerian *Borago officinalis* tested in this work showed that this plant, especially her flavonoïds extract, had a remarkable inhibitory effect on resistant respiratory pathogens.
Further studies are needed to develop formulations for pharmaceutical use based on these extracts to fight these pathogens.

### List of abbreviations

*B. officinalis*: *Borago officinalis L.*
GC-MS: Gas Chromatography- Mass Spectrophotometry
HPLC: High Performance Liquid Chromatography
DPPH: 2,2-diphenyl-1-picrylhydrazyl
MIC: Minimal Inhibitory Concentration
$EC_{50}$: Efficient Concentration 50
$R_t$: Retention time
C.E.: Crude extract
AMP: Ampicilin
IMI: Imipenem
GENT: Gentamicin
AZITH: Azithromicin
CIP: Ciproflaxin
VAN: Vancomycin
OXA: Oxacilin
TET: Tetraciclin
ERYT: Erythromicin
PENI: Penicilin
AMO: Amoxicilin
CEF: Cefazolin
LEV: Levoflaxin

### Authors' contributions

This work is a part of preparation of a PHD thesis and was achieved by AFIF CHAOUHE Thanina, with ARAB Karim and BENDAHOU Mourad as advisers. Advisers contributed by counseling and writing of this manuscript.

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