

## Evaluation of biochemical, antioxidant and antibacterial activities of garlic extracts (*Allium sativum. L*) grown in five Moroccan eco-regions

S. Ouroadi<sup>(a,b)\*</sup>, A. Hasib<sup>(b)</sup>, F. El Mahi<sup>(b)</sup>

<sup>(a)</sup> Laboratory for Sustainable Innovation and Applied Research, Technical University of Agadir, Technopôle d'Agadir, Qf Tilila, Agadir 80000, Morocco

<sup>(b)</sup> Agro-industrial and Environmental Process Team, GEEAI Laboratory, Faculty of Sciences and Technology, Sultan Moulay Slimane University, BP523, Beni-Mellal, Morocco

\* Corresponding author:

[Siham.ouroadi@e-polytechnique.ma](mailto:Siham.ouroadi@e-polytechnique.ma)

Received 29 Nov 2022,

Revised 10 Sept 2022,

Accepted 10 Sept 2022

### Abstract

Garlic (*Allium sativum. L*) is an essential vegetable throughout the world not only as a spice but also a traditional medicine. In the present research garlic as a raw material was explored to its chemical composition as well as its mineral analysis. The current study proved that garlic contained (59 %-67%) of moisture, (32,99±0,08-41,36±0,08) of dry matter, (0,67±0,24-2,15±0,37) of crude fiber content, (14,07±0,21-17,007±0,06) of protein on dry weight basis, while it was found that garlic contained a low amount of fat. whilst, for mineral composition, it is found that garlic had highest amount of potassium and sodium (2773,8 to 45225,56 and 66,85 to 132.92 mg/100g) respectively whereas the other mineral content were in traces. Garlic is a good source of antioxidant contents. These compounds reveal activity effectiveness not less than synthetics. The present study tested an aqueous extract of garlic in vitro for its antibacterial activity against *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae*. The aqueous extract of garlic showed increased inhibitory effect. The maximum antibacterial activity was observed against *Klebsiella pneumoniae* and minimum activity against *Escherichia coli* and *Staphylococcus aureus*. The results show that the size of the inhibition zone varies widely from one area to another and quantitative assessment of the minimum inhibitory concentrations for bacteria showed a concentrations ranging from 8,33 mg/ml and 16,6 mg/ml for *Klebsiella pneumoniae*, 16,6 mg/ml and 33,3 mg/ml for both *Escherichia coli* and *Staphylococcus aureus*.

**Keywords:** Garlic (*Allium Sativum*), Antioxidant activity, Antibacterial activity, Inhibitory effect

## 1. Introduction

Garlic (*Allium sativum*. L) is a perennial plant of the Alliaceae related to onions, chives, shallots, and leeks. It is an indigenous herb of Western Asia and Mediterranean where it has been cultivated for centuries. The major garlic growing countries includes Korea, China, India, USA, Spain, Argentina, and Egypt, among which China is by far the largest producer. It is mainly used as a food flavoring agent and condiment in various foods and spices such as kimchi, mayonnaise, salad dressing, spaghetti, pickles, etc. [1] The garlic plant is made up of fleshy edible cloves that are encased in a white or pink, thin coat. It has leaves, stem, and flowers located on the head that are also edible. It is easy to grow and can be grown all year round. The leaves are long, narrow and flattened (figure 1: a,b,c). It is cultivated in temperate and tropical climates. Garlic plant grows well in well-drained soil and requires a cool and moist period during growth and a relatively dry period as it matures. It is one of the world's oldest medicines and has been used not only for flavoring but also as a medicinal herb for its prophylactic and therapeutic properties. Garlic and garlic supplements are consumed in many cultures on account of their beneficial effects [2]. It has also been known as a medicinal plant applied as a medication for lowering blood pressure, reduction of serum cholesterol and triglycerides and inhibition of platelet formation [3]. The bioactive components of garlic are mainly responsible for the healing properties [4]. Main pharmacological effects of garlic are attributed to its organosulphur compounds [5]. It also contains many other sulfur containing compounds such as allin, ajoene, diallylsulfide, dithin, S-allylcysteine, and enzymes, vitamin B, proteins, minerals, saponins, flavonoids, and maillard reaction product, which are non-sulfur containing compounds [6]. However, Sulphur and polyphenols present in garlic respond to the antibacterial, antifungal and antioxidant activity was carefully studied in previous reports [7]. Polyphenolic compounds are commonly bound in both edible and inedible plants and they have been reported to have multiple biological functions such as antioxidant, anti-inflammatory, anti-cancer and anti-microbial activities [8,9,10]. These compounds reveal activity effectiveness not less than synthetics [11]. Garlic components have a bacterial effect on germs causing tuberculosis, cholera, typhoid and paratyphoid fever [12]. Moreover, garlic extracts exhibited activity against both gram negative (*Escherichia coli*, *Salmonella* and *Citrobacter*, *Enterobacter*, *Pseudomona* and *Klebsiella*) and gram positive (*Staphylococcus aureus*, *Streptococcus* and *Bacillus anthrax*) all of which are cause of morbidity worldwide [13]. Previously conducted researches confirmed that garlic is not only effective against many gram positive and gram negative bacteria but also possess antiviral and antifungal activity [14]. The aim of the present study is to measure and compare the antioxidant activity of garlic and tested the antibacterial activity in vitro of aqueous extract of fresh garlic of five area of Morocco against *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae*.



**Figure 1:** (a) Garlic (*Allium sativum*) in the growth

## 2. Material and methods

## 2.1 Plant material

Garlic bulbs (*Allium sativum*) were collected from five locations in Morocco: Fez (Ain Cheggag), Meknes(Agouray), Ben Hmed, Tetouan (Bni Hssan), and Marrakesh (Ait Ourir). These areas are best known for the production of garlic in Morocco.

## 2.2 Chemical and mineral composition of garlic

### 2.2.1. Chemical composition

#### Moisture

Moisture, contents of garlic samples was established according to (AOAC, 1990) method [15]; the ground samples were dried in the oven at 105°C for 24 h. The samples were taken out of the oven, cooled in a dessicator and weighed. Percent moisture content is measured as the weight lost during drying and is expressed as a percentage of the wet sample:

$$\% \text{ Moisture} = [(weight \text{ wet sample} + container) - weight \text{ dry sample} + container] \times 100 / (weight \text{ wet sample} + container) - (weight \text{ empty container})$$

#### Dry matter

The dry matter was determined by difference [15]:

$$\% \text{ Dry Matter} = 100 - \% \text{ Moisture}$$

#### Crude Fat

The crude fat was obtained by exhaustively extracting 3g of each sample in a Soxhlet apparatus using hexane as the extractant.

#### Protein

Proteins content was determined from the nitrogen content by Kjeldahl method according to AACC (2000) method No. 984-13 [16]. Garlic was digested with concentrated H<sub>2</sub>SO<sub>4</sub> by using digestion mixture (K<sub>2</sub>SO<sub>4</sub>:FeSO<sub>4</sub>:CuSO<sub>4</sub> i.e. 100:5:10) until the color was light greenish. The digested material was diluted up to 250 ml in volumetric flask. 10 ml of 40% NaOH as well as 10 ml of digested sample was taken in distillation apparatus where liberated ammonia was collected in beaker containing 4% boric acid solution using methyl red as an indicator. This resulted in formation of ammonium borate that was used for nitrogen determination in sample. Thus percentage of nitrogen in sample is assessed by titrating distillate against 0,1N H<sub>2</sub>SO<sub>4</sub> solution till color is light golden. Crude protein content was estimated by multiplying nitrogen percent (N %) with factor (6,25).

$$N (\%) = Vol. \text{ of } 0,1N \text{ H}_2\text{SO}_4 \times 0,0014 \times Vol. \text{ of dilution (250ml)} \times 100 / Vol. \text{ of Distillate taken} \times weight \text{ of sample}$$

$$Crude \text{ protein } (\%) = Nitrogen (\%) \times 6,25$$

#### Crude fiber

The garlic sample was subjected to crude fiber content by elaborating method AOAC 982,29 [16]. Fat free sample was digested with 1,25% H<sub>2</sub>SO<sub>4</sub> followed by 1,25% NaOH. After filtration and washing with distilled water reaming residues was weighed and ignited in muffle furnace at temperature of 550-650°C till grey or white ash was obtained. The crude fiber percentage was estimated according to the expression given below.

$$Crude \text{ fiber} = weight \text{ loss (g)} \times 100 .$$

All the analysis was carried out in triplicate for each sample.

### 2.2.2. *Mineral composition*

For mineral analysis, samples were incinerated in muffle furnace at 550°C for 12h. Ashes were quantified gravimetrically. The ashes were diluted with hydrochloric acid 0,1N and with distilled and demineralised water. The dosage of the studied trace elements (Ca, Mn, Al, Si, K, Mg, Na, Cr, Cu, Fe, Zn and Se) was created by the water method regia and analysis by spectrometric Plasma Atomic Emission Inductively Coupled argon (ICP-AES). The water method regia's policy sample dissolution in a mixture of hydrochloric and nitric acid, then analyzed by ICP-AES. The used apparatus was an ICP-AES (Varian-Vista), equipped with an ultrasonic nebulizer Cetac. The main analytical parameters of the device are:

Rf power: 0.7 - 1.5 kilowatt (kW 1.2-1.3 for axial);

Flow rate of plasma gas (Ar): 10.5- 15 l / min (radial), 15 l /min (axial);

Auxiliary gas flow (Ar): 15 l / min (axial);

Viewing size: 5-12 mm;

Copy and playback time: 1-5 s (maximum 60 s);

Copy Time: 3 s (maximum 100 s).

## 2.3 *Antioxidant activity*

### 2.3.1. *Chemicals and Reagents*

The solvents and the chemicals used were of analytical grade, methanol was used as solvent for extraction of antioxidants compounds. DPPH, ABTS, potassium persulphate, sodium carbonate, Folin-Ciocalteu, gallic acid, aluminium trichlorid, sodium acetate were stored at prescribed conditions in the laboratory.

### 2.3.2. *Extracts preparation*

1 g of garlic bulbs were extracted using a method of maceration with 10 ml of methanol 80% (MeOH) for 76 h at room temperature. After the maceration, the extracts were collected, filtered through a filter system vacuum. The filtrate was evaporated under reduced pressure in a rotary evaporator at 35°C until the extracts became completely dry. After evaporation, the residues were dissolved in methanol and stored at 4°C until use. The extraction process was carried out in triplicate for each sample.

### 2.3.3. *Determination of DPPH-radical scavenging capacity*

The antioxidant activity was assessed on basis of the radical scavenging effect of the stable DPPH (1,1- diphenyl-2-picryl-hydrazyl) radical and was determined by the method described by Otunola and Afolayan [17]. Briefly, 0.5 ml of the extract was added to 2.5 ml of methanolic solution of DPPH, shaken vigorously and incubated for 30 min in the dark at room temperature. The absorbance of the reaction mixture at 517 nm was measured with a spectrophotometer. The percentage of free radical scavenging activity was calculated as follows:

$$I\% = [(Abs\ control - Abs\ sample) / Abs\ control] \times 100$$

Where Abs (control) is the absorbance of DPPH radical + methanol, and Abs (sample) is the absorbance of DPPH radical + sample extract or standard.

The 50% inhibition concentration (IC<sub>50</sub>) was then obtained from a linear regression plot of percentage inhibition against concentration of the extract.

### 2.3.1. *ABTS radical scavenging activity*

The method described by Otunola and Afolayan [17] was used to determine the ABTS scavenging activity. Two stock solutions of 7 mM ABTS and 2.4 mM potassium persulphate v/v were mixed together, allowed to react for 12 h at room temperature in the dark and used as the working solution. This was further diluted by mixing in 1 ml of freshly prepared ABTS solution to obtain an absorbance of  $0.706 \pm 0.001$  units at 734 nm using the spectrophotometer. The extract was allowed to react with 1 ml of the ABTS+ and the absorbance was read at 734 nm after 7 min. the percentage ABTS+ inhibition was calculated as follows:

$$\% \text{ ABTS + scavenging activity} = \frac{\text{Abs (control)} - \text{Abs (sample)}}{\text{Abs (control)}} \times 100$$

Where Abs (control) is the absorbance of ABTS radical + methanol, and Abs (sample) is the absorbance of ABTS radical + sample extract or standard.

The 50% inhibition concentration (IC<sub>50</sub>) was then obtained from a linear regression plot of percentage inhibition against concentration of the extract.

## **2.4. Antibacterial activity of aqueous extract**

### **2.4.1. Chemicals and Reagents**

The bacterial cultures used in this study were obtained from the Laboratory of Bioprocedy and Biosurface, Faculty of Sciences and Techniques, Beni Mellal, Morocco. These are *Staphylococcus aureus* (ATCC 25923), *Escherichia.coli* (ATCC 25922), and *Klebsiella pneumoniae* (ATCC 13883).

### **2.4.2. Aqueous extract of garlic preparation**

100 mg of peeled garlic was washed first by distilled water and then by 95% ethanol. The samples are left in the open air to evaporate the residual ethanol on surface and they were grinded with 100ml of sterile distilled water using mortar and pestle. The crude aqueous extract was centrifuged at 6000rpm for 20mn. The supernatant was collected and filtered through a filter system vacuum to make 100% extract. The extract was collected in a sterile container and stored at 4°C until use. Extracts were further diluted to make different concentrations such as 100%, 75%, 50%, and 25% by mixing with appropriate volumes of distilled water.

### **2.4.3. Testing of antibacterial activity using agar well diffusion method**

Resistant bacterial strains were inoculated into 10 ml of sterile nutrient broth and incubated at 37 °C for 18 h. Each culture (106 CFU/ml) was swabbed on the surface of sterile nutrient agar plate and left for 5 to 15 minutes until solidification. In each agar plate, four wells were prepared with the help of sterilized cork borer of 5 mm diameter. 100 µl of each concentration already prepared (100%, 75%, 50% and 25%) were added in their corresponding wells. The plates were incubated in an upright position at 37°C for 24 hours. The diameter of inhibition zones was measured in mm and the results were recorded. All the tests were confirmed by triplicate assays.

### **2.4.4. Minimal inhibitory concentration (MIC)**

For MIC determinations, serial dilutions of aqueous garlic extract in 5 ml of broth inoculated with 50 µl of fresh pre-cultures (inoculums ~106 UFC/ml) were prepared. The tubes were shaken and incubated at 37°C overnight and the highest dilution in which there were no growths was recorded as the MIC. All MICs were confirmed by triplicate assays.



### 3. Results and discussion

#### 3.1 Chemical and mineral composition

The results obtained by the biochemical analysis of garlic in five Moroccan areas are presents in table 1. The data showed that dry matter ranged from  $32,99 \pm 0,08$  to  $41,36 \pm 0,08$ ). Meknes and Ben Hmed cultivar have relatively higher value ( $41,36 \text{g}/100\text{g}$  and  $38,47 \text{g}/100\text{g}$ ) respectively, while a low levels were found in Fez cultivar with  $32,99 \text{g}/100\text{g}$ . These results are similar to those found in the literature [18, 19]. The results of moisture data shows that they vary widely from one area to another and ranges from 59 % to 67% respectively.

**Table 1. Chemical composition of garlic (g /100 g dry weight basis) from different geographical areas in Morocco.**

	Dry matter	Fiber	Proteins	Fat	Moisture %
Marrakesh	$33,517 \pm 0,14$	$12,151 \pm 0,36$	$16,607 \pm 0,02$	$0,036 \pm 0,007$	65
Tetouan	$33,149 \pm 0,58$	$1,693 \pm 0,87$	$14,33 \pm 0,05$	$0,17 \pm 0,01$	65
Meknes	$41,357 \pm 0,08$	$30,673 \pm 0,23$	$15,213 \pm 0,02$	$50,344 \pm 0,03$	59
Ben Hmed	$38,472 \pm 0,4$	$1,177 \pm 0,22$	$17,007 \pm 0,05$	$70,341 \pm 0,03$	59
Fez	$32,997 \pm 0,08$	$41,304 \pm 0,15$	$14,07 \pm 0,21$	$0,227 \pm 0,02$	67

The fiber content ranged from  $0,67 \pm 0,24$  to  $2,15 \pm 0,37$  of dry matter. Marrakesh cultivar records the highest value ( $1,7 \text{g}/100\text{g}$ ). While a low level ( $0,673 \text{g}/100\text{g}$ ) was found in Meknes cultivar which is in accordance with the literature [20, 21, 22, 23, 24]. Ben Hmed and Marrakesh cultivar records the highest value of proteins compared to other regions and the low amount was found in Fez cultivar (Table. 1).

**Table 2. Minerals content (mg 100 g-1) of garlic from different geographical areas in Morocco**

	Fez	Marrakesh	Meknes	Ben Hmed	Tetouan
<b>Al</b>	1,156	2,691	2,832	2,331	5,012
<b>K</b>	2773,803	3881,595	3698,562	8233,176	45225,559
<b>Ca</b>	64,887	95,622	62,142	74,379	86,599
<b>Cr</b>	0,578	2,239	0,244	3,245	1,384
<b>Mn</b>	1,042	1,433	1,002	1,583	0,892
<b>Fe</b>	6,225	14,336	5,293	19,819	9,424
<b>Cu</b>	0,731	0,931	0,821	1,116	0,785
<b>Zn</b>	3,190	4,498	4,651	4,889	5,041
<b>Se</b>	0,404	0,619	0,318	0,368	0,000
<b>Na</b>	96,109	89,317	66,847	127,526	132,924
<b>Mg</b>	96,547	105,123	83,419	109,949	103,224
<b>Si</b>	3,719	5,799	14,348	4,731	5,824

This agrees with the results of the previous studies [22, 23, 25]. The fat content in all cultivar was found in lowest quantity (under  $1 \text{g}/100\text{g}$ ). These results are comparable to those obtained by Favier et al., 1995, Soucis et al., 1994 [22, 23]. According to the present data, mineral profile of garlic (Table 2) showed that it contains potassium as a major mineral in a maximum quantity ( $2773,8$  to  $45225,56 \text{mg}/100\text{g}$ ) followed by sodium ( $66,85$  to  $132,92 \text{mg}/100\text{g}$ ), calcium ( $62,14$  to  $95,62 \text{mg}/100\text{g}$ ), iron ( $5,29$  to  $19,81 \text{mg}/100\text{g}$ ), and magnesium ( $83,41$  to  $109,95 \text{mg}/100\text{g}$ ).

Furthermore, other minerals like zinc, manganese and copper were present in lowest quantities 3,19-5,041 mg/100g, 0,89-1,58 mg/100g, 0,731-1,11 mg/100g respectively. Extensive research has been carried out to estimate the amount of mineral elements present in garlic. The results obtained from the previous findings of Otunola *et al.* (2013) [17] reported that potassium ( $54,00 \pm 1.40$  mg/100g) being the most abundant element in garlic followed by the calcium ( $26,30 \pm 0,14$  mg/100g) phosphorous ( $10,19 \pm 0,26$  mg/100g) iron ( $5,29 \pm 0,08$  mg/100g) sodium ( $4,10 \pm 0,14$  mg/100g) and magnesium ( $4,10 \pm 0,14$  mg/100g) in considerable amount, while zinc, copper and manganese were in lowest quantity ( $0,34 \pm 0,17$  mg/100g) ( $0,001 \pm 0,00$  mg/100g) and ( $0,001 \pm 0,00$  mg/100g) respectively. According to Andreini *et al.* (2008) [26], some transition metals including iron, zinc, manganese, and copper are very essential for life through their function as both structural and catalytic cofactors for proteins. Zinc supplementation for children between three months and five years reduces frequency and severity of diarrhea and respiratory illnesses (Aggarwal *et al.*, 2007) [27]. Selenium functions as a dietary antioxidant and thus has been studied for its possible role in chronic diseases [28].

### 3.2 Antioxidant activity

The antioxidant activity of plant extracts varies with assay methods Therefore a single assay may be inadequate [29]. For this reason, we cross checked antioxidant activities of extracts with two antioxidant activity assays namely DPPH and ABTS radical scavenging activity. In the DPPH assay, and as shown in table 3, all the cultivars were found to possess the higher antioxidant activity, and the high value is revealed on Tetouan cultivar (60,85 %) followed by Fez (59,79 %) while the low value (51 %) is shown on Ben Hmed cultivar. These results are higher than those found by G.A. Otunola and Afolayan [17] with mean value of 20 % and 35 %. The IC<sub>50</sub> (concentration of sample needed to scavenge 50% of DPPH) was calculated by linear regression of plots and it ranges from 1,94 mg/ml to 16,33 mg/ml.

**Table 3. DPPH antiradical activity in five areas of Morocco**

	Fez	Meknes	Marrakesh	Ben Hmed	Tetouan
DPPH %	59,79±0,67	53,41±0,55	53,72±0,23	51,51±0,62	60,85±0,11
IC <sub>50</sub> mg/ml	4,43	17,70	11,23	16,33	1,94

**Table 4. ABTS antiradical activity in five areas of Morocco**

	Fes	Meknes	Marrakech	Ben Ahmed	Tetouan
ABTS %	1.27±0,56	2.95±1,01	29.96±2,68	2.95±0,97	7.17±1,27
IC <sub>50</sub> mg/ml	3,43	5,36	4,90	4,13	1,30

For the second method based on the ABTS assay, the IC<sub>50</sub> ranges from 1,3 mg/ml to 5,36 mg/ml. The activities of the extracts from all areas against ABTS<sup>+</sup> are significantly high to the DPPH assay and these trends are not far to those reported in the literature [11]. The high value is recorded in Fez cultivar with 1,27 % followed by Tetouan cultivar with 7,17 % while the low value is revealed on Marrakesh cultivar (Table 4). Good antioxidant activity of garlic extracts towards ABTS may result from the fact that, according to literature, one of polyphenol compounds in garlic is quercetin treated as a very good scavenger of ABTS cation-radicals [30, 31].

### 3.3 Antibacterial activity of aqueous extract

The results demonstrate that aqueous garlic extract has shown the best activity at all concentrations up to 25% concentration. This indicates that aqueous garlic extract has the potential of activity against both gram-positive

(*Staphylococcus aureus*) and gram-negative bacteria (*Escherichia coli* and *Klebsiella pneumoniae*). This finding corresponded well to previous reports [32, 33, 34]. As shown in table 5, we can see the variation in the size of the inhibition zone among the different group of bacteria. The diameter of the zone of inhibition varied between 14 and 22 mm for *klebsiella pneumoniae*, between 16 and 20 mm for *Escherichia coli* and between 17 and 20 mm for *Staphylococcus aureus*. Iwalokun et al. [35] reported that antibacterial activity of aqueous garlic extract by well diffusion method was characterized by inhibition zones of 20,2 to 22,7 mm for gram-positives and 19,8 to 24,5 mm for gram-negatives. Moreover, Mukhtar et al. [14] found that aqueous garlic extract had the best inhibitory activity showing maximum zone of 22 mm against *Escherichia coli*. This agrees with the results of the present study. The results demonstrated that the size of the inhibition zone varies widely from one area to another. Fez cultivar records the highest values that range from 20 and 22 mm against the three pathogenic bacteria: *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae*, followed by Tetouan cultivar that the size of the inhibition zone varies from 17 to 19 mm. while the values obtained from Marrakesh, Ben Hmed and Meknes cultivar, are smaller comparatively to than of Fez and Tetouan cultivar. This variation in the size of the inhibition zone may be due to the lipid content of the membranes of the different groups of the microorganisms and the permeability of allicin and other garlic constituents. The diameter of the zone of inhibition also varies depending on the origin of the samples. This may be due to planting conditions, harvesting, method of irrigation, soil type and storage conditions.

**Table 5. Antibacterial activity of different concentrations of AGE of different region against *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae***

Origin of samples	Mean diameter of inhibition zone (mm)											
	Staphylococcus aureus				Escherichia coli				Klebsiella pneumoniae			
	100%	75%	50%	25%	100%	75%	50%	25%	100%	75%	50%	25%
Fez	20	17	15	12	20	16	15	12	22	20	17	14
Marrakesh	21	15	14	14	18	16	15	15	18	16	15	12
Tetouan	17	14	11	8	19	15	14	10	19	17	16	14
Ben Hmed	19	18	11	6	16	12	11	7	12	-	-	-
Meknes	18	14	12	10	18	15	14	12	14	-	-	-

**Table 6. MIC values of Aqueous Garlic Extract of different region against *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae***

Origine of samples	MIC mg/ml		
	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Klebsiella pneumoniae</i>
Fez	16,6	16,6	13,3
Marrakesh	16,6	25	8,33
Tetouan	16,6	25	16,6
Bni Hmed	33,3	33,3	16,6
Meknes	33,3	33,3	16,6



**Table 7. MIC of Aqueous Garlic Extract of different region against *Escherichia coli***

	25 %	20 %	15 %	10 %	8 %	5 %	3 %
Fez	-	-	-	-	-	+	+
Marrakesh	-	-	-	-	-	-	+
Tetouan	-	-	-	-	+	+	+
Meknes	-	-	-	-	+	+	+
Ben Hmed	-	-	-	-	+	+	+

**Table 8. MIC of Aqueous Garlic Extract of different region against *Klebsiella pneumoniae***

	25 %	20 %	15 %	10 %	8 %
Fez	-	-	-	-	+
Marrakesh	-	-	-	+	+
Tetouan	-	-	-	+	+
Meknes	-	+	+	+	+
Ben Hmed	-	+	+	+	+

**Table 9. MIC of Aqueous Garlic Extract of different region against *Staphylococcus aureus***

	25 %	20 %	15 %	10 %	8 %
Fez	-	-	-	-	+
Marrakesh	-	-	-	-	+
Tetouan	-	-	-	-	+
Meknes	-	+	+	+	+
Ben Hmed	-	+	+	+	+

+: Indicates growth of bacteria

-: Indicates inhibition of bacteria

The minimum inhibition concentrations (MIC) or lowest densities of garlic aqueous extract that prevented the growth of microorganisms were determined in the present study. The table 6 represents the MIC values of bacterial strains studied for each region. The MIC ranges between 8,3 mg/ml and 16,6 mg/ ml for *Klebsiella pneumoniae* and between 16,6 mg/ml and 33,3 mg/ml for *Escherichia Coli* and *Staphylococcus aureus*. This shows that garlic is effective for all bacterial strains studied with more action against *Klebsiella pneumoniae*. We also note that the minimum inhibition concentrations vary from one region to another as indicated in the following tables (7,8,9).

## Conclusion

This study revealed that garlic contains a range of chemicals compound that play an important role in the maintenance of human health and disease prevention. It was found that it is a source of numerous minerals like copper, zinc, iron, calcium, manganese, magnesium, aluminum and selenium although their quantities may vary depending on their provenance. Moreover, this study researched and compared the antioxidant activity of 5 cultivars from different areas in Morocco for their acclaimed health benefits. A large variability in these contents was observed among the cultivars which allow us to create a differentiation on the groups. The fives cultivars represent a good source of natural antioxidants, and they could be considered as useful sources of materials for human health. However, further research

is required to investigate the organosulphur compounds which are also responsible for the healing properties of garlic. The result of the Antibacterial activity study demonstrated that *Staphylococcus aureus*, *Escherichia.coli* and *Klebsiella pneumoniae* were effectively inhibitory in aqueous garlic extract. Hence, it is concluded that the aqueous garlic extract can be used to produce new therapeutics so it can be used to develop new antimicrobials. Further research is required to investigate the bioactive molecules of garlic and his properties against other gram-positive and gram-negative bacteria.

## References

- [1] S-N. Lee, N-S. Kim, D-S. Lee, Anal Bioanal Chem. 377 (2003) 749–756 DOI
- [2] E. Anifantaki, E. Touloupakis, D. F. Ghanotakis, J Food Biochem. (2010) 1745-4514
- [3] R. sariri, A. aliakbar, D B. Mesdarghi and A. Rezazadeh. Asian J Chem. 14 (2002) 1197-1202 [4] SG. Santhosha, P. Jamuna, S.N. Prabhavathin, Food Biosci. 3 (2013) 59 – 74 10.1007/s00216-003-2163-z
- [5] M. Arzanlou, S. Bohlooli, Food Chem. 120 (2010) 179–183
- [6] M. Gulfraz, M. Imran, S. Khadam, D. Ahmed, M. J. Asad, K. S. Abassi, M. Irfan, S. Mehmood, Afr J of Plant Sci. 8 (2014) 298-306
- [7] Y S. Queiroz, E Y. Ishimoto, D H M. Bastos, G R. Sampaio, Food Chem. 115 (2009) 371-374
- [8] H M. Womeni, F T Djikeng, B Tiencheu, M Linder, Adv Biol Chem. 3(2013) 304-313
- [9] SA. Salhi, A. Bouyanzer, A. Chetouani, S. El Barkany, H. Amhamdi, I Hamdani, A. Zarrouk, B. Hammouti, J.M. Desjobert, J. Costa, Mor. J. Chem. 5 N°1 (2017) 59-71
- [10] A. Sawafta, B. Mansour, S. Amereih, A. Zarrouk, A. Chetouani, I. Warad. Mor. J. Chem. 7 N°4 (2019) 758-764
- [11] Y. El Ouadi, A. Beladjila, A. Bouyanzer, Z. Kabouche, H. Bendaif, F. Youssfi, M. Berrabah, R. Touzani, A. Chetouani, B. Hammouti, Mor. J. Chem. 5 N°1 (2017) 139-152
- [12] VD. Nikolic, M. Stankovic, LB. Nikolic, DM. Cvetkovic, DU. Skala, Chem. Ind. 58 (2004)109-113
- [13] D. Deresse, Asian J Med Sci. 2 (2010) 62-65
- [14] S. Mukhtar, I. Ghor, Int J of Appl Bio Pharm technology. 3 (2012).
- [15] AOAC, "Official methods of analyses", Association of Official Analytical Chemists: Washington, (1990).
- [16] AACC. 2000. **Approved methods of the American Association of Cereal Chemists, 10th Ed. AACC, St. Paul, MN, USA.**
- [17] G.A.Otunola, A.J. Afolayan, J Appl Bot Food Qual. 86 (2013) 66–70, DOI:10.5073/JABFQ.2013.086.010
- [18]G.P. Sharma, Suresh Prasad. J Food Eng 75 (2006) 441–446.
- [19] P. S. Madamba, R. H. Driscoll & K. A. Buckle. J Food Eng 23 (1994) 309-319.
- [20] S.H.Omar, J. Nat. Prod, 2013. pp. 3661–3696.
- [21] H.A.R. Suleria, et al. Asian Pac J Trop Dis, (2015), vol 5, pp 271–278.
- [22] J.C. Favier, J. I. Ripet, C. Toque, M. Feinberg, Répertoire général des aliments Table de composition INRA, CNEVA, Ciquel Tec et Doc /Paris, 1995, 897.
- [23] S.W. Souci, W. Fachmann, H. Krant, Tableaux de valeurs nutritives, 5ème édition medpharm, CRC preo/Germany, 1994, 1091.
- [24] S.G. Santhosha, P. Jamuna, S.N. Prabhavathin, A review. Food Bioscience; (2013), 3, 59.
- [25] L.D.Lawson., C.D. Gardner, J. Agric. Food Chem.,(2005), 53, 6254–6261.
- [26] C. Andreini, I. Bertini, G.Cavallaro, G. L Holliday, and J. M Thomson, J Biol Inorg Chem (2008) 13: 1205-1218.
- [27] R. Aggarwal, J. Sentz, and M. A. Miller, Pediatrics (2007) 119 (6): 1120-1130.

- [28] Boosalis, M. G. Nutr Clin Pract (2008) 23 (2): 152-160.
- [29] GC. Yen, PD. Duh, HJ. Su, Food Chem. 89 (2005) 379-385.
- [30] P A. Sekher, T S. Chan, P J. O'Brien, C A. Rice-Evans, Biochem and Biophys Res Commun. 282 (2001) 1161–1168.
- [31] A M. Nuutila, R. Puupponen-Pimiä, M. Aarni, K-M. Oksman-Caldentey, Food Chem. 81 (2003) 485–493.
- [32] K. E. Giles, D. Hide, D M. Salmon, Giles KE, D Hide, D M. Salmon, Microb Ecol Health Dis. 12 (2000) 81–84.
- [33] P. Saravanan, V. Ramya, H. Sridhar, V. Balamurugan, S. Umamaheswari, Glob Vet. 4 (2010) 519-522.
- [34] YA. Jassim, M J. Nushia, Journal of Babylon University/Pure Appl Sci. 22 (2014).
- [35] B.A. Iwalokun, A. Ogunledun, D.O. Ogbolu, S.B. Bamiro, J. Jimi-Omojola, J Med Food. 7 (2004) 327- 333.
- 
- (2022) ; <https://revues.imist.ma/index.php/morjchem>