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Contribution to the Evaluation of Antibacterial Activity of Allium Sativum L. (Garlic) Essential Oil and Fresh Juice

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Abstract: The research aims to evaluate in vitro the antibacterial activity of the essential oil and fresh juice of Allium sativum L. (garlic), harvested from the region of El Harrouche, Skikda (Algeria). The antibacterial effect of essential oil and fresh juice was assessed by the Aromatogram method against three Gram-clinical bacterial strains (*Escherichia coli*, *Pseudomonas aeruginosa* and Klebsiella pneumoniae) and a Gram + strain (*Staphylococcus aureus*) in addition to two reference strains *Escherichia coli ATCC25922* and *Staphylococcus aureus* ATCC29213. The minimum inhibitory concentration of the essential oil and fresh juice was determined by the solid dilution method. The results showed that the essential oil had an antibacterial effect only on *Staphylococcus aureus* strains (19 and 18mm), whereas the fresh juice exerted an antibacterial effect against all the tested strains, with inhibition zones of 17, 18,19,18, 16 and14 mm for *Staphylococcus aureus*, Staphylococcus aureus ATCC 29213 , *Escherichia coli*, *Escherichia coli ATCC25922* Pseudomonas aeruginosa and *Klebsiella pneumoniae* respectively. Significant statistic differences were recorded between the effects of different juice dilutions ($p = 0.004$) where the tested strains were completely resistant at the dilution 5%. The minimum inhibitory concentration of the essential oil was 640 μg/ml for *Staphylococcus aureus*, while those of fresh juice were 640μg/ml for Escherichia coli strains, 1280μg/ml for *Staphylococcus aureus* strains and 2560μg/ml for *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*.

Keywords: *Antibacterial activity, allium sativum L., Aromatogram, essential oil, fresh juice, minimal inhibitory concentration*

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I. INTRODUCTION

Antibiotics are not totally inoffensive for the organism. In addition to their antimicrobial activity, they may provoke undesirable effects [1]. Antibiotics are also treated by the appearance and spread of multiresistant bacteria [2]. Faced with this problem, the use of herbal medicine is more and more relevant [3]. Phytotherapeutics are extracted from medicinal plants and their active ingredients may relieve symptoms and even cure diseases [4]. Allium sativum L. is one the olds medicinal plants used as condiment, aliment or for therapeutic purposes [5]. Garlic has been used for centuries in various societies to combat infectious diseases. Historically, it is believed that Louis Pasteur described the antibacterial effect of

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garlic in 1858 for the first time, although no reference is available [6, 7]. Garlic is characterised by an antiviral, antifungal and antiparasitic activities [8, 9, 10, 11]. It has a wide broad of antibacterial activities against Gram positive and Gram negative bacteria [8]. In order to continue to exploit the medicinal plants growing in Algeria and famous for their antimicrobial phytopharmaceutical properties, we tried to detect and highlight the antibacterial power of garlic harvested from the region of El Harrouch, the state of Skikda (Algeria) but mainly to compare the effect of essential oil and fresh juice.

II. MATERIAL AND METHOD

A. *Isolation and Identification of Bacterial Strains*

Pathogenic bacteria were isolated from clinical samples. Gram negative bacteria were isolated on Hektoen medium while Gram positive bacteria were isolated on Chapman medium. Negative bacteria were identified using API 20E system. Gram positive bacteria were identified using the catalase and coagulase tests. The reference strains *Escherichia coli ATCC25922* and *Staphylococcus aureus ATCC29213* were provided by Dr. Becheker Imane.

B. *Extraction of Garlic Essential Oil*

Essential oil of garlic was extracted using an hydrodistillator in the laboratory of soil chemistry, Skikda university. 100 grams of dry garlic were firstly macerated one hour in 300 ml of distilled water. The homogenate was then put in the hydrodostillator during 2 to 3 hours. Two phases were obtained, the organic one is the essential oil and the aqueous one is the aromatic water. Anhydride magnesium or sodium sulfate was added to eliminate all water particles. Essential oil was them conserved in small glass bottles at 4°C.

Fresh juice was obtained after grinding 60 g of the garlic bulbs and filtration trough a gauze. The filtrate was them centrifuged at 3000g during 20 mn and the supernatant was recovered. The fresh juice was then diluted in order to obtain the following dilutions: 100%, 75%, 50%, 20%, 10% and 5% [12] and conserved at 4°C.

C. *Chemical Composition of Garlic Essential Oil*

Chemical composition of garlic essential oil collected from El Harrouch region was previously determined by [13] using a chromatograph coupled to a mass spectrometer (MS) type Shimadzu equipped with a capillary column OV17X .

D. *Determination of Antibacterial Activity of the Garlic Essential Oil and Fresh Juice*

Antibacterial activity was determined using the method of discs diffusion in solid medium (Aromatogram). The inoculum was prepared from a 18 hours culture on a Mueller Hinton broth (opacity equivalent to 0.5MacFarland) with a first dilution then a second dilution (1/100) to get 106CFU/ml [14]. Filter paper discs (6mm) impregnated with 15 ul of essential oil were deposited on Muller Hinton medium previously inoculated with 1 ml of the bacterial suspension 106CFU/ml . A disc impregnated with distilled water and a second one impregnated with tween 20, ethylic alcohol and distilled water were used as control discs. For the fresh juice the filter discs were impregnated with 5 ul of each dilution. A supplemented disc impregnated with 5 ul of distilled water was used as control disc. Antibacterial activity was determined according to [15]. A supplemented disc impregnated with 5 ul distilled water was used as control disc. Petri dishes were then incubated at 37C for 24h.

E. *Determination of Minimal Inhibitory Concentration of Essential Oil and Fresh Juice*

1) Preparation of the mother solution: Mother solutions of 2560 ug/ml are good for most of the sensitivity standard ranges [16]. In a sterile flask containing a volume of distilled water, we added a quantity of essential oil to get the concentration 2560 ug/ml [14]. 2% (v/v) of ethylic alcohol of 95% and 0.05% of Tween 80 were added to the mother solution to get an homogenate solution.

2) Dilution in solid medium: The technique of dilution in solid medium was used. 4 ml of the initial solution of each extract were diluted in 2ml of sterile distilled water. Serial dilutions were then prepared from each extract: 1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128, 1/256. 2ml of each dilution were then added to Petri dishes containing 18 ml of Muller Hinton medium and inoculated with 1 ml of the bacterial suspension (106CFU/ml) previously prepared .

F. *Bacterial Sensitivity to Antibiotics*

The antibacterial activity of garlic essential oil and fresh juice was compared with the activity of the following antibiotics: IPM, CIP, GEN, CL, DO, FF C, FOX, AMC, TIM, AMX, PF, VA, GEN, E, SP, FC, MY, P, OX, AM, CL, C, CTX, FOX, AZM, IPM, K, TOB, ATM, CN, OF, TIM (Appendix) .

A bacterial inoculum was firstly prepared. Four to five bacterial colonies were put in 5 ml of sterile physiological water (0.9%) and well homogenised to get an opacity of 0.5 Mac Farland equivalent to an optical density of 0.08 to 0.1 at 625 nm. Muller Hinton medium

previously versed in Petri dishes was then inoculated with the prepared inoculums using the swabbing method. The bacterial sensitivity was determined according to CA-SFM [17].

G. Statistical Analysis

Statistical analysis was done using One Way ANOVA test with Greenhouse Geisser Correction at $p < 0.05$. Data were treated using an IBM SPPS software.

III. RESULTS AND DISCUSSION

A. Microbiological Analysis

During this study we worked on 4 pathogenic bacterial strains. They were identified using macroscopic , microscopic and biochemical characters as *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and Staphylococcus aureu in addition to two reference strains *Escherichia coli ATCC25922* and *Staphylococcus aureus ATCC29213*.

B. Characteristics of Garlic Extracts

The organoleptic characteristics of garlic essential oil showed that it is of liquid aspect, pungent odour, and a yellow colour. The yield of essential oil extracted from El Harrouch region garlic was 0.02% whereas that of fresh juice was 2.16% (Table 1).

TABLE 1
YIELDS OF GARLIC EXTRACTS (ESSENTIAL OIL AND FRESH JUICE)

	Essential Oil	Fresh Juice
Weight of Garlic (G)	100 G	60g
Yield of Extraction (%)	0.02%	2.16%
Extraction Method	Hydrodistillation	Grinding And Centrifugation

Essential oils of garlic are not easily extractable and need extraction under vacuum which is not available. In addition to that this plant is poor in essential oils [18]. The obtained yield was less than that obtained from garlic of Mustaghanem, El Taref and Biskra regions (0.2, 0.3, and 1% respectively) [12, 19, 20]. These variations in garlic essential oil yields are explained by the maturity degree of garlic bulbs, the interaction with the environment (soil, climate) , the time of harvest and the method of extraction [21].

C. Evaluation of Garlic Extracts Antibacterial Activity

Essential oil of garlic was only active against *Staphylococcus aureus* strains with inhibition zones of 18mm and 19mm (Table 2).

TABLE 2
CLASSIFICATION OF THE BACTERIAL STRAINS SENSITIVITY ACCORDING TO MOREIRA ET AL. (2005)

Extract	Essential Oil	Fresh Juice (100%)	Fresh Juice (75%)	Fresh Juice (50%)	Fresh Juice (20%)	Fresh Juice (10%)	Fresh Juice (5%)
Bacterial Strain							
Staphylococcu Aureus	18mm (++)	17mm (++)	15mm (++)	13mm (+)	12mm(+)	6mm (-)	0mm (-)
Staphylococcus Aureus Atcc 29213	19mm (++)	18mm (++)	17mm (++)	15mm (++)	13mm (+)	6mm (-)	0mm (-)
E.Coli	0mm (-)	19mm (++)	18mm (++)	15mm (++)	12mm(+)	0mm (-)	0mm (-)
E.Coli Atcc 25922	0mm (-)	18mm (++)	16mm (++)	15mm (++)	13mm (+)	0mm (-)	0mm (-)
Klebsiella Pneumoniae	0mm (-)	16mm (++)	13mm (+)	12mm (+)	7mm (-)	5mm (-)	0mm (-)
Pseudomonas Aeruginosa	0mm (-)	14mm (++)	12mm (+)	11mm (+)	10mm (-)	7mm (-)	0mm (-)

Not Sensitive (-) : Diameter < 8mm , Sensitive (+) :9mm < Diameter < 14mm , Very Sensitive (++) : 15mm < Diametre < 19mm

This activity was higher than that obtained by [12, 18, 22] who reported inhibition zones of 15, 8 and 12mm respectively. According to [23, 24, 25, 26] high resistance rates were recorded within Gram negative bacteria in comparison with Gram positive bacteria. This may be due firstly to the action of garlic's allicine on Gram positive bacteria [27] and secondly to the presence of lipopolysaccharidic layer in the wall of Gram negative bacteria [28, 29]. [14] however, reported that 56,6% of *Pseudomonas aeruginosa* strains were sensitive to garlic essential oil of El Harrouch region. On the other hand fresh juice was highly active towards the six stains with inhibition zones of 17, 18, 19, 18, 16 and 14mm for *Escherichia coli*, *Escherichia coli ATCC 25922*, *Staphylococcus aureus* ,*Staphylococcus aureus ATCC 29213*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* respectively. Strika and his collaborators [30] demonstrated that domestic fresh juice was more effective against staphylococci strains (31-29 mm) in comparison with Gram negative bacteria. [31] showed that garlic juice effectively inhibited the growth of both *Pseudomonas sp* and *S. aureus* (16mm for each). Statistical analysis showed significant differences between the effects of the different fresh juice dilutions ($p = 0.004$). Bacteria were totally resistant at the dilution 5%. Essential oil of Allium sativum L. did not show an important antibacterial activity in comparison with fresh juice. This may be explained by the loss of volatile components during the extraction and/or storage of essential oil and during the incubation period [32].

D. *Determination of Minimal Inhibitory Concentration*

The minimal inhibitory concentration of essential oil towards the two strains of *Staphylococcus aureus* was 640 μg/ml (Fig 1).

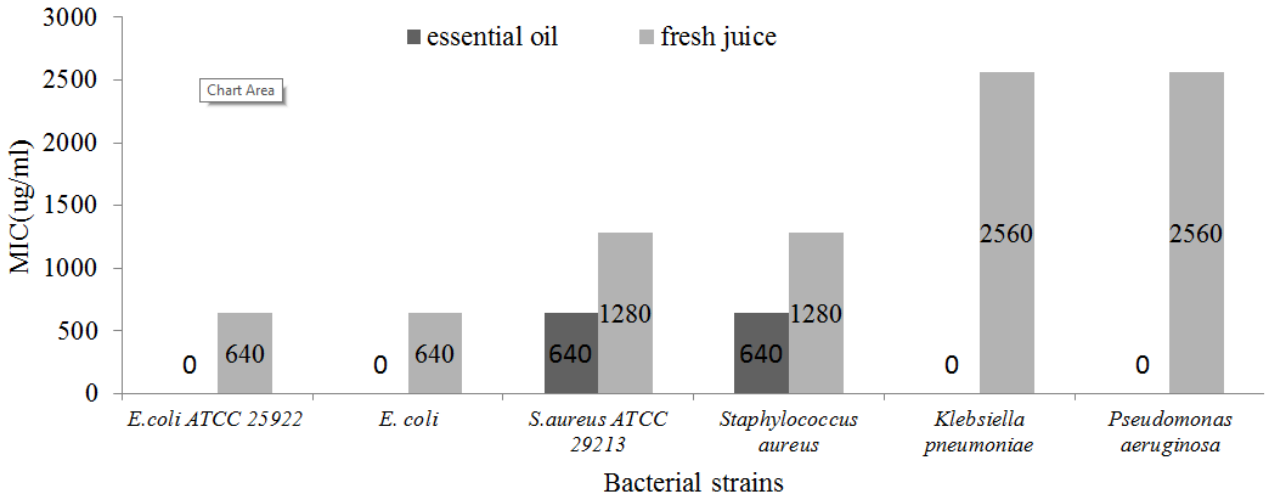

Fig. 1. The minimal inhibitory concentration Of essential oil and fresh juice (Ug/Ml)

The weakest minimal inhibitory concentration of fresh juice was recorded in case of Escherichia coli strains (640 μg/ml) whereas the highest one was recorded with *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* (2560 μg/ml). [31] reported minimal inhibitory concentrations of 1 and 100 mg/ml for *Pseudomonas aeruginosa* and *Staphylococcus aureus* respectively. The minimal inhibitory concentrations obtained in our study are of high values. This is may be explained by the chemical profile of garlic which is poor in trisulfide diallyl (1.47%) and tetrasulfide diallyl (4.92%) [13]. These two compounds are demonstrated by many authors as the most active compounds in Allium sativum L [33, 34].

E. *Comparison of Antibacterial Activity of Antibiotics and Garlic Extracts*

1) Staphylococcus auresu ATCC 29213: The reference strain *Staphylococcus aureus ATCC 29213* is a wild strain (Fig 2). It was very sensitive to the tested antibiotics and very sensitive to essential oil (19mm) and to fresh juice (18mm).

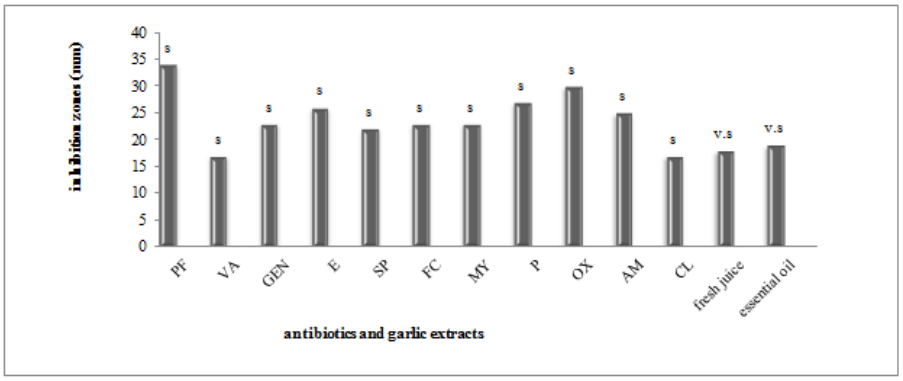

Fig. 2. Comparison between the effect of garlic extracts and antibiotics against *staphylococcus aureus atcc 29213*

2) Staphylococcus aureus: Staphylococcus aureus was highly sensitive to the garlic extracts, which showed large inhibition zones (18 and 17 mm for the essential oil and the fresh juice respectively) (Fig 3). Garlic essential oil and fresh juice had better activity than the antibiotics P,AM,OX and CL to which the strain was resistant.

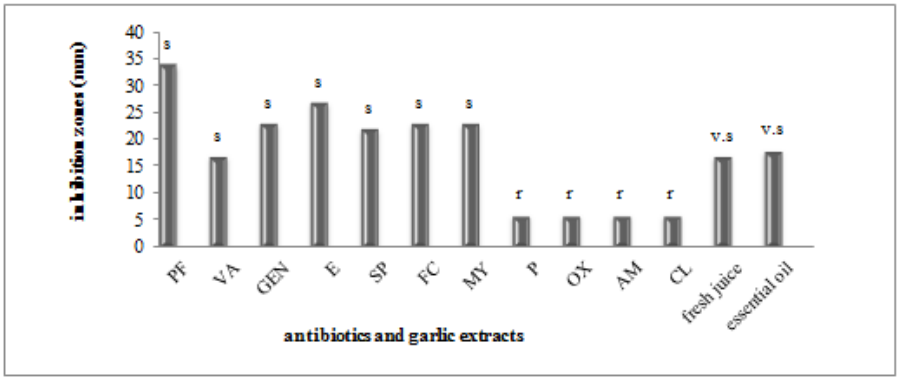

Fig. 3. Comparison between the effect Of garlic extracts and antibiotics against *staphylococcus aureus*

3) Escherichia coli ATCC 25922: The reference strain *Escherichia coli ATCC 25922* which is a wild strain was sensitive to all the tested antibiotics and highly sensitive to the fresh juice (18mm) (Fig 4).

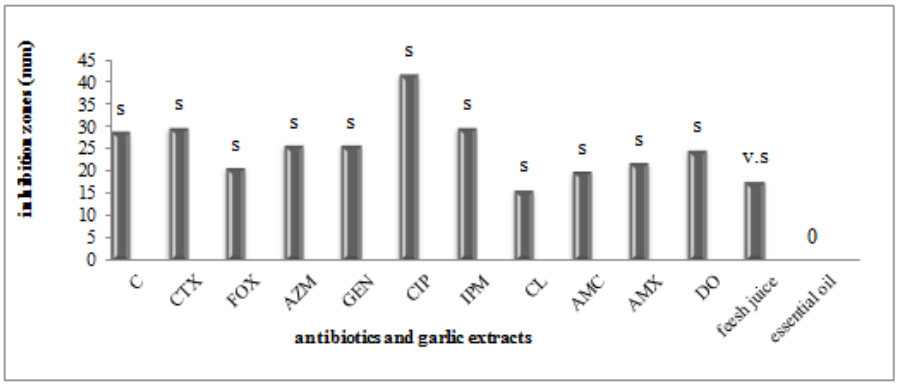

Fig. 4. Comparison between the effect of garlic extracts and antibiotics against *escherichia coli atcc 25922*

4) Escherichia coli: Escherichia coli has demonstrated high sensitivity toward the fresh juice (17 mm) in comparison with the antibiotics AMC,AMX, DO and CL to which it has developed an antibioresistance (Fig 5).

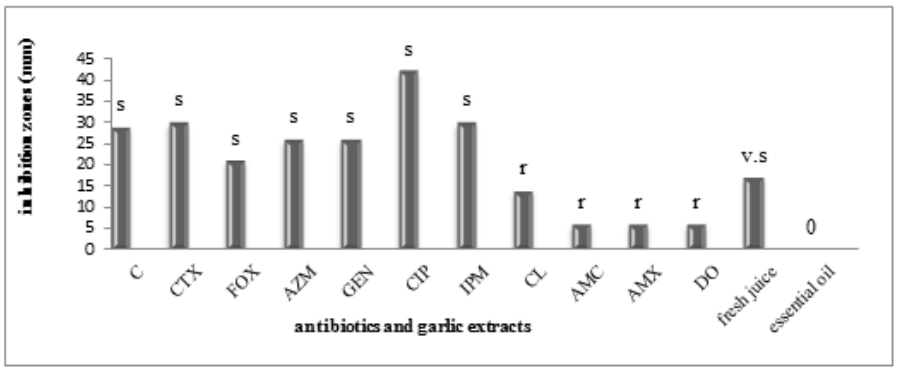

Fig. 5. Comparison between the effect of garlic extracts and antibiotics against *escherichia coli*

5) Klebsiella pneumoniae: The obtained results (Fig 6) revealed that *Klebsiella pneumoniae* was very sensitive to fresh juice (16 mm) which had a notorious inhibitory effect in comparison with the majority of the tested antibiotics TOB,CTX,CL,AMC,CN,OF and TIM whose *Klebsiella pneumoniae* was resistant.

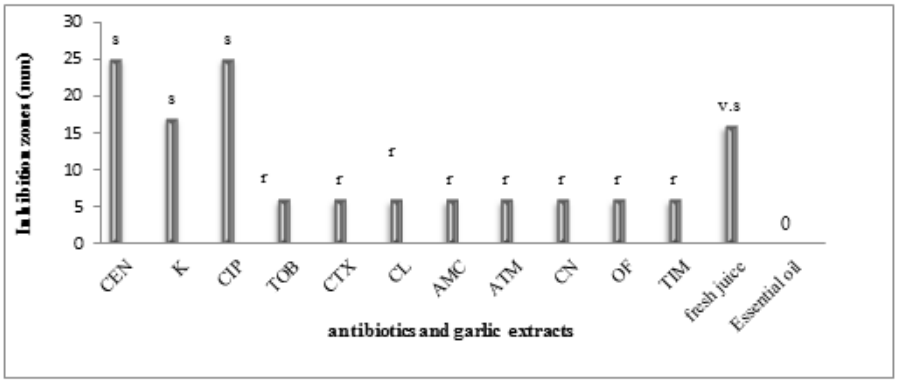

Fig. 6. Comparison between the effect of garlic extracts and antibiotics against *klebsiella pneumoniae*

The advantage of plant extracts is that they don't push the microbes to develop resistance toward them. One of the raisons is that the classical antibiotics are constituted of one active molecule. which makes the microbes easy to synthesis an enzyme able to neutralize or destruct it. For exemple, the essential oil of Satureja montana L. is composed of different molecules, to develop a resistance toward this essential oil, the microbes need to synthesize an enzyme able to degrade all of the active molecules which is impossible or dozen of different enzymes at the same time which is also impossible [35].

IV. CONCLUSION

The study of garlic antibacterial activity suggests that this plant is a natural source of chemical molecules that may have important antibacterial activity. This activity differs in function of the extract and the method of extraction which may influence the nature of the extracted components. The fresh juice constitutes an excellent antibacterial product in comparison with essential oil. The obtained results may have possible applications in the treatment of infectious maladies. Garlic fresh juice and essential oil provide a less expensive source of biomolecules which may be used as complementary drugs with antibiotics, so that they help to face the multiresistant bacteria.

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Appendix

APPENDIX 1: LIST OF THE TESTED ANTIBIOTICS

Code	Antibiotic
Am	Ampicillin
Amc	Amoxicillin+Clavulanic Acid
Amx	Amoxicillin
Atm	Aztreonam
Azm	Azithromycin
C	Chloramphenicol
Cip	Ciprofloxacin
Cl	Colistin
Ctx	Cefotaxim
Do	Doxycyclin
E	Erythromycin
Fc	Fusidic Acid
Ff	Fosfomycin
Fox	Cefoxitin
Imp	Imipenem
Gen	Gentamicin
K	Kanamycin
Of	Ofloxacin
My	Lyncomycin
Ox	Oxacillin
P	Penicillin
Pf	Pefloxacin
Sp	Spiramycin
Tim	Ticarcilin
Tob	Tobramycin
V	Vancomycin