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Evaluation of antibacterial activity of aqueous extract and essential oil from garlic against some pathogenic bacteria

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Abstract

The antibacterial effects in vitro were evaluated from aqueous extracts (AE) and essential oils (EO) of garlic *Allium sativum* of local production (Algeria) and import (China). Different concentrations are used on three bacterial strains: *Staphylococcus aureus, Escherichia coli* and *Klebsiella oxytoca*. The inhibitory power seems to be proportional to the concentration. All the bacterial isolates were susceptible to both extracts, EO and AE of both garlic, Algerian and Chinese. We noted that *K. oxytoca* seems to be more sensitive among other strains to the effect of the fresh extract and essential oils with inhibition zone of 22, 23 mm and 22, 21 mm for Algerian and Chinese garlicAE and EO, respectively. The highest inhibition is achieved by both EO and AE. Natural spices of garlic possess effective anti-bacterial activity against multi-drug clinical pathogens and can be used for prevention of drug resistant microbial diseases and further evaluation is necessary.

Introduction

The use of herbal drug is increasing. One of these plants used most intensively and widespread is garlic. Historically, garlic has been used for centuries worldwide by various societies to combat infectious disease. Garlic can be provided in the form of capsules and powders, as dietary supplements, and thus differ from conventional foods or food ingredients. Louis Pasteur was the first to describe the antibacterial effect of onion and garlic juices. *Allium* vegetables, particularly garlic (*Allium sativum* L.) exhibit a broad i) antibacterial activity against Gram-negative and Gram-positive bacteria, including multidrug-resistant enterotoxicogenic strains of *Escherichia coli*; ii) antifungal activity, particularly against *Candida albicans*; iii) antiparasitic activity, including some major human intestinal protozoan parasites such as *Entamoeba histolytica* and *Giardia lamblia*; and iv) antiviral activity (Ankri and Mirelman, 1999). besides other beneficial effects i.e i) reduction of risk factors for cardiovascular diseases, ii) reduction of cancer risk, iii) antioxidant effect, iv) antimicrobial effect, and v) enhancement of detoxification foreign compound and hepatoprotection (Bayan *et al*., 2014). Among the *Alliums*, garlic has the most powerful and penetrable smell. The first presentations on the physical properties and chemical structure of the principal compound and antibacterial fragrant garlic were cited in Benmeddour *et al.* (2015). The antibacterial principle of *A. sativum* was identified as diallylthiosulfinate and named "Allicin". Allicin is produced during the crushing of garlic cloves by the interaction between the amino acid alliin and the enzyme alliinase; allicin is a precursor of a number of secondary products formed in crushed garlic preparations and possesses various biological activities (Balestra *et al*., 2009). Garlic has attracted particular attention of modern medicine because of widespread belief about its effects in maintaining good health. In some Western countries, the sale of garlic preparations ranks with those of leading prescription drugs. There is appreciable epidemiologic evidence that demonstrates therapeutic and preventive roles for garlic. Garlic can be consumed as fresh and has also its pills, capsules and extracts. While, it is safe, when taken in carful amounts, it can lacerate stomach, when consumed in excessive amounts (Ayaz and Alpsoy, 2007). Garlic, with more than 200 chemical substances in its body, such as volatile oils consisting on sulfur (allicin, alliin and ajoene), enzymes (alliinase, peroxidase and miracynase), carbohydrates (sucrose, glucose), minerals (germanium, selenium, zinc), amino acids (cysteine, glutamine, isoleucine and methionine), flavonoids (quercetin, cyaniding, allistatin I and II), vitamins (C, E, A, B1 and B2)

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and β-carotene (Goncagu and Ayaz, 2010), has the capacity of protecting human body against many illnesses. Although, it is said that garlic should be consumed as fresh for it can be effective, some researches argue that in some situations its cooked and waited extracts and oils can provide better protection against free radicals and infections than fresh garlic (Ayaz and Alpsoy, 2007). Garlic cloves include a mixture of mono and polysulfides smelling very heavy (Goncagu and Ayaz, 2010). The objective of this study is to evaluate the antibacterial potential of extracts ("AE" and "EO") from local garlic (Algeria) and import garlic (China). The inhibitory potency of such extracts is tested on three different pathogenic bacterial strains which were antibiotic resistant. As this extracts are not tested on other non resistant bacterial strains.

Materials and Methods

Plant material

About 1 kilogram of fresh garlic (*Allium sativum* L.) bulbs from local production (Algeria) and import (China) were purchased from a weekly market (Ain Assel, El-Tarf. Algeria).

Aqueous garlic extract preparation

The cloves on fresh bulbs from the two samples were separated and peeled to obtain the edible portion. One kilogram of the edible portion was crushed under aseptic conditions. The homogenate was recovered by filtration through double layer of sterile fine mesh cloth and collected to make 100% extract. This was collected in an eppendorff tube and stored at 4°C until used.

Garlic essential oil extraction

In order to isolate essential oils by hydrodistillation, the fresh plant material (cloves) was packed in a still and a sufficient quantity of water is added and brought to a boil; alternatively, live steam is injected into the plant charge. Due to the influence of hot water and steam, the EO was freed from the oil glands in the plant tissue. The vapor mixture of water and oil was condensed by indirect cooling with water. From the condenser, distillate flows into a separator, where oil separated automatically from the distillate water. The hydrodistillation was carried out in apparatus during 4 hours; EO obtained were stored at 4°C in hermetically sealed dark eppendorff tube until they were used.

Microbial culture growth conditions

Micoorganisms used included the following Gram-positive bacteria: *Staphylococcus aureus* (urinate), and Gram-negative ones, *Escherichia coli* (urinate), *Klebsiella oxytoca* (urinate). All microorganisms were isolated from hospital and are antibiotic resistant *E. coli* and *K. oxytoca* resistant to vancomycine, *S. aureus* resistant tocolistine. The isolates were obtained from the Microbiology Laboratory of Ibrahim Marzougui Hospital, El-Tarf, Algeria. Bacteriologically, the isolates were purified and then identified.

Selection of dilution solvent and disk diffusion method

Several solvents were tested for their antibacterial activity using the disk diffusion method, whereas, several studies showed that the solvent dimethylsulfoxide (DMSO) was the most solvent used for antimicrobial EO studies (Ouibrahim *et al.*, 2004; Kheyar *et al.*, 2014). In fact the DMSO showing no antimicrobial activity, so, it was selected as a diluting medium for the oils. This solvent also served as the control. Each EO was used at different concentrations: pure oil or undiluted oil was taken as dilution 1, diluted oil in DMSO to make dilutions of 1/2, 1/4, 1/8, 1/16, 1/32 and 1/64. The same dilutions were made with the AE but in distilled water.

Antibacterial analysis

Discs of 6mm in diameter, previously sterilized, were used. The selected strains of bacteria were inoculated into 10 mL of sterile nutrient broth, and incubated at 37°C for 8 hours. The cultures were swabbed on the surface of sterile nutrient agar plates using a sterile cotton swab. Under aseptic conditions and using a micropipette, 100 µl of different concentrations of garlic extracts (EO and AE) was put on each disc and placed on agar. A witness disc (soaked in DMSO) was incubated under the same conditions to ensure that DMSO was devoid of antibacterial activity. After incubation for 24 h in an incubator at 37°C, reading was done. The effect of EO and AE on bacteria was estimated by the appearance of clear zones around the discs. The diameter of the halo of growth inhibition was measured and expressed in mm (including the diameter of the disc of 6 mm).

Results and Discussion

EO yield

The yields of essential oils from the fresh matter of garlic were 0.3 and 0.5% for local garlic and Chinese one, respectively. These results are different from those found in other regions around the world. The yield of Tunisian garlic found by Chekki *et al.*

(2014) was 0.15%, according to Benmeddour *et al.* (2015), the yield recorded in Algerian garlic was 1%. These differences may result from the high moisture that characterizes the study's area; because it is known that maximum yields are obtained by dry weather.

In vitro *antimicrobial activity*

Antibiotics commonly used for therapeutic purposes, as well as antibiotics added to animal feedstuff for increasing animal flesh production, contribute to the extensive spread of resistance. Antibiotic resistance has also been shown in plant pathogenic bacteria. Furthermore, hospital, industrial, and domestic wastes worsen the global situation. Resistant microorganisms may pass on to other hosts in different ways or their mutations may give new multiplying bacterial generations. Horizontal gene transfer between microorganisms has a great effect on increasing bacterial pathogens. In order to safeguard public health, an alternative interesting approach for reducing pathogen transmission should be to use EOs.

Garlic has been known for ages to have anti-infective properties against a wide range of microorganisms. The present study has further demonstrated the antimicrobial potency of garlic against local multidrug-resistant bacteria from Algeria.

The *in vitro* antibacterial activity of the EOs and AEs against the tested microorganisms (Gram-positive and Gram-negative bacteria) was assessed by the disc diffusion method by measuring the inhibition zones. According to the results presented in Tables 1, 2 and 3 the different extracts with the highest antibacterial effects produced inhibition zones larger than 20 mm diameter.

Both extracts (EO and AE) have an antibacterial activity against strains tested and the activity was a linear function of concentration. EOs were more effective as compared to AEs. At 100% (dilution 1), the maximum zone of inhibition was observed against *K. oxytoca*, a Gram-negative organism and the minimum was against *E. coli* a Gram-negative and *S. aureus* a Gram-positive organism, for the EO; whereas for the AE at 100% (dilution 1), the maximum zone of inhibition was observed against *E. coli*, a Gram-negative organism and the minimum was against *S.aureus* a Gram-positive organism.

The results presented in Table 3, clearly demonstrate that among the strains tested, *K. oxytoca* was the most sensitive to both Algerian and Chinese garlic EO, showing inhibitory zones of 23.0 and 21.0 mm, respectively. We noted that the Chinese EO was active until dilution of 1/64 (10 mm), which is interesting in antibacterial treatment knowing the toxicity of concentrate EO, whereas at the same dilution *K. oxytoca* was resistant to the Algerian EO. In this context, the EO from Chinese garlic is more effective than the Algerian garlic EO. These results are in agreement with those of Tsao and Yin (2001) on Chinese EO garlic, *K. pneumonia* was extremely

Table 1. Antibacterial activity of different concentrations of Algerian and Chinese garlic against *E. coli* by disk diffusion method

Diameter of inhibition zone (mm) including well diameter of 6 mm				
Concentration	Algerian garlic		Chinese garlic	
	EO*	AE**	EO	AE
1	10 (+)	**24 (+++)**	**20 (+++)**	**18 (++)**
1/2	9 (+)	12 (+)	14 (+)	16.5 (++)
1/4	8 (-)	10 (+)	14 (+)	15 (++)
1/8	8 (-)	6 (-)	10 (+)	13 (+)
1/16	7.5 (-)	6 (-)	10 (+)	9 (+)
1/32	7 (-)	6 (-)	10 (+)	7 (-)
1/64	6 (-)	6 (-)	7 (-)	6 (-)

EO*: Essential oil; AE**: Aqueous Extract
+ and – values represent the degree of sensitivity of the strains according to Ponce *et al.*(2003): not sensitive : diameters < 8 mm; sensitive (+): 9 < diameters <14 mm; very sensitive (++):15 < diameter <19 mm and extremely sensitive (+++): diameters > 20 mm.

Table 2. Antibacterial activity of different concentrations of Algerian and Chinese garlic against *S. aureus* by disk diffusion method

Diameter of inhibition zone (mm) including well diameter of 6 mm				
Concentration	Algerian garlic		Chinese garlic	
	EO*	AE**	EO	AE
1	10 (+)	11 (+)	**19.5 (+++)**	8 (-)
1/2	9 (+)	9 (+)	9 (+)	7 (-)
1/4	8 (-)	8 (-)	8 (-)	6 (-)
1/8	6 (-)	7 (-)	7 (-)	6 (-)
1/16	6 (-)	6 (-)	6 (-)	6 (-)
1/32	6 (-)	6 (-)	6 (-)	6 (-)
1/64	6 (-)	6 (-)	6 (-)	6 (-)

EO*: Essential oil; AE**: Aqueous Extract
+ and – values represent the degree of sensitivity of the strains according to Ponce *et al.*(2003): not sensitive : diameters < 8 mm; sensitive (+): 9 < diameters <14 mm; very sensitive (++):15 < diameter <19 mm and extremely sensitive (+++): diameters > 20 mm.

Table 3. Antibacterial activity of different concentrations of Algerian and Chinese garlic against *K. oxytoca* by disk diffusion method

Diameter of inhibition zone (mm) including well diameter of 6 mm				
Concentration	Algerian garlic		Chinese garlic	
	EO*	AE**	EO	AE
1	23 (+++)	22 (+++)	21 (+++)	22 (+++)
1/2	18 (++)	20 (+++)	19 (++)	20 (+++)
1/4	15 (++)	15 (++)	17 (++)	18 (++)
1/8	12 (+)	10 (+)	16 (++)	13 (++)
1/16	10 (+)	8 (-)	14 (++)	10 (+)
1/32	8 (-)	6 (-)	12 (++)	8 (-)
1/64	7 (-)	6 (-)	10 (+)	6 (-)

EO*: Essential oil; AE**: Aqueous Extract
+ and – values represent the degree of sensitivity of the strains according to Ponce *et al*.(2003): not sensitive : diameters < 8 mm; sensitive (+): 9 < diameters <14 mm; very sensitive (++):15 < diameter <19 mm and extremely sensitive (+++): diameters > 20 mm.

sensitive to EO showing an inhibition zone of 24 mm and with those of Fournomiti *et al*. (2015)

However, as shown in table 2, *S. aureus* was extremely sensitive to the Chinese EO with an inhibition zone of 19.5 mm, and was only sensitive to Algerian EO. On the other hand, this bacterium was resistant to the Chinese AE and sensitive to the Algerian AE with a weak inhibition zone diameter of 11 and 9 mm for the two first dilutions (1 and 1/2). *E. coli* was extremely and very sensitive to the Algerian and Chinese AE with an inhibitory zone of 24 and 18 mm, respectively. This sensitivity disappears at dilution of 1/32 and 1/16 for Algerian and Chinese garlic, respectively. For the EOs, *E. coli* was extremely sensitive to Chinese EO (20 mm) even at dilution of 1/32 and only sensitive to the Algerian EO (10 mm) and the sensitivity disappear at dilution of 1/4. On their study, Chekki *et al.* (2014) recorded an inhibition zone of 7 mm with Tunisian garlic ethanolic extract and no activity for the EO.

Sah *et al.* (2012) tested the effect of garlic AE from Sultanate Omane on the same bacterial genera tested in this study, the last recorded inhibition zone of 26, 23 and 21 mm for *K. pneumoniae, E. coli* and *S. aureus*, respectively. Inhibitions zones of 14.3, 19.3 and 15.6 mm were recorded for *E. coli, S. aureus* and *K. penumoniae,* respectively by Gull *et al.* (2012) for aqueous garlic extract from Pakistan, whereas, an inhibition zones of 21.5 and 20 mm were recorded for *E. coli* and *S. aureus,* respectively for aqueous garlic extract from Sokoto, Nigeria (Garba *et al.*, 2013).

These results demonstrate that both Gram positive and negative bacteria are inhibited by the antimicrobial components of garlic to varying degrees. The three bacterial strains tested in this paper were sensitive to aqueous extract of garlic at least on one of the different dilutions prepared. Similar to this study, other researchers also observed this inhibitory effect against bacteria on broth medium; thus, the results of the present study are in agreement with other studies (Ankri and Mirelman, 1999; Elsom *et al.*, 2000).

Hovana *et al.* (2001) showed that when the concentration of garlic aqueous, methanol and ethanol extracts increased in disks (125 - 500 mg/ml), zones of inhibition were generated for *S. aureus* and *E. coli.* The inhibition zones of garlic extracts in this study were correlated with the high concentration of each disc. According to the low concentration of garlic extracts disk in our study, the zone of inhibition could not be observed. The reported activity of garlic is thought to be due to allicin and other thiosulfinates. Allicin, ajoenes and other thiosulfinates found in garlic have a wide spectrum of antimicrobial activity and this is probably due to the thiosulfinate reacting with thiol groups of various bacterial enzymes. The thiosulfinate structure [S(=O)S] appears to be essential for the bactericidal, antifungal, and antiprotozoal properties of garlic, likely reacting with SH-containing enzymes of these pathogens (Willis, 1956; Tsao and Yin, 2001) such as alcohol dehydrogenase, thioredoxin reductase, and RNA polymerase (Elsom *et al*., 2000). Rabinkov *et al.* (1998) have stated that in addition to allicins antioxidant activity another major biological effect of allicin was shown to be its rapid reaction with thiol group containing proteins.

Conclusion

Garlic is a well know spice consumed as food in many parts of the world either in the raw form or added to cooked food. The qualitative assessment of the antibacterial effect shows that most of the bacterial strains resistant to antibiotics are sensitive to the EO and the AE of garlic from local production and import. Varying diameter of the inhibition zones were observed in at least one of different test dilutions. However, activity was usually observed for the crude extract. Comparing the susceptibility of the studied pathogenic, Gram positive bacteria are more susceptible to the action of EO and AE than Gram negative bacteria, which is consistent with many studies in this regard. The antibacterial effect is proportional to the concentration of the extracts

(EO and AE). It could be concluded that Chinese garlic can be utilized as new cultivars in Algerian cultivation. Furthermore, according to the results, we can recommend taking one to two drops of garlic extracts diluted form (in a vegetable oil or water) daily as a preventive treatment. This investigation was carried out as a model system with a limited number of isolates and further investigations are planned using a range of clinical isolates of sensitive species to ascertain the therapeutic potential of aqueous and essential oil garlic extract.

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